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SUMMARY

FRUIT GROWING

VITICULTURE AND OENOLOGY

VEGETABLE GROWING

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARCHITECTURE

MISCELLANEOUS

FRUIT GROWING


EX·TERRA·AVRVM

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EVALUATION OF THE ENVIRONMENTAL IMPACTS OF FRUIT PRODUCTION USING LIFE CYCLE ASSESSMENT (LCA) (REVIEW)

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Abstract

One important tool for the evaluation of the environmental impacts of fruit production is Life Cycle Assessment (LCA). More than 150 papers published in peer-reviewed scientific journals were selected and reviewed to provide an integrated perspective. In-depth analyses of the methods used were presented according to the specific topics. Conventional and organic technologies were compared for different fruit species highlighting the environmental impacts of their components such as the carbon footprint. Specific topics were reserved for horticultural inputs (fertilizers, phytosanitary protection products) and also for seedlings respectively planting material. The Life Cycle Assessment proved to be a suitable tool to visualize the effects of a decision on the environmental system during the entire life cycle of a horticultural product.

Key words*: carbon footprint; organic technology; horticultural product life cycle.*

INTRODUCTION

Life cycle assessment (LCA) is the comprehensive analysis of a product's entire life cycle in terms of sustainability (Golsteijn, 2020). Hunt & Franklin (1996) mentioned in their study, regarding the origin of LCA in the USA, that the idea of environmental life cycle assessments (LCA) was conceived in the late 1960s and early 1970s, in the same period as in Europe. Harry E. Teasley, Jr., in 1969, elaborated the first formal analytical scheme that was to become LCA, while he was managing the packaging function for the Coca-Cola Company. He presented a study in which the energy, material, and environmental consequences of the entire life cycle of a package from the extraction of raw materials to disposal were quantified. The term LCA was used since 1990 in the U.S.A., the historical term for these environmental life cycle studies being resource and environmental profile analysis (REPA).

Volkwein & Klöpffer (1996) presented the general principles of LCA beginning with the most general basis for evaluation criteria deduced from human rights. The most important human right is the right to life, guaranteed to every person through a series of international regulations. Their thesis stated that the protection of individuals against operations of a third person is indirectly existent because a state cannot tolerate the threat of the life of a person by environmental pollution caused by a third person. This human right is the benchmark of a comprehensive environmental policy.

According to the right to life, the precautionary principle has to be considered by the definition of environmental goals and the valuation of the ecological importance of the impact categories.

The concept of sustainable development including the precautionary approach is found in all the documents since the "Earth Summit" in Rio 1992: Agenda 21 (UN 1992), Rio Declaration (UN 1992), Statement of Forest Principles (UN 1992), Convention on Climate Change (UNEP/WMO, 1992), and Convention on Biological Diversity (UNEP 1992), but also in the most of the documents issued after, like Sustainable Development Goals of Agenda 2030, European Green deal, European Bioeconomy Strategy, European Food and Nutrition Security Strategy - Food 2030, New

DG Agri strategy on Agricultural Research and Innovation, The Common Agricultural Policy (CAP) and CAP 2020+, The European Union Climate and energy package action, European Biodiversity Strategy.

The task of the evaluation procedure in LCA follows the general principles. Each impact can be judged by a set of criteria explicitly or implicitly answering questions regarding the impact on the right of family and private life, the right to the enjoyment of the highest attainable level of health of the population, or the impact on the future generation. There is also an evaluation of the level of danger of the impact or the request of the priority of the use of renewable resources.

Purposes and use cases of LCA. Life cycle thinking aims to increase the sustainability of a product or system along its entire value chain by reducing environmental impacts and at the same time increasing socio-economic performances (UNEP 2020). LCA primarily focuses on environmental impacts alone (ISO 14,040/14,044), assessing quantitatively the environmental impacts of products and services along their value chains. Environmental impacts in the LCA context refer to adverse impacts on the areas of concern such as ecosystem, human health, and natural resources (Peña et al., 2021; Viere et al., 2020; Muralikrishna & Manickam, 2017; Lee & Inaba, 2004).

The leading standards for Life Cycle Assessment are (Finkbeiner, 2013; Klöpffer, 2012; Lee & Inaba, 2004; www.iso.org/standard):

- ISO 14040:2006. Environmental management - LCA - Principles and framework with AMD 1:2020;
- ISO 14044:2006. Environmental management - LCA - Requirements and guidelines with AMD 1:2017 and AMD 2: 2020;
- ISO/TR 14047:2012. Environmental management - Life cycle assessment - Illustrative examples on how to apply ISO 14044 to impact assessment situations
- ISO/TS 14048:2002. Environmental management - Life cycle assessment - Data documentation format;
- ISO/TR 14049:2012. Environmental management - LCA- Illustrative examples on how to apply ISO 14044 to goal and scope definition and inventory analysis.

Muralikrishna & Manickam (2017) presented life cycle phases and processes according to ISO ISO 14,040/44 framework (Figure 1):

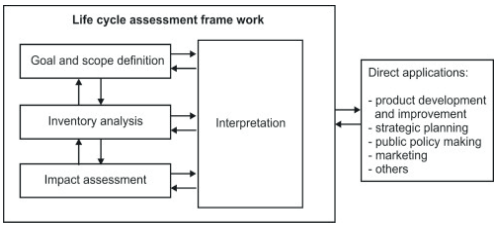

Figure 1. Life cycle phases and process according to ISO ISO 14,040/44 framework (Muralikrishna & Manickam, 2017)

Goal and Scope definition. The goal for LCA has to respond to what are the objectives of performing the LCA study, what are the application areas of the LCA results, and who are the potential audience.

The scope has to present clear descriptions, including the product system, the function of the product system, the product system boundaries, and data category (Lee & Innaba, 2004; Klüppel, 1997; ISO 14040:2006).

The life cycle inventory analysis scheme is presented in Figure 2 (Lee & Innaba, 2004), this stage being focused on data collection and aggregation (Reap et al., 2008; Klüppel, 1997; Neitzel, 1996; Owens, 1996).

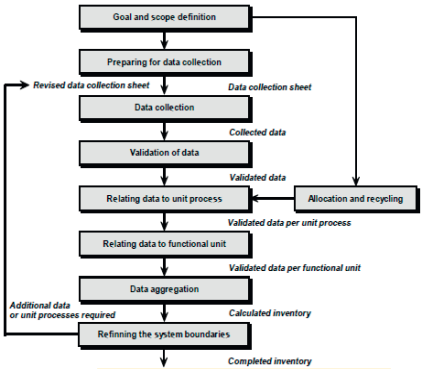

Figure 2. Life cycle inventory analysis (Lee & Innaba, 2004)

The life cycle impact assessment scheme is presented in Figure 3 according to Lee & Innaba (2004).

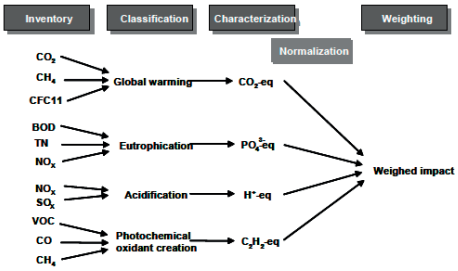

Figure 3. Life cycle impact assessment (Lee & Innaba, 2004)

Life cycle interpretation contains three key elements as defined by ISO 14044: (1) the identification of key issues, (2) the evaluation (including checking completeness, sensitivity, and consistency), (3) development of conclusions together with recommendations (Lee & Innaba, 2004; ISO 14044:2006).

LCA involves a complex analysis beginning with data collection, processing, and analysis. Viere et al. (2020) presented a comprehensive scheme with the software used in LCA (Figure 4).

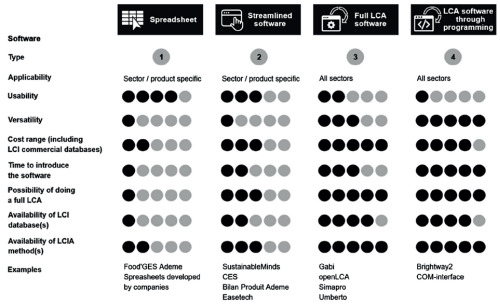

Figure 4. Software used in LCA (Viere et al., 2020)

The objective of this review was to present the evaluation of the environmental impacts of fruit production using LCA through published articles inventory.

ENVIRONMENTAL IMPACTS OF FRUIT PRODUCTION USING LIFE CYCLE ASSESSMENT

There were several topics analysed, components of the evaluation of the environmental impacts of fruit production using Life Cycle Assessment: (A) agricultural ecosystem, land use, crop species choice, specific methodology; (B) pesticide and fertilizers evaluation; (C) fruit species crop cultivation and (D) fruit residues.

For the first topic, a selection of articles focused on the agricultural ecosystem, production, evaluation of agro-food products, details regarding the nutritional function of food, land use impact with some soil quality indicators in the context of life cycle assessment was made (Table 1).

Table 1. Studies on environmental impacts of agricultural ecosystems (mainly fruit production) using life cycle assessment

A. agricultural ecosystem, land use, crop species choice, the specific methodology	
agricultural production	Cowell & Clift, 1996; Li et al., 2019
urban agriculture	Peña and Rovira-Val, 2020
fruit and vegetable production and evaluation of impact mitigation practices, Spain	Martin-Gorriz et al., 2020
products from agro-based companies	Mfitumukiza et al., 2019
agri-food, Portugal	Morais et al., 2016
field emissions in the carbon footprint of agricultural products	Peter et al., 2016
nutritional functional units in commodity-level life of agri-food system	McAuliffe et al., 2020
nutrition function of foods	Weidema & Stylianou, 2020
food consumption and nutrition	Nemecek et al., 2016
consequences of diet	Carlsson-Kanyama et al., 2003
review, environmental product declarations	Schau & Fet, 2008
transport	Sim et al., 2007
agricultural strategic development planning	Tsangas et al., 2020
Agri-food process and circular economy	Stillitano et al., 2021
land-use through kiwifruit production	Coelho & Michelsen, 2014
land use impacts on the natural environment	Cowell & Lindeijer, 1999; Koellner & Scholz, 2008; Milà i Canals et al., 2007; Müller-Wenk & Brandão, 2010; Koellner et al., 2013
soil compactation indicator	Garrigues et al., 2013
soil acidification and nutrient management	Peters et al., 2011

In the second topic (pesticide and fertilizers evaluation), are highlighted researches regarding pesticide-related toxicity impacts of crops, comparing nutritional value and yield as functional units in the environmental assessment of horticultural production with organic or mineral fertilization; distribution, and budget of nutrients in a commercial apple orchard; environmental and agronomical assessment of three fertilization treatments applied in horticultural open field crops; horticulture and orchards as new markets for manure valorisation with less environmental impacts; fertilizers production and human health impacts of more pesticides (Table 2).

Table 2. Studies on environmental impacts of pesticide and fertilizers in fruit production

B. Pesticide and fertilizers evaluation	
pesticide emissions for LCA of agricultural products	Hauschild, 1999
organic waste treatments yielding the most relevant organic amendments and fertilizers, France	Avadí, 2000
human health impacts	Fantke & Jolliet, 2016
pesticide assessment	Hellweg & Geisler, 2003; Rosenbaum et al., 2015
production	Gaidajis & Kakanis, 2021; Wu et al., 2021; Muñoz et al., 2018a,b
manure for orchards and horticulture	Fangueiro et al., 2021

In the third topic, more studies regarding the evaluation of fruit crop impact on the environment using LCA are listed.

Production systems, semi-intensive and intensive, extensive and intensive production, determining cropping patterns with emphasis on optimal energy consumption, organic conversion, organic farming is also presented.

For different species like almond, apricot, apple, blueberry, peach, pear, pomegranate, raspberry, strawberry, walnut, the impact of their cultivation technology was evaluated. Several studies focused on different fruit species evaluation, comparing apples with oranges; pistachio, almond, and apple production in Greece; pistachio, nectarine, peach, and apple in Iran; apple, banana, orange, peach, pear in China. In the end, two studies on the assessment of fruit residues are listed (Table 3)

Table 3. Studies on environmental impacts of fruit production using life cycle assessment

C. Fruit species crop cultivation	
Almond	
almond production, California, USA	Marvinney & Kendall, 2021
Apricot	
comparison orchard systems, Italy	Pergola et al., 2017
Apple	
apple-growing, Switzerland	Mouron et al., 2006a, b
apple production, New Zeeland	Milà i Canals et al., 2015
conventional and organic apple production in Nova Scotia, Canada	Keyes et al., 2015
intensive versus semi-extensive apple orchards, France	Alaphilippe et al., 2016
conventional and organic apple production, China	Zhu et al., 2018
organic and low-input apple production, France	Alaphilippe et al., 2013
comparison between ancient apple and Golden delicious production, Italy	Cerutti et al., 2013
comparing domestic versus imported apples, Europe	Milà i Canals et al., 2007
the carbon footprint of southern hemisphere fruit exported to Europe (apple from Chile to the UK)	Iriarte et al., 2021
mulch using in apple orchards, Canada	Bamber et al., 2020; Bamber et al., 2021
drying methods for the production of apple powder, Italy	Marco et al., 2015
apple consumption in Belgium	Goossens et al., 2019
Blueberry	
the carbon footprint of organic blueberry production, Chile	Cordes et al., 2016
organic blueberries, Chile	Rebolledo-Leiva et al., 2016
Citrus	
Comparison between organic and conventional, Spain	Ribal et al., 2017
Peach	
overview of environmental and economic assessments in the fruit sector; red peach production system, Sicily	Ingrao et al., 2015
potential pollutant-induced, China	Wang & Zhao, 2019
packaging, transportation, Japan	Sasaki et al., 2021
production, Portugal	Pires Gaspar et al., 2018

Pear	
the environmental burdens of pear production systems; environmental mitigation; further mitigate environmental impacts by improved nutrient management	Wang et al., 2020
fossil energy use and greenhouse gas emissions in pear production, China	Liu et al., 2010
fertilizer application	Vatsanidou et al., 2020
Pomegranate	
production, Peru	Vázquez-Rowe et al., 2017
Raspberry	
fruit production, Chile	Vásquez-Ibarra et al., 2021
Strawberry	
production, Spain	Romero-Gámez & Suárez-Rey, 2020
protected strawberry productions, Italy	Ilari et al., 2021
Walnut	
seedlings production	Cambria & Pierangeli, 2011.
Comparative studies on fruit species	
pistachio, almond, and apple production, Greece	Bartzas et al., 2017
pistachio, nectarine, peach, and apple, Iran	Ordikhani et al., 2021
review on the farm stage	Bessou et al., 2013; Cerutti et al., 2014; Cerutti et al., 2011
oil palm fruits from Indonesia, and small citrus from Morocco	Bessou et al., 2016
apple and orange, Netherlands	Tyszler et al., 2014
apple, banana, orange, peach and pear, China	Yan et al., 2016
D. Residues	
fruit waste utilization	Srivastava et al., 2021; Tedesco et al., 2019

CONCLUSIONS

Life Cycle Assessment is a relatively new instrument in evaluating the environmental impacts of the product or service from the very first to the very last or from cradle to grave.

Although in the beginning, there were few studies regarding the impact of fruit growing on the environment, our study highlighted the extension of these researches, both specific to a fruit species and on different technology details. Being an efficient instrument for environmental product declarations, connecting the LCA results and the socio-economical needs of producers is important for acceptance by the agricultural sector. Producers can operate with LCA as a tool for increasing their competitiveness.

Further actions are needed to communicate in the form of improvement opportunities, that can be a useful addition to changing producer behaviour and reducing environmental emissions.

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A LITERATURE REVIEW OF LIFE CYCLE COST ANALYSIS TECHNIQUE APPLIED TO FRUIT PRODUCTION

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Abstract

This research aimed to carry out a literature review of the use of life cycle cost analysis (LCCA) in the fruit production sector by analysing its evolution in the last twenty years period. Life cycle cost analysis (LCCA) was an economic evaluation technique valuable that enabled to estimate of the total cost of owning and operating over a given period. At the same time, it provided additional information to supplement LCA-based decision-making. Articles published in peer-reviewed scientific journals were selected and reviewed. They were refined according to references and organized in several groups. Specific topics were selected such as organic and conventional technologies. Cost-hot spots along product life cycle stages were identified and improvement actions were presented.

Key words*: cost-hot spot; life cycle stages; organic technology.*

INTRODUCTION

Life cycle cost analysing (LCCA) is part of the life cycle evaluation methods, highlighting the overall economic cost of a specific product, service, or system (Davis et al., 2005; Perera et al., 2009). It is defined by the ISO standard, Buildings and Constructed Assets, Service-life Planning, Part 5: Life-cycle Costing as an economic assessment, considering all the significant and relevant cost flows of a specific project (ISO 15686-5:2017; RICS, 2016). LCCA is a method for assessing the total cost of owning and operating a product, facility, or a system over a period of time. The life cycle costs are the sum of the direct, indirect, recurring, nonrecurring, and other related costs incurred or estimated to be incurred during the useful life span (Fuller & Petersen, 1996; Woodward, 1997; Fuller, 2010; Gram & Schroeder, 2012; Bosona et al., 2019; Kambanou & Sakao, 2020).

This method can be a powerful technique that enables to making of the most cost-effective decisions at different life cycle stages. The LCCA should be performed early in the design process, giving the possibility to refine the design to ensure a reduction in life-cycle costs (Fuller & Petersen, 1996; Hunkeler & Rebitzer, 2003; Estevan & Schaefer, 2017; Bosona et al., 2019).

As a method, LCCA precedes the extension of environmentally oriented lifecycle thinking or sustainability (Hunkeler et al., 2008; Kambanou & Sakao, 2020). LCCA was first used in the United States by the Department of Defense in the mid-1960s. They applied LCC in the procurement of military equipment, as they found that acquisition costs only accounted for a small part of the total cost for the weapons systems while operation and support costs comprised as much as 75% (Asiedu & Gu, 1998; UNEP LCA Training Kit, 2013; Estevan & Schaefer, 2017). Since then, LCCA was widely used also in the construction sector and nowadays in green public procurement (Perera et al, 2009; Directorate-General for Environment & ICLEI, 2016; Clement et al., 2016; Bucea-Manea-Țoniș et al., 2021). It becomes essential in sustainable public procurement (SPP) (www.ec.europa.eu). A general appreciation for LCC sustain is

important in changing the procurement and budgeting mindset from "the best value for money" to the "best value across the asset life-cycle." Green public procurement is expected to reduce environmental impacts and save resources. Environmental vocabularies for green contract identification were highlighted (Yu et al., 2020). An interesting evolution of the value of green contracts percentage from total contracts was observed. In 2012, Ireland (25%), Netherlands (22%), United Kingdom (19%), Slovakia (17%), Slovenia (10%), Bulgaria (10%), Danmark (9%), Cyprus (9%), Greece (8%), and Hungary (8%) were first top 10 countries (Estevan & Schaefer, 2017). In 2018, France, Switzerland and Ireland had over 35% value of the green contract from all contracts, followed by Sweden, Denmark, Norway, United Kingdom (Yu et al, 2020).

An integrated life cycle evaluation study could combine more methods: Life cycle assessment (ISO 14040:2006; https://eplca.jrc.ec.europa.eu), Life cycle cost analysis, and social Life cycle assessment (the methodology is still underdeveloped) (Swarr et al., 2011; Fiedler et al., 2018).

LCC and LCA are designed to provide answers to different questions. Life Cycle Assessment evaluates the relative environmental performance of alternative production systems for providing the same function. Life Cycle Cost compares the cost-effectiveness of alternative investments or business decisions from the perspective of an economic decision-maker (Norris, 2001; Gluch & Baumann, 2004).

UNEP LCA Training Kit (2013) detailed three approaches for aligning environmental and economic dimensions: defining LCA compatible with LCC, defining LCC compatible with LCA, or a mix of the previous ones. Combined LCA/LCC results help specify eco-efficiency or environmental cost-effectiveness of decisions, as 'cost per unit of environmental improvement'.

Specific instruments were developed for both methods, IT tools and methods (Langdon, 2005), separately or combined.

The objective of this study was to present a review of the Life cycle cost analysis technique applied to fruit production.

LIFE CYCLE COST ANALYSIS TECHNIQUE APPLIED TO FRUIT PRODUCTION

LCC involves methods of financial evaluation that calculate and analyse simple payback, net present value (NPV), and internal rate of return (IRR) (Fuller & Petersen, 1996; Durairaj et al., 2002; Bosona et al., 2019). It implies details about cost categories, bearers, models, and aggregation (UNEP LCA Training Kit, 2013).

For the cost categories, details for economics (cost regarding budget, market, collective, alternative, social, etc.), life cycle stages (R&D, primary production, manufacturing, use, disposal, etc.), and activity types (design, transport, sales, manufacturing, etc.) should be provided.

The cost bearer like producers, supply chain, owner, the user (not the owner), life cycle (all involved), country's society, global society can be evaluated.

Between cost models can be listed steady-state models, comparative static equilibrium models, static optimization models, quasi-dynamic models, dynamic optimization models, dynamic models, system dynamic models, etc.

Net present value, average yearly cost, steady-state cost, annuity, pay-back time, or benefit-cost ratio are used as methods (UNEP LCA Training Kit, 2013).

Very few articles were found on the Life cycle cost analysis method applied to fruit production (selection in Table 1), in accordance with França et al. (2021), Lampridi et al. (2019), de Luca et al. (2017).

Sottile et al. (2020), combined LCA with LCC analyzes and presented the ecological and economic indicators for orchard renewal in Sicily at almond (*Prunus dulcis* L.). Environmental impact categories for modern and traditional almond orchards were evaluated, higher values for global warming and non-renewable energy parameters at the modern one being registered. For the economical evaluation, the best results were registered for modern almond orchids, for all net present values per hectares invested scenario. Interesting results for stakeholders perception evaluation on the relevance of different categories of assessment showed the maximum relevance for LCCA categories (investments, operational costs and rentability)

and minimum for LCA for growers. And minimum relevance for LCCA indicators but maximum for LCA for territorial governance.

Pruning to energy is an important topic for several years for renewable energy resources. Bosona et al. (2019) for almond, apple, olive, vineyard, and peach evaluated the economic impact. The life cycle costs varied from 50.06 €/tw.b. (vineyard chips), 54.67 €/tw.b. (vineyard bale), 65.85 €/tw.b. (olive chips), 71.37 €/tw.b. (apple bale), 94.49 €/tw.b. (peach chips) to 108.90 €/tw.b. (almond chips) in a specific scenario of transport (50 km). The operational cost was about 73% of the total life cycle cost while investment cost represented the remaining 27%. Dyjakon et al. (2020) stated that under 25 km distance between plant and farm is the optimum value for profitable activity in the apple orchard.

A harvester for recovering wood biomass from apple orchards was evaluated using LCCA by Nati et al. (2018).

Tamburini et al. (2015) included in their study apple and pear between the dominant five crops in the Emilia Romagna region, Italy, using LCC combined with LCA. Potential environmental impacts due to the agricultural phase for the production of 1 kg of selected crops was evaluated at 9.70×10^{-2} for apple and 3.76×10^{-1} for pear GWP_{100} (kg CO_2 eq.) and at 7.95 €cent/kg to apple respectively 42.96 €cent/kg to pear for total costs of the life cycle. When quantified of externalities costs deriving from fertilizers and pesticides use, integrating LCA with LCC, externalities calculated from fertilizers emissions were 4.23 €cent/kg (apple) and 6.59 €cent/kg (pear) and for pesticides emissions 7.05 €cent/kg (apple) and 4.06 €cent/kg (pear).

Using a photovoltaic irrigation system was assessed in India for the banana crop (Narale et al., 2013) or in Greece (Taousanidis & Gavros, 2016) for olive orchards. Both studies found the LCC for the PV system lower than the diesel one, being a more economical choice.

A comparison between organic and conventional systems combined with the LCA method was made for several fruit species like bergamot, olive, lemon, and orange. At bergamot, the organic system presented a higher performance (NPV of 91,421.60 € ha^{-1}), rather than in a conventional system (71,921.06 € ha^{-1}). IRR was also 28% greater in organic than conventional system. Orange and lemon organically produced had lower LCC costs than conventional ones (Pergola et al., 2013).

More studies on olive crop economical evaluation were made. The profitability of olive cultivation was higher in the organic system mainly due to the subsidies (Mohamad et al., 2014; Stillitano et al., 2018; Iofrida et al., 2020). Hot spots were identified, along with each phase of the production process, in order to suggest management strategies to reduce production costs and to increase production efficiency (Stillitano et al., 2016).

Weeds control in olive orchards was modeled and using reduced herbicide applications in combination with the no-tillage scenario was the less expensive solution, compared with conventional farming system and zero chemical weeding (De Luca et al., 2018a; De Luca et al., 2018b).

Several studies were focused to assess the profitability of fruit crop species, being an important instrument in policy decisions. In Calabria Region, Southern Italy, economical evaluation through LCC methods showed fig crop more suitable than vineyard and olive (Stillitano et al., 2017).

Table 1. LCCA methods applied in the fruit growing sector

Species	Topics	Source
apple	pruning-to-energy, Poland	Dyjakon et al., 2020
almond*	comparison modern with traditional farms, Italy	Sottile et al., 2020
banana	solar PV water pumping system for irrigation, India	Narale et al., 2013
bergamot*	comparison between conventional and organic cropping systems, Italy	Strano et al., 2017
fig	production "Dottato" cultivar, Italy	Stillitano et al., 2017
olive	photovoltaic irrigation system, Greece.	Taousanidis & Gavros, 2016
olive*	weed control, Italy	De Luca et al., 2018a; De Luca et al., 2018b
olive*	comparison between organic and conventional olive systems, Italy	Mohamad et al., 2014; Iofrida et al., 2020
olive	comparison between organic and conventional olive systems, Italy	Stillitano et al., 2018

Species	Topics	Source
olive	economic feasibility assessment of different olive farming investments in order to identify the key elements to optimize their economic performance	Stillitano et al., 2016
almond, apple, olive, vineyard, and peach	pruning-to-energy	Bosona et al., 2019
lemon and orange*	production in organic and conventional farming, Italy	Pergola et al., 2013
tomato, apple, pear, wheat, and chicory*	environmental and economic impacts of the agricultural production of the dominant five crops in the project area, Emilia Romagna region, Italy	Tamburini et al., 2015
fruit waste	review of evaluation methods in LCC	De Menna et al., 2018

*includes LCA method

CONCLUSIONS

Life cycle cost analysis, besides Life cycle assessment and social life cycle assessment, are decision tools. Knowing and applying them lead to sustainable decisions and investments. LCCA is also a powerful instrument to be included in most of the research and technology transfer projects for universities or research institutes.

Combining LCC with LCA increases the knowledge, even if calculating the costs of the effects of environmental degradation are difficult and some are still in discussion on how to be quantified.

Further researches are required in agriculture and especially in fruit growing production for a better understanding of horticultural systems and better investment decision.

An in-depth analysis could allow integrating policy tools into effective packages that will increase the supply of desired environmental and social goods, ensuring at the same time farmers' economic sustainability.

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INFLUENCE OF CONTROLLED ATMOSPHERE STORAGE ON QUINCE (*CYDONIA OBLONGA*) CULTIVARS QUALITY

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Abstract

The aim of this study is to determine the C.A. storage conditions that can preserve better the organoleptic attributes of quince (Cydonia oblonga Miller, Rosaceae family), depending on the level of CO_2. The three quince cultivars studied were 'Ekmek', 'Bereczki' and 'Tinella', monitored for 350 days. The samples were stored in C.A. conditions with: T: 1°C, RH: 95%, O2-3% and different levels of CO_2, in the Research Center for Studies of Food Quality and Agricultural Products, of the UASMV Bucharest. The following physiological and biochemical measurements: respiration and transpiration rate, maturity index, ascorbic acid content, were compared with organoleptic attributes. After 350 days of storage, from obtained results it was observed a decrease of the ascorbic acid content between 51,4% (for 'Tinella' cv. in 5% CO_2) and 70% (for 'Ekmek' cv. in 5% CO_2). For respiration rate were observed increases between 85% (for 'Ekmek' cv.in 2% CO_2) up to almost 3 times (for 'Tinella' cv. in 0% CO_2), between the initial and final moments. As expected, the CO_2 content preserved better the quality of quinces compared with control (0% CO_2), based on the results obtained from the sensory analysis.

Key words*: ascorbic acid, C.A., mass loss, quince, respiration rate.*

INTRODUCTION

Quince (*Cydonia oblonga* Mill.), is among the oldest cultivated plants, native to Central Asia (Rop et al., 2011), the crop being found mainly in the Mediterranean countries, Germany, Great Britain, Russia, Eastern European countries and South America (Kuden et al., 2009).

Like apple and pear, quince belongs to the subfamily Pomoideae, family Rosaceae, being known for its distinct, sour and astringent fruit taste (Dehghannya, 2018; Karar, 2014; Szychowski, 2014), rich in sclereids (Bădulescu, 2016). It is one of the least cultivated climacteric fruit species in the temperate zone (Rop et al., 2011).

Quince are known as important sources of polyphenols, vitamins and minerals and with high antioxidant capacity (Karar, 2014; Legua, 2013), their consumption having a positive impact on health (Stojanović, 2017). Consumed less fresh (Rop et al., 2011), quinces are of great interest for processing in the form of jams, juices and are used in pastries. Dried quinces are also used as an ingredient in traditional Iraqi (Wojdylo et al., 2014) and iranian food in the form of stew or quince soup (Dehghannya et al., 2018).

The new post-harvest technologies main objective is to find the optimal storage method with maintaining the fruits organoleptic qualities (Oltenacu et al., 2015).

The attributes with the greatest importance in sensory analysis are fruit firmness, perceived by consumers as an indicator off freshness (Cortellino et al., 2017) and taste that is correlated with maturity index (Yoon et al., 2005). One of the most common methods for the preservation of fruit and vegetables is the controlled atmosphere (C.A.). C.A. helps to maintain the organoleptic characteristics during storage (Oltenacu et al., 2013) and prevent loss of fruit quality (Bessemans et al., 2016).

Changes in fruit quality indicators were observed during storage: increased values for dry matter content and maturity index (TSS/TA) and decresed values for firmness (Jan et al., 2012) and ascorbic acid, the final value for each quality indicator depending on the cultivar (Lemmens et al., 2020).

The aim of this study is to determine the C.A. storage conditions that can preserve better the organoleptic attributes of quince (*Cydonia oblonga* Miller, Rosaceae family), depending on the level of CO_2.

MATERIALS AND METHODS

The three quince cultivars studied were 'Ekmek', 'Bereczki' and 'Tinella', monitored for 350 days. The samples were stored in C.A. conditions with: T: 1°C, RH: 95%, O_2:3% and different levels of CO_2, in the Research Center for Studies of Food Quality and Agricultural Products, of the UASMV Bucharest. The physiological and biochemical measurements like respiration and transpiration rate, maturity index, ascorbic acid content, were compared with organoleptic attributes.

Before sensory analysis sessions the evaluators were trained to recognize the basic characteristics of each variety. The sensory analysis questionnaire contain questions in order to evaluate organoleptic attributes like: exterior appearance, color, smell, taste, and texture (crunchiness).

Respiration rate was determined with a static, closed system, in containers with hermetic closure with a volume of 1180 ml with Lambda T NDIR Monitor, ADC BioScientific LTd.. The respiration rate was measured and the results were expressed in mg CO_2/kg/hour (Enciu, 2020; Stan, 2020).

The transpiration rate was determined using a gravimetric measurements (Fante, 2014; Bezdadea-Cătuneanu, 2019) and the results were expressed in g water/100 g f.w./hour.

The quinces total titratable acidity was determined by titration with NaOH 0.1N to 8.2 pH, using the automatic titrator TitroLine. The firmness of the quinces was determined using a piston of 11 mm diameter (Bessemans, 2016; Rizzolo, 2010) of an electronic penetrometer Turoni TR and the results were expressed in kg/cm^2. The total soluble solids content of the quinces juice was obtained with refractive device Kruss DR301-95 (%Brix).

The maturity index was calculated using the formula: TSS/TA, and it was correlated with the values of the taste. The firmness of the samples was correlated with the values of the texture. In this study, one of the main purpose was to compare the organoleptic attributes, provided through trained evaluators, for the three quinces cultivars stored for 350 days in C.A.

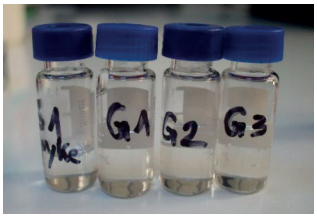

Figure 1. Samples for ascorbic acid evaluation from quince fruits

Ascorbic acid content was determined using the same method described by Bezdadea-Cătuneanu et al. (2017) and Popa et al. (2019). Statistical analyses were performed using Excel, like: mean, standard deviation, ANOVA single factor, T Test and correlations (Pomohaci, 2017; Bezdadea-Cătuneanu, 2019).

RESULTS AND DISCUSSIONS

Ekmek cv. registered a total score of 4.44 points (Figure 2, Table 1) out of maximum 5 points, for the initial moment, for all five organoleptic attributes. After 350 days of storage in C.A. without CO_2, the scores drop at: 2.9 points. Quinces stored in 2% CO_2, could no longer be analyzed. The quinces behavior stored in 5% CO_2 was different, the score drop at: 4.03 points out of maximum 5 points, after 350 days, for all five organoleptic attributes.

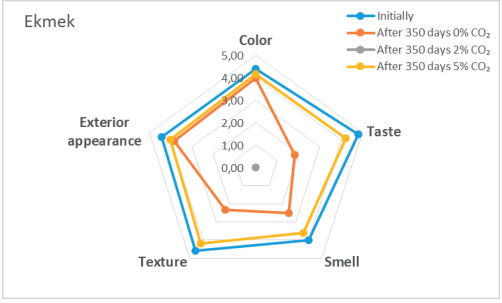

Figure 2. Variation of organoleptic attributes during storage in C.A. conditions for 'Ekmek' cultivar

'Bereczki' cv. registered a total score of 3.84 points (Figure 3, Table 1) out of maximum 5 points, for the initial moment, for all five organoleptic attributes. After 350 days of

storage in C.A. without CO_2, the scores drop at: 2.83 points. Quinces stored in 2% CO_2, recorded a decrease to 3.03 points. The quinces score, stored in 5% CO_2, drop at: 3.38 points out of maximum 5 points, after 350 days, for all five organoleptic attributes.

Figure 3. Variation of organoleptic attributes during storage in C.A. conditions for 'Bereczki' cultivar

'Tinella' cv. registered a total score of 4.56 points (Figure 4, Table 1) out of maximum 5 points, for the initial moment, for all five organoleptic attributes.

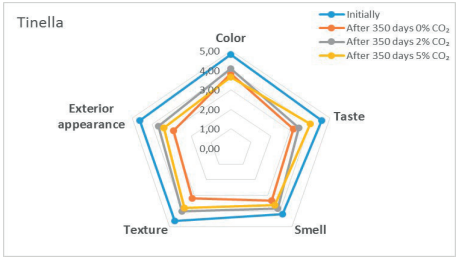

Figure 4. Variation of organoleptic attributes during storage in C.A. conditions for 'Tinella' cultivar

After 350 days of storage in C.A. without CO_2, the scores drop at: 3.28 points. Quinces stored in 2% CO_2, recorded a decrease to 3.80 points. The quinces score, stored in 5% CO_2, drop at: 3.69 points out of maximum 5 points, after 350 days, for all five organoleptic attributes.

All three quince cultivars storage in C.A. without CO_2, presented dehydrated peel after storage.

For 'Ekmek' cultivar, the taste and maturity index (TSS/TA) (Table 5) are negatively correlated. But no correlation was observed between firmness and texture.

Slightly negative correlations were reported for 'Bereczki' cultivar between taste of quinces and maturity index (TSS/TA).

For 'Tinella' cultivar, the taste of the fruit is weakly correlated negatively with maturity index. For 'Tinella', the firmness of the quinces was not correlated with texture.

Respiration rate (Table 2) showed significant differences ($P<0.05$) for samples stored in 0% CO_2, after 210 days, for 'Ekmek' and 'Tinella' cultivars. The tendency to grow maintained up to 350 days. The quince cultivars behaved differently, registering some significant increases compared to the initial moment, thus for 'Ekmek' and 'Tinella', maximum value of respiration rate being registered at 210 days, compared to the initial moment.

Transpiration rate (Table 2) of the samples stored in 0% and 5% CO_2, showed increases after 210 days, followed by a decrease in values after 350 days of storage.

In 2% CO_2, the behaviour of transpiration rate was similar, decreasing at 350 days compared to 210 days, for the 'Ekmek' cultivar and an increase for 'Bereczki' and 'Tinella' cultivars. The values had a decreasing trend at 350 days compared to 210 days.

For 5% CO_2, the transpiration rate increased at 210 days followed by a decrease at 350 days.

Table 1. Variation of organoleptic attributes during storage in C.A. conditions for quince cultivars

			Color	Taste	Smell	Texture	Exterior appearance	Total
Ekmek	Initially		4,40	4,80	4,00	4,60	4,40	4,44
	After 350 days	0% CO_2	4,00	1,83	2,50	2,33	3,83	2,90
		2% CO_2				-		
		5% CO_2	4,17	4,20	3,60	4,20	4,00	4,03
Bereczki	Initially		4,00	3,60	4,20	4,00	3,40	3,84
	After 350 days	0% CO_2	3,83	2,00	2,83	2,33	3,17	2,83
		2% CO_2	4,00	2,17	3,00	2,50	3,50	3,03
		5% CO_2	3,92	3,00	3,60	3,00	3,40	3,38
Tinella	Initially		4,80	4,60	4,20	4,60	4,60	4,56
	After 350 days	0% CO_2	3,83	3,17	3,33	3,17	2,92	3,28
		2% CO_2	4,08	3,42	3,83	4,00	3,67	3,80
		5% CO_2	3,67	4,00	3,60	3,80	3,40	3,69

Table 2. Variation of respiration and transpiration rates during storage in C.A. conditions for 'Ekmek' cultivar

Ekmek		Respiration rate		Transpiration rate	
		Average	Std. Dev.	Average	Std. Dev.
Initially		27,3303	1,7753	0,1153	0,0176
0% CO_2	210 days	33,1916	7,2097	0,0206	0,0097
	350 days	43,0413	12,3647	0,0041	0,0006
2% CO_2	210 days	45,1075	6,7359	0,0248	0,0014
	350 days	23,1412	2,5880	0,0139	0,0014
5% CO_2	210 days	50,4807	2,6739	0,0368	0,0096
	350 days	23,7853	0,9661	0,0289	0,0204

Table 3. Variation of respiration and transpiration rates during storage in C.A. conditions for 'Bereczki' cultivar

Bereczki		Respiration rate		Transpiration rate	
		Average	Std. Dev.	Average	Std. Dev.
Initially		24,4890	8,9511	0,1132	0,0093
0% CO_2	210 days	49,7721	12,7657	0,0131	0,0017
	350 days	49,2052	1,1895	0,0091	0,0022
2% CO_2	210 days	43,7863	6,1143	0,0156	0,0103
	350 days	63,0061	1,4962	0,0214	0,0000
5% CO_2	210 days	54,3866	15,7530	0,0479	0,0064
	350 days	40,8656	11,7781	0,0096	0,0089

Table 4. Variation of respiration and transpiration rates during storage in C.A. conditions for 'Tinella' cultivar

Tinella		Respiration rate		Transpiration rate	
		Average	Std. Dev.	Average	Std. Dev.
Initially		27,3582	1,9520	0,0505	0,0318
0% CO_2	210 days	20,8385	3,0589	0,0205	0,0062
	350 days	78,9048	12,5439	0,0302	0,0087
2% CO_2	210 days	36,5109	3,6134	0,0263	0,0041
	350 days	36,0108	3,8295	0,0318	0,0010
5% CO_2	210 days	60,2983	32,7145	0,0304	0,0011
	350 days	37,6364	3,8392	0,0230	0,0001

Table 5. Variation of maturity index during storage in C.A. conditions for quince cultivars

Cultivars		TSS/TA	
		Average	Std. Dev.
Ekmek	Initially	24,58	3,74
	0% CO_2	50,55	1,72
	2% CO_2	45,88	2,84
	5% CO_2	47,90	0,84
Bereczki	Initially	23,38	0,66
	0% CO_2	36,53	3,52
	2% CO_2	38,16	4,26
	5% CO_2	41,09	1,34
Tinella	Initially	22,23	2,51
	0% CO_2	33,11	0,48
	2% CO_2	49,09	5,64
	5% CO_2	40,59	1,50

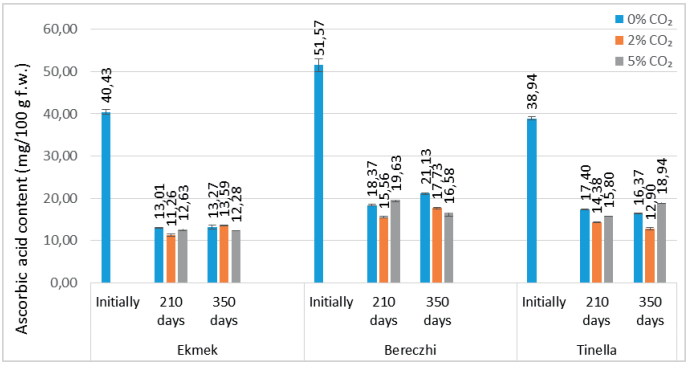

Figure 5. Variation of ascorbic acid content during storage in C.A. conditions for quince cultivars

After 350 days of storage, from obtained results it was observed a decrease of the **ascorbic acid content** between 51.4% (for 'Tinella' cv. in 5% CO_2) and 70% (for 'Ekmek' cv. in 5% CO_2). For respiration rate were observed increases between 85% (for 'Ekmek' cv. in 2% CO_2) up to almost 3 times (for 'Tinella' cv. in 0% CO_2), between the initial and final moments. The total ascorbic acid content of 'Ekmek' quince cultivar (Figure 5), recorded 40.43 mg/100 g f.w., similar value of ascorbic acid content with values registered for blueberries, at the initial moment (Shi et al., 2017). The total ascorbic acid content of 'Ekmek' stored in 0% CO_2 recorded significant differences during storage, decreasing after 210 days (13.01 mg/100 g f.w.), followed by an insignificant increase 13,27 mg / 100 g f.w. after 350 days. In 2% CO_2, 'Ekmek' quinces registered a significant decrease in the total ascorbic acid content, the value being 11.26 mg / 100 g f.w. at 210 days. For 'Ekmek' cultivar stored in 5% CO_2, the maximum value was recorded at 210 days (12.63 mg/100 g f.w.), and the minimum value at 350 days (2.777 mg/100 g f.w.).

For 'Tinella' cultivar (Figure 5), the total ascorbic acid content recorde 38.94 mg/100 g f.w. for the initial moment, progressive decrease at 210 days (17.40 mg/100 g mv) and 350 days (16.37 mg/100 g f.w.). In 2% CO_2, quinces had a significant progressive decrease for total ascorbic acid content, the value being 14.38 mg/ 100 g f.w. at 210 days, decreasing to 12.90 mg/ 100 g f.w. at 350 days. For quinces stored in 5% CO_2, the maximum value was recorded at 350 days of storage (18.94 mg / 100 g f.w.), and the minimum value at 210 days (15.80 mg/100 g f.w.). Rop et al. (2011) recorded values between 41.12 mg / 100 g f.w. and 78.90 mg/100 g f.w. similar values with the initial moment, for all three quince cultivars studied.

CONCLUSIONS

The CO_2 content preserved better the quality of quinces compared with control (0% CO_2), based on the results obtained from the sensory analysis.

The total ascorbic acid content decreased sharply during storage for all cultivars, under all conditions.

Respiration rate increased for 'Ekmek' cultivar in 0% CO_2 and decreased in the 'Ekmek' cultivar in 2% and 5% CO_2. For 'Bereczki' and 'Tinella' cultivars, respiration rate increased in all storage conditions. The transpiration rate decreased for all cultivars, under all conditions.

However, further research it requires for the changes appeared between physiological and biochemical, to highlight the influence of temperature on quinces.

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THE EVOLUTION OF THE ALMOND CROP TECHNOLOGY - A REVIEW

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Abstract

The almond (Amygdalus communis L.) is one of the first plants to be cultivated by man since prehistory. Often the almond is mentioned in the ancient religious texts, being appreciated for its tasty and healthy fruits, but also the plant as a whole is known as a symbol of hope. The Greeks and the Romans can be considered the first almond growers, spreading this species in their colonies. Nowadays, this crop evolved from solitary trees through the vineyards, to intensive orchards and more recently, to high and super high-density orchards. New resistant cultivars to specific climatic conditions, specific rootstocks, and technology contributed to the development of almond crop technology. In this review, we point out the evolution of the cultivation technologies for the almond crop with their specific characteristics like the planting density, the rootstock vigour, the cultivars specificity, the training, and the pruning systems, the irrigation, the soil management, the fertilizers, the plant protection, etc. This paper can be a useful tool for anybody interested in almond crop technology.

Key words*: Prunus dulcis, rootstock, cultivar, canopy, pruning system.*

INTRODUCTION

The almond (*Amygdalus communis* L.) is a deciduous tree native to South-West Asia. Almonds were collected into the wild, some 10,000 years ago, and were among the first plants to be domesticated by man, around the third millennium BC (Albala, 2009). The kernels are very nutritious and relatively non-perishable food, with many pharmaceutical uses, being among the medicinal plants to be prescribed and mentioned in the ancient books of medicine and religious texts.

This review is about the evolution of almond crop technology, from the beginning to present times.

Although not much written information succeeded until our days, about how the almond orchards were managed from the ancient times to the late modern period, there is clear evidence that in the Mediterranean basin and Asia, almonds were an important crop. Almond clonal propagation by grafting has been known since ancient Greek and Roman times, dating back to more than 2,000 years ago, from Columella (Batlle et al., 2017). Also, many important ancient medicinal texts from Hippocrates, to Charaka Samhita, Suśruta-saṃhitā, to the medieval period of Avicenna or Hu Sihui, or all the recipes mentioned in the ancient and medieval cookbooks like De re coquinaria, Kitab al-Tabikh, The forme of cury and others (Socias et al., 2017), indicates the importance of the almonds. It is important to state that the almonds are mentioned in Greek mythology, The Bible, The Torah, The Quran. In conclusion, almonds were and are an important part of human's life, in a socio-economic manner, spiritual, medicinal, culinary, art, etc. (Socias et al., 2017)

Little is known about how the almond orchards were managed in the past, that is why this paper will examine how the technology evolved in the last century.

The paper will try to examine the traditional, intensive, and super high density (SHD) almond orchard systems, with their particularrities, and what differentiates them. What rootstocks and varieties are used for each orchard system, the planting distances, the type of training and pruning, how the pests and diseases, the soil and the row weeds are managed, the irrigation system how the trees are fertilized and the fruits are harvested. Pointing out the different almond orchard systems, could offer a better understanding indicating how to choose this crop or another, a technology crop or the other, how a farmer can improve himself, etc.

At a worldwide level, in North America and Australia, this crop gained a lot of popularity

among the farmers. In Europe, Africa, and Asia, the tendencies are recording a decrease of the cultivated surface, with several exceptions.
In Romania, the quantities produced and the land occupied by the almond crop also decreased in the last 30 years.
According to FAOSTAT (Figure 1), in the last twenty years, the almond production with shell doubled and the area harvested with almonds extended by 25%.

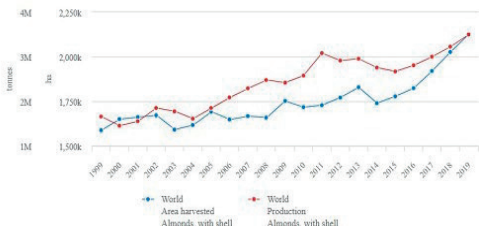

Figure 1. World production of almonds and the area harvested with almonds (1999-2019 period)

The most recent advances in the almond crop technology could represent an opportunity to disseminate information in the scientific world and farmers, that could lead to satisfying the Romanian market needs for almonds. Low vigor rootstocks, late and very late blooming cultivars, mechanical pruning, automatized irrigation systems, mechanical harvesting, and other advanced management practices could inspire the farmers to adopt this crop.
The review aims to highlight the new almond orchard systems and technologies.

MATERIALS AND METHODS

To achieve the goal there will be an extensive description of each crop technology taken into consideration, namely traditional, intensive, and super high-density almond orchards.

RESULTS AND DISCUSSIONS

Traditional almond orchards

The traditional almond orchards are to be found, in general, in Europe, Northern Africa, and Asia. The traditional almond orchards are included among "traditional agricultural landscapes", popular in the Mediterranean basin. The traditional agricultural landscapes are described by the use of land that has led to substantial changes to the original conditions, but has also maintained the functionality of the natural systems or has replaced it with other systems that are compatible with the local environmental conditions (Antrop, 2005).
Frattaroli et al. (2014), argues that in Italy, the traditional almond orchards were mixed, sometimes with olive trees, or were planted in a pure crop. The ones that were pure crop, are regular, with more than 50 trees per hectare, or irregular, with less than 50 trees per hectare. In Italy, it is still the most widespread almond crop technology (Sottile et al., 2014).
In Spain, some of the traditional almond orchards are also pastures for animals, as the Spanish call it "Dehesa de Almendros" (Font et al., 2010), or non-irrigated orchards with low-density planting distances like 8 m × 8 m or even wider, to make more efficient the use of the limited rainwater (Gradziel et al., 2017).
In the Middle East, generally, the almond grows under rain-fed conditions, water being hard to access resource.
In Romania, the almond growing zone is quite the same as the grapevine, that is why, traditionally, the almond trees are found through vineyards (Cociu, 2011).
In the traditional orchard system, the grafted almond trees were produced using seedlings as rootstocks (Cordeiro & Monteiro, 2002).
Most of the varieties used were local selections.
The traditional almond orchards are characterized by a low density, planting distances being 8 m x 8 m, or wider (Frattaroli et al., 2014).
The training and pruning are summary or not being done at all (Sottile et al., 2014). Pests and diseases control, soil management, inter or intra row weed management, in most of the cases, is not realized or is briefly done (Frattaroli et al., 2014). The trees are irrigated under rain-fed conditions and in most cases, are not fertilized. The harvest is done manually (Frattaroli et al., 2014).
The traditional almond growing system is less important for the profitability of this crop or the almond yields, but still, the almonds cultivated traditionally, are offering ecosystem services and producing fruits in deserted areas where not many fruit trees can live.

Intensive almond orchards

The intensive almond orchards are the most common ways this crop is cultivated

worldwide. It is also the most widespread system for almond cultivation in Romania since all the recommendations are for wide-spacing planting (Cociu, 2011).

The development of the intensive almond orchards is correlated with the presence of the almond breeding programs, thus countries with important cultivated surfaces of intensive orchards had or have successful breeding programs. The recent almond breeding programs developed superior rootstocks and varieties, better adapted to local pedo-climatic conditions. In general, the breeding programs objectives varied from zone to zone. The main objectives of the almond breeding programs around the world were nut quality, late-blooming, self-compatibility, drought tolerance, resistance to different fungi, bacterial diseases, and insects, etc. (Segura et al., 2017).

Generally, the rootstocks used in the intensive almond orchards are GF-677, Garnem, Felinem, Nemaguard, seedlings, and others. In Romania, Felix and Tomis 1 are the rootstocks used (Şcheau, 2013).

A large number of varieties with commercial significance and different traits are being used, like Nonpareil, Texas, recently Butte and Monterey in the USA (Almond Board of California) and Nonpareil in Australia (Almond Board of Australia), Filipo Ceo, Tuono, and Cristomorto in Italy, Ferragnes and Ferraduel in France, Desmayo Largueta and Marcona, more recently Antoneta and Guara in Spain, Mărculeşti 3/51, Tohani 17, and more recently, Ana, April, Mirela and Veronica in Romania (Gavăt et al., 2015). Most of the varieties mentioned above are self-sterile, have an early or medium-late blooming, being frequently affected by the spring frosts, and need to be pollinated. Also, it is important to know the chilling and heat requirements of a variety, before choosing it for a new orchard (Gaeta et al., 2018).

In the last decades, it became a necessity to realize intensive orchards with self-fertile and late-blooming varieties like Tuono, Antoneta, Guara, and other varieties.

Planting distances in Europe are ranging from 8 m x 8 m to 5 m x 4 m, from 156 trees to 500 trees per hectare (Cociu, 2011), while in California the common distance is 7.3 m x 7.3 m or even wider (Hendricks, 1996).

The fruit trees canopy is trained as an open vase and most of the pruning is done manually, with the possibility to apply the topping pruning, mechanically (Cociu, 2011).

Pests and diseases are managed manually or mechanically by spraying pesticides, using pheromone traps, and other cultural practices (Perju, 2002). Regular monitoring of the pest populations can predict potential problems. Keeping records of the monitoring results will help forecast pest outbreaks and schedule cultural practices. Table and graphs of pest counts can help identify population patterns (Flint, 2002).

Soil management is done mechanically. The space between rows is maintained tilled or with cover crops like leguminous and other species. Ramos et al. (2010) argue that cover crops in semi-arid conditions improve soil quality, compared to frequently tilled managed soil by increasing the organic matter content, improving the chemical and physical fertility of the soil, and enhancing the soil biology activity, in a wide-spaced 7m x 14 m rainfed almond orchard. Becerra et al. (2010) point out that soil compaction resulting from tractor traffic, increases soil cone index and soil bulk density, decreasing soil porosity, indicating that almond orchard soil is unable to limit subsoil compaction under moderate traffic intensity.

Weed control is realized with herbicides or mechanically by tilling with inter-row rotary harrow or hoe or other devices through tillage, burning/flaming the weeds, or mowing. Intra row weed control it's done with herbicides (Ludwig et al., 2020). A study about mulching as an alternative technique for weed management draws attention to black geotextile and almond husks as organic mulch, which are successful alternatives to glyphosate applications for managing weeds (Verdu & Mas, 2007).

Most of the intensive almond orchards are not irrigated in Europe, Africa, and Asia. Some researchers have demonstrated that regular deficit irrigation and sustained deficit irrigation are financially feasible alternative methods to full irrigation, in semi-arid zones where water price is higher and hard to obtain. These irrigation treatments could help farmers to preserve production levels while ensuring the feasibility of the almond plantation investment

(Alcon et al., 2013). It is worth adding that Lipan et al. (2019) observed that in regular deficit irrigation and sustained deficit irrigation, there aren't significant differences in the lipid and minerals content, moreover in some regular deficit treatments there were discovered higher fat, potassium, and unsaturated fatty acids content. Another study observed that during the 3-year experimental period and two successive cycles of harvest-period irrigation deprivation resulted in yield reductions that were associated with reduced shoot growth in severely watered stressed trees. The water stress did not influence flowering and fruit set on established spurs, nor did it accentuate the spur mortality (Esparza et al., 2001). In California, nearly 80% of the almond orchards are using micro-irrigation (Schwankl et al., 2017). Some authors argue that in some parts of the world the production increases exponentially about the amount of irrigation water, being able to reach 3,000 kg of kernels/ha.

Fertilization is a base practice in managing an intensive orchard. Fertilization is done by incorporating manure 30 to 40 tons per hectare, once every three to five years, and complex NPK fertilizers, every winter (Cociu, 2011). For the young orchards, it is recommended that the annual dose of NPK be distributed as follows: in the autumn, after the leaves have fallen, the full P dose, half of the K dose, and of ¼ the annual N dose. The rest it's applied in the spring, when the shoots start to grow, half of the N annual dose, and half of K annual dose. The rest of ¼ N, it's applied at the beginning of June, when the buds start differentiating (Davidescu & Davidescu, 1992). It is important not to apply higher doses than necessary of N, after sampling the soil and leaf status, because the susceptibility of hull rot increases (Saa et al., 2016). It has been demonstrated that boron is an important micronutrient that increases fruit set if applied before the flowering period of a plant (Hanson et al., 1985), in some cases the increase can reach 100% like in the case of sour cherry, with post-harvest and pre-bloom foliar boron fertilization (Hanson, 1991). Nyomora (1997) observed that the fruit set was increased by 130% and the yield by 53% for the Butte cultivar, after applying B in the fall.

Also, it is recommended to apply fertilizers along with the foliar spraying of pesticides.

The harvesting is done manually or mechanically. Mechanically is done by shakers, with at least two workers being used (Pascuzzi & Santoro, 2017). Shakers have the disadvantage of injuring the almond tree trunks during the harvest, which results in serious damage to tree vigour, health, and longevity. Injuries where the bark is crushed or torn from the trunk immediately attract a variety of insects, several of which are known vectors (Connell et al., 2005).

The intensive almond growing system is the most widespread and the first option when farmers choose to develop a new almond orchard. The initial investment, popularity, long-term certitude of the profitability, the evidence that others succeeded in realizing intensive orchards, etc., are important points of view when farmers choose not to risk applying newly developed technologies (Almond almanac, 2020). The intensive system's weaknesses are the necessity of specialized workers, trees training and pruning, harvesting, and so on. The need for irrigation in Europe, Asia, and Africa, on contrary, the excessive use of water in California for producing almonds became a state problem. The rootstocks and the varieties used, in many parts of the world, are pretty old and an advance towards renewing them would be a benefit for the production.

Super high-density almond orchards

The technology is advancing in horticulture and almond crop has known it recently, too.

Super high density (SHD) is said to be the future of the almond orchards. In the last decades, different researchers in Europe worked to develop a new almond crop technology (Iglesias, 2019a). More recently, the development of low vigor rootstocks had allowed testing higher trees densities per hectare. The novelty of SHD almond orchards consists of enabling to fully mechanize the orchard management operations (Iglesias, 2019b).

The mechanization reduces and solves the deficit of labor in horticulture, especially specialized workers. Also mechanizing the work results in lower costs saves time, improves workers' safety, reduces labor requirements

and production costs, and increases the quality of the products (Carbo et al., 2017).

The long-term effects are currently unknown, that is why at the moment, this new crop technology is still tested. Although California has the highest area harvested with almonds in the world and is the no.1 producer, the academics and the farmer's perspective over the SHD orchard system was conservatory over implementing it. In Spain, Portugal, and also Italy, the SHD model is developing more quickly (Maldera et al., 2021).

A study realized in 2018 and 2019, in an experimental almond orchard in Spain showed that the fruit set rates were similar in both SHD and open-center training systems. The average almond in shell weight was significantly higher in an intensive orchard where trees were trained in the open-center system, while the fruit yield was significantly higher in the SHD one. It follows, then, that the SHD training system would be more efficient, although less productive than the open-center training system (Gascón et al., 2019). In the same study, it is argued that the almond trees are prone to an alternate-bearing pattern (i.e., previous year fruit-bearing harms subsequent year flowering), which can be more or less pronounced depending on the specificity of the cultivar. That is said because two years were not enough to formulate appropriate conclusions.

It is believed that the SHD model offers advantages in the effectiveness of the phytosanitary treatments, in the management of water savings in irrigation, minimizing the soil maintenance, early yields, the possibility of harvesting with over-the-row machines or robotic harvester, the labour reduction, therefore results in an improvement of the profitability of the crop (Gascón et al., 2019).

The hedgerow is fully developed between the 3rd to the 5th year, this is the moment when the maximum production is attained while, in the intensive system, the maximum production is attained only after 10 to 12 years (Cociu, 2011). Thus, results that the initial investment is recovered more quickly, also higher profitability than in the intensive system.

The rootstocks recommended for SHD almond orchards are the low vigor ones, like Irta-1, Rootpac 20, Rootpac 40, Krymsk 86, Ishtara, or GF-677 (Duval, 2016). Low vigour rootstocks could be associated with a higher yield efficiency that is making it ideal for high-density orchards with the benefits of reducing labour costs, especially for pruning and harvest (Yahmed et al., 2016). Gascón et al. (2019), argues that the low vigour rootstocks are favouring smaller and more efficient canopies, and at the same time, reduce production costs due to better efficiency of water and fertilizers, better accessibility to the canopy, and easier mechanization. Since the market doesn't offer enough possibilities of low vigour rootstocks at the moment, a good alternative is GF-677. GF-677 is known as one of the best rootstocks for the almond crop and its medium-high vigour and excessive growth will be tempered by the high-density planting. The varieties used have high commercial significance. Independence, in the USA (Almond Board of California), Tuono and Supernova in Italy, Lauranne in France, Vayro, Marinada (Vargas et al., 2009), Penta (Dicenta et al., 2009), Belona (Dias et al., 2018), Soleta and Makako in Spain (Dicenta et al., 2018). Most of the varieties mentioned have good agronomic characteristics, are self-fertile, have medium or low vigor, have a late to very late blooming time, and are very productive (Socias et al., 2017).

Planting distances are ranging from 4 m x 2 m to 3 m x 1 m, from 1,250 trees/ha to 3,333 trees per hectare. The planting distances can differ from country to country and are based upon the latitude of the area of planting. For example, for 40° latitude North, 3.35 m between rows is an optimum distance. In Romania, for 45° latitude North, it is required 4.0 m between rows (Iglesias & Torrents, 2020).

The fruit trees canopy is trained as a single central axis. The pruning is done mechanically by limiting the height and the width of the fruit trees row, creating a hedgerow. Trees planted more densely are smaller being easier to fill the canopy space. They are less likely to have scaffold breakage problems regardless of how they are trained Gascón et al. (2019).

Pests and diseases are managed mechanically by spraying pesticides, using pheromone traps, and other cultural practices (Perju, 2002). Based on phenological observations, growers could easily monitor developmental stages and schedule timely various agronomic manage-ments such as frost protection, pollination, fruit

thinning, irrigation, fertilization, pruning, pests and diseases management, and harvesting (Sakar et al., 2019).

The soil is managed mechanically. The space between rows can be maintained by no-till, tilled, or covered with green crops like leguminous and other species, soil work being reduced to a minimum. On the row, weed control is realized with herbicides or mechanically by tilling with intra row rotary harrow or hoe (Socias et al., 2017).

It is a necessity to irrigate the SHD almond orchards, even with a deficit of water, the most recommended being drip irrigation.

Fertilization is a necessity in a SHD almond orchard. Fertilizers application is preferred to be done when the plant has the most need of them. If the soil is not tilled, fertilization is required to be done by fertigation also by applying fertilizers with foliar spraying of the pesticides. (Muhammad et al., 2017)

The harvesting is done mechanically by machines, such as the over-the-row harvesters of olive hedgerows and grapevine's trellis. This innovation offers a better fruit quality. The fruit does not touch the ground, thus contamination risks by aflatoxins and salmonella are avoided (Carbo et al., 2017).

The SHD system is the last almond crop technology improvement, and to summarise its advantages, less human labour is needed, rapidly entry into full production, higher effectiveness, on the long term could be more profitable by paying less on human labour, on water, and pesticides, also new and more qualitative and efficient rootstocks and varieties are being used, fully mechanized pruning and harvesting, and better harvesting machines. As for disadvantages, for the moment, it is an orchard technology still in test, in the long term all the good and bad effects can't be anticipated. It is unknown exactly how a SHD orchard will behave 20, or 30 years after being planted. What would be the yields, how the yields evolve in relation with the almond predisposition to alternate-bearing, long-term management, and evolution of the pests and diseases in an SHD orchard. Another disadvantage is the higher cost of orchard establishment, plus not enough nurseries and rootstocks diversity (Socias et al., 2017).

To better view and understand the differences between each crop technology, Table 1, is presented, the summarized characteristics of the main almond orchard systems, like what rootstocks are being used, the varieties characteristics, the planting distances, the trees training, the pruning, the pests, diseases and weeds management, the irrigation, the fertilization, and the harvesting.

Like a quick insight into the strengths and weaknesses of each crop technology, traditional, intensive, and SHD.

Table 1. The summary of the almond orchard systems characteristics

Orchard system	Traditional	Intensive	Super high density
Rootstocks used	Seedlings	Medium to high vigour	Low to medium vigour
Varieties characteristics	Local selections	Self-sterile; Early to medium-late blooming	Medium-late to very late blooming
Planting distances (m)	8 x 8 or higher	8 x 8 to 5 x 4	4 x 2 to 3 x 1
Training	Rarely done	Open vase	Central axis
Pruning	Rarely done	Manual to semi mechanized	Mechanical
Pests & Diseases	Rarely done	Mechanically	Mechanically
Soil Management	Rarely done	Mechanically	Mechanically
Weed management	Rarely done	Mechanically	Mechanically
Irrigation	Rain fed	Rain fed to drip irrigation	Drip irrigation
Fertilization	Rarely done	Mechanically	Mechanically
Harvesting	Manual	Done with shakers	Done with over-row harvesters

CONCLUSIONS

The traditional almond orchards are less and less an option for today's farmers. The traditional orchards offer important ecosystem services but are not concentrated on almond production. It is believed that traditional almond orchards are one of the last options when a farmer is interested in developing a new orchard.

At present, most of the producing almond orchards are managed in an intensive system. This crop technology is the farmer's first option when choosing to develop a new almond orchard. Along the time the possibility of mechanizing increased, resulting in higher profits and larger yields. Until clear evidence of the SHD system's superiority and profitability, it will remain the farmer's first option for the almond orchards.

SHD is a new almond crop technology, developed in the last decades. The SHD model attracts more and more interest in the academic world. Until now the science confirmed that this crop technology offers the possibility to fully mechanize the work, rapidly enter into full production, higher effectiveness than the intensive orchards, lower water and pesticide use, less human labour needed. More studies are needed to confirm its higher profitability over the intensive crop system. The orchard establishment costs, the prediction in time over the stability of the orchard in terms of pests and diseases management, prediction on the long term of yields and profitability, are serious questions that somehow are still unanswered in comparison with the intensive crop system.

The SHD crop system is necessary to be studied more. This model could represent an important option, in the future, for farmers around the world and from Romania, also.

Scientific progress is necessary to evolve, for horticulture, in general, and for the almond crop, particularly.

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PHENOLOGY OF SWEET CHERRY CULTIVARS UNDER THE CLIMATE CHANGE EVENTS AT BISTRITA FRUIT REGION OF NORTHERN TRANSYLVANIA, ROMANIA

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Abstract

The cherry crop is one of the most important horticultural crops both nationally and internationally. In terms of production, Romania is in the top ten countries worldwide, more precisely it ranks ninth, with a production of 682.010 tons recorded in the last ten years. At Fruit Research and Development Station Bistrita, 12 varieties with different ripening periods were monitored during the last three years (2018-2020). Objective of the research was to assess the phenological response to climate change and the behavior of some Romanian and foreign bred sweet cherry cultivars. Observations were made throughout the year for each phenophase. Following the data obtained, the number of days assigned to each phenophase was determined, for each year studied (2018-2020). Results reveals that the instability of climatic factors directly influenced the vegetation period of the cherry crop. Data showed that in the inflorescence emergence and flowering period due to climate change events there is a possible risk for late frost events. Phenology datasets in relationship with weather data modelling are bioindicators for climate change events and are key elements in fruit crop technology.

Key words: *crop, cherry, climatic factors, fruits.*

INTRODUCTION

Sweet cherry (*Prunus avium* L.) is a diploid species, with origins in the Black Sea and the Caspian Sea region, native to south-eastern Europe and western Asia, being a fruit crop with a worldwide economic importance, due to the exceptional nutritional, technological and commercial value of its ovoid or heart-shaped fruits.

We assist nowadays to climate change phenomena throughout the world in the fruit producing areas, which dramatically influences yields, quality of fruits, economical sides of crop production, incomes and marketing prices and therefore the market is directly affected by these fluctuating negative events. The agro-climatical adaptability, ecological plasticity of cherry cultivars, are key elements of both successful research and farming.

Several researches were made in Romania some years ago regarding phenology and frost resistance of trees (Budan et al., 1995; Asanica et al, 2014) in the south zone of the country, but in the northern part of the country there is a lack of recent updated studies regarding phenology and weather impact on cherry fruit crop. Previous researches in Pitesti and Bucuresti area showed the climatic stress in some cultivars which occurred during 2005-2006 and had a great impact on the cherry crop (Budan et al. 2007). Adequate phenological models together with climatical modelling can show predictions regarding relationship of weather and cropping of cherry cultivars in local conditions.

The generative buds of cherries are very sensible and susceptible of freezing, injuring and frost. Frost resistance depends on many factors including age, genetical factors, tissue content of water, sap and soluble solids, environmental conditions (Proebsting, 1982; Melba R. Salazar & Gutierez, 2014). The main problem of cherry cultivation in Romania nowadays is the high age of plantations, with less frost resistance and the new plantations that are not yet in the fruiting period because a great amount of new cherry orchards were established during 2015-2020 in the PNDR reconversion program of Ministry of Agriculture and Rural Development.

Several researches studied the cherry and sour cherry crop and simulated predictions, charts and graphs on bud-brake and flowering dates

(Ladanyi et al., 2009; Apostol et al., 1990; Chitu et al., 2005, 2006), respectively on other woody plants (Davarnejad et al., 1993-apple, 1996; Davarnejad et al., 1996-pear; Hrotko, 1985-Prunus mahaleb; Rachko,1985-pear). Studies provided insights into how plant growth can be affected by such conditions but also possible outcomes of management options (Soltesz, 2004; Spano et al., 2002). The key element of the studies are the sum of the degree days (GDD) initially established by Baggiolini in the early 1952`s (Baggiolini, 1952) and several other studies contributed to the wider understanding of plant physiology terms of dormancy, thus the phenology models mostly had the basis that budding and flowering occurs after the chilling effect during the dormancy and subsequently after an amount of specific heat accumulation.

Objective of the research was to assess the phenological response to climate change and the behaviour of some Romanian bred and foreign-bred sweet cherry cultivars.

MATERIALS AND METHODS

The research was carried out at Fruit Resarch and Development Station Bistrita, in geomorphological unit of Bistrita hills, the climate is cold and temperate. There is significant rainfall during a year (757 mm) in the Bistrita area, the climate is considered Dfb according to Köppen-Geiger classification, with an average annual temperature of 9.6°C (1993-2019). Observations were made in the field throughout the year for each phenophase. Following the data obtained, the number of days assigned to each phenophase was determined, for each year studied.

Extreme climate change events (climatical accidents, hail), which occurred in the study period were assessed visually and weather data registration was made electronically by Adcon Telemetry weather station. Sum of the active temperatures were counted, based on existent weather data from the Bistrita Meteorological Station. We calculated the sum of daily active temperatures, growing degree days (GDD) taken above the basic temperature (5°C) and cumulated from the endodormancy until the begin of flowering. Twelve varieties with different maturation periods were monitored especially during the last three years (2018-2020) but also with a broader analysis of the last 10 years flowering data. The analysed cultivars were, 'Timpurii de Bistrita', 'Bigareau Burlat', 'Rosii de Bistrita', 'Negre de Bistrita', 'Uriase de Bistrita', 'Rubin', 'Germersdorf', 'Jubileu 30', 'Stela', 'Van', 'Kordia', 'Ana'.

RESULTS AND DISCUSSION

Woody fruit species synchronize their physiology including blooming and their annual growth patterns in accordance with environmental conditions, mainly the temperature factor. Stone fruit species are the earliest in the blooming process.

Figure 1 shows the accumulated sum of active temperatures above 5°C between 2012-2020, according the Julian day of the year until the beginning of flowering.

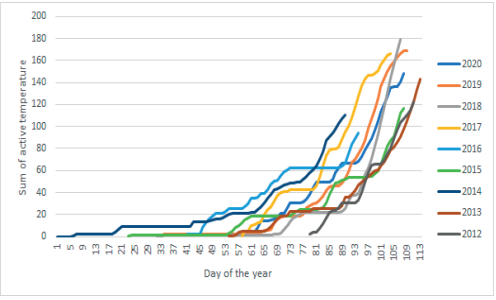

Figure 1. Relationship of the sum of active temperatures and Julian days of the year until flowering

We see very different degree day accumulation patterns with very fast uprising of cumulated temperatures (2012) starting at the 79th day of the year, much later than the coming years. This means that the early 2012-2013 year winter periods were harsher in comparison with the 2018-2020 period. Smoother, progressive accumulation of GDD`s were observed in 2014 (90th Julian day), 2015 (108th Julian day), 2016 (94th Julian day) with much earlier flowering in these years.

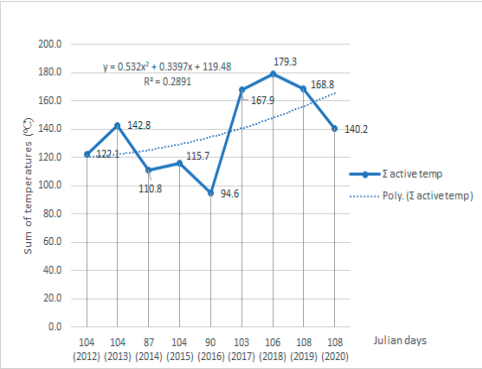

Figure2. Dynamics of the sum of active temperatures in the study period (2012-2020)

Figure 2 shows higher active temperature values until flowering in the last 4 years (2017-2020) at 103-108 Julian days, values ranged from 140.2 to 179.3°C. In the period of 2012-2016 flowering occurred at lower sum of temperatures with a minimum value of 94.6°C at the 90th Julian day.

Data showed in Figure 3 presents the relationship between the Julian day of the year and the average daily temperatures at which flowering occurred. Data showed that values varied between from 87 to 115 Julian days with average of daily temperatures ranging 6.9°C to 12.6°C, average beginning of flowering in research period was at 103 days at 10°C.

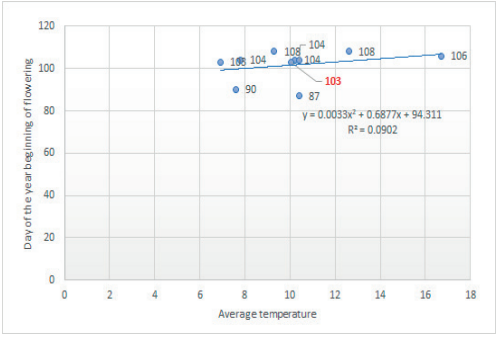

Figure 3. Average daily temperatures and the day of the year at the beginning of flowering

Phenology data for the early variety 'Timpurii de Bistrita' showed (Figure 4) a great variation in duration, in 2014 and 2016 flowering begun early at the end of March and beginning of April. With the coming years study showed that the flowering was shifted from March to the middle and end of April in Northern Transylvania in Bistrita Fruit Region, mainly with 16-17 April during 2018-2020.

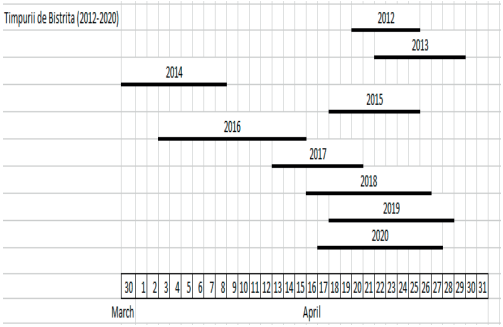

Figure 4. Phenology data for 'Timpurii de Bistrita' cherry cultivar during 2012-2020 at FRDS Bistrita

When analysing the whole range of 12 cultivars, results showed that in 2018 at early cultivar 'Timpurii de Bistrita' and 'Bigareau Burlat' flowering begun on 16th of April and had a duration of 11 days until 26th of April, with 'Rosii de Bistrita' and 'Negre de Bistrita' cultivars, which seem to be in the same flowering category of early cultivars, but with a shorter flowering period (5 days for 'Rosii de Bistrita').

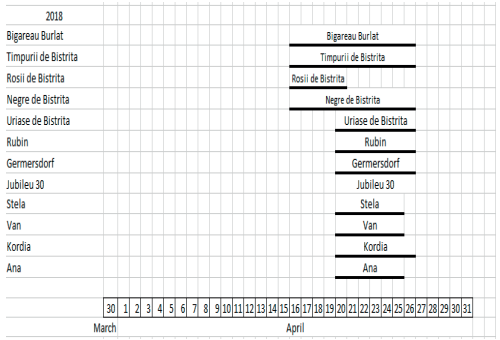

Figure 5. Phenology data for cherry cultivars during 2018 at FRDS Bistrita

Late maturation cultivars begun flowering at 20.04.2018 with a duration of 7 days of flowering. In 2019 (Figure 6) flowering occurred between 17-28th of April for the early cultivars ('Bigareau Burlat', 'Timpurii de Bistrita', 'Rosii de Bistrita', 'Negre de Bistrita') and some of the late maturation cultivars ('Jubileu 30', 'Stela', 'Van' , 'Kordia', 'Ana'). The late maturation cultivars

begun the flowering 2 days later ('Uriase de Bistrita', 'Rubin', 'Germersdorf').

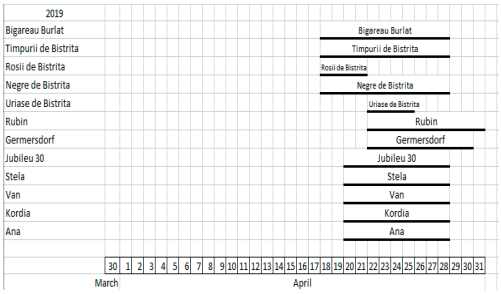

Figure 6. Phenology data for cherry cultivars during 2019 at FRDS Bistrita

Significant changes occurred in 2020 regarding flowering (Figure7), early ripening cultivars begun the flowering almost in the same time period as in 2018-2019 period (17-27th of April) but the late maturation cultivars 'Uriase de Bistrita', 'Rubin', 'Germersdorf', begun the flowering much later, between 27th of April and05th of May.

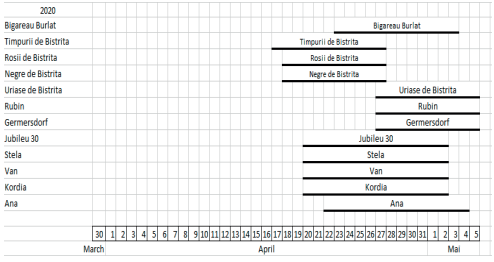

Figure 7. Phenology data for cherry cultivars during 2020 at FRDS Bistrita

A frost event occurred in April 2020 in April, and the early cultivars were seriously affected, yield being significantly reduced. Oppositely, the late maturation cultivars 'Uriase de Bistrita', 'Rubin', 'Germersdorf' begun the blooming later, so they escaped the early frost events, yield was not affected at these cultivars. In the last three experimental years (2018-2020), the blooming of cherry cultivars was between 16-28th of April and had a duration of 11-13 days, with average blooming date of 20th April. When analysing the 2012-2017 period, we can observe that the blooming occurred much earlier, between 30th March – 08th of April in 2014 and between 03-15th of April in 2016; the years 2012, 2013, 2015, 2017 had overlapping blooming periods between 13-29th of April, and in these years the average blooming date was 16th of April. The general analysis on the whole experimental period showed that the average blooming date for time period 2012-2017 (16.04) shifted with about four days (20.04) in the last 3 years (2018-2020) which clearly is due to climate change phenomena. This tendency will be more pronounced, if we will assist to a much more heat accumulation and warming up phenomena of the weather, especially in the winter period, before blooming in spring.

CONCLUSIONS

This study shows the shifting process of flowering into more lately dates, especially for late cultivars which bloomed till the beginning of May, climate changing events, such as, warmer winter periods influenced the blooming, promoting the appearance of first flowers in later Julian days. The great variation of temperatures in spring affects mainly the early cultivars, which has an impact on market prices and the investment costs of farmers in the technology. Temperature factors cannot be influenced directly, thus appropriate establishment of orchards with cultivars according flowering period, detailed phenology modelling is the key for proper choosing of the optimum cultivars in a specific geographic area.

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PRELIMINARY RESEARCH ON THE LEAVES MICROBIOTA OF EDIBLE CLIMBING ROSE UNDER ORGANIC MANAGEMENT

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Abstract

Little is known about the fungal communities of rose leaves, the interactions between and with the main pathogens or their response to abiotic and biotic stress. In this context, a preliminary study on microbiota of healthy and infected leaves by Diplocarpon rosae and Podosphaera pannosa was carried out in the organic edible climbing rose plantation of Faculty of Horticulture - USAMV Bucharest. The composition and incidence of fungal isolates was variable in time as well as in presence or absence of pathogens. The fungal community of rose leaves was represented by Alternaria, Aureobasidium, Cladosporium, Chaetomium, Epicoccum, Fusarium, Penicillium and Sordaria isolates. Our study highlights the presence of some well knowns natural antagonists as Chaetomium globosum, Epicoccum nigrum, Sordaria fimicola and Aureobasidium pullulans isolates. Further, presently ongoing studies shall reveal the antagonistic properties of our isolates and their colonizing ability (in vitro and in vivo assays). Our further studies will contribute to a better understanding of the composition and diversity of rose leaves fungal community and how the microbiota can play a role in plant protection

Key words*: edible rose, leaves fungal microbiota, organic management.*

INTRODUCTION

Rose is one of the most important ornamental horticultural crops all over the world, being historically associated with beautiful gardens. However, there is a worldwide trend of using roses as an ingredient in various spices and functional foods (Park et al., 2005), as raw material for anti-inflammatory drugs or as a potential source of antioxidant compounds promoting human health (Ge et al., 2013; Lee et al., 2015; Choi et al., 2015; Haejo & Shin, 2017; Butcaru et al., 2019).

Rose is susceptible to a large number of diseases that impact negatively on overall plant performance, reduce flower yield, quality, marketing and production costs (Chalova et al., 2017).

Podosphaera pannosa (powdery mildew), and *Diplocarpon rosae* (black spot) are the most common and damaging fungal pathogens in roses (Debener & Byrne, 2014; Byrne et al., 2019). Both diseases affect susceptible cultivars worldwide (Gochomo et al., 2006; Munnenkhoff et al., 2017), leading to development of immature and stunted plants, yellowing and premature defoliation or reduced rose flower production.

Organic edible roses are grown using environmentally friendly practices. Cultivation of healthy plants in organic farming is a challenge due to the lack of resistance of *Rosa damascena* to the main diseases and pests (Chalova et al., 2017). Cultural practices as planting in well - drained soil amended with organic matter, providing air circulation, avoiding overhead watering or maintaining good sanitation are basic guidelines for disease management. Also, organic products (biological control agents, plant extracts) are available and effective in rose disease control, having a preventive rather than eradicating function (Gupta & Dikshit, 2010). However, some botanical pesticides may express certain levels of toxicity and therefore extensive research is needed prior to their practical application (Chalova, 2017).

To protect edible roses against fungal infections it is important to explore novel strategies. Leaves has been recognized as an important habitat for microorganisms, including bacteria, yeasts and filamentous

fungi. Knowledge of leaves microbiota is important for the management of foliar diseases. Indigenous fungal and bacterial populations have been found efficient in limiting pathogen population, and thereby minimizing the disease severity in different crops (Suman, 2008).

There are several studies thar have been reported a wide range of beneficial effects of microbiota members on plant health including disease suppression, induction of systemic resistance, increased nutrient acquisition, increased tolerance to abiotic stresses or adaptation to environmental variations (Hassani et al., 2018).

Despite the increasing interest in leaves microbiota, little is known about the diversity and community structure of fungi associated with *Rosa* spp. as well as its ecological roles. Also, little is known about the fungal communities of edible rose leaves, the interactions between and with the main pathogens or their response to abiotic and biotic stress. In this context, a preliminary study on microbiota of healthy and infected edible climbing rose leaves by *Diplocarpon rosae* and *Podosphaera pannosa* was carried out.

MATERIALS AND METHODS

Biological material.

A survey of black spot and powdery mildew was conducted in an organic edible climbing rose plantation established in 2015 at University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture.

Typical symptoms of black spot that include circular dark brown spots with irregular margins measuring up to 15 mm were observed.

Powdery mildew was identified as a white to grayish spots or patches and powdery growth on the upper surface of the leaves.

Isolation of microbiota from healthy and infected leaves.

Healthy and infected rose leaves with black spot and powdery mildew symptoms (Falstaff cultivar) were collected in June and November 2020.

The leaves were cut into small pieces (0.5 cm), surface sterilized using 70% ethanol and rinsed for three times. Tissues were blotted dried and plated on potato dextrose agar (PDA). Plates were incubated at 22°C until growing of colonies. Fifty leaves/variant have been collected and one hundred fragments have been plated. The experiments were repeated twice.

Results are expressed as isolates incidence (%). Fungal isolates were identified based on their macroscopic (colony colour, pigmentation) and microscopic characteristics.

RESULTS AND DISCUSSIONS

Fungal community associated with healthy or infected edible rose leaves was assessed in July and November 2020.

In July, the fungal community of edible climbing rose leaves has been represented by *Alternaria*, *Aureobasidium*, *Cladosporium*, *Chaetomium*, *Epicoccum*, *Fusarium*, *Penicillium* and *Sordaria* isolates. Bacterial strains were also present.

The composition and incidence of fungal isolates was variable in time as well as in presence or absence of pathogens (Figure 1).

In healthy leaves, the highest incidence was recorded for *Chaetomium globosum* isolates (48%) in July, followed by *Aureobasidium pullulans* (17%) and *Sordaria fimicola* isolates (13%). All isolates belong to species that are recognized as natural antagonists. In November, the fungal community associated with healthy leaves was represented by *Alternaria alternata* (25%), *Epicoccum nigrum* and *Cladosporium cladosporioides* (3%) isolates. Bacterial colonies were also present (3%).

In July, in leaves with black spot symptoms, the highest incidence was recorded for *S. fimicola* isolates (22%). Other isolates detected were *A. alternata* (18%), *Fusarium* sp. (8%), *C. globosum* (6%) and *Aureobasidium pullulans* (6%). In November, *A. alternata* isolates was recorded with the highest incidence (84%). Like for healthy leaves, isolates of *E. nigrum* have been recorded, but with low incidence (3%). *Penicillium* sp. isolates (3%) and bacterial colonies (7%) were also been present in leaves with black spot symptoms (Figure 1).

The leaves community associated with powdery mildew attack have been represented

by *S. fimicola* isolates (49%), *A. pullulans* (28%), *C. globosum* (21%), *A. alternata* (18%), *Fusarium* sp. (14%), and *Monilia (Neurospora) sitophila* (10%). Bacterial strains were also present (8%). In November, from leaves with powdery mildew symptoms only *A. alternata* (67%) and *E. nigrum* isolates (27%) have been detected and isolated (Figure 1).

Different microorganisms have been isolated from rose phylloplane (leaf surface) with powdery mildew symptoms, but by a different method than ours (wash leaf method). Kumar and Chandel (2018) reported the presence of *Fusarium*, *Botrytis*, *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria*, *Trichothecium*, *Trichoderma* and two bacterial isolates (*Bacillus* sp. and *Pseudomonas* sp.). Some of these isolates have been recorded in our study, too.

Gosh & Shamsi (2014) reported five fungal species that have been associated with black spot symptom of *Rosa centifolia*. The associated fungi were *C. cladosporioides, C. oxysporum, Marssonina rosea*, *Penicillium* sp. and *P. guepinii*.

Our study highlights the presence of *C. globosum*, *E. nigrum, S. fimicola* and *A. pullulans* isolates with potential antagonistic properties.

Chaetomium globosum isolates have been detected only in July, both in healthy and infected leaves (Figure 2), with the highest prevalence in healthy tissues. *Chaetomium globosum* is a saprophytic fungus which is widely distributed on plant, soil, straw and dung as well as an endophytic one. *Chaetomium* species have been documented to be potential antagonists of several soil- and seed-borne plant pathogens (Yue et al., 2018).

Epicoccum nigrum isolates were detected only in November (Figure 2), with low incidence in healthy and black spoted leaves and with a higher prevalence in leaves with powdery mildew symptoms. *Epiccocum nigrum* is a ubiquitous saprophytic hyphomycete found on various substrates (soils and plants). Also, it was isolated as an endophytic fungus. *Epicoccum* species are famous for their application in the biocontrol of many fungal pathogens but also for their capability to produce biologically active compounds with medical applications (antioxidant, antimicrobial, and anticancer agents) and pigments with industrial application (Ogórek & Pląskowska, 2011; Elkhateeb & Daba, 2019).

Sordaria fimicola isolates have been detected only in July, in healthy and infected leaves (Figure 2) with higher incidence in leaves with powdery mildew and black spot symptoms compared to healthy leaves.

The genus *Sordaria* has important saprophytic species that grow well and fast on organic materials (Kavak, 2012). The main habitat of *S. fimicola* is decaying organic matter and dung of plant-eating animals. This fungus, a model for studying genetics and meiosis in ascomycetes has been also isolated as endophyte or from the surface of necrotic leaves colonized by fungal pathogens (Kavak, 2012; Newcombe et al., 2016). Recent studies focusing on novel biocontrol agents, reported the antagonistic activity of *S. fimicola* in wheat (Er, 2010) and maize (Abdallah et al., 2018) against *Fusarium graminearum*.

Isolates of *A. pullulans* have been detected only in July (Figure 2), with a low prevalence in healthy leaves. A higher incidence, compared to healthy leaves was observed in leaves with powdery mildew symptoms followed by those with black spot attack. *Aureobasidium pullulans* is a saprophytic yeast-like fungus, well documented, found in plants, soil, rocks and water which is well known for his antagonistic activity, especially against postharvest pathogens (Bozoudi & Tsaltas, 2018).

These observed dynamics/increase of indigenous beneficial populations in diseased leaves could be the result of the pathogens activity or a direct relationship between them and pathogen. The host plant also provides metabolic capabilities which leads to the adaptation of niche specialized inhabitants (Thrall et al., 2007). Effective colonization, large population size and the viability of beneficials are important for successful biocontrol of plant diseases. Increasing the size of a constituent of the indigenous microbiota of leaves when the constituent is used as a biocontrol agent is one of our further research objectives. When the size of indigenous beneficial population is low, application of bio-products based on biological control agents could lead to a better control of diseases.

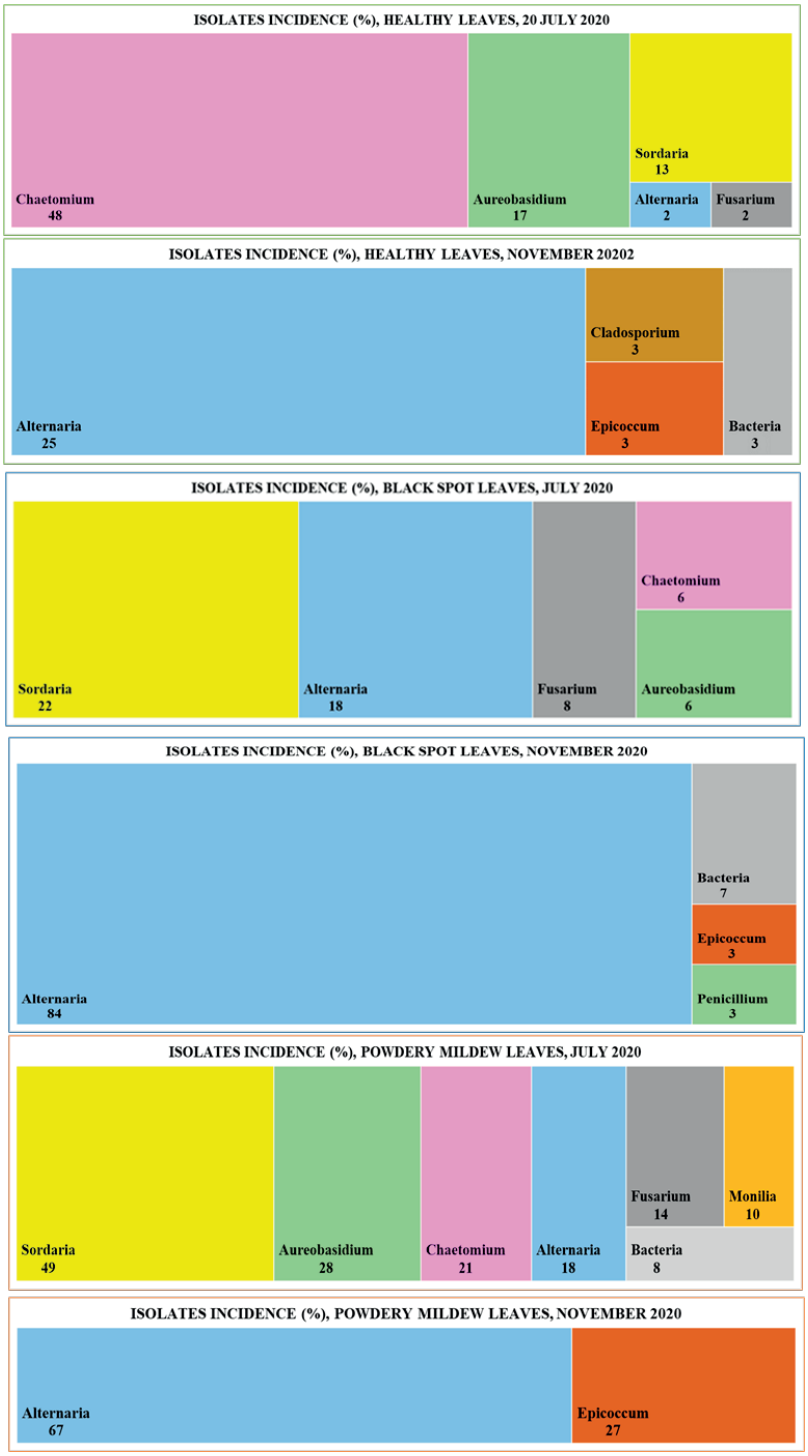

Figure 1. Incidence of detected isolates on organic climbing rose leaves, healthy or affected by black spot and powdery mildew

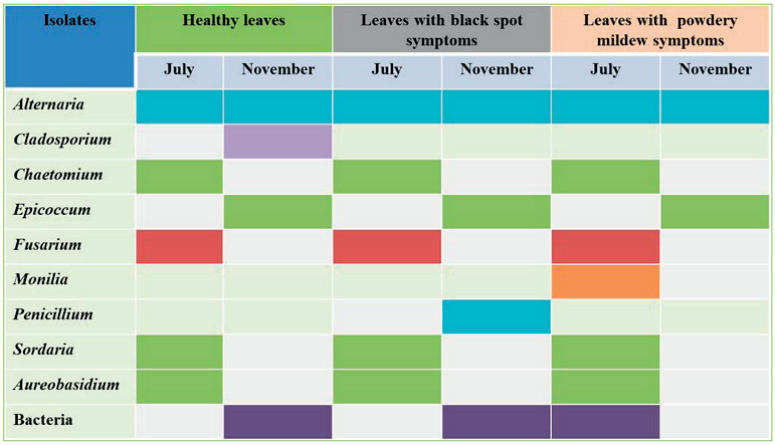

Figure 2. Occurrence of detected isolates in July and November on organic climbing rose leaves, healthy or affected by black spot and powdery mildew

Among the other isolates that have been detected, we mention those belonging to *A. alternata*, which have been present both in July and November, in all variants (Figure 2). In healthy leaves, the prevalence of *A. alternata* isolates was low in July and higher in November. In leaves with black spot or powdery mildew symptoms, the incidence of *A. alternata* isolates was higher than in healthy leaves, in July. In November, these tissues have been colonized mostly by *A. alternata* isolates.

CONCLUSIONS

Our preliminary results provide a first glimpse into the microbial community associated with healthy or diseased leaves of organic edible climbing rose. The microbiota composition and incidence of isolates was variable in time as well as in presence or absence of *D. rosae* or *P. pannosa* pathogens.

Since leaves of organic edible climbing rose are naturally colonized by resident microorganisms known for their antagonistic activity such as *A. pullulans*, *C. globosum, E. nigrum*, and *S. fimicola* the present challenge is to understand their biocontrol potential and how such microorganisms can be successfully integrated in the control main diseases.

Further studies are needed to better understand how the composition and diversity of edible rose microbial community can interacts or influence the pathogens. Research is currently underway to test their antagonistic properties and colonizing ability on the phylloplane (*in vitro* and *in vivo* assays). Also, studies are underway for the identification of recurring patterns in the dynamics of the microbial populations, and the acquisition of knowledge on the mechanisms that generate these patterns.

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START OF THE BLUEBERRY BREEDING PROGRAM AT THE UNIVERSITY OF AGRONOMIC SCIENCES AND VETERINARY MEDICINE OF BUCHAREST

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Abstract

Blueberry varieties are one of the central decision and part of the orchard success when it comes to the investor's choice. Therefore, even it is remarked a steadily increase of fruits demand from the global blueberry market, the consumer and producers claim new varieties of blueberries with new and better quality traits such as firmness, flavor, shelf life, storability, mechanical harvest, resistance to biotic and abiotic stress and even decorative values enhancement. In this trend, our endeavor at the Faculty of Horticulture Bucharest is focused to start breeding cultivars for the next blueberry generation. Beside the general breeding goals set for blueberries, at the University of Agronomic Sciences and Veterinary Medicine of Bucharest we are targeting additional objectives such as earliness in ripening, fruit color variability and decorative appearance. For the spring of 2021, we choose the following blueberry varieties as genitors: 'Simultan', Hannah's choice', 'Early blue', 'Duke', 'Chandler', 'Pink lemonade', 'Spartan', 'Blue ribbon', 'Hortbleu petite', 'Peach sorbet', V. angustfolium. First hybridization results are promising and new crossing and evaluations are about to be done in the next years.

Key words*: Vaccinium corymbosum, Vaccinium angustifolium, crossing, variety.*

INTRODUCTION

In the range of the berry crops, blueberries are ones of the most appreciated and desired fruits by consumers. Beside the well-known nutraceutical proprieties (Min S et al., 2017) and benefits for human consumption (Kalt W. et al., 2019), the blueberries are expecting to continue grow in interest for farmers and investors worldwide.

In this respect, the breeders must develop new blueberry varieties with superior traits (Pluta S. & Zurawicz E., 2014) that match both the actual and future consumer preferences and growers' expectations (Gilbert J. et al, 2014). The difficulties in choosing the right traits for the new blueberry cultivars come from the relevance of these traits in different parts of the world including adaptation, resilience (Lobos G. & Hancock J.F., 2015) and industry priorities among consumers demands (Gilbert J., 2016). So, some of the plant and fruit characteristics represent common goal for many breeders to achieve but some of them still define the local or regional particularities.

Nevertheless, fruit quality remains a strong target in the business. For instance, firmness, sweetness (Gilbert J. et al., 2015), flavour (Sater H., 2020; Farneti B. et al., 2017), shelf life and overall appearance are most relevant traits to be addressed (Gallardo K. et al., 2018).

On the other side, for the large-scale production, the emerging blueberry varieties need to be ready for mechanical harvesting and in this regard, additional traits of plants and fruits are needed such as plant architecture, compact ripening period, excellent fruit firmness, easy detachment from stalks etc.

Modern techniques (Cappai F. et al., 2020) as marker-assisted breeding method (Mengist, M.F. et al., 2021) are developed and extended to be predictable and to have a better selection efficiency in the breeding activity. For this, special logistics and knowledge is required.

Not for long time ago, few breeding companies aimed to create blueberry varieties with

ornamental value or mixt valorisation of plants, opening a new direction for blueberry breeding programmes (Kobelt M., 2020) and enlarging the genetic datasets.

Famous breeding companies as Fall Creek started specific breeding programme for northern highbush blueberries in Europe (Fresh Plaza, 2020) and many of the latest cultivars became already well-known and appreciated.

For the producers it is very important to start a new plantation with a high value genetic material, and to influence the market and trends for fresh blueberry consumption. This is one of the reasons to increase and speed the breeding work for the upcoming period.

For Romania, the Research Institute for Fruit Growing Pitesti is the single institution that own a breeding programme for blueberry and in more than 30 years of activity in this field, fifteen great Romanian blueberry varieties have been bred (Mladin P. et al., 2012) and are available for growers (Ancu I. et al., 2013).

At the Faculty of Horticulture in Bucharest, a great number of blueberry varieties have been collected and studied in the past 10 years. Also, since 2016, in the frame of the Laboratory for sensorial analyses of the Research Centre for Studies of Food Quality and Agricultural Products, we organized yearly tasting sessions with more than 60 blueberry varieties.

The great interest of the consumers and farmers for the national and international blueberry assortment indicate us the opportunity to start a new breeding programme and bring our contribution to the next blueberry generation.

The current paper is presenting the first steps of our effort in developing a long lasting and fruitful breeding programme in the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

MATERIALS AND METHODS

For the first year of breeding activity at the Faculty of Horticulture Bucharest, beside the general breeding goals set for blueberries, at the University of Agronomic Sciences and Veterinary Medicine of Bucharest we are looking for additional objectives such as earliness in ripening, fruit size, colour variability and decorative plant traits.

In the spring of 2021, we choose the following blueberry varieties to work with as genitors: 'Simultan' (RO), 'Hannah's choice', 'Early blue', 'Duke', 'Chandler', 'Blue ribbon', 'Hortbleu petite', 'Peach sorbet' and *V. angustifolium*.

From the nine blueberry varieties, 16 cross combinations have been done between 17.04.2021 and 6.05.2021 (Table 1).

Table 1. Dates of hybridizations made in 2021 and genitors used in crossings

No	Pollination date	Cross combination
1	20.04.2021	Simultan x Duke
2	20.04.2021	Simultan x Hannah's choice
3	20.04.2021	Simultan x Hortbleu petite
4	6.05.2021	Simultan x Blue Ribbon
5	20.04.2021	Duke x Simultan
6	17.04.2021	Duke x Early blue
7	20.04.2021	Duke x Hannah's choice
8	20.04.2021	Duke x Chandler
9	17.04.2021	Hannah's choice x Duke
10	20.04.2021	Hannah's choice x Simultan
11	17.04.2021	Early blue x Duke
12	1.05.2021	Blue ribbon x Simultan
13	20.04.2021	Chandler x Duke
14	6.05.2021	V. ang x Hortbleu petite
15	6.05.2021	V. ang x Peach sorbet
16	6.05.2021	Peach sorbet x Hortbleu petite

We aim to harvest the seed from other varieties that cannot be used this year for controlled hybridization such as: 'Pink lemonade', 'Spartan', 'Toro', 'Pink breeze', 'Legacy'.

Each plant container was utilized for only one cross combination (Figure 1).

Figure 1. Blueberry hybridization plot in the experimental field of the Faculty of Horticulture Bucharest

The mother plants have been prepared in advance (Figure 2):

- selecting the right flower clusters,
- flowers were protected by special paper to avoid accidental or foreign pollination.
- emasculation of flowers
- reintroducing the flower clusters into the paper bags

The father plants were used to harvest the pollen from the suitable flowers and moment. Pollen drops were captured in the Petri vessels and regularly shacked about 24h-36h until the full release of the pollen. After 1-2 days, the paper bags were opened, and the pollen was gentle placed with the brush on the top of the stigma. Then the number of pollinated pistils were counted and bag resealed.

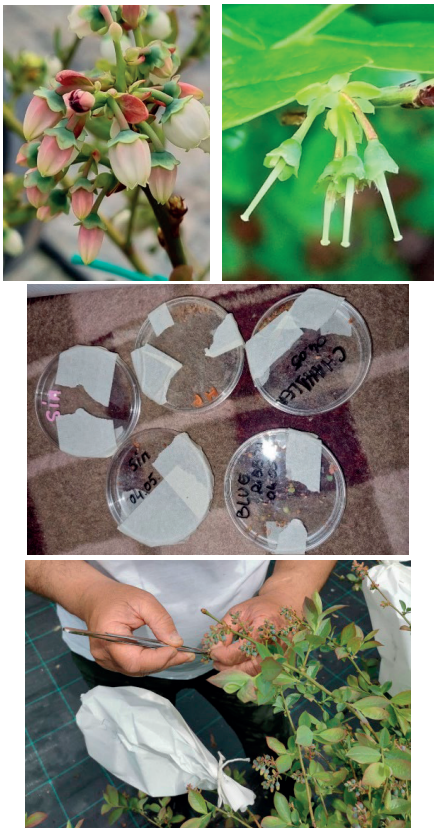

Figure 2. Blueberry breeding steps

After three weeks, the control was made for each bag and cross combination. The fruits set was observed (Figure 3), and the hybrid fruits counted.

Figure 3. Hybrid fruits set (Early blue x Duke)

RESULTS AND DISCUSSIONS

From the 16 cross combinations (Figure 4), 756 flowers were pollinated in 2021 and 653 hybrid fruits were set up. In this respect, the percentage of 86.38% of fruit set is considered a promising one.

Some examples of different hybrid fruits obtained are in Figures 5, 6, 7 and 8.

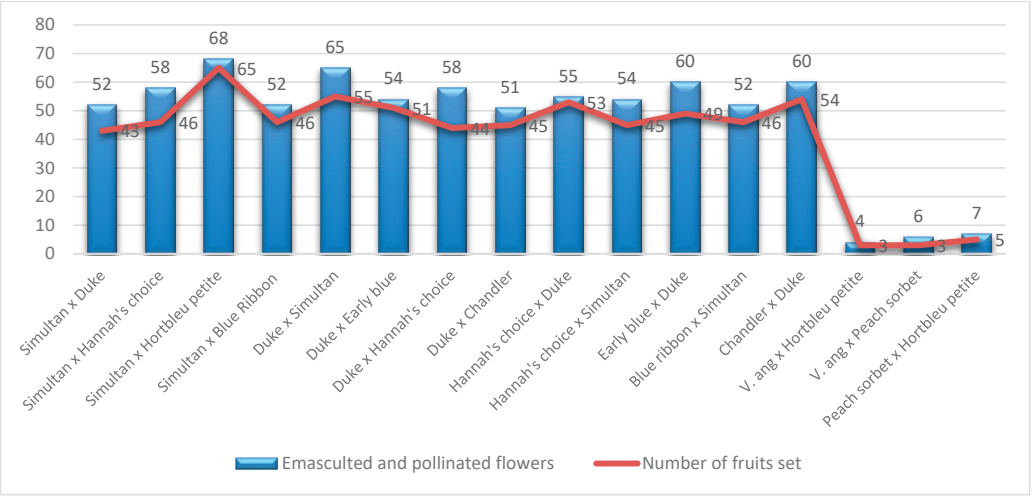

Figure 4. The results of blueberry varieties cross combinations made in 2021

Figure 5. 'Duke' x 'Simultan'

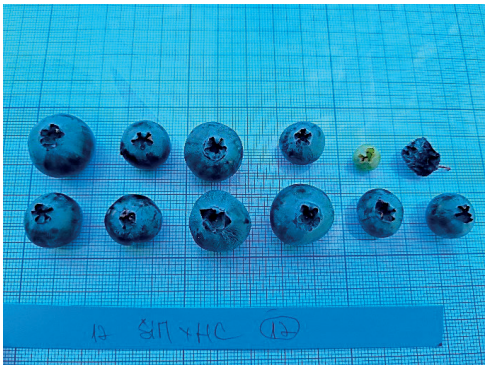

Figure 6. 'Simultan' x 'Hannah's choice'

Figure 7. 'Simultan' x 'Duke'

Figure 8. 'Chanticleer' x 'Duke'

Analysing in depth each cross combination (Figure 9), we can observe that most of the varieties exceed 80% of fruits set. The highest percentage of 90.00% was achieved by 'Chandler' followed by 'Hannah's choice' and 'Blue ribbon'. The lowest share of fruit sets (60%) was remarked at *Vaccinium angustifolium*.

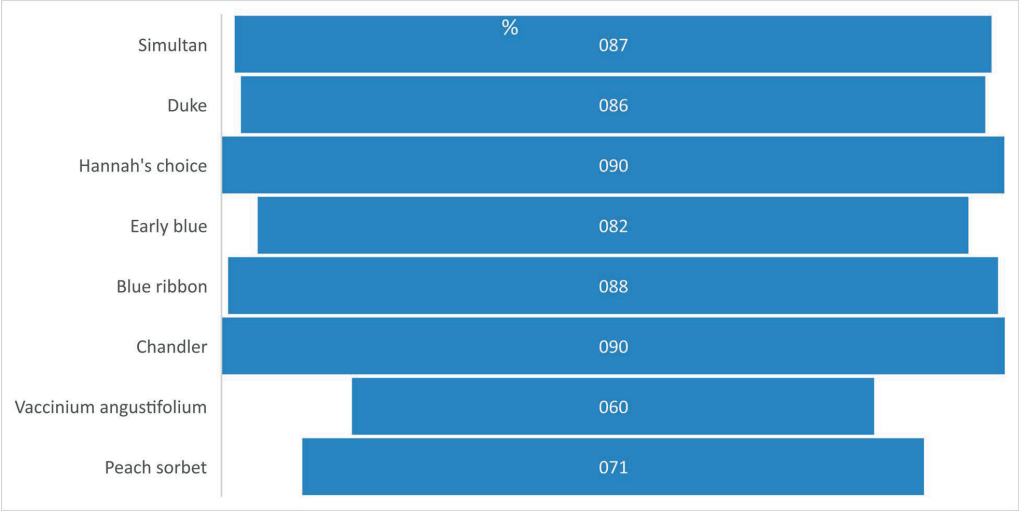

Figure 9. The fruit set percentage of the mother blueberry varieties regardless the male genitor

The *V. angustifolium* and 'Peach sorbet' variety choose for the decorative purpose has encountered lower percentages. Although, the fruits set up from the interspecific combinations (*V. angustifolium* x *V. corymbosum*) allow us to follow the hybrid seeds in further breeding process.

CONCLUSIONS

First hybridization results are promising, and new crossing and evaluations are about to be done in the next years.

Interspecific hybridization results in a lower number of fruits set than intraspecific crossings. The highest number of fruit sets percentage (90%) was calculated at 'Chandler' x 'Duke' combination.

Early varieties combinations range between 81.67% and 88.46% fruit set.

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THE OUTBREAK OF *SPILONOTA OCELLANA* (DENIS & SCHIFFERMÜLLER), THE EYE-SPOTTED BUD MOTH ON APPLE TREES IN BISTRIȚA REGION

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Abstract

In the period 2019-2020 at the Fruit Research & Development Station Bistrița observations have been made constantly in apple and other fruit trees orchards. As a consequence, besides the usual apple pests and diseases such as: the codling moth, the San Jose scale, woolly apple aphid, scab, powdery mildew and brow rot, new threatening pests were detected. They are a part of the Lepidopterae order and did not represent a major problem in the past. Male adults of Spilonota ocellana (eye-spotted bud moth) were caught on pheromone traps placed in a nearby plum orchard, for monitoring the flight of Cydia funebrana, in the years 2019-2020. The number of captures was 53 in 2019 and 159 in 2020 per trap. In 2019 the damage was sporadically encountered both on leaves and fruits but in 2020 the damage was significantly higher. On some apple cultivars the fruits were damaged up to 23,23%. Data suggest the need for control measures to be taken especially in the second part of the summer.

Key words*: Spilonota ocellana, apple, damage, flight pattern .*

INTRODUCTION

The climatic conditions are globally changing and so they do in Bistrița area, leading, alongside the modifications in the life cycle of the traditional pests of the apple orchards, to some new damaging insects to appear. *Spilonota ocellana* (Denis & Schiffermüller) (Figure 1) is a well-known defoliator of different trees and shrubs, but up until now, has not been a significant threat for fruit trees at Fruit Research & Development Station Bistrița (F.R.D.S.B.).

Figure 1. Adult male of the eye-spotted moth on pheromone traps, F.R.D.S. Bistrita, 2020

Spilonota ocellana sin. *Tmetocera ocellana* is an insect of the Torticidae family, *Spilonota* genus that can be encountered in all cultivation regions of the apple in the northern hemisphere throughout the vegetative growing stage (Figure 2). It is a highly polyphagous species that has a lot of host plants both cultivated or spontaneous. Some of the host plants genus are: *Alnus* spp., *Querqus* spp., *Juglans* spp., *Larix* spp., *Crataegus* spp., *Malus* spp., *Prunus* spp., *Pyracantha* spp., *Pyrus*., *Rubus* spp., *Sorbus* spp., *Salix* spp., while in orchards it prefers apples (*Malus*) and also sweet cherry (*Prunus*). (http://idtools.org/id/leps/tortai/Spilonota_ocellana.htm)

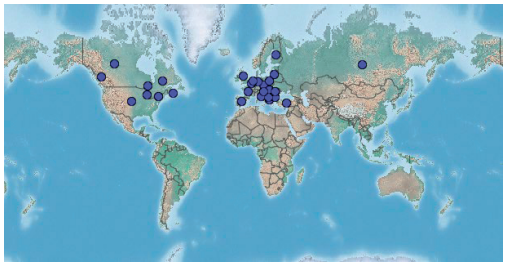

Figure 2. Distribution map of the eye-spotted moth. Source https://www.cabi.org/isc/datasheetreport/51015

There have been studies that underline the possible problems that this pest can cause in different fruit tree species. In the 1970-1971

seasons the eye-spotted bud moth caused up to 45 % damage to the pear and peach untreated orchards in Saragossa province in Spain (Cabezuelo Perez & Hernandez Esteruelas, 1975). But the same study reveals that in treated orchards the damaged buds represented only 3.5%. In Romania the damage on buds previously recorded was around 15% (Perju, 2002) and we found no data on the damage on fruits. More recent studies have been made in Canada, the Okanagan and the Sinukameen regions on apples as a consequence of some Spilonota ocellana outbreaks (Swain, 2016; Swain et al., 2017)

The entomologic literature mentions that the eye-spotted moth completes one generation per year, the adults being present in orchards from June to August (Perju, 2002). The female lays the eggs on leaves and the new born larvae feed primarily on it. The pest usually overwinters as fifth and sixth instar larvae, sometimes fourth instar (Mcbrien & Judd, 2004), that constructs a hibernaculum, often in a spur crotch. In spring they emerge and resume feeding on fruiting buds, flowers and leaves. They form tubular chambers by rolling leaves or by webbing the space between two leaves. By webbing the leaves, the larvae form their pupation nest near the feeding spot. In apples, damage can also occur on fruits in different growth stages, the larvae externally feeding on them. Late instar larvae are approximately 9-14 mm long with a grey to dull reddish-brown abdomen. The head and prothoracic shield are reddish-brown to black, sometimes with dark mottling. Prothoracic legs are dark brown (Figure 3).

Figure 3. *Spilonota ocellana* larvae.

MATERIALS AND METHODS

Weekly readings of the captures of adult males on pheromone traps (Figure 4) were performed, in order to obtain the flight curve for *Spilonota ocellana*.

Figure 4. Captures of adult males of the eye-spotted moth on pheromone traps, 2020

The pheromone used was produced by the Research Institute in Chemistry Raluca Ripan in Cluj and is composed by 2 components: Z8-dodecen-1-yl acetate and E8-dodecen-1-yl acetate. This combination is used for capturing *Cydia funebrana* adult males. The pheromone traps were placed in a plum orchard adjacent to two apple orchards in which the damage was evaluated at the end of the season 2019 and 2020. The eye-spotted moth being a pest the records have been registered although it was not the main concern in that particular orchard. Data recorded along the two years (2019 and 2020) were grouped on seven days intervals, so it can be compared using graphic representation.

During the flight period of the eye-spotted bud moth there were 8 insecticide sprays on each year (Table 1), in the treated orchard and none in the untreated control orchard where the damage was assessed.

The meteorological data has been recorded daily with the aid of a computerised system so that the appearance of the adults and the flying curve were corelated with the evolution of temperatures and rainy days through the vegetative season.

At the end of the season 300 fruits/orchard and 200 leaves/orchard from the two apple orchards were analysed to determine the frequency of the attack of the eye-spotted bud moth.

The significance of the differences between the years was analysed by Oneway Anova test and graphic analysis.

Table 1. Treatments with insecticides made in 2019 and 2020 in the treated orchard

Spray	2019		2020	
	Data	Active ingredient (concentration)	Data	Active ingredient (concentration)
T1	09.05	Dimetoat 400 g/l	07.05	Acetampirid 200 g/kg
T2	22.05	Lambda cihalothrine 50 g/l	20.05	Dimetoat 400 g/l
T3	04.06	Tiacloprid 480 g/l	30.05	Deltametrin 250 g/kg
T4	17.06	Chlorpyriphos metil 225 g/l	18.06	Acetampirid 200 g/kg
T5	01.07	Chlorpyriphos metil 225 g/l	06.07	Dimetoat 400 g/l
T6	18.07	Dimetoat 400 g/l	16.07	Spirotetramate 100g/l
T7	06.08	Lambda cyhalothrin 50 g/l	27.07	Acetampirid 200g/kg
T8	22.08	Tiacloprid 480 g/l	18.08	Tiacloprid 480 g/l

RESULTS AND DISCUSSIONS

The readings of the pheromonal traps in 2019 show a low level of flight throughout the summer with an increase in number of moths during the warmer months. as the Figure 5 shows.

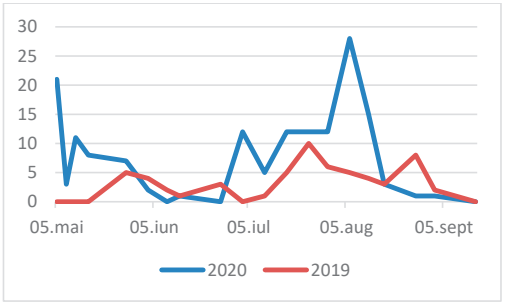

Figure 5. The flight of male adult moths caught in the summer of 2019 and 2020

We used a combination of pheromones that we usually use to capture *Cydia funebrana* adult males with very good results. The pheromone traps Atrafun seem to be very efficient for *Spilonota ocellana* too, which is interesting and suggests that one of the two compounds is attractant for both these species. The distribution of the captures was also verry different in the 2 years (Figures 6 and 7). This is an aspect that indicates that the evolution of this pest is quite different in different years.

The number of adult moths caught in the two years in May and June varied very much. While in May 2019 the number of the captures represented just 9% from the total number of male moths caught that season, in 2020, 33% of the total number of moths were caught in May. It is also very interesting that the adults appeared very early in May 2020 and continued to fly all through the summer until 02.09.2020. This indicates a large amount of variability of the distribution of overwintering instars or even a second generation considering the fact that the diapause necessity has been less studied.

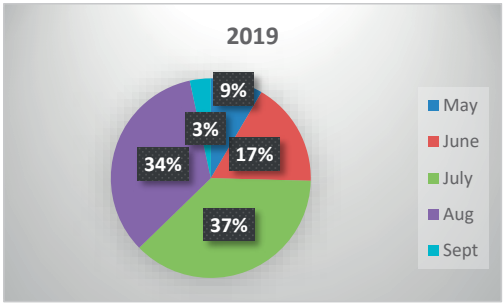

Figure 6. Distribution of the male adult moths caught in the summer of 2019

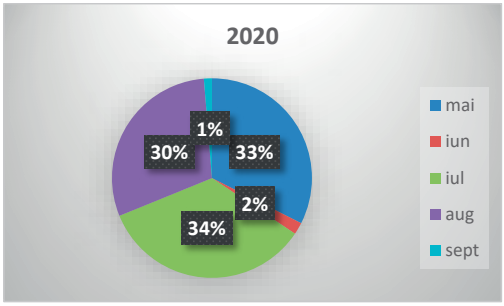

Figure 7. Distribution of the male adult moths caught in the summer of 2020

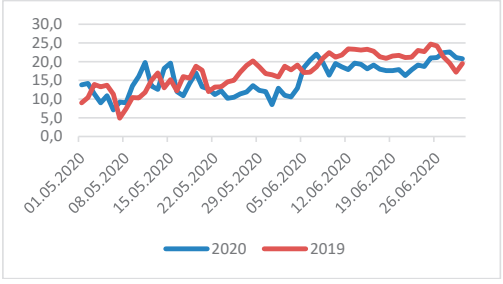

Figure 8. Evolution of average temperatures in May-June in the years 2019 and 2020

In June, on the other hand, 17% of the moths were caught in 2019 and only 2% in 2020. The situation in June corelates to the evolution of the meteorological conditions in the two years. Even though the differences are not statistically significant for the average temperature, they are statistically significant for the number of rainy days (Fcalc ≤ Fcrt; Fcalc. = 0.72; Fcrt. = 18.51282051 Df = 3, α = 0.05) (Table 2). As a consequence of the big number of days with rain the number of moths caught was significantly smaller on one hand and on the other it was impossible to enter the orchard to apply the necessary insecticide treatments from 29.05.2020 to 18.06.2020.

Table 2. Average temperatures and number of rainy days in May and June 2019 and 2020

Year	Average temperatures		Rainy days	
	May	June	May	June
2019	13.74	20.89	16	10
2020	12.64	18.28	17	19

The meteorological conditions in June, however were probably very suitable for the evolution of the eggs laid by the females that appeared in May.

On July and August, the distribution is not significantly different but the number of moths caught in 2020 was much higher than in 2019. Their number was much higher in the summer of 2020 when we compared the number of the moths caught on each summer month as the Table 3 shows.

Table 3. Number of individuals caught on the pheromone traps in 2019 and 2020

Month	2019	2020
May	5	50
June	10	3
July	22	53
Aug	20	46
Sept	2	2
Total	**59**	**154**

The increased number of moths active in the orchards on the second part of the summer of 2020 leaded to a big number of larvae that produced a lot of damage both on leaves and on fruits. They usually web leaves together and feed on the them (Figure 9).

Figure 9. *Spilonota ocellana* damage on Generos apple leaves, 2020

Or they web a leaf on a fruit and feed on the fruit staying well protected by the leaf. An obvious discolouration of the fruit occurs at the spot where the leaf was attached and there are multiple feeding spots that diminish the quality of the crop (Figures 10, 11).

Figure 10. Florina apple fruits damaged by the eye-spotted bud moth larvae, 2020

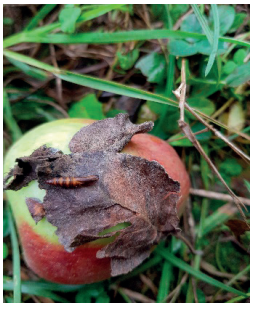

Figure 11. Florina apple fruit and leaves webbed together and eye-spotted bud moth pupae, 2020

The frequency of the damaged leaves and fruits was much higher in 2020, the year of the outbreak, than in 2019 (Figures 12, 13).

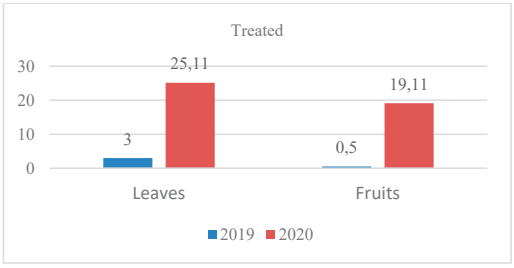

Figure 12. Percentages of damage frequency in the treated plot in 2019 and 2020

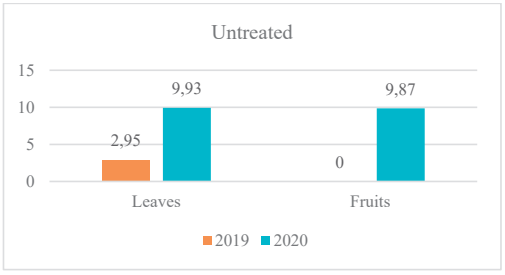

Figure 13. Percentages of damage frequency in the untreated plot in 2019 and 2020

The frequency of the damaged fruits and leaves were higher in the treated orchard than in the untreated orchard in both years. The attack on fruits in the treated orchard was very small, while in the untreated orchard was not even spotted on 2019, with significant presence in the year of the outbreak, 2020. The smaller frequency of the damage in the untreated plot could be possibly due to the mechanisms of regulation of the ecosystems in untreated environments or to the higher presence of other Tortricidae moths such as *Cydia pomonella*, *Hedya nubiferana* and *Choristoneura* spp. It is also possible that the smaller frequency of damage in the untreated orchard is because the eye-spotted bud moth has increased in numbers in the last two years and the future evolution could be quite different. This is an aspect that will be further evaluated in future studies.

The damage was highest on Florina apples and leaves but had an important negative impact on all the other varieties (Figure 14).

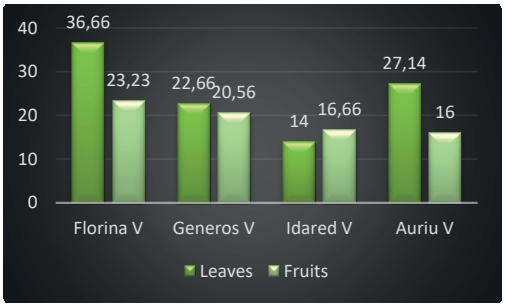

Figure 14. Percentages of damage frequency on the apple varieties from the treated plot at F.R.D.S. Bistrita, in 2020

The increased damage frequency in 2020 in spite of the insecticide sprays indicates that the substances used or the timing of the sprays was not suitable for controlling *Spilonota ocellana*. But we must consider the fact that this species was not a target due to the sporadic damage caused in 2019 and to the fact that it was not a threat for apples in this region.

CONCLUSIONS

In 2020 a new challenge for the protection of crops appeared in two of the apple orchards of Fruit Research-Station Bistrița.

The damage caused by the eye-spotted bud moth had a significant negative impact on the quality of the fruits. Therefore, a specific and integrated pest management program for this species must be developed and applied.

In the years that follow the study should continue in order to clarify the aspects concerning the pheromones that attract Spilonota ocellana and the ecologic mechanisms that make the untreated biotops more resilient to the outbreaks of this pest.

ACKNOWLEDGEMENTS

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NEW TYPE OF ANTIHAIL, ANTIRAIN AND ANTIFROST SYSTEM FOR PROTECTION OF ORCHARDS

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Abstract

The present paper presents a review regarding the use of nets and other plastic covers for the orchard protection against hail, rain and late frosts. The installation of the protection systems creates a modified microclimate that influences many orchard parameters and most of these modifications brings benefits for the farmers, as indicated by many studies made in the last decades. Two key factors that seem to be influenced in a positive manner by the plastic covers are the late frosts protection and the earlier maturation of the fruits. In the sweet cherry orchard from the Experimental field of USAMV Bucharest a roof plastic cover has been installed in order to study the evolution of the plants inside and outside the covered area. The new system which has been patented in 2020, assures protection against late frosts, hail and rain, sweet cherries being very susceptible to cracking caused by rain during the ripening season. During the study, will monitor the effectiveness of the protection as well as the impact of the system on the fruit set, the earliness of fruit production, yield quality and quantity, diseases impact and the general evolution of the cherry trees.

Key words*: fruit protection, rain cover, plastic cover, orchard protection, hail net, orchard protection.*

INTRODUCTION

Climate change is a fact that cannot be contested anymore. Its effects are being felt all around the world. We witnessed devastating storms, draughts, warm winters and cold springs, snow falls in areas where they have never been seen before, hail storms and so on.

The impact of these phenomena on agriculture is huge, because they affect all the aspects of the plants' life. If we consider the horticulture, the impact is even bigger, as the horticultural crops are generally of higher value and many of them are perennial, so one phenomenon can affect the plants for the next years.

In this context, the use of protection systems for horticultural crops is a key factor in assuring the quality and quantity of the production. For vegetable production the best way for crop protection is to use greenhouses. They are very effective in protecting the plants against late frosts and strong enough to resist hail storms. But the cost of building greenhouses is so high that, they do not represent a viable solution when we speak about fruit orchards, or even vegetables grown on field. Therefore, there is a need for a feasible, cost effective alternative that can be used to protect fruit trees and that can be used for big surfaces of land.

The present paper makes a review of the systems used worldwide to protect the fruit trees and presents also a new protection system that was developed and patented in Romania. The system combines the characteristics of the standard hail and rain protection systems with the covering and screening of greenhouses and protects the crops against late frosts, hail, rain and wind.

Review of the orchard protection systems

The orchards protection against hail by covering them with nets of various meshes and colours is one of the most effective means of protection. Besides assuring an almost perfect protection, this system brings other benefits, such as better fruit quality (Kiprianovski et al., 2016), improved yield and even improved efficiency of photosynthesis of the shaded leaves (Bosančić et al., 2021), reduction of sunburn on fruits etc.

In the last years there have been made tests with coloured, photo-selective nets. As any type of net cover will decrease the light transmission, this can adversely affect vegetative and reproductive plant growth, but

each type of colour net can improve the quality and colouration on specific cultivars (Blanke, 2009).

The anti-hail nets are used usually after blossom, till late after harvest period, when the risk of hail storm persists. Anyway, they do not protect the orchards against late frosts or rains during the harvesting period.

There are fruit species susceptible to cracking caused by rain coming in the pre-harvesting period. The most common example is the sweet cherry tree. For the protection against rain there have been proposed many solutions. All of these solutions have the same base principle which is to cover the trees with a plastic roof. One main aspect about these covers is that they can be affected and even destroyed by the wind gusts. Therefore, most of these systems can only be used during a short period of time (2-3 weeks), before and throughout the harvesting period. All the systems used against the rain proved good effects, even if the fruit cracking was not 100% avoided. According to Measham et al., (2014) "the development of apical and stem end cracks are induced by skin surface wetting, while deep cracks on the side of the fruit are induced by water moving via the vascular system". The vast majority of rain plastic shelters discharge the water in the space between the row trees, therefore there is rain water coming through the roots towards the fruit, so the second type of cracks cannot be avoided. The trees cultivated under the so called "high-tunnels" or inside greenhouses can be totally protected against cracking, but these constructions are very expensive and have other disadvantages, like low ventilation, that make them unfit for tree growers.

Using structures that permitted the plastic sheets to be used for longer periods gave the opportunity to conduct studies about the influence these covers on the various aspects of fruit cultivation. According to Lang et al., (2016), "Protective covering systems can modify many variables in the orchard production environment and ecosystem, including rain, wind, frost, and some pests and diseases, resulting in more consistent sweet cherry cropping with higher quality fruit and healthier trees in less-than-optimal growing regions."

One of the most important benefit of plastic shelters was the protection given against late spring frosts. Covering the orchard with plastic roofs "increased the temperature by approximately 1-2ºC, resulting in decreased frost damage occurring during the bloom stage" (Vávra et al., 2019, p. 503). Studies of the plastic covers on sweet cherry orchards, conducted in Germany in nineties, proved that even if they did not offer 100% protection against cracking, they "gave an efficient protection against frost during flowering in 1997" (Balmer, 1998).

Plastic covering of the canopy also proved useful for reducing the leaf diseases associated to rain water and damages caused by hail (Kjaer et al., 2016). Tests have been made to apple trees and there have been less infections and consequently less treatments. On sweet cherry orchards plastic shelters "used to cover trees from bloom throughout the growing season, can reduce some key diseases (cherry leaf spot and bacterial canker), and it may be feasible to increase certain pesticide spray intervals due to less loss of protective residues to rain or UV light breakdown" (Lang, 2014).

Because the plastic covering alters the light characteristics, it modifies the fruits maturation and colouring. Studies conducted in Vignola Region of Italy showed that the characteristics of the polyethylene used in plastic covers have different influences on the ripening date of cherry trees. Some types accelerate fruit ripening, while other induce a delay in maturation (Grandi et al., 2017). This suggested that by using different plastic covers and eventually sequential opening of the covers, some early cultivars will maturate even earlier, while the harvesting period of some late cultivars can be delayed for some days. This method can bring important benefits for farmers, as cherry prices are very high off-season.

The fruits quality grown under the plastic shelters is another key aspect that has to be considered. According to the type of covers, the influence on the quality can be different. If the system does not assure a good ventilation the fruits will have "less surface colour, reduced red juice colour and lower percent of soluble solids" (Børve and Meland, 1998) and they will "have a higher risk of infection of different

diseases" (Meland and Skjervheim, 1998). But, with shelters that assured a good ventilation, the fruits were of a very good quality, with diameter and weight higher than the ones grown in open fields (Lang, 2014).

Besides the beneficial aspects of using plastic covers for orchards there is also a big issue: pollination. Pollination using European honeybees (*Apis mellifera*) is affected negatively by plastic covers, "since these covers change the light spectral quality that honeybees use for effective navigation" (Lang, 2014). A good solution to this problem is the use of bumblebee hives (*Bombus* spp.). Bumblebees seem to be less affected by the conditions under the covers (Lang, 2014) and therefore the pollination of the flowers is good.

The effect of opening the plastic covers early in the spring on the maturation date of the fruits has been extensively studied in researches conducted all over the world. On sweet cherry trees the results have been contradictory. In some cases, the earliness of the production was spectacular: "when roof plastic covers are used during the whole season, trees bloom 6 to 13 days earlier, harvest is advanced by 12 to 19 days, and soluble solids concentration increase in comparison with uncovered controls" (Wallberg and Sagredo 2014, *apud.* Blanke and Balmer, 2005). In other cases, the results have been exactly the opposite, a "delayed ripeness from 4-7 days" (Børve and Meland, 1998), or 1 to 5 days delay (Meland and Skjervheim 1998, *apud.* Zbinden, 1988). Most probably the properties of the plastic which was used, as well as the geographical conditions of place were the studies have been made have had an impact on the results, so there is a need to replicate these studies in the Romanian conditions and with different types of plastic.

The use of plastic shelters was extended to many other species beside the sweet cherry. Raspberries grown in China, under the plastic rain shelters have a bigger height and diameter and the harvesting can be done even during rainy days, which results in a higher amount of marketable fruits and a lower percentage of lost fruits. Also, the harvesting season of primocane fruiting raspberry has been prolonged late in the autumn (Dai et al., 2020).

Kiwifruit "could be harvested 15-20 days earlier under plastic house culture than in the field without any quality deterioration" (Cho et al., 2016).

Under the rain covers the mean yield of mango fruit increased by a staggering 60.2%, while the quality of the fruits was not affected and more, the fruits decay after harvest was diminished significantly (Xu et al., 2019).

Used in apple and pear orchards, plastic shelters can reduce the incidence of the rain induced diseases, with a direct impact on the lower number of treatments. These shelters can also protect the plants against hail and sun, eliminating the burns caused by the high solar irradiance (Kjaer et al., 2016).

Apricots, peaches and nectarines grown in temperate climate are very susceptible to be affected by late frosts. In Romania, almost regularly there are several nights with freezing temperature during middle and late spring. Studies conducted at the R.S.F.G. Constanța between 2012 and 2014 showed that as much as 90% of some peach cultivars have been lost because of the frosts (Moale et al., 2016). In 1991 there has been made a study for apricot grown inside plastic houses. The results were very promising as there were obtained higher yields and earliness of 15-20 days compared to apricots grown on open fields (De Salvador et al., 1991).

MATERIALS AND METHODS

In 2020 we developed a protection system using plastic sheets linked to a structure of cement posts. The sheets covered each row of trees and the lateral sides were linked from one row to the next one with elastic strings. The resulting space between the sheets gives the air the possibility to exit the system in such a way that it does not create pressure on the structure itself. Therefore, the system can be used from early spring until late autumn, protecting the crop from late frosts, hail, rain, sunburn. If we do not want that rain water to enter inside the system, we can mount a plastic gutter that will transport the rain water outside the system (Figure 1). The system has been patented and has been already installed on about 20 hectares of different crops, like sweet cherries, raspberries, strawberries and blackberries.

As part of this study, we installed the system on 14 rows of sweet cherries orchard located

inside the USAMV Experimental field in Bucharest. The plot contains the following cultivars: 'Ferrovia', 'Lapins', 'Celeste', 'Vega', 'Skeena', 'Early Red', 'New Star', 'Kordia', 'Mora di Vignola', 'Firm Red', 'Giant Red', 'Katalin', 'Ulster', 'Sam', 'B. Burlat', 'Boambe de Cotnari', 'Hedelfinger, Germersdorf', 'Van', 'Rivan', 'Regina', 'Giorgia', 'Rubin' and 'Severin' grafted on PHLC, Colt, CAB6P, CAB11E and *Prunus mahaleb* L. The opening of the covering system has been made on March 31, 2021. We opened the system on about half of the total surface, in order to have control trees to compare.

As there are many cultivars with different canopy types and the plants are not of the same age, we identified those cultivars whose position permits to have plants both inside and outside the opened system. In this way, we can study the evolution of the covered plants by comparison with the ones that remained uncovered. The cultivars that will be studied are: 'Early Red', 'Ferrovia', 'Kordia', 'Regina', 'Rubin' and 'Severin'. In order to monitor the temperature and wind speed we installed temperature sensors inside the covered area as well aș outside. Our goal is to compare the evolution of the trees that are covered by the plastic shelter with the ones outside. The aspects that will be investigated are: effect of low night temperatures, pollination by insects, fruit development and maturation dates, fruit colouration, fruit cracking caused by rain and the general evolution of the trees.

RESULTS AND DISCUSSIONS

Several days after the system opening, the temperatures dropped significantly during the night between the 8th and 9th of April. Our sensors registered -3.8ºC outside the covered area and -2.1ºC inside. The temperature difference of about 1.5ºC is consistent with the measurements we made during the last years and with measurements made by other researchers in similar conditions (Vávra et al., 2019). In the morning of the 9th of April the whole field was covered by hoar-frost, with the exception of the sheltered area. Actually, the hoar-frost remained on the plastic sheets and did not fall on the trees (Figure 2). The 'Early Red' trees were in full blossom and we could see the effect of the hoar-frost on the flowers that have been exposed. In the following days we checked these flowers and it was clear that they have been affected (Figure 3). The covered 'Early Red' trees did not have affected flowers with the exception of some flowers which were positioned in the area were the plastic sheets were linked and they were exposed to the hoar-frost. This proved that the system can be successfully used to protect trees against hoar-frost and even against negative temperatures in spring time. On another hand, if the temperature drops significantly, the plastic covers themselves are not enough to eliminate the damages. Additional heat sources are needed, which, with the help of the covers, will increase sufficiently the temperature. In the next season we will install such heat sources and we will test how the system influences the loss of heat, as well as the fuel consumption needed to keep the positive temperature inside the covered area.

The modification of light parameters inside the covered areas gives a concern regarding the effectiveness of honeybee (*Apis mellifera*) pollination. Lang (2014) speaks about this problem and proposes as an alternative solution the use of bumblebees (*Bombus* spp.). In our research area we installed three honeybee hives situated inside the covered space and one bumblebee hive. In the first days the bees did not seem interested to visit the flowers of the sweet cherry trees, neither covered, nor situated in the open area. Most probably this was caused by the fact that there were few blossomed trees inside the experimental field and in close proximity there are some parks where there were other blossoming trees. When the majority of the trees started to blossom, the bees activity inside the experimental field increased. We could see that the honeybees preferred the trees situated outside of the covered space. There were honeybees also inside the covered space, but not in big numbers. The bumblebees however, did not seem to make differences between the inside and outside of the covered area. The pollination is ongoing during these days, so we will have the results in the coming weeks. We will further investigate also if the roof plastic covering has any effect on the fruit maturation as well as on the fruit quality and quantity.

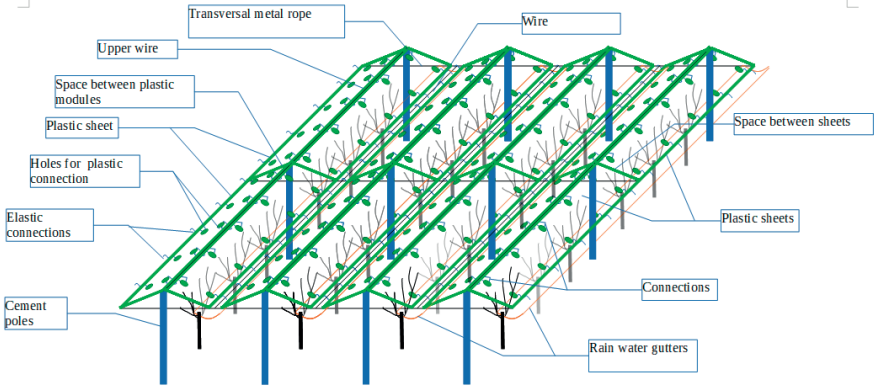

Figure 1. Roof plastic cover

Figure 2. Hoar-frost outside and inside the roof plastic cover

Figure 3. Flowers damaged by hoar-frost outside the roof plastic cover

CONCLUSIONS

The impact of climate change on fruit production is huge and cannot be underestimated anymore. The very low temperatures of winter combined with the high temperatures during late winter – early spring and with sudden drops of temperature below zero degrees in late spring are causing big problems for fruit growers in temperate areas, like Romania (Asănică et al., 2012; Asănică et al., 2014). Hail storms destroy fruit crops already on regular basis. Severe draughts alternate with heavy rains coming during the harvesting periods. There is no other solution for avoiding the damages caused by all these phenomena but to use the protected crop environments. Besides the use of greenhouses and high-tunnels, there must be found other cheaper solutions that can be afforded by the regular farmer. One such solution is the use of roof plastic covers, that can cover big areas, have a good cost-benefit ratio, can be adapted to various types of crops and provide protection against late frosts, hail and rain, besides other benefits like fruit earliness or disease protection.

All these aspects will be thoroughly studied in the next period on the specific climatic conditions of Romania. Further results of this study will be published.

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INFLUENCE OF PRE AND POST-HARVEST CONDITIONS ON QUALITY INDICATORS AND MICROBIOTA OF ORGANIC *ARONIA MELANOCARPA*

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Abstract

Organic chokeberries (Aronia melanocarpa L.) are plants with unique health-promoting properties, due to its high content of antioxidants in fruits. The present study focuses on the incidence of fungal pathogens and the quality parameters of organic chokeberry in different storage conditions. Two organic chokeberry varieties, 'Nero' and 'Nero Eggert', were harvested in August 2020 from the University of Agronomic Sciences and Veterinary Medicine of Bucharest experimental orchard, stored in normal and controlled atmosphere conditions, and analysed during the storage period. In pre-harvest field, for both varieties of chokeberry it were performed two different fertilization scheme: control (untreated) and treated variant with organic fertilizers applied on soil or by foliar sprays. Aronia melanocarpa L. fruits were monitored in terms of: shape index, firmness, total soluble solids, total titratable acidity, dry matter content, total phenolic content, antioxidant activity and fungal fruit pathogens during storage. Total phenolic content and antioxidant activity showed similar behaviour during storage period for all samples. Penicillium, Botrytis and Alternaria were the main fungal pathogens identified on organic chokeberry fruits.

Key words*: chokeberry, controlled atmosphere, DPPH, fungal pathogens, storage condition.*

INTRODUCTION

Black chokeberry (*Aronia melanocarpa*) is a plant native to North America and was transferred to Europe about a century ago (Chrubasik & Li, 2010) and is a member of *Rosaceae* family, *Maloideae* subfamily (Howard et al., 2013).

The cultivation of *Aronia* for the food industry started in Russia in 1900s in the cold areas of Siberia and, afterwards the crops spread all over Russia. In the first half of the 20th century, the plant was introduced also to the other European countries like Poland, Germany, Finland, Sweeden and Norway (Jannick et al, 2008). At the beginning of cultivation in Europe, chokeberry was used for domestic production of juices and jams, but in the recent years, *Aronia* berries was cultivated more as an industrial crop (Hardin, 1973; Strigl et al., 1995).

Previous studies show that chokeberry is one of the richest fruits in phenolic compounds, they have antioxidant potential, bringing multiple health benefits for the consumers (Denev et al., 2012). However, due to its astringent taste and high tannin content, chokeberries is not a very popular table fruit, despite its qualities. (Chrubasik et al., 2010)

Recent studies have shown, regarding the maximum reach levels of fruits anthocyanin content and weight, the optimal harvest period is at the end of August, beginning of September (Andrzejwska et al., 2015).

Postharvest environmental conditions have a major impact on visual and compositional quality of fruits. The most important component of postharvest environmental is temperature that has a great impact on the quality of fresh fruits (Cheng et al., 2013). It is estimated that about 25% of the harvested fruits are decayed by pathogens during postharvest (Droby, 2006).

During the early stages of growth, the pathogens remain in the fruit tissues and remain there for all the maturation period. After harvesting, the diseases will be visible during the storage period (Passey et al., 2017).

MATERIALS AND METHODS

Samples

Two varieties of organic chokeberry, 'Nero' and 'Nero Eggert', were harvested in 2020 from the experimental field of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. For both varieties of chokeberry it were performed two different fertilization scheme: control, untreated (noted with M) and treated variant (noted with TR) with organic fertilizers applied on soil or by foliar sprays. They were stored at 1° C and 95% relative humidity (RH) for one day, after which they were transported to Postharvest Technologies Laboratory from Research Center for Studies of Food Quality and Agricultural Products**.**

After the initial analyses were performed, the fruits were evenly distributed and stored in three different conditions: 1) normal atmosphere (NA) with 1°C and 95% RH, 2) controlled atmosphere conditions with 1˚C, 95% RH, 3% and 5% CO_2 (CA 5% CO_2), and 3) controlled atmosphere conditions with 1°C, 95% RH, 1.5% O_2, and 10% CO_2 (CA 10% CO_2).

Organic chokeberries samples were analysed in 5 different moments, initial (noted with 0-zero), after 1, 3, 6, 7 months of storage (noted with 1, 3, 6, 7).

Chemicals

To determine antioxidant activity, DPPH (1,1-diphenyl-2-picrylhydrazyl) and Folin & Ciocalteu's reagent were purchased from Sigma-Aldrich Chemie GmbH (Riedstrasse, Steinheim).

Methanol used was bought from Honeywell (Riedel-de Haën, Seelze, Germany).

Gallic acid was purchased from Carl Roth and Trolox (6 - hydroxy - 2, 5, 7, 8 - tetramethylchroman - 2 - carboxylic acid) from Acros Organics, Fisher Scientific (Geel, Belgium). Sodium hydroxide 0.1N was from Cristal R Chim S.R.L. (Bucharest, Romania) and anhydrous sodium carbonate was purchased from Lach-Ner, s.r.o. (Neratovice, Czech Republic).

Ultrapure water used it was made with a Milli-Q equipment (Millipore, Bedford, MA).

Potato dextrose agar (PDA) used in microbiological analyses was purchased from Scaharlau S.A. (Germany).

Quality indicators

Quality parameters were represented by firmness, total titratable acidity (TTA), pH, dry matter (DM) and total soluble solids (TSS), methods of analysis being described forward.

Firmness results were obtained and expressed in N/cm² using a digital penetrometer (53205 TR Italy) equipped with a 3 mm piston.

TTA and pH analysis were realised using the TitroLine automatic titrometer, equipped with pH electrode. The analysis consist in mixing 5 g of fresh homogenized sample with 25 mL of distilled water, measuring the initially pH values and then titration with 0.1N NaOH until the final pH is 8.1° T according with AOAC Official Method 942.15. For TTA, results were expressed in g malic acid /100g of fresh fruit similar with Stan et al. (2020).

Dry matter results were obtain using UN110 Memmert oven and drying approximately 1 g of sample at 105°C (Stan et al., 2020) until constant weight. The analysis of total soluble solids (TSS) were performed using Kruss DR301-95 digital refractometer (Cătuneanu et al., 2017).

Phenolic content and antioxidant activity

For total polyphenol content (TPC) quantitative determination was used the Folin–Ciocâlteu method adapted after Georgé et al., (2005) protocol. Samples extraction consist in trituration of 1 g fresh sample with 10 mL of 70% methanol and incubated overnight at room temperature (aprox. 22°C) and dark. Extraction continue next day through 500 rpm homogenization for 1 h and, then centrifugation for 5 min at 4°C and 7000 rpm. The supernatant was recovered and the residue re-extracted two more times and the final volume of extract was 30 mL. By mixing 0.5 mL of extract with 2.5 mL of Folin-Ciocâlteu reagent and incubated for 2 minutes at room temperature (aprox. 22°C) is the first step in total polyphenol content determination. Second step is represented by adding 2 mL of 7.5% sodium carbonate solution (Na_2CO_3) and incubate the obtained mix for 15 minutes at 50°C. The final step is based on the absorbance read at Specord 210 Plus UV-VIS spectro-photometer (Analytik Jena, Jena, Germany) at the 760 nm wavelength. Results are expressed in mg GAE/100 g fresh weight and Gallic. Gallic acid is used as standard solution.

Antioxidant activity determination was used the DPPH (2,2-diphenyl- 1-picrylhydrazyl) method, similar as Bujor et al. (2016) with modifications presented forward. Mixing 0.4 mL of extract with 2 mL of 0.2 mM solution of DPPH in methanol and incubated for 30 minutes, in dark with continuous homogenising. The absorbance was measured at 515 nm wavelength. Results were expressed as mg Trolox/100 g FW. The blank reference was realised with methanol.

Pathogens identification

The experiments were realised in the Plant Protection Diagnostic Laboratory. Organic chokeberries were analysed in 3 different moments: after 3, 6 and 7 months of storage in all 3 conditions. The samples were incubated at 22°C for 48 hours on PDA (potato-dextrose agar) culture medium, followed by macroscopic identification of pathogens.

The preparation of PDA culture in the pathogen development was made by following the existing literature (Hulea et al., 1969). Petri dishes with 65 mm diameter were used.

Statistical analysis

Statistical analysis of obtained data was performed using Microsoft Excel for standard deviation, represent the average of three replicates with independent sample preparation.

RESULTS AND DISCUSSIONS

Quality indicators

Both varieties of chokeberries registered quality indicators variation for all of the three storage conditions.

Experiment were performed during 7 months of storage, but physiological disorders appears after 3 months of storage in NA conditions, and 5 months of storage in CA conditions (1.5% CO_2, 16% O_2, 1°C, 95% RH and 1.5% CO_2, 16% O_2, 3°C, 95% RH).

The initially TTA values of 'Nero' variety untreated 0.97 ± 0.07 g malic acid/100 g FW (Table 1), after 6 months of storage, the TTA values decrease to 0.86 ± 0.01 g malic acid/100 g FW, which means the acidity of chokeberries increases after 6 months of storage.

Table 1. Variation of pH, total titratable acidity (TTA), total soluble solids (TSS), and dry matter (DM) content during storage of 'Nero Eggert' variety

Variety/ Treatment	Storage conditions	Analysis moment (months)	pH	TTA (g malic acid/100 g FW)	Total soluble solids (%)	Dry matter (%)	Firmmnes (kg/cm²)
Nero/ untreated	NA with 1°C, 95% RH	0	3.51 ±0.03	0.97 ±0.07	16.45 ±2.45	22.07 ±0.46	0.33 ±0.07
		1	3,43 ±0.06	1.11 ±0.02	18.87 ±1.05	23.94 ±1.43	0.453 ±0.098
		3	3.29 ±0.00	1.08 ±0.01	19.79 ±0.94	24.15 ±0.21	0.315 ±0.047
		6	3.69 ±0.01	0.86 ±0.01	19.95 ±1.60	27.46 ±0.76	0.393 ±0.063
		7	3.73 ±0.01	0.91 ±0.01	22.17 ±1.41	26.38 ±1.02	0.410 ±0.106
	CA 1.5% CO_2, 16% O_2, 1°C, 95% RH	1	3.41 ±0.02	1.03 ±0.01	17.89 ±2.10	24.44 ±1.42	0.403 ±0.061
		3	3.37 ±0.05	1.01 ±0.01	18.85 ±0.65	23.34 ±0.27	0.405 ±0.064
		6	3.73 ±0.01	0.88 ±0.03	20.69 ±1.92	30.57 ±1.22	0,388 ±0,051
		7	3.64 ±0.02	0.94 ±0.01	23.44 ±1.39	29.09 ±2.08	0.422 ±0,089
	CA 1.5% CO_2, 16% O_2, 3°C, 95% RH	1	3.37 ±0.05	1.07 ±0.01	19.14 ±2.30	23.64 ±1.83	0.450 ±0,094
		3	3.38 ±0.07	1.03 ±0.04	20.45 ±0.99	23.12 ±1.32	0,391 ±0,049
		6	3.72 ±0.01	0.88 ±0.03	19.39 ±2.56	22.46 ±2.11	0412 ±0.062
		7	3.70 ±0.03	0.87 ±0.02	21.25 ±1.77	27.19 ±1.45	0.355 ±0.051
Nero/ treated	NA with 1°C, 95% RH	0	3.47 ±0.02	1.11 ±0.004	18.21 ±1.20	22.75 ±0.46	0.37 ±0.05
		1	3.36 ±0.05	1.09 ±0.01	19.65 ±1.42	23.55 ±0.68	0.415 ±0.043
		3	3.40 ±0.07	1.04 ±0.04	20.49 ±0.98	26.11 ±0.51	0.357 ±0.075
		6	3.74 ±0.02	0.80 ±0.01	21.48 ±0.77	27.10 ±0.89	0.376 ±0.064
		7	3.77 ±0.01	0.87 ±0.001	21.88 ±1.77	28.57 ±0.00	0.396 ±0.086
	CA 1.5% CO_2, 16% O_2, 1°C, 95% RH	1	3.39 ±0.08	1.06 ±0.03	19.50 ±1.12	25.21 ±1.09	0.442 ±0.052
		3	3.31 ±0.02	1.03 ±0.03	18.97 ±0.87	21.68 ±0.20	0.341 ±0.045
		6	3.66 ±0.01	0.95 ±0.01	21.11 ±1.18	27.88 ±1.42	0.408 ±0.048
		7	3.68 ±0.06	0.94 ±0.01	20.82 ±2.89	27.69 ±2.22	0.442 ±0.089
	CA 1.5% CO_2, 16%O_2, 3°C, 95% RH	1	3.37 ±0.06	1.06 ±0.01	19.21 ±0.67	25.36 ±0.51	0.349 ±0.086
		3	3.41 ±0.07	1.05 ±0.01	20.05 ±1.26	23.28 ±0.27	0.414 ±0.088
		6	3.66 ±0.09	0.95 ±0.01	22.01 ±1.41	27.41 ±0.39	0.410 ±0.084
		7	3.63 ±0.03	0.90 ±0.02	22.38 ±1.09	27.20 ±1.50	0.443 ±0.054

Nero treated variety show similar variation of TTA after 6 months of storage. Similar behavior were observed at 'Nero Eggert' variety (Table 2), both treated and untreated, in all 3 storage conditions.

TSS values showed constant increases to all samples, in all storage conditions, fruits dehydrating considerable after 3 months of storage, which increase the concentration of total soluble solids. During the storage period, the value of TSS for the fruits stored in NA with 1°C, 95% RH increase much more compared to the fruits stored in CA 1.5% CO_2, 16% O_2, 1°C, 95% RH and CA 1.5% CO_2, 16% O_2, 3°C, 95% RH. Dry matter values showed significant increases for all samples, in all storage conditions, after 6 months of storage.

The firmness values of 'Nero' variety, both treated and untreated showed an increase after 1 month of storage, in all storage conditions. After 3 months of storage the firmness showed a small decrease to all samples.

'Nero Eggert' showed a constant decrease of firmness values (Table 2) after one month of storage, with exception of untreated sample stored in NA with 1°C, 95% RH, variation being similar to Nero variety.

Table 2. Variation of pH, total titratable acidity (TAA), total soluble solids (TSS), and dry matter (DM) content during storage of 'Nero Eggert' variety

Variety/ Treatment	Storage conditions	Analysis moment (months)	pH	TAA (g malic acid/100 g FW)	Total soluble solids (%)	Dry matter (%)	Firmmnes (kg/cm²)
Nero Eggert/ untreated	NA with 1°C, 95% RH	0	3.49 ±0.01	0.97 ±0.01	17.78 ±2.06	24.7 ±0.80	0.343 ±0.045
		1	3.44 ±0.03	0.96 ±0.02	19.26 ±2.06	25.08 ±0.81	0.442 ±0.067
		3	3.32 ±0.02	0.92 ±0.012	19.51 ±1.53	24.14 ±0.19	0.338 ±0.064
		6	3.80 ±0.01	0,61 ±0.011	20.82 ±1.12	26.59 ±0.53	0.300 ±0.083
		7	3.71 ±0.01	0.89 ±0.004	19.77 ±2.27	27.45 ±0.38	0.567 ±0.085
	CA 1.5% CO_2, 16% O_2, 1°C, 95% RH	1	3.42 ±0.03	0.96 ±0.002	17.43 ±1.63	24.25 ±0.94	0.407 ±0.064
		3	3.42 ±0.11	0.90 ±0.03	19.19 ±1.83	22.54 ±0.15	0.374 ±0.067
		6	3.73 ±0.01	0.78 ±0.003	17.91 ±2.61	26.84 ±1.25	0.376 ±0.105
		7	3.69 ±0.01	0.80 ±0.01	21.50 ±1.91	28.50 ±0.66	0.342 ±0.087
	CA 1.5% CO_2, 16% O_2, 3°C, 95% RH	1	3.44 ±0.03	0.93 ±0.01	18.15 ±1.15	23.55 ±0.68	0.359 ±0.081
		3	3.36 ±0.03	0.91 ±0.02	19.44 ±1.26	24.17 ±1.04	0.374 ±0.067
		6	3.72 ±0.02	0.78 ±0.003	21.51 ±1.23	25.24 ±1.00	0.338 ±0.077
		7	3.71 ±0.01	0.78 ±0.02	20.27 ±0.84	26.92 ±0.90	0.407 ±0.057
Nero Eggert/ treated	NA with 1°C, 95% RH	0	3.50 ±0.01	1.00 ±0.02	20.36 ±1.42	23.19 ±0.72	0.408 ±0.05
		1	3.42 ±0.06	1.00 ±0.006	18.47 ±1.34	23.46 ±0.72	0.416 ±0.067
		3	3.36 ±0/02	0.93 ±0.02	19.03 ±1.60	23.32 ±0.25	0.348 ±0.055
		6	3.71 ±0.01	0.81 ±0.02	20.90 ±0.74	25.66 ±0.01	0.371 ±0.067
		7	3.71 ±0.02	0.79 ±0.01	19.61 ±1.25	26.97 ±0.43	0.442 ±0.086
	CA 1.5% CO_2, 16% O_2, 1°C, 95% RH	1	3.43 ±0.03	1.02 ±0.02	18.90 ±0.81	22.86 ±0.49	0.414 ±0.070
		3	3.37 ±0.02	0.99 ±0.01	18.90 ±1.87	22.64 ±0.43	0.367 ±0.065
		6	3.74 ±0.04	0.85 ±0.02	20.63 ±1.42	27.81 ±0.42	0.388 ±0.051
		7	3.71 ±0.01	0.87 ±0.004	22.63 ±1.42	30.03 ±0.35	0.418 ±0.073
	CA 1.5% CO_2, 16% O_2, 3°C, 95% RH	1	3.46 ±0.03	1.00 ±0.03	18.68 ±1.19	21.26 ±0.15	0.416 ±0.047
		3	3.38 ±0.04	0.96 ±0.01	18.73 ±1.26	22.59 ±0.52	0.341 ±0.060
		6	3.70 ±0.01	0.85 ±0.02	19.97 ±1.96	24.12 ±1.37	0.375 ±0.066
		7	3.68 ±0.04	0.82 ±0.01	22.05 ±2.31	26.43 ±0.67	0.395 ±0.099

Phenolic content and antioxidant activity

Total phenolic content were determined from whole fruit and showed similar behavior for both varieties in all tested storage conditions. For Nero variety, initially TPC values was 1274.5 mg GAE/100 g FW, results similar with those obtain by Catană et al. (2017), which demonstrate important increases during storage after 3 and 6 months of storage, up to 1667.1, respectively 1812 mg GAE/100 g FW for fruits stored in NA with 1°C, 95% RH. The increases are similar to the samples stored in CA 1.5% CO_2, 16% O_2, 1°C, 95% RH and CA 1.5% CO_2, 16% O_2, 3°C, 95%.

TPC was better preserved in CA 1.5% CO_2, 16% O_2, 1°C, 95% RH during storage period, for all varieties (Figure 1).

All samples present a high antioxidant activity, initially value being registered at 699.78 mg Trolox Eqiv/100 g FW for 'Nero' variety untreated, 742.84 mg Trolox Eqiv/100 g FW for 'Nero' variety treated, 682,675 mg Trolox Eqiv/100 g FW for 'Nero Eggert' variety untreated and 723.59 mg Trolox Eqiv/100 g FW for 'Nero Eggert' variety treated, results similar to Kapci et al. (2013). After 1 month of storage all samples stored in all condition have showed important increases of antioxidant activity, but after 3 months, chokeberries stored in NA with 1°C, 95% RH, showed the higher increase of antioxidant activity. After 6 months, all samples stored in all condition showed a high increase of antioxidant activity, up to 1002.11 mg Trolox Eqiv/100 g FW on 'Nero' variety treated stored in NA with 1°C, 95% RH (Figure 2).

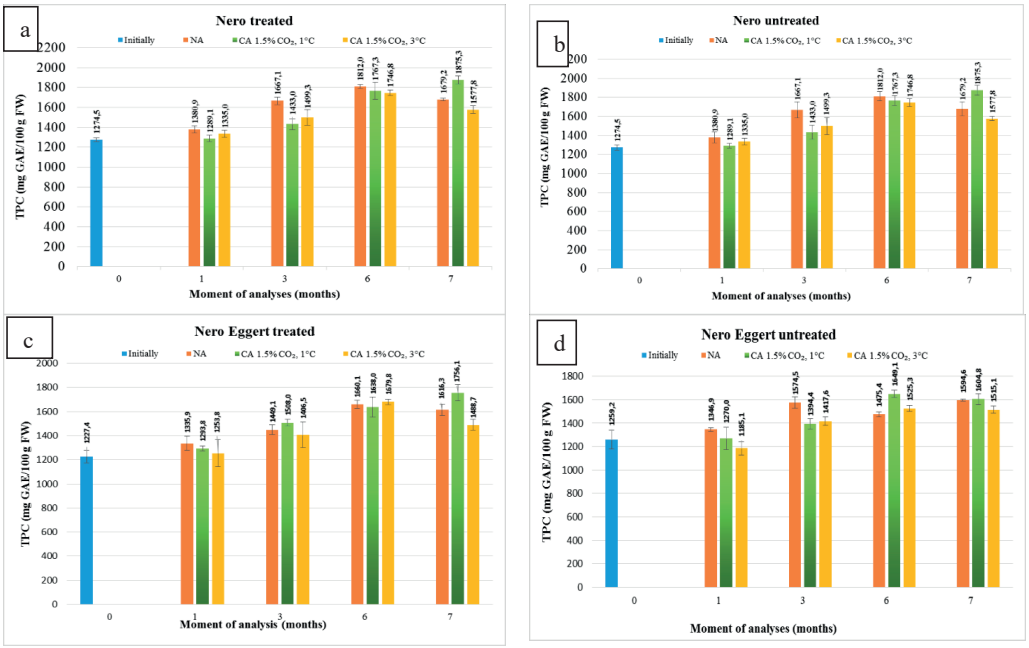
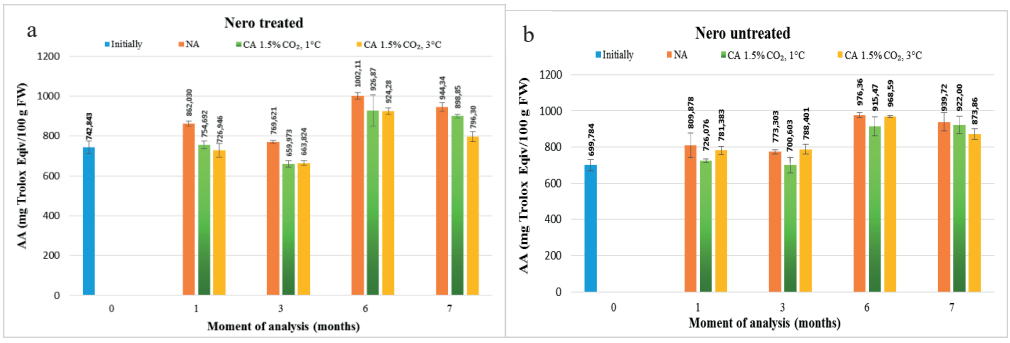


Figure 1. Total phenolic content variations for organic 'Nero' and 'Nero Eggert' chokeberries, registered during storage period (a-'Nero' variety treated; b-'Nero' variety untreated; c-'Nero Eggert' variety treated; d-'Nero Eggert' variety untreated)



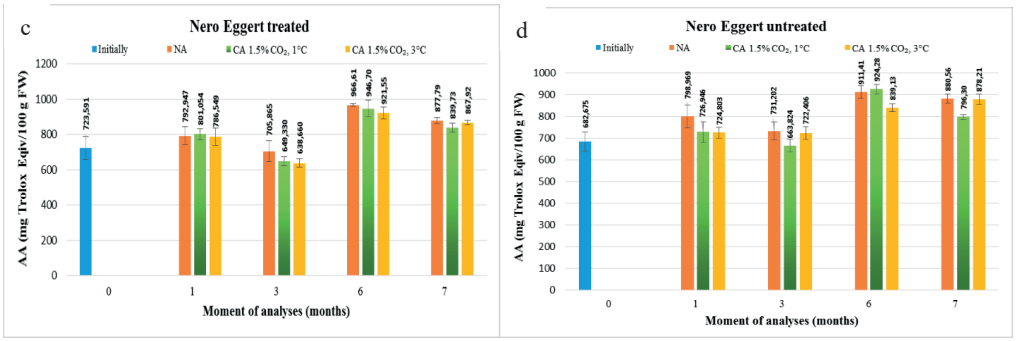


Figure 2. Antioxidant activity variations for organic 'Nero' and 'Nero Eggert' chokeberries, registered during storage period (a-'Nero' variety treated; b-'Nero' variety untreated; c-'Nero Eggert' variety treated; d-'Nero Eggert' variety untreated)

Pathogens identification

Studying the pathogens found on the harvested fruits, it was found that the microflora present in the samples consisted of genus fungi species like *Fusarium* spp., *Penicillium* spp., *Cladosporium* spp. and *Alternaria* spp. After 3 months of storage, *Penicillium* spp. were found on all the samples, exception being 'Nero' variety untreated stored in CA 1.5% CO_2, 16% O_2, 3°C, 95% RH (Tables 3, 4).

High incidence of *Alternaria* spp. were observed on untreated samples, comparative with treated samples, in all storage conditions, in all moments of analyses. *Botrytis* spp. incidence were higher in NA with 1°C, 95% RH than in both CA 1.5% CO_2, 16% O_2, 1°C, 95% RH and CA 1.5% CO_2, 16% O_2, 3°C, 95% RH. *Cladosporium* spp. was detected only on samples stored in CA 1.5% CO_2, 16% O_2, 1°C, 95% RH and 'Nero Eggert' variety treated stored in NA with 1°C, 95% RH. *Fusarium* spp. was only detected on 'Nero Eggert' variety untreated stored in NA with 1°C, 95% RH.

Table 3. Identification of fungal pathogens in 'Nero' variety

Variety/ Treatment	Storage conditions	Analysis moment (months)	*Peniciullium* spp.	*Fusarium* spp.	*Alternaria* spp	*Cladosporium* spp.
Nero/ Untreated	NA with 1° C, 95% RH	3	+	-	-	-
		6	+	+	-	-
		7	+	+	-	-
	CA 1.5% CO_2, 16% O_2, 1°C, 95% RH	3	+	-	-	+
		6	+	-	-	+
		7	+	-	-	+
	CA 1.5% CO_2, 16% O_2, 3°C, 95% RH	3	-	-	-	-
		6	-	-	-	+
		7	-	-	-	+
Nero/ Treated	NA with 1°C, 95% RH	3	+	-	-	-
		6	+	-	-	-
		7	+	-	-	-
	CA 1.5% CO_2, 16%O_2, 1°C, 95% RH	3	+	-	+	+
		6	+	-	+	+
		7	+	-	+	+
	CA 1.5% CO_2, 16% O_2, 3°C, 95% RH	3	+	-	-	+
		6	+	-	-	+
		7	+	-	-	+

Table 4. Identification of fungal pathogens in 'Nero Eggert' variety

Variety/ Treatment	Storage conditions	Analysis moment (months)	*Peniciullium* spp.	*Fusarium* spp.	*Alternaria* spp	*Cladosporium* spp.
Nero Eggert/ Untreated	NA with 1° C, 95% RH	3	+	-	-	-
		6	+	-	-	-
		7	+	-	-	-
	CA 1.5% CO_2, 16% O_2, 1°C, 95% RH	3	+	+	-	-
		6	+	+	-	-
		7	+	+	-	-
	CA 1.5% CO_2, 16% O_2, 3°C, 95% RH	3	-	-	-	+
		6	-	-	+	+
		7	-	-	+	+
Nero Eggert/ Treated	NA with 1°C, 95% RH	3	+	-	-	-
		6	+	-	-	-
		7	+	-	-	-
	CA 1.5% CO_2, 16% O_2, 1°C, 95% RH	3	+	-	-	+
		6	+	-	+	+
		7	+	-	+	+
	CA 1.5% CO_2, 16% O_2, 3°C, 95% RH	3	+	-	-	+
		6	+	-	-	+
		7	+	-	-	+

CONCLUSIONS

Organic fertilization did not significantly influence the properties of chokeberry fruits, all quality indicators are similar between treated and untreated fruits.

The results show that the fruit retains its firmness better in both controlled atmosphere conditions comparative to normal atmosphere storage.

The longest storage period had the varieties stored in CA 1.5% CO_2, 16% O_2, 1°C, 95% RH, but to keep the antioxidant capacity at higher values, it is recommended to store the fruits for a maximum 6 months.

RECOMMENDATIONS

'Nero' variety stored in NA with 1°C, 95% RH, had the highest concentration of TPC after 6 months of storage, which is the maximum storage period, to keep the phenolic content at the optimal level.

'Nero' variety stored in CA 1.5% CO_2, 16% O_2, 1°C, 95% RH, showed constant firmness values during storage, being the most performing variety of chokeberries.

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MODIFIED ATMOSPHERE INFLUENCE IN ORGANIC 'TITA' PLUMS QUALITY

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Abstract
The modified atmosphere is intensively studied as a fruit storage method due to consumers growing demands regarding the fresh fruit quality. The aim of this study was to extend the organic plums shelf life, by packing them in biodegradable, eco-friendly trays, and to ensure that the physicochemical and nutritional characteristics are maintained throughout the storage period. The experiments were performed in 2019 and 2020 with organic 'Tita' plums harvested at the end of July, packed immediately and cold stored at 1 ± 0.5 °C and 85 ± 5 % RH. The modified atmosphere was realised in passive way through respiration and transpiration processes of organic plums. As control, unpacked plums were kept in same storage conditions. The results showed small decreases for physicochemical characteristics of organic 'Tita' plums stored in modified atmosphere, after more than 40 days, for both years of study. Based on the physicochemical and nutritional properties we can state that organic 'Tita' plums packed in biodegradable and compostable trays were better preserved.
Key words*: antioxidant activity, biodegradable package, cold storage, passive MA, respiration.*

INTRODUCTION

Modified atmosphere packaging is a technique used for prolonging the shelf-life period of fresh or minimally processed foods (Sandhya, 2010). Modified atmosphere packaging (MAP) is defined as "the packaging of a perishable product in a changed atmosphere and different by air concentration". This preservation technique is based on air surrounding the food in the package is changed to another gaseous composition and the initial product freshness may be prolonged.

Modified atmosphere packaging (MAP) technology is largely used for minimally processed fruits and vegetables (Sandhya, 2010) and can be realized as an active or passive dynamic process (Jalali et al., 2017). Passive MAP relies on natural initial gaseous composition as well as the interaction between the respiration rate of the produce, and packaging material gases permeation, while in active MAP gaseous composition is additionally flushed into the package in order to achieve faster equilibrium atmosphere (Jalali et al., 2017; Caleb et al., 2012; Farber et al., 2003; Mahajan et al., 2007).

Plums (*Prunus domestica* L.) are climacteric fruits with highest diversity in native cultivars in Romania (Stan et al., 2020; Butac et al., 2019). Due to their climacteric characteristic, after harvesting plums continue the respiration and transpiration processes, which can be used in passive modified atmosphere packaging. One of the most common plum cultivars is 'Tita' variety, wich belong to *Prunus domestica* L. species. In the last years the passive or active MAP utilization as postharvest technologies was more extesively studied, with the main scope of prolonging the freshness and shelf life of fruits, especially for plums. The MAP efficacy in reducing chilling injury symptoms in some plums varieties was studied in several papers (Díaz-Mula et al., 2011a; Cantín et al., 2008; Guan and Dou, 2010) and it was observed that ripening parameters like dehydration, softening, respiration rate, colour changes, total soluble solids increasing, and acidity losses are delayed (Díaz-Mula et al., 2011b).

The aim of this study was to extend the organic plums shelf life, by packing them in biodegradable, eco-friendly trays, and to ensure that the physicochemical and nutritional

characteristics are maintained throw the storage period.

MATERIALS AND METHODS

Chemicals

Chemicals used in experiments were purchased from different producers: Gallic acid from Carl Roth; Trolox from Acros Organics, Fisher Scientific (Geel, Belgium); Folin-Ciocâlteu's reagent and DPPH (1,1-diphenyl-2-picrylhydrazyl) from Sigma-Aldrich Chemie GmbH (Riedstrasse, Steinheim); anhydrous sodium carbonate from Lach-Ner, (Neratovice, Czech Republic); sodium hydroxide 0.1N from Cristal R Chim S.R.L. (Bucharest, Romania); methanol from Honeywell (Riedel-de Haën, Seelze, Germany) and ultrapure water was obtained with Milli-Q water equipment (Millipore, Bedford, MA).

Samples

Organic plums from 'Tita' variety were harvested in July 2019 and 2020 from ICDP Pitesti (Research Institute for Fruit Growing from Pitesti, Romania), and stored at 2°C, 90% relative humidity (RH) until were transported to the Research Center for Studies of Food Quality and Agricultural Products (USAMV of Bucharest) in the Postharvest Technologies Laboratory. Plums were packed immediately after reception and cold stored at 1 ± 0.5°C and 85 ± 5% RH. The modified atmosphere was realized in passive way through respiration and transpiration processes of organic plums. As control, unpacked plums were kept in same storage conditions. Organic plum samples were evaluated during 41 days of storage in modified atmosphere.

Quality indicators

Quality parameters represented by pH, total titratable acidity (TTA), total soluble solids (TSS), dry matter (DM), and firmness; were analyzed during storage period, their methods being described below.

TTA analysis was performed with the automatic system TitroLine, equipped with pH electrode. 5 g of fresh sample were mixed with 25 mL of distillate water and titrated with 0.1N NaOH up to 8.1 pH according with Saad et al. (2014) and AOAC Official Method 942.15. Results were expressed in g malic acid / 100 g of fresh fruit similar with Stan et al. (2020). pH was obtained by measuring the pH values of sample before titration. TSS analysis was realized with digital refractometer Kruss DR301-95, in accordance with Brix reading (Turmanidze et al., 2017). Dry matter content was obtained by oven drying (UN110 Memmert) 1 g of sample at 105°C (Ticha et al., 2015) until constant weight. Firmness was expressed in N/cm² and performed with digital penetrometer 53205 TR Italy and 8 mm piston (Stan et al., 2020). During storage period the passive modified atmosphere was monitorized by measuring oxygen and carbon dioxide concentrations with OxyCheck equipment.

Bioactive compounds

Bioactive compounds like total polyphenol content (TPC) and antioxidant activity were also monitored during experiment development. TPC quantitative determination using Folin-Ciocâlteu method was realised besed on a previous developed protocol (Bădulescu et al., 2019). Extraction consist in 1 g fresh sample mixed with 10 mL of 70% methanol, incubated in dark at room temperature (approx. 21°C), then homogenized for 1 h and 500 rpm, followed by centrifugation for 5 min at 4°C and 7000 rpm, with supernatant recovering and re-extracting the residue for two more times. The final volume was 30 mL. Sample preparation for spectrophotometric measurements: 0.5 mL of extract was mixed with 2.5 mL of Folin-Ciocâlteu reagent and incubated at room temperature (approx. 21°C) for 2 minutes. Then 2 mL of 7.5% sodium carbonate solution (Na_2CO_3) were added and incubated at 50°C for 15 min. Spectrophotometric measurements of the samples were realized using the Specord 210 Plus UV-VIS spectrophotometer (Analytik Jena, Jena, Germany) at the 760 nm wavelength. Results were expressed in mg GAE/100 g fresh weight.

Antioxidant activity was determined using the DPPH (2,2-diphenyl- 1-picrylhydrazyl) method (Bujor et al., 2016). Therefore 0.2 mL of extract were mixed with 2 mL of 0.2 mM solution of DPPH in methanol and incubated in dark with continuous homogenising for 30 minutes. The Specord 210 Plus UV-VIS

spectrophotometer (Analytik Jena, Jena, Germany) was used to measure the sample absorbance at 515 nm wavelength. Results were calculated as mg Trolox eq./100 g fresh weight.

Statistical analysis

All data were obtained from average of three independent replicates and were statistically analysed with standard deviation using Microsoft Excel.

RESULTS AND DISCUSSIONS

Quality indicators

Quality indicators of organic 'Tita' plums were monitored during 41 days of cold storage and modified atmosphere. Analysis were performed initially, after 28, respectively 41 days for both plum storage conditions. In Table 2 are presented oxygen and carbon dioxide variation during storage of organic 'Tita' plums. It can be observed that after packaging, due to respiration and transpiration processes oxygen concentration decrease and carbon dioxide increased in both years of study. In Table 1 it can be observed that translucency appear after 28 days of both organic 'Tita' plums storages (packed in modified atmosphere and bulk). Same physiological disorder were observed by Stan et al. (2020) for organic 'Tita' plums stored controlled atmosphere conditions with 5% CO_2, respectively 10% CO_2. After 41 days of storage in 2019 the organic 'Tita' plums stored as bulk presented a more sever decay appearance comparing with those from 2020 experiment. When organic 'Tita' plums packed in modified atmosphere were analyzed, it was observe that translucency also appear after 28 days of cold storage and after 41 days similar results like in the case of bulk plums storage were noted. The translucency disorder was present after 28 days of storage (passive modified atmosphere and bulk), comparing with same organic plums from Stan et al., (2020) paper, stored in controlled atmosphere with 3% O_2 and 5% CO_2, respectively with 1.5% O_2 and 10% CO_2 when disorder appear after 5 weeks of storage (35 days). It can be see that a high concentration of carbon dioxide and low oxygen prolong the shelf life of organic 'Tita' plums. In the case of organic 'Tita' plums packed in passive modified atmosphere, the carbon dioxide and oxygen concentrations were high, therefore the storage capacity was considerably lower.

In Table 3 the following quality indicators are present TTA, pH, TSS, DM and firmness. The TTA values of organic plums registered a similar variation for both storage methods used and for both studied years. Similar results were observed by Majeed & Jawandha (2016) and Stan et al. (2020). Dry matter content registered increases during storage periods in 2019 with 51% for plums packed in passive MA and with 93% for those stored bulk after 41 storage days. TSS shown smiliar variations for both studied years. In 2019 experiment, firmness registered decreases of 19% for plums packed in passive MAP comparing with those stored bulk, which registered decreases with 57% after 41 storage days. These result are in accordance with those observed by Manganaris & Crisosto (2020) in their study were they mentioned that fruit stored in air are less firm than those stored in CA and temperatures above 0°C.

Bioactive compounds

Bioactive compounds were represented by total phenolic content and antioxidant activity and present similar variations during storage period for both storage conditions and years. In 2020 study the organic 'Tita' plums showed smaller values comparing with those obtained in 2019, but kept a similar trend for both TPC and AA.

For plums packed in passive MAP, higher values for TPC - 128.11 mg GAE/100 g FW comparing with bulk stored - 117.27 mg GAE/ 100 g FW after 41 days of storage were observed. Comparing with the initial moment of analysis, the TPC values registered increases in both study years (Figure 1).

An increase in AA values for plums packed in passive MA in 2019 (1468.09 mg Trolox eq./ 100 g FW) after 41 storage days was observed when compared to the initial results (1255.10 mg Trolox eq./100 g FW). On a contrary basis, in 2020 a small decrease (1033.58 mg Trolox eq./100 g FW) was observed after 41 storage days, comparing with the initial analysis (1205.99 mg Trolox eq./100 g FW) (Figure 2).

The obtained variation for both TPC and AA are small, less than 10% for passive MA in both years, and for bulk plums in 2020, except

for the AA for bulk plums in 2019, which showed a decrease of 14.08%. This suggest that passive MA stored plums can be stable for at least 41 days without high degradation of TPC and AA.

Table 1. Influences of modified atmosphere and cold storage on organic 'Tita' plums appearance

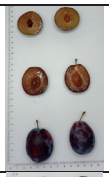
Year	Moment of analysis (weeks) / Storage conditions	0	28 days	41 days
2019	Before storage		n/a	n/a
	Modified atmosphere	n/a		
	Bulk	n/a		
2020	Before storage		n/a	n/a
	Modified atmosphere	n/a		
	Bulk	n/a		

Table 2. Oxygen and carbon dioxide variation during storage of organic 'Tita' plums

Variety	Storage conditions	Analysis moment (weeks)	Oxygen concentration (%)	Carbon dioxide concentration (%)
'Tita'/ 2019	N/A	**0**	20.9 ±0.001	0.74 ±0.05
	Modified atmosphere	**28**	19.04 ±0.0074	6.90 ±0.003
		41	18.54 ±0.008	6.37 ±0.004
'Tita'/ 2020	N/A	**0**	20.89 ±0.01	0.78 ±0.02
	Modified atmosphere	**28**	19.18 ±1.01	6.06 ±3.06
		41	20.075 ±0.7	5.90 ±0.007

Data represent mean ± standard deviation of five replicates.

Table 3. Variation of quality indicators during storage of 'Tita' plums in modified atmosphere and bulk in cold storage

Variety	Storage conditions	Analysis moment (weeks)	pH	TAA (g malic acid/100 g FW)	TSS %	DM %	Firmmnes (N/cm²)
'Tita'/ 2019	N/A	**0**	3.42 ±0.06	1.16 ±0.01	17.85 ±1.10	8.13 ±1.47	15.14 ±1.86
	Modified atmosphere	**28**	3.39 ±0.05	1.17 ±0.00	15.17 ±1.72	14.06 ±1.27	8.46 ±1.73
		41	3.43 ±0.03	0.89 ±0.01	13.94 ±1.93	12.3 ±0.90	12.15 ±4.87
	Bulk	**28**	3.34 ±0.05	0.95 ±0.01	15.93 ±2.29	14.38 ±0.36	7.54 ±1.73
		41	3.60 ±0.20	0.74 ±0.04	16.87 ±2.14	15.74 ±0.87	6.50 ±2.31
'Tita'/ 2020	N/A	**0**	3.25 ±0.02	0.96 ±0.01	13.31 ±2.06	14.89 ±5.18	18.05 ±4.85
	Modified atmosphere	**28**	3.26 ±0.02	0.83 ±0.003	13.04 ±0.27	11.36 ±0.88	8.97 ±3.16
		41	3.43 ±0.14	0.75 ±0.01	13.49 ±0.99	10.46 ±1.57	9.00 ±1.68
	Bulk	**28**	3.20 ±0.05	0.94 ±0.005	15.90 ±2.06	14.94 ±1.13	10.62 ±3.29
		41	3.28 ±0.03	0.77 ±0.01	14.73 ±0.38	14.04 ±0.56	7.32 ±2.05

Data represent mean ± standard deviation of three replicates.

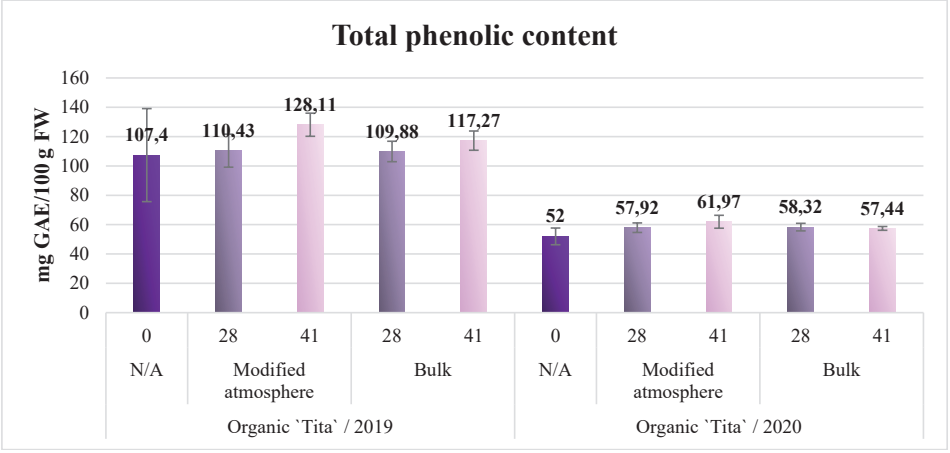


Figure 1. TPC variation for organic 'Tita' plums in modified atmosphere and cold storage

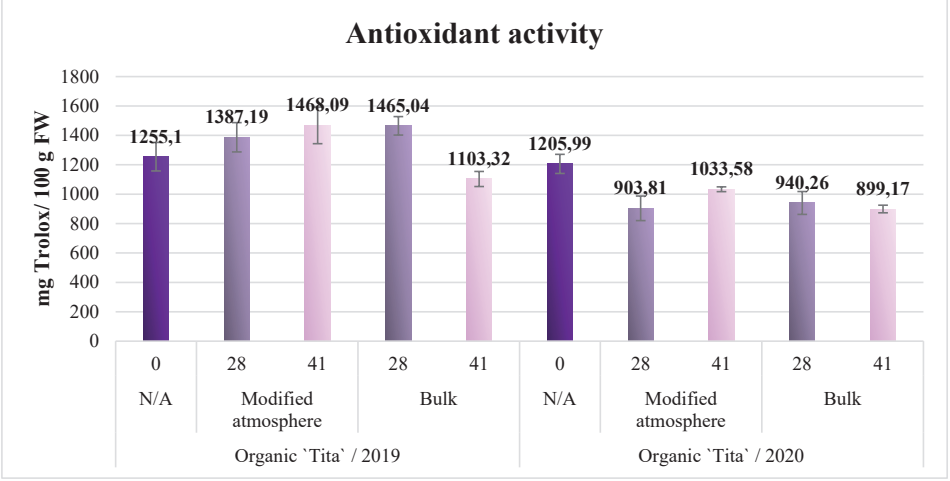


Figure 2. AA variation for organic 'Tita' plums in modified atmosphere and cold storage

CONCLUSIONS

The results showed firmness decreases of organic 'Tita' plums stored in modified atmosphere and bulk, after 41 days, for both years of study.
Even if the translucency disorder appear after 28 days both storage methods, the quality indicators present better values for organic 'Tita' plums packed in passive MA than those stored as bulk. Moreover the bioactive compounds of organic 'Tita' plums packed in biodegradable and compostable trays were better preserved.

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JUJUBE PROCESSING: METHODS, PRODUCTS AND NUTRACEUTICAL VALUE

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Abstract

Chinese jujube (Ziziphus jujuba Mill.) is the most important species of Rhamnaceae family and one of the oldest cultivated fruit trees in the world. In Romania, it was introduced for the first time in Dobrogea region, some 2000 years ago. The first cultivated varieties were introduced in 1997 at the Faculty of Horticulture, in Bucharest and nowadays the first commercial orchards are planted. This review aims to highlight the nutritional importance of jujube fruit and its high potential for the development of new and valuable food products. Moreover, jujube can be incorporated into different food formulations to improve their nutraceutical quality. A huge number of products can be obtained from fresh and dried jujube fruits: juice, compote, tea and coffee, liqueur, wine, brandy, but also gems, fruit bars and cakes. Jujube fruits can be processed in different ways, dehydration being utilized for most of the varieties. Besides that, fruits can be transformed into non-alcoholic fermented beverages such as juices, tea, coffee, energy drinks, or distilled beverages.

Key words*: benefits, food products, jujube fruits, nutritional importance, Ziziphus jujuba Mill.*

INTRODUCTION

Chinese jujube (*Ziziphus jujuba* Mill.), also called Chinese date, is one of the oldest cultivated fruit trees in the world and is the most important species in the large cosmopolitan family *Rhamnaceae* in terms of its economic, ecological, and social importance. Its utilization and cultivation history can be traced back to the Neolithic age, 7000 years ago (Qu & Wang, 1993; Guo et al., 2010; Liu et al., 2020).

Chinese jujube is a new fruit species for Europe with a high potential to be planted on arid and semiarid areas on marginal, poor, and even salty soils. Being a multi-millennial fruit crop in China, jujube has high importance in the Chinese diet due to its complex nutraceutical properties.

Nearly 1,000 varieties and local genotypes are cultivated in China on over two million hectares on low input production systems.

In Romania, jujube populations were found in the Dobrogea region (Ciocârlan, 2000), between the Danube and the Black Sea in the neighbourhood of antique sites as Greek, Roman and Byzantine ruins at Ostrov, Jurilovca and Mahmudia.

Probably, those old civilizations had an important role in the introduction of this Asian plant to the area (Stănică, 2000; Stănică, 2009; Stănică et al., 2018).

The plant, nearly unknown, is named Dobrogea olive by the locals and the fruits are rarely used for eating. Only Ostrov type, a real *Ziziphus jujube* tree, has interesting fruit for fresh and dry consumption (Ciocârlan, 2000; Stănică, 2009; Stănică et al., 2018).

Even it was introduced in the Dobrogea region, some 2,000 years ago by the Greek and Roman colonists, jujube plants and fruits are nearly unknown, as it happens in other countries from the Mediterranean basin.

The first cultivated varieties were introduced at the Faculty of Horticulture in Bucharest from Shanxi Province, China, within a common research project in 1997 (Stănică, 2019).

A superfruit species for the future should simultaneously meet the diverse needs of growers, consumers, marketers, governments, and society.

Generally, growers prefer fruit trees that crop early, reach high and stable yields quickly, and have light pest pressure, easy management, low cultivation costs, and high economic benefits (Stănică, 2019).
Consumers like fruits that are delicious and nutritious and that appeal to appearance and status. Marketers prefer fruits that can be easily transported from production areas and have long shelf lives and large markets (Liu et al., 2020).
The government and society pay more attention to ecological friendliness, the efficient use of land resources, and the social and economic advantages for rural farmers in marginal regions. By meeting all these expectations, jujube deserves to be considered a super fruit for the future (Liu et al., 2020).
This review aims to highlight the nutritional importance of jujube fruit and its high potential for the development of new and valuable food products.

MATERIALS AND METHODS

1. Health benefits

Traditional medicinal uses and potential health benefits of jujube are found also in the modern medical industry.
The jujube leaf, which is the main by-product of jujube, has also been used in traditional Chinese medicine (TCM) for thousands of years to improve sleep, nourish the heart and soothe the nerves, and reduce haemorrhaging (Damiano et al., 2017).
Based on Iranian traditional medicine, local traditional healers (Hamedi et al., 2016) used powders of stem bark and leaves of jujube to cure wounds and oral wounds as aphthous. Hamedi et al. (2016) also discovered that fruits are widely used in Iranian folk medicine as antitussive, laxative agents, and blood pressure reducers.
Jujube has also been used in Chinese medicine (TCM) for many years for its various and numerous health benefits such as anti-inflammatory (Yu et al., 2012), anti-cancer (Plastina et al., 2012), gastrointestinal protective, anti-oxidant, anti-insomnia, and neuroprotective (Yoo et al., 2010).
In recent years, food scientists and nutrition specialists agree that jujube fruits, consumed daily, contribute to reducing risks of certain diseases, including cancer and cardio and cerebrovascular diseases (Liu et al., 2000).
Different parts of jujube can be used for curing different kinds of illness such as diabetes, diarrhea, skin infections, liver complaints, urinary disorders, obesity, fever, pharyngitis, bronchitis, anaemia, cancer, insomnia, and of course for blood purification and the gastrointestinal tract (Shahrajabian et al., 2019).

2. Nutritional value

Jujube is an interesting plant to prevent several diseases. Fruits are one of the major dietary sources of various antioxidant phytocompounds for humans.
Jujubes have a high nutritional value for the human health (Stănică, 2016; Stănică, 2019).
Taking all this into consideration, the jujube fruits were included in the category of new "super fruits" that should be introduced on large scale production in Romania but in Europe, also (Stănică, 2016; Dicianu et al., 2017; Liu et al., 2017; Zhao et al., 2017; Stănică, 2019; Stănică et al., 2020a).
The various antioxidants (polyphenol, ascorbic acid, carotenoids, and tocopherols) present in fruits contribute to these beneficial effects (Peschel et al., 2006).
These antioxidants prevent diseases by scavenging radicals or by suppressing the formation of free radicals by binding to metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen (Guroo et al., 2017).
Jujube has a high nutritional value due to the presence of a large number of nutrients and phytochemicals, such as fibres, proteins, fat, carbohydrate, vitamins (ascorbic acid, thiamine, and riboflavin), phenolics, and minerals (Chen et al., 2019; Rashwan et al., 2020).
First of all, its dietary fibres (Li et al., 2007) and fructose (Gao et al., 2012) contents may contribute to regulating blood sugar levels by slowing digestion, with its fibres content also contributing to controlling calories intake by its satiating effect.
To a lesser extent, jujubes are a source of healthy, essential fatty acids because jujube fruit is rich in unsaturated fatty acids (68.54-72.44% of the total fat in jujube fruits). There are 33 fatty acids identified from the dried pulp of *Ziziphus jujuba* Mill. (Gusakova et al., 1999).

The chain length of those components was from 7 to 28 carbon atoms. Sixteen fatty acids, with a dominance of 16:1 (Guo et al., 2010) and 16:1, (Pawlowska et al., 2009) are partly responsible for the fragrance of the fruit.

Fatty acid profiles of the fruits were influenced by their developmental stage (Guil-Guerrero et al., 2004).

The predominant fatty acids in jujube selections were oleic, linoleic, palmitic, and palmitoleic acids (San & Yildirim, 2010).

Jujube fruits are rich in lipids, especially linoleic acid (omega-6), that the human body is not capable of producing (Simopoulos et al., 2008).

Great interest has developed for jujubes because of their high content of vitamin C, which makes them an important source of this vitamin for human nutrition.

Moreover, the jujube, although to a lesser extent, is a source of several other vitamins, such as thiamine, riboflavin, niacin, vitamin B6, and vitamin A (Chen et al., 2019).

In this part, we comprehensively discussed the jujube nutrients and phytochemicals that were divided into three categories: macronutrients, micronutrients, and bioactive compounds.

The content of macronutrients, micronutrients, and bioactive compounds in jujube fruits. (*Ziziphus jujuba* Mill.) (Rashwan et al., 2020) is present in Table 2.

2.1. Macronutrients

Generally, macronutrients are the main nutrients in the foods that we consume, such as proteins, fat, carbohydrate, fibres, and moisture. According to the National Research Council (1989), jujube is a rich source of macronutrients.

Chinese jujube contains eighteen kinds of amino acids including eight essential amino acids. In addition, protein, sugar, and fat content ranged from 3.3 ~ 4.0, 50.3 ~ 86.9, and 0.2 ~ 0.4 g/100 g DW (dry weight), respectively (Food & Board., 1989).

Sheng and his team (2002) found that the jujube Chinese winter fruit contained moisture 74.08%, soluble protein 0.307%, total sugar 18.46%, soluble solid 27.0%, and fibres 1.37%.

In contrast, a study on five Chinese jujube cultivars made by Li et al. (2007) reported that jujube contained carbohydrates ranged from 80.86 to 85.63%, reducing sugar 57.61-77.93 %, soluble fibres 0.57-2.79%, insoluble fibres 5.24-7.18%, protein 4.75-6.86%, lipid 0.37-1.02%, and moisture 17.38-22.52 % (Li et al., 2007).

Afterwards, Uddin and Hussain (2012) uncovered that the moisture, total solids, total soluble solids, total sugar, proteins, fat, and ash contents of jujube fruit were 83%, 17%, 8.1°Brix, 6%, 1.6%, 0.2%, and 0.7%, respectively.

Instead, a study on four Spanish jujubes by Hernández et al. (2016) unveiled that Spanish jujube contained moisture amount from 78.3 to 82.1%, TSS (total soluble solids) 14.6 to 18.4 °Brix, total sugars 10.8 to 19.2 g·100 mL^{-1}, protein 3.7 to 5.8%, and crude fibres 0.7 to 1.0 g·100 g^{-1} DW.

A recent study on three Chinese jujubes by Chen et al., uncovered that the moisture content, total dietary fibres, protein, and total sugar of jujube fruit were ranged from 64.31-76.50, 4.85-7.32, 1.87-3.97 g/100 g, and 28.68 to 31.7% (FW), accordingly (Chen et al., 2019).

These studies confirmed that jujube fruits have large amounts of macronutrients and the contents were varied depending on location, cultivars, and detection method (Rashwan et al., 2020).

2.2. Micronutrients

Micronutrients are important substances that are found in small amounts in food, often indicated as minerals and vitamins. Micronutrients are vital elements for healthy growth and development and disease prevention.

Previous studies indicated that jujube fruits were a rich source of micronutrients including vitamins, macroelements (N, K, Mg, Ca, P, etc.), and microelements (Fe, Mn, Cu, Zn, etc.) (Chen et al., 2019; Choi et al., 2016). Sheng's team (Sheng et al., 2002) reported that Chinese winter jujube fruit contained higher vitamin C, approximately 379.4 mg/100 g, which was about 80 to 100 times higher than that of apple.

On the other hand, they also found that jujube fruit contained a moderate amount of minerals, such as phosphorus, potassium, calcium, magnesium, iron, manganese, copper, zinc and sodium. In addition, Li et al. (2007) evaluated the range of micronutrients in five Chinese

jujube cultivars such as ascorbic acid (192-359 mg/100 g), thiamine (0.04-0.08 mg/100 g), and riboflavin (0.05-0.09 mg/100 g). According to their study, the range of minerals contents (mg/100 g FW) in jujube cultivars were as follows, potassium contents (79.2 to 458, phosphorus 59.3 to 110, calcium 45.6 to 118, manganese 24.6 to 51.2, iron 4.68 to 7.90, sodium 3.22 to 7.61, zinc 0.35 to 0.63, and copper 0.19 to 0.42 (Li et al., 2007; San et al., 2009).

Jujube fruit contained about 71.92% of the nitrogen-free extract, which contained calcium and potassium approximately 72.14 and 899.82 mg/100 g, respectively (Kim et al., 2011). In the case of four Spanish jujubes cultivars, the range of vitamin C content was found to be 0.41-0.64 (g 100 mL^{-1}), while the range of macroelements content such as potassium, calcium, magnesium, and sodium were 11.9-17.3, 0.23-0.72, 0.40-0.77, and 0.11-0.43 g kg^{-1} DW, respectively.

Furthermore, the range of microelements content such as iron, zinc, copper, and manganese were 10.2-17.1, 4.0-5.1, 0.5-1.2, and 0.2-2.9 mg kg^{-1} DW, accordingly (Hernández et al., 2016).

In two Korean jujubes cultivars, the vitamin C content ranged from 29 ~ 37.67 mg/100 g FW, and the minerals content such as calcium, phosphorus, and iron were ranged from 11.58 to 14.69, 32 to 29.83, and 0.3 mg/100 g FW, respectively (Choi et al., 2016). Analysis of vitamin C content in three Chinese jujube cultivars showed the significantly higher content ranged from 162.50 to 244.58 mg/100 g FW (Chen et al., 2019).

In summary, it is confirmed that jujube fruit is a potential source of micronutrients (Rashwan et al., 2020).

2.3. Bioactive compounds

Jujube is considered a great source of bioactive components, including polyphenols, triterpenic acids, polysaccharides, nucleosides, and nucleobases. Jujube is thus recognized as one of the rich sources of functional food (Wojdyło, et al., 2016). For example, in the pulp of jujube fruit, total phenolic ranged from 1.1 to 2.4 g/100 g DW, and flavonoids contents ranged from 0.7 to 1.8 g/100 g DW.

Furthermore, the jujube fruits contained several flavonoids compounds, such as procyanidin B2, epicatechin, quercetin – 3 – O – rutinoside, quercetin – 3 – O – galactoside, kaempferol glucosyl – rhamnoside (Choi et al., 2011).

Jujube contained a low amount of triterpenic acids, nucleosides, and nucleobases. A study by Guo et al. (2015) reported that the total triterpenic acids in six stages of growth of jujube fruit ranged from 166-6126 (μg/g of DW), whereas the total nucleosides and nucleobases contents ranged from 253-481 (μg/g of DW). In the case of fresh jujube, titratable acids content of three Chinese jujube cultivars ranged from 1.98 to 3.12% FW, cAMP ranged from 20.35 to 87.5 (μg/g FW), and total flavonoids ranged from 41.21 to 62.72 (mg/g FW) (Chen et al., 2019). On the other hand, the polysaccharide is one of the minor bioactive compounds of jujube fruit (Zhan et al., 2018). A recent study confirmed that the maximum crude polysaccharide yield obtained from jujube fruit was 7.9%.

According to their research, the main components of jujube polysaccharides were arabinose, galactose, glucose, mannose, rhamnose, and galacturonic acid (Liu et al., 2020; Rashwan et al., 2020).

Table 1. Nutritional value of fresh jujube fruit

Nutritional value per 100 g	
Energy	79 kcal
Carbohydrates, by difference	20.23 g
Fat	0.2 g
Protein	1.2 g
Ash	0.51 g
Water	77.86 g
Vitamins	
Vitamin A	2 μg
Thiamine (B1)	0.02 mg
Riboflavin (B2)	0.04 mg
Niacin (B3)	0.9 mg
Vitamin B6	0.081 mg
Vitamin C	69 mg
Minerals	
Calcium (Ca)	21 mg
Iron (Fe)	0.48 mg
Magnesium (Mg)	10 mg
Manganese (Mn)	0.084 mg
Phosphorus (P)	23 mg
Potassium (K)	250 mg
Sodium (Na)	3 mg
Zinc (Zn)	0.05 mg
Copper (Cu)	0.073 mg

Source: USDA, 2019

The nutritional value of fresh jujube fruit according to (USDA, 2019) is present in Table 1. This makes jujube a premier choice for anyone looking to live a healthier life or even simply bolster their immune system.

3. Processing

The common purpose of food processing is to avoid food spoilage during the storage period, which is caused by the development of bacteria, yeasts and fungi. It also makes the seasonal products available for a long period.

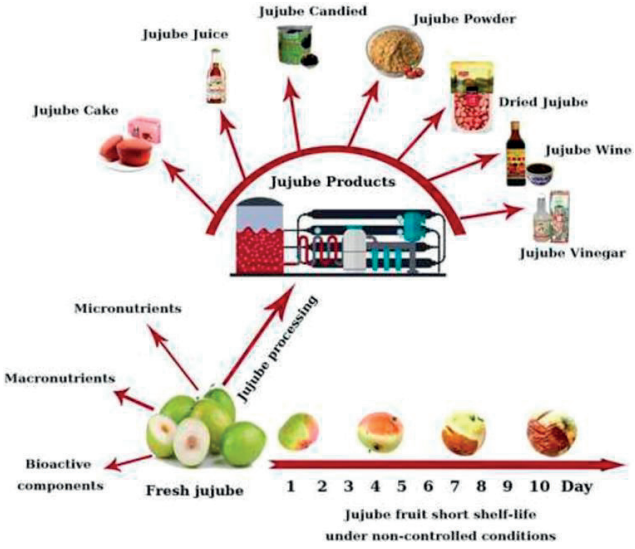
Suitable product processing allows maintenance of typical organoleptic and nutritional features of foods, which is also beneficial for human health (Krška & Mishra, 2009).

Normally, jujube fruit is harvested in autumn, and their postharvest shelf-life is very short (Stănică et al., 2020b). It can be stored for no more than ten days under non-controlled conditions (Zozio et al., 2014a).

Thus, it is very important to explore jujube processing and/or preservation strategies for extending its shelf-life.

Shin and co-authors studied the diverse processing methods for jujube fruits. They found that dried fruits, nectar, jam, fruit extracts, and powdered tea were the most promising processing methods. Evaluation of these products was conducted by sensory evaluation and chemical analysis (Shin et al., 1992).

Besides, Krška & Mishra (2009) studied the different ways of processing jujube fruits like cloying with honey, preservation in sweet-sour infusion vinegar, conservation in sweet infusions like compote and dry jujube fruits. Furthermore, another study investigated the processing and preservation of jujube fruits via developing various products formula such as jam, jelly, chutney and pickles (Rashwan et al., 2020; Uddin & Hussain, 2012). In Figure 1. processing possibilities of jujube can be observed.



Source: Rashwan et al., 2020

Figure 1. Processing possibilities of jujube fruit

3.1. Dried jujube

The drying technique is one of the most substantial methods for the preservation of foods, which has been used for a long time. Drying can assist in transportation and storage of jujube by providing some features such as lighter weight and smaller volume compared to fresh products.

In addition, it can prolong the product's shelf-life via minimizing and/or removing moisture-mediated deteriorative reactions.

Hence, it can prevent the growth and reproduction of the microorganisms that cause putrefaction (Brasiello et al., 2013).

Dried jujubes have a chewy texture and taste similar to dates. Besides, dried jujube products

(e.g. whole fruit, slices, jujube chips, and powder, etc.) are popular among consumers owing to great taste and high nutritional value (Hao et al., 2019; Wang et al., 2016; Wojdyło et al., 2016; Wojdyło et al., 2019).
In Figure 2, can be observed dried jujube in different forms.

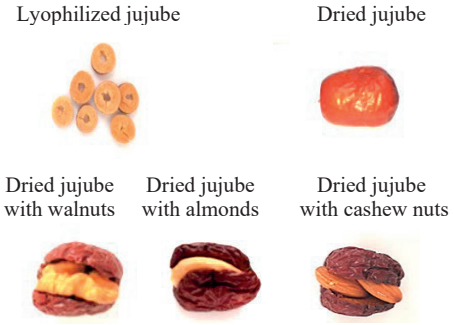


Figure 2. Dried jujube in different forms

3.2. Candied jujube

Candied fruit is known as crystallized fruit or glacé fruit, where the whole fruit or smaller pieces of fruit/peel are placed in heated sugar syrup, which absorbs the moisture from the fruit and eventually preserves it (Kuwabara, 1988). Candied jujube is a famous product in China. Haiying (2006) reported that candied jujube with nutritional value can meet more consumer's needs (Figure 3). Jujube with low-sugar syrup type can be prepared through substituting xylitol for sugar, where can be reduced saccharifierous quantity of the preserved fruit.

Figure 3. Candied jujube

3.3. Jujube jam, jelly and cakes

Jams are thick sweets prepared by the cooking of crushed or chopped fruits with enough amount of sugar, which tend to hold their shape but are less coherent than jelly (Rashwan et al., 2020).
Jelly is very similar to jam but is produced by boiling/cooking fruit juice with a large amount of sugar, with or without the addition of pectin and food acids depending on the pectin and acid content in the fruit (Zhao, 2012).
Jujube fruits have a large number of nutritional components especially polysaccharides that is considered as one of the bioactive compounds. Therefore, jujube jams and jelly are considered promising processed foods (Zhao et al., 2012).

Figure 4. Jujube cakes

3.4. Jujube fruit as a food additive

The jujube powder is used in many food product formulations (Najjaa et al., 2020). The ingredients added to food to enhance the nutritional value, improve the taste, maintaining the flavour and appearance of food, etc., are known as food additives. Recently, the application of food additives was of great importance in the food industries throughout the world.
However, there are increasing concerns about the harmful health effects (hazard) of synthetic food additives, including colourings, flavourings, and preservatives (Liu and Hill., 2015).
Therefore, the use of natural food additives is the better alternative to overcome the health risks of synthetic food additives. Jujube fruits are considered as one of the natural food ingredients, which is applied both in food and herbal medicinal (Rashwan et al., 2020) because of their good health benefits.
The powder of jujube (Figure 5) has a higher availability of phenolic compounds; possesses antioxidant, anticancer antimicrobial, and antianemia activity (Addo et al., 2019).

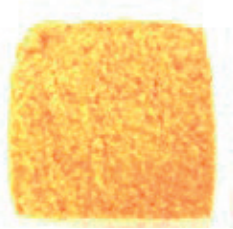


Figure 5. Jujube powder

For example, the addition of jujube extract to the bread dough showed an increased water absorption rate.
In addition, the dough development time, stability, and degree of the extension were decreased, while the degree of weakness, degree of resistance, and resistance/ extensibility were increased (Lee et al., 2005).

3.5. Jujube beverages

Any drinkable liquid prepared for human consumption except water is called a beverage. Recently, the consumption of fruit beverages has increased throughout the world due to the rich source of natural nutrients.
Like other fruits beverages, jujube beverages such as juice, tea, wine, and others, are also available in the market due to their rich source of bioactive compounds (Rashwan et al., 2020). China is one of the top jujube producers, which is about 90% of the total world production. Interestingly, the production amount of jujube in China has been increasing significantly from 4 to 15 million tons over the last 10 years (Guo et al., 2018).

3.6. Jujube juice

Jujube juice (Figure 6) contained a large number of bioactive components, is a rich source of vitamin C, and possesses antioxidant properties. (Vithlani et al., 2010).
Nowadays, jujube juice is consumed as a food ingredient and supplement due to potential sources of bioactive components (Rajauria & Tiwari., 2018; Zhang et al., 2012).
Jujube juice can be successfully combined with other fruits.

Figure 6. Jujube juice

3.7. Jujube wine

Jujube wine (Figure 7) is considered one of the important jujube fruit beverages. However, studies on wines production from jujube fruits are limited.
Based on the scientific study by Liu & Zhao, (2011), the optimum fermentation conditions for jujube wine were as follows: initial sugar (18%), pH value (4.0), fermentation temperature (24°C) and inoculum concentration of dry yeast *Saccharomyces cerevisiae* (0.3%) (Rashwan et al., 2020).

Figure 7. Jujube wine

3.8. Jujube brandy

VOCs (Volatile Organic Compounds) play an important role in brandy aroma, whereas the presence, absence, or different proportions of VOCs can be greatly influenced by processing methods (Rashwan et al., 2020). Fresh jujube brandy (Figure 8) is a popular alcoholic beverage that is produced by the distillation of fermented broth achieved via continuous fermentation of jujube fruits (Li et al., 2016).

Figure 8. Jujube brandy

This alcoholic beverage is characterized based on the presence of volatile organic compounds which are produced during the fermentation, distillation, and storage stages (Xia et al., 2020).

CONCLUSIONS

Jujube fruit is a dietary supplement with high contents of bioactive compounds such as dietary fibers, mineral, and natural antioxidant compounds. Nevertheless, fresh jujube has a short shelf-life. Thus, converting fresh jujube to processed products is the best way for preserving it for a long-time. Moreover, jujube can be incorporated into different food formulations to improve their nutraceutical quality. A huge number of products can be obtained from fresh and dried jujube fruits:

juice, compote, tea and coffee, liqueur, wine, brandy, but also gems, fruit bars and cakes. Jujube fruits can be processed in different ways, dehydration being utilized for most of the varieties. Besides that, fruits can be transformed into non-alcoholic fermented beverages such as juices, tea, coffee, energy drinks, or distilled beverages.

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KIWIFRUIT PROCESSING. A REVIEW

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Abstract

Kiwifruit (Actinidia sp.) is native to Asia and the first commercial plantations were established in New Zealand, in the early twentieth century. In our country, the first commercial orchard was planted at Ostrov Agricultural State Enterprise (CT) in 1993, by the efforts of Nicolae Cepoiu, Corneliu Petrescu and Florin Stănică. Kiwifruit has become popular worldwide for fresh consumption due to its high sensory and nutritional properties. It contains high levels of bioactive compounds such as vitamin C, vitamin E, flavonoids, antioxidants, carotenoids, minerals and fibbers. Being cultivated both in North and South hemisphere, and having a good storage life (over 6 months), fresh fruits are available through the year. Even so, kiwifruit is largely processed as juices, nectars, syrups, alcoholic drinks (cider, liqueur, brandy), candies and fruit bars, jam and marmalade, patisseries (cakes, cookies), dehydrated and lyophilized products. The aim of this paper is to present a review of the main processing possibilities for kiwifruit.

Key words*: Actinidia sp., benefits, fruits products, nutritional properties.*

INTRODUCTION

Kiwifruit is botanically known as *Actinidia deliciosa* (Beutel et al., 1990). Also known as 'China's miracle fruit', 'the horticultural wonder of New Zealand' and 'Chinese gooseberry (Nirmal et al., 2018; Iliescu et al., 2019).

This species was first found along the border of the Yangtse River valley in China and later, in 1847 and 1904, the first plants were sent to England and United States, respectively (Yerex et al., 1983).

Kiwifruit is an example of recent success in the domestication and commercialisation of a plant for food (Young et al., 1995).

According to official records, the major centre of diversity for *Actinidia* genus is the hilly region of South-Western China (Litz 2005; Nirmal et al., 2018; Stănică et Zuccherelli, 2009), but the first commercial orchards were established in New Zealand (Ferguson and Bollard, 1990; Warrington, 1990; Young et al., 1995) in 1950's (Barboni et al., 2010; Iliescu et al., 2019).

The expansion of this species throughout the world began to take place in the 1960s with the export of New Zealand plants and seeds to destinations such as Germany, Italy, Spain, India, South America, Morocco, Israel and South Africa (Yerex et al., 1983; Pinto T., 2018).

By the 1980's, other countries around the world began to produce and export kiwi.

At present, commercial growth of the fruit has spread too many countries including the United States, Italy, Chile, France, Greece, India and Japan. (FAO., 2018; Teresa Pinto, 2018).

In Romania, the first commercial orchard was planted at Ostrov Agricultural State Enterprise (CT) in 1993, by the efforts of Nicolae Cepoiu, Corneliu Petrescu and Florin Stănică, on an area of 2 ha. Hayward, Kramer, Katiuscia, Tomuri cultivars and three new selections obtained by Vitroplant Cesena: AD 20, AD 24 and AD 25 were planted (Stănică, 2009; Stănică et Zuccherelli, 2007).

MATERIALS AND METHODS

Nutritional value

This new fruit is considered a superfood and is widespread throughout the world, due to his high nutritional value characteristic flavour and high antioxidant and anti-inflammatory properties (Sanz et al., 2020).

Kiwifruit are some of the most nutrient-dense fruit and compared with other commonly consumed fruit, are particularly rich in vitamins C, E, and K, folate, carotenoids, potassium, fibre, and contain a range of phytochemicals (Ferguson and Ferguson, 2003). Both green and gold kiwifruit contain almost double the amount of vitamin C found in oranges and strawberries; traditionally known as good sources of vitamin C (Scăeţeanu et al., 2019).

In addition, kiwifruit have been shown to be a significantly better delivery vehicle for replenishing depleted vitamin C at tissue levels, compared with supplemental vitamin C, in a mouse model (Vissers et al. 2011).

Green kiwifruit has a higher total dietary and insoluble fibre content than other commonly consumed fruit (Scăeţeanu et al., 2019).

Its soluble fibre content is lower than that of oranges, but compares well with apples, bananas, and strawberries.

Both green and gold kiwifruit contain significant levels of two fat-soluble vitamins, vitamin E and vitamin K (as phylloquinone).

Kiwifruit compares well with avocado (1.5 compared with 2.07 mg vitamin E/100 g), the only other fruit high in vitamin E (USDA 2011).

It has been assumed that vitamin E in kiwifruit is restricted to the seeds and therefore not bioavailable (Ferguson and Ferguson, 2003).

However, this seems to be a myth, as Fiorentino et al. (2009b) showed that α-tocopherol is found in the flesh of the kiwifruit, and consumption of both green and gold kiwifruit resulted in increased plasma vitamin E concentrations (Chang and Liu, 2009; Hunter et al., 2012).

The potassium in kiwifruit is comparable with that of bananas, well known for their high potassium content, and more than double that of other fruit. Gold kiwifruit are a good source of folate; similar to that of oranges, but higher than other fruit. (Scăeţeanu et al., 2019).

Apart from oranges, both green and gold kiwifruit are better sources of carotenoids, including β-carotene, lutein, and zeaxanthin, than other fruit. The carotenoids contribute to the colour of the kiwifruit, but the unique green colour of green kiwifruit is attributed to the retention of chlorophyll during ripening (1 mg of chlorophyll/100 g), which masks the yellow colour of the carotenoids (McGhie et al., 2002; Nishiyama, 2007).

Kiwifruit also contains a range of other phytochemicals/polyphenols, although many of the phenolics and flavonoids in kiwifruit are yet to be identified, as to date they have been un-extractable (Tarascou et al., 2010).

The taste of kiwifruit is influenced by the balance of sugar and organic acids (Welma et al., 2012).

According to the Institute of Medicine, the recommended daily fibre intake for adults is 25 grams for women and 38 grams for men (Anderson et al., 2009; Lindsey et al., 2020).

However, average fibre intake for US children and adults are less than half of the recommended levels (Wong et al., 2007).

Table 1. Nutritional references for 100 g fresh fruit for the main species of *Actinidia*

Nutritional value per 100 g	UM*	*A. deliciosa*	*A. chinensis*	*A. arguta*
Energy	kcal	61 [22]	63 [22]	32 [11]
Water	g	83,1 [22]	82,4 [22]	88,0 [11]
Carbohydrates	g	14,66 [22]	14,23 [22]	9,20 [11]
Sugars	g	8,99 [21]	10,98 [21]	3,9-9,6 [1; 3; 24; 17]
Protein	g	1,14 [5]	1,23 [5]	1,70 [11]
Fiber	g	2,13-3,39 [15; 19]	2,0 [19]	2,9-4,1 [14]
Saturated fats	g	0,029 [8]	0,149 [8]	13.90-30.50 [10; 14]
Vitamins				
Vitamin A	µg	87,0 [22]	23,0 [22]	37,3-84,5 [12]
Thiamine (B1)	mg	0.027 [22]	<0.01 [22]	0,01-0,05 [9; 10; 7;17]
Riboflavin (B2)	mg	0.025 [22]	0.074 [22]	0,02-0,11 [9; 10; 7; 17]
Niacin (B3)	mg	0.341 [22]	0.231 [22]	0,50-1,55 [9; 10; 7; 17]
Vitamin B6	mg	0.63 [22]	0.079 [22]	1,10-1,90 [9; 10; 7; 17]
Vitamin C	mg	92,7 [4; 16; 21; 23]	105,4 [4; 16; 21; 23]	22,8-43,0 [16; 17; 25]
Vitamin E	mg	1,46 [4; 21; 23]	1,49 [4; 21; 23]	4.6–5.3 [6]
B9 (Folic acid)	µg	25 [22] - 29 [4; 21; 23]	34 [22]	-
Minerals				
Magnesium, Mg	mg	17[22]	12.0 [22]	10,0-23.2 [5; 20]
Phosphorus, P	mg	34 [22]	25 [22]	31,7-80,2 [5; 20]
Potassium, K	mg	300 [5; 21]	250-400 [18]	162,7-382 [5; 20]
Calcium, Ca	mg	34 [22]	17.0 [22]	51,5-120,1 [5; 20]
Copper, Cu	mg	0.13 [22]	0.15 [22]	0,05-0,16 [1 a,b; 13]
Iron, Fe	mg	0.31 [22]	0.21 [22]	0,31-1,15 [1 a,b; 13]
Zinc, Zn	mg	0.14 [22]	0.08 [22]	0,18-1,45 [1; 13]
Source				

[1] Bieniek, 2012a; [2] Bieniek, 2012b; [3] Boyes et al., 1996; [4] Chew et al., 2012; [5] Drummond, 2013; [6] Ferguson et Ferguson., 2003; [7] Gan et al., 2004; [8] Henare, 2016; [9] Jiang, 2011; [10] Jin et al., 2014; [11] Jo et al., 2007; [12] Kim et al., 2014; [13] Latocha et al., 2015; [14] Latocha, 2017; [15] Lintas et al., 1991; [16] Nishiyama et al., 2004; [17] Nishiyama et al., 2008; [18] Samadi-Maybodi et Reza Shariat, 2003; [19] Schakel et al. 2001; [20] Sivakumaran et col., 2016; [21] Stonehouse et col., 2013; [22] US Department of Agriculture, 2019; [23] Vasile Scăeţeanu et al., 2019; [24] Wojdyło et al., 2017; [25] Xiao, 1999.

Kiwifruit is known to contain approximately 2% to 3% dietary fibre comprised of one-third soluble fiber and two thirds insoluble fibre (Mishra et al., 2012; Lindsey et al., 2020). Nutritional references of kiwifruit is summarized in Table 1.

This makes kiwifruit a premier choice for anyone looking to live a healthier life or even simply bolster their immune system.

Health benefits of kiwifruit consumption

Food scientists and nutrition specialists agree that fruits and vegetables, consumed daily, contribute to reducing risks of certain diseases, including cancer and cardio and cerebro-vascular diseases (Liu et al., 2000).

The various antioxidants (polyphenol, ascorbic acid, carotenoids, and tocopherols) present in fruits and vegetables contribute to these beneficial effects (Peschel et al., 2006).

Recent studies also have confirmed the health benefits associated with its consumption (Baranowska-Wojcik´ et Szwajgier, 2019; Lopez-Sobaler´et al., 2016). Antioxidants contained in kiwifruit reduce oxidative stress and support the cardiovascular system (Leontowicz et al., 2016). In addition to its low caloric content, wealth of vitamins and its high phenolic content (Baranowska-Wojcik´ and Szwajgier, 2019) provide protection against heart diseases, cancer, diabetes, vascular diseases and central nervous system diseases (Tyagi et al., 2017), making this fruit a valuable component of a healthy diet and may also be used as a dietary supplement (Baranowska-Wojcik´and Szwajgier, 2019; Sanz et al., 2020).

As a source of ascorbic acid and polyphenols, the kiwifruit aids in lowering the risk of arteriosclerosis, cardiovascular diseases, and some forms of cancer (Puri et al., 2018) in irritable bowel syndrome (Chang et al., 2010) and also protect the cells *in vitro* from oxidative DNA damage (Teresa., 2018).

There is growing evidence that kiwifruit have beneficial effects on digestive health and general wellbeing, a potentially important characteristic in the light of the increasing proportion of the elderly population in ageing societies that experience impaired bowel function, changes in gastrointestinal function and gastrointestinal discomfort (Donaldson et al., 2018).

A consumption of kiwifruit plays a protective role against reactive oxygen species (ROS) due to the presence of great content of antioxidants (Duo et al., 2009; Skinner et al., 2013), enhance iron retention (Stonehouse et al., 2013), have beneficial effects on digestion (Lintas et al., 1991) and may decrease cardiovascular disease incidence (Stonehouse et al., 2013; Scăeţeanu et al., 2019).

Kiwifruit have also been proposed to decrease gastric symptoms of indigestion such as bloating (Chan et al., 2007).

Kiwifruit have been shown to relieve constipation in several clinical trials (Eady et al., 2019; Chan et al., 2007; Barbara et al., 2005).

The laxative effect of kiwifruit has been attributed to its fibre content, which is approximately 2-3% of its dry weight.

As described earlier, the fibre content of kiwifruit is approximately two-thirds insoluble and one third soluble fibre (Mishra et al., 2012; Lindsey et al., 2020).

Allergens

Although the popularity of kiwifruit is constantly growing due to its pleasant taste, low caloric value and beneficial effects on health, there are also some contra-indications addressed especially to people who are more sensitive, allergic or intolerant to some fruit compounds (Lucas et al., 2003; Vasile Scăeţeanu et al., 2019).

According to some studies, people who are allergic to birch pollen are also allergic to kiwi (Eriksson et al., 2003); and approximately 12-17% of people allergic to natural rubber latex may develop an allergy to kiwifruit (Kim and Hussain, 1999; Lucas et al., 2003).

The main allergens identified in kiwifruit are actinidine (Act d 1), protein kiwellin (Act d 5), thaumatin-like proteins (Act d 2) and oxalates (Bublin et al., 2004; Hassan and Venkatesh, 2015; Henare, 2016; Maddumage et al., 2013; Nguyen, 2012).

Most patients with allergic reactions to kiwifruit experience localized symptoms of irritation and discomfort in the oral mucosa, but more severe systemic reactions such as rash, redness, and hives have also been observed (Ferguson et al., 2003; Lucas et al., 2007; Lucas et al., 2014; Nishiyama et al., 2004;

Pastorello et al., 1998; Popovic et al., 2013). Most allergic symptoms occur within minutes of eating kiwi fruit (Bublin, 2013).

Some studies (Watanabe and Takahashi, 1998) have shown that *A. arguta* fruits have a lower oxalate content than those of *A. deliciosa* species.

According to Chen et al. (2006), thermal processing reduces the risk of causing allergic reactions in people who are sensitive or intolerant to the consumption of fresh kiwi.

Storage possibilities

Being cultivated both in North and South hemisphere and having a good storage life (over 6 months), fresh fruits are available through the year when harvested at correct maturity and at optimal storage conditions (temperature, gas composition, relative humidity, ethylene concentration). In Figure 2. processing possibilities of kiwifruit can be observed.

Kiwifruits that are intended for sale within 3-4 months are stored under normal atmospheres (Brigati and Donati, 2003), whereas fruits that are to be kept for longer times are stored under modified atmospheres. Earlier MAP with 3% O_2 and 3% CO_2 was used, but afterwards oxygen concentrations reduced and CO_2 concentrations were increased owing to the reason that higher CO_2 concentrations reduce fruit respiration and hence ripening (Sozzi et al., 1980).

Presently kiwifruits are stored under 4.5% to 5.0% CO_2 and 1.8% to 2.0% O_2 in CA storage (Brigati and Donati, 2003). ULO (Ultra Low Oxygen) technique have also been used but it did not seem to be suitable for kiwifruit as it results in development of off-flavours and thus reduced storage life (Brigati and Donati, 2003).

Within cool storage relative humidity is maintained above 94% to 95% that helps to reduce weight loss by 2% to 7% (Nardin and Galliano, 1988).

Kiwifruits are sensitive to ethylene and concentrations as low as 0.1-1.0 ppm can induce softening (Monzini and Gorini, 1986). In cool storage, ethylene concentrations are kept below 0.05 ppm in order to prevent softening (Guroo et al., 2017).

Processing

Kiwifruits are mostly eaten fresh, although some kiwifruits are also processed into juices, alcoholic beverages (cider, liqueur, brandy), purees, candied fruit and bars, jam and marmalade, dehydrated and lyophilized products, cakes or pastries, kiwifruit leathers (Cassano et al., 2007; Guroo et al., 2017). In Figure 2, processing possibilities of kiwifruit can be observed.

The freeze-drying process, using lower temperatures and reduced pressure, is more expensive, but allows a much better retention of bioactive ingredients (Morais et al., 2018; Tylewic et al., 2020).

Actinidia deliciosa - the green kiwifruit is not usually processed due to the fact that the chlorophyll responsible for the attractive green colour gets destroyed during processing (Torreggiani et al., 1994).

Also, the characteristic flavour of green kiwifruit gets lost. *Actinidia chinensis* - the golden kiwifruit has become an alternative to food processors. In terms of processing, 'Jintao' variety has shown good results, the yellow colour of the fruit survives well in processed products like juices and jams (Guroo et al., 2017).

However, high prices currently fetched by yellow-fleshed kiwifruit reduces the processing options. For the fruits that do not meet quality standards of the fresh fruit market, processing can be an alternative for adding value to the product.

Small quantities of fruit that do not meet grade standards are used for cosmetics or nutraceuticals (Guroo et al., 2017).

Although in our country the foods based on kiwi fruit are quite few, worldwide the range of commercial products is very wide. In Figure 1 some of the products found on the shelves of Chinese stores are presented: a) kiwi cider; b) bars and jellies; c) 1-3 jam; d) - i) candied or dehydrated fruits; j) kiwi honey; k) lyophilized fruits.

Source: Lavinia Iliescu, 2019

Figure 1. Kiwifruit products, China 2019

The fruits of *Actinidia deliciosa* are less used in the production of products, such as juices or jams, than those of Actinidia chinensis, due to chlorophyll, which is destroyed during processing (Torreggiani et al., 1994).

Guine and Seabra (2017), created two types of kiwi-based nutritional bars (plain and walnut) and analysed consumer preferences. They capitalized on the pulp of kiwi fruit not in accordance with marketing and consumption fresh (in terms of shape and size).

To evaluate the sensory profile, the following attributes were analyzed on a 5-point hedonic scale: consistency, texture, intensity of kiwi flavor, kiwi taste, acidity, sweet taste and homogeneity.

A preference test was also performed to identify the preferred sample of consumers. The conclusions of the study were - simple kiwi bars were much more appreciated, especially in terms of color, consistency, texture and homogeneity (Guinea and Seabra, 2017).

In order to assess the sensory properties of some dehydrated kiwi fruits, at different temperatures (50°C, 60°C, 70°C and 80°C), descriptive sensory profile tests were performed, the evaluated sensory attributes being: colour intensity, aroma intensity, sweet taste, sour taste and bitter taste, texture, general appearance (Correia et al., 2017). Chakraborty et al. (2020), using a hedonic scale of 9 points, determined the degree of consumer acceptance for freeze-dried kiwi fruit and obtained the following results: for sweet taste an acceptance rate of 68.18%, for sour taste - 90.91%, salty taste - 100%, bitter taste - 100%, aroma - 95.45%, texture - 77.27% and for the overall impression - 81.82%.

Hussein et al. (2017) analyses consumers' perception of natural kiwi juice, which can be considered a functional drink, due to its high content of antioxidants and vitamins. Consumer preferences on the colour, taste, aroma and overall appearance of natural kiwi juice indicate the possibility of widespread marketing of this product.

For kiwi products such as jams, marmalades, jams, the combination with other types of fruit can improve the colour and flavour of dishes.

In studies on consumer perception, Reddy et al. (2015), analysed parameters such as - general appearance, taste, aroma, texture, colour, consistency, aftertaste, overall acceptability.

Regarding the drinks obtained from kiwifruit, Hande and Chavan (2019) mentioned in their study some results on consumer preferences regarding colour, appearance, consistency, aroma and overall appreciation of products.

A major impact on consumers' perception of products is also the packaging or the form of presentation, which can influence the association between colour and flavour (Wan et al., 2014).

Figure 2. Processing possibilities of kiwifruit

According to Soufleros et al. (2001), the sensory evaluation of kiwi wines and the statistical analysis of the results showed that: the sweetness is significantly affected only by the amount of sugars, while the role of alcohol is marginal. Sensory alcohol is significantly influenced by sugars, alcohol and CO2, while carbon dioxide, viewed as a sensory indicator, was not found to be statistically influenced by any chemical factor examined.
The conclusions of the studies showed that the acceptability of kiwi wines is higher if they contain 10% vol. alcohol, more than 30 g/l sugars and 0.5 bar CO_2 (Soufleros et al., 2001).

CONCLUSIONS

Kiwifruit contains high levels of bioactive compounds such as vitamin C, vitamin E, flavonoids, antioxidants, carotenoids, minerals and fibbers, he becoming popular worldwide for fresh consumption due to its high sensory and nutritional properties.
Even fresh fruits are available through the year (being cultivated both in North and South hemisphere, and having a good storage life – over 6 months), kiwifruit is largely processed. The main processing possibilities for kiwifruit worldwide are juices, nectars, syrups, alcoholic drinks (cider, liqueur, brandy), candies and fruit bars, jam and marmalade, patisseries (cakes, cookies), dehydrated and lyophilized products.

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EVALUATION OF THE VITICULTURAL POTENTIAL FROM THE PIETROASA WINE-GROWING REGION IN THE CONTEXT OF CURRENT CLIMATIC CHANGES

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Abstract

Pietroasa wine-growing region is characterised by their particular natural environment, such as climate, soil properties, and a human factor, deciding on the use of grapevine cultivars and viticulture practices. The database underlying this study includes climatic factors and indicators that are considered to be defining for the climatic suitability of a geographic area. They are: solar insolation (hours/1.04-30.09), annual average temperature (°C), the hottest month average temperature (°C), the sum of fractions of average daily temperatures above 10°C for the period from 1st April to 30 September (Σtu°C), average rainfall for the period from 1st April to 30 September (mm), the length of bioactive period (mm), the heliothermal index (IHr), the bioclimatic index (Ibcv) and the index of oenoclimatic aptitude (IAOe). Significant changes were noticed when comparing recent climatologic period (2010-2020) to the reference climatological period (1958-2009).

Key words*: viticulture; climate change, bioclimatic indices.*

INTRODUCTION

Climate change is a global phenomenon, triggered at the beginning sec. XX, against the background of the increase of the industrial activity. It manifests through increasing the air temperature, changing the precipitation regime, the increase of insolation and solar radiation, as well as the intensification of the phenomena extreme weather conditions (IPCC, 2013).

Climate is the critical component of *terroir* limiting grape and wine production, which also determine suitability of particular grapevine (*Vitis vinifera* L.) varieties for wine production in the particular winegrowing region (Gladstones, 1992). Impact of change climate on viticulture is major; the change in phenology vines; the growth alcoholic potential and decrease in the total acidity of the must; lower predictability of output size and quality wine; earlier maturation of grapes, with altered colour and their aromatic profile; profile change well-known organoleptic of wines.

Several bioclimatic indices are commonly used in vineyard zoning and in aim to describe suitability of climate of different winegrowing regions (Jones et al., 2009). Bioclimatic indices are also useful metrics to provide the information about climate changes impact on viticulture (Malheiro et al., 2010). One of the most widely used indices is temperature - based Winkler index (WI), using a growing degree base of 10°C (growing degree-days; GDD), to place viticulture in the context of climate suitability (Winkler, 1944).

The Cool Night Index (CI), which accounts for minimum temperatures during grapevine maturation period, is also one of the strictly thermal indices (Tonietto, 1999; Tonietto & Carbonneau, 2004). Using a degree-day approach, with the inclusion of a day-length factor as a proxy for radiation, the Huglin Heliothermal Index (HI; Huglin, 1978) allows assessing the thermal potential of a given region. HI can help in determination of the thermal demands for the ripening of each grape variety, also reflecting the potential grape sugar content.

MATERIALS AND METHODS

For this study, there were used weather date recorded for a period of 10 years (2010-2020) and use the database for period 1961-2020. Bioclimatic indices were calculated: the average temperature of the growing season

(AvGST), Growing degree-days (GDD or WI) according to Winkler et al. (1974), Huglin index according to Huglin (1978), Cool night index (CI) according to Tonietto (1999) and Tonietto and Carbonneau (2004) and maximum/minimum temperature and the amount of precipitation in growing season.

Average growing season temperature according to Jones (2006) (AvGST):

$$1/N \sum ((Tmax + Tmin)/2)$$

Growing degree-days (GDD or WI) according to Winkle et al. (1974):

$$WI=\sum(Tmax+Tmin)/2-10^0C ;$$

Tmax – maximum daily temperature

Tmin – minimum daily temperature

Huglin Index (HI) according to Huglin (1978):

$$\sum((Tavg-10^0C)+(Tmax-10^0C))/2*k ;$$

Tavg - average daily temperature

k – Latitude, daylight adjustment factor

Cool night index (CI) according to Tonietto (1999) and Tonietto and Carbonneau (2004):

$$CI= 1/N \sum Tmin;$$

N – number of days in the period

To calculate the agro-climatic indices, the database of the Pietroasa Research Station was used.

Bioclimatic indices were evaluated based on the listed class levels:

Index	Period/classes definition April-October
Average growing season temperature (TGS) Jones (2006)	Too cool < 13°C Cool 13 - 15°C Intermediate 15-17°C Warm 17-19°C Hot 19-21°C Very hot 21-24°C Too hot > 24°C
Growing degree - days (GDD or WI) Winkler et al. (1974)	Region I < 1390 Region II 1391 - 1670 Region III 1671 - 1940 Region IV 1941 - 2220 Region V > 2220
Index	Periode/classes defination April-September
Huglin index (HI) Huglin (1978)	Very cool (HI-3) < 1500 Cool (HI-2) 1500 - 1800 Temperate (HI-1) 1800 - 2100 Temperate warm (HI+1) 2100 - 2400 Warm (HI+2) 2400 - 2700 Very warm (HI+3) > 2700
Index	Periode/classes defination September
Cool night index (CI) Tonietto (1999)	Very cool nights(CI+2)<12°C Cool nights (CI+1) 12 - 14°C Temperate nights (CI-1) 14 - 18°C Warm nights (CI-2) > 18°C

In addition to bioclimatic indices, it was take into account:

Maximum temperature in growing season T^0C April-October period;

Precipitation in growing season (mm) April-September period.

RESULTS AND DISCUSSIONS

Temperature is a primary environmental factor that plays a key role in affecting several plant physiological processes including phenology, vegetative growth, flowering and fruit set, crop development, yield and quality.

The wine-growing Pietroasa region belonged to intermediate climate based on AvGST for reference period 1961-1990, ranging from 16°C to 16.69°C. Regarding the period from 1990-2010 values ranged from 16.69°C to 17.63°C meaning that region moved to another variety favourability class fall into warm climate according to AvGST. The growing season temperature increased by 0.72°C from 16.69°C between 1961-1990 to 17.42°C between 1990-2020

Recent climatological period 2010-2020, AvGST values ranged from 17.07°C (2010) to 18.71°C (2012), thus maintaining region in warm climate (Figure 1).

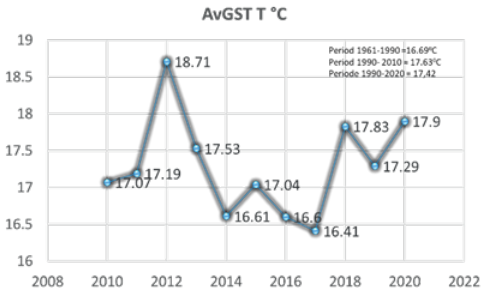

Figure 1. AvGST in period 2010-2020

The Winkler Index, sometimes known as the Winkler Scale or Winkler Regions, is a technique for classifying the climate of wine growing regions based on heat summation or growing degree-days. Regarding climatological period 2010-2020, values ranged from 1662 to 1825 GDD, which means that Pietroasa belongs to Winkler region III. These results show that the region is favourable for high production of

standard to good quality table wines (Figure 2). Along with local varieties in Pietroasa wine-growing region are found favourable conditions for international varieties 'Chardonnay', 'Pinot noir', 'Sauvignon Blanc', 'Riesling', 'Cabernet Sauvignon', 'Merlot', 'Semillion', 'Syrah', 'Chardonnay', 'Tempranillo', 'Grenache', 'Barbera'.

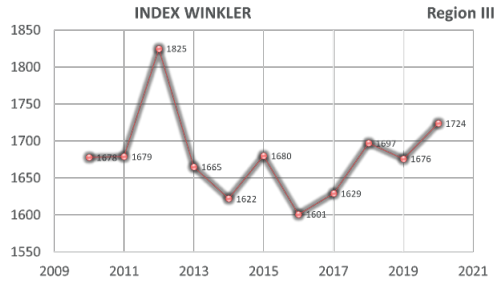

Figure 2. Index Winkler
in period 2010-2020

The Huglin heat sum index (or after Huglin respectively is warmth index or short Huglin index,) in which the temperature sum over the temperature threshold of 10°C is calculated and then summed for all days from beginning of April to end of September. The calculation uses both the daily average temperatures and the maximum temperatures and slightly modifies the calculated total according to latitude. Each grape variety needs a certain amount of heat in order to be cultivated successfully in the long term in a given area. According to the Huglin index for the period 2010-2020, Pietroasa belongs the area temperate warm (HI+1). In ranging from 2243 (2016) and 2345 (2020). In the year 2012 the index Huglin was 2640 which indicates the change of region in warm (HI+2) (2400 - 2700) (Figure 3). Considering HI, there are certain limits regarding growing of different grape varieties. Varieties such as 'Cinsault', 'Syrah', 'Grenache', 'Carignan', 'Mourverdre' needs to be grown in temperate warm (HI+1) climate to reach maturity.
Regarding this, such varieties are suitable for growing in Pietroasa wine region, important aspect for zoning and other varieties in this wine area.

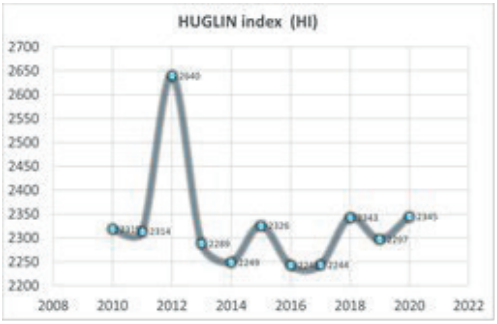

Figure 3 Index Huglin in period 2010-2020

Cool night index ranged from 15.8°C to 20.0°C in period 2010-2020 which shows us that they are temperate nights CI-1 there is an intermediate condition between viticulture climates cool nights and warm nights. The cool night index has an upward trend (Figure 4).
It is important monitoring this index for the next years for evaluation the qualitative potentials of wine-growing Pietroasa region, especially in relation to the secondary metabolites of grapes (polyphenols, aromas), responsible for the colour and aromas of grapes and wine.

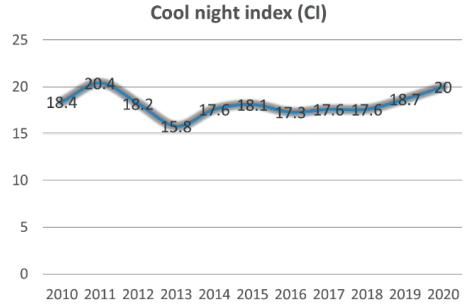

Figure 4. CI in period 2010-2020

In wine-growing Pietroasa region, the maximum temperatures in the summer often reach 33-39 °C for growing season (Figure 5). This high level causes the photosynthesis process to be blocked and the respiration and evapotranspiration processes to intensify. The heat also hastens ripening, producing grapes with bolder flavours, more sugar, and wine with more alcohol and short on taste and aroma.

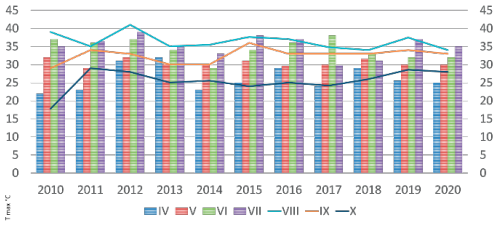

Figure 5. Maximum temperatures in period 2010-2020

While climate and humidity play important roles as well as the soil, a typical grape vine needs 635 to 890 mm of water a year, occurring during the spring and summer months of the growing season, to avoid stress. If much of the vines water needs are met by rainfall, the distribution rather than the total amount of rainfall is important. In wine-growing Pietroasa region, the precipitation has been increasing since 1961. In period 1961-1990 multiannual average of precipitation in growing season was 373 mm, in period 1991-2010 multiannual average of precipitation in growing season was 377 mm and in period 2010-2020 multiannual average of precipitation in growing season was 394 mm.

The precipitation within a wide range, uring the growing season (April 1st- September 30th) slightly increased between 1961-2020, with an average of about +21 mm.

The results obtained in the period 2021-2020 (Figure 6) show that there is a tendency to increase precipitation during flowering (late May to early June) and the installation of excessive drought starting with August during the ripening of the grapes.

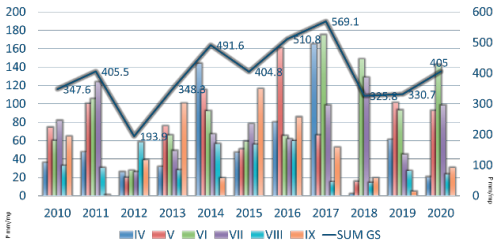

Figure 6. Amount of precipitation (mm) in period 2010-2020

Although the amount of annual precipitation is optimal, their uneven distribution during in growing season period causes them to lead to a physiological stress of the vine.

CONCLUSIONS

This study provides detailed analysis of bioclimatic indices in Pietroasa winegrowing regions. The growing season temperature increased by 0.72°C from 16.69°C between 1961-1990 to 17.42°C between 1990-2020. In period 2010-2020 values ranged from 1662 to 1825 GDD, which means that Pietroasa region belongs to Winkler region III.

According to the Huglin index for the period 2010-2020, Pietroasa belongs the area temperate warm (HI+1). In ranging from 2243 (2016) and 2345 (2020). In the year 2012 the index Huglin was 2640 which indicates the change of region in warm (HI+2) (2400 - 2700)

The precipitation in growing season, increased by +21 mm between 1961-2020

Regardless of the geographical region and the type of climate specific to it, climate change is expected to alter the specificity of vineyards and will change the boundaries of DOC wine production areas

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STUDY ON THE PHENOLOGY AND FERTILITY ELEMENTS OF SOME VINE VARIETIES DURING THE VEGETATION PERIOD

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Abstract

In the 2019 year, the phenology of the white and black grape varieties for wine was studied, from Medgidia centre and the fertility elements for each variety were determined. Also, the climatic elements specific to 2019 compared to the multiannual average were studied. The year 2019 recorded values close to or higher than the multiannual average for almost all climatic elements, except for the sum of annual rainfall from the vegetation period, where the values are much lower, this year being a deficit in rainfall. According to the value of the Real Heliothermic Index (IHr) of 3.13, the year 2019 registered optimal values for the vine. The study shows that: white varieties for wine, sprouted early, between 15 April ('Chardonnay') and 17April ('Muscat Ottonel'); black varieties, sprouted between 15 April ('Merlot') and 18 April ('Cabernet Sauvignon'); the white varieties bloomed on 5 June ('Merlot') and 7 June ('Cabernet Sauvignon'); the ripe, occurred on different dates for different varieties; full maturity was in 8 September for white varieties and between 17-21 September for the black. For the varieties studied, the largest number of fertile shoots were for 'Muscat Ottonel' (22).

Key words*: fruit elements, phenology, ripe, thermal balance.*

INTRODUCTION

Cernavoda, Murfatlar and Medgidia vine centers are part of the Murfatlar vineyard, the only vineyard located in the south-eastern part of Dobrogea spreading on both sides of the Carasu valley. The research in the viticultural field has shown that external ecological factors act on the vine plants. Therefore, there are analysed: - climatic and atmospheric factors, which include: the light, air temperature, atmospheric humidity, precipitation and winds; - pedological factors: soil temperature, soil humidity and air, and physico-chemical characteristics of the soil; - orographic factors: the relief, slope of the land, exposure and altitude; -biotic soil factors composed of: phytocenosis, zoocenosis, microbocenosis, fungocenosis, parasitocenosis. Ecological conditions listed above, influence each phenophase in each period of the annual biological cycle of the vine (Oslobeanu et al., 1980; Olteanu, 2000; Dejeu, 2004; Bucur, 2011). In many wine-growing areas, were recorded more changes in the climate as a result of the high average temperatures, due to low precipitations (Stroe and Cojanu, 2018; Bucur et al., 2019; Bădulescu et al., 2020).

MATERIALS AND METHODS

The climatic factors evolution in the ecosystem of Medgidia wine center (like temperature, rainfall, humidity, insolation and so on) was monitored using the Weather Master 2000 performance meteorological station; all climate factors in 2019 were compared with the multiannual average from 1999-2018;

The working material was represented by four vine varieties: 'Chardonnay' and 'Muscat Ottonel'- grapes for white wine and 'Cabernet Sauvignon' and 'Merlot'- grapes for red wine. All these varieties are grafted on the rootstock Berlandieri x Riparia Oppenheim Selection 4 and planted at 2.2 m distances between rows and 1.1 m between plants in a row; the area of a vine nutrition is 2.42 m^2; the vine number/ha 4,132; rows orientation N-S; support system: concrete pillars; number of wires: 6 (two load-bearing wires and two double rows for off shoots directing); trunk highness: semi-tall with 70 cm; driving form: double Guyot;

The phenological behaviour of the above-mentioned varieties from the Medgidia wine center and the calendar delimitation of the phenophases were followed in the existing plantations (BBCH Monograph, 2018);

For twenty-five vines of each variety, it was performed the fertility elements statistic (total shoots/vine, fertile shoots, sterile shoots, total number of inflorescences, percentage of fertile shoots (FS%), relative and absolute fertility coefficients and relative and absolute productivity indices). The relative fertility coefficient was calculated according to the formula (Oslobeanu et al., 1980; Bucur, 2011; Bădulescu et al. 2020):

$$\frac{\text{no. of inflorescences on the vine plant}}{\text{total no. of shoots (fertile + sterile)}} \neq 1$$

and absolute fertility coefficient:

$$\frac{\text{nr. of inflorescences on the vine plant}}{\text{total nr. of fertile shoots on the vine plant}} \geq 1$$

In order to calculate the productivity indices, the fertility coefficients were multiplied by the weight of a bunch of grapes (in grams) for each variety (Bădulescu et al., 2020);
The standard deviation was calculated using the formula:
=STDEV (number 1; number 2).

RESULTS AND DISCUSSIONS

Climate characterization of the 2019 year:
The vintage 2019 year started with an average temperature over the normal average of the period. There was an absolute minimum of -10.5°C, which did not affect the fruit buds and the viability being 100%.
Temperatures over 10°C higher than normal were recorded throughout the vegetation period, which led to a normal growth and development of the vine (Table 1).

Table 1. Thermal regime in air and in soil in 2019 compared with the multiannual average (1999-2018)

Month	Air temperature ^{0}C				Soil temperature ^{0}C			
	average		Abs. max	Abs. min.	average		Abs. max	Abs. min.
	Multiann average	2019			Multiann average	2019		
I	0.5	2.5	16.9	-10.5	0.7	2.5	22.4	-15
II	1.3	5.8	20.8	-5.6	3.4	6.6	33.5	-8.6
III	4.2	10.8	27.0	-5.5	8.1	13.2	49.6	-8.4
IV	10.5	12.5	27.4	-4.50	13.2	17.1	53.9	-8.2
V	16.2	20.11	32.8	7.5	20.8	26.0	64.9	4.1
VI	20.4	27.0	38.1	14.9	27.2	33.9	69.7	12.3
VII	22.6	26.7	38.9	14.4	31.3	34.6	70.1	10.2
VIII	22,6	24.4	39.0	14.5	29.6	36.6	69.5	10.2
IX	17.6	22.8	36.1	7.0	21.6	28.5	67.5	2.7
Annual average	12.8	16.9	39.0	-10.5	17.3	22.1	70.1	-15.0

During the vegetation period, the thermal balances (global, active and useful) registered values were above the normal average of the year (Table 2).

Table 2. The thermal balances (global, active and useful) in 2019 during the vegetation period compared with the multiannual value (1999-2018)

Month	Global (Σ°C)		Active (Σ°C)		Useful (Σ°C)	
	Multiann value	2019	Multiann value	2019	Multiann value	2019
IV	369.7	374.2	219.8	331.5	53.8	81.5
V	513.7	623.4	513.7	623.4	203.7	313.4
VI	620.1	811.2	620.1	811.2	328.1	511.2
VII	726.3	829.0	726.3	829.0	416.3	519.0
VIII	671.0	853.1	671.0	853.1	361.0	543.1
IX	521.2	685.8	521.2	685.8	252.7	385.8
Σ°C	**3,422**	**4,176.7**	**3,303.6**	**4,134**	**1,615.6**	**2,435.4**

In the table 2 it is observed that, in 2019, the global thermal balance was 4,176.7°C compared to multiannual value of 3,422.0°C; the active thermal balance was 4,134.0°C, with 830°C higher than the multiannual value, and the useful thermal balance was almost 820°C higher than the multiannual value. The main climatic elements of 2019 compared to the multiannual average (1999-2018) are presented in the table 3.

Table 3. Synthesis of the main climatic indices of 2019 year compared to multiannual averages (1999-2018)

The climatic elements analysed	Multiann average	2019
Global thermal balance (Σt°g)	4,790.7	5,058.6
Active thermal balance (Σt°a)	4,300.7	4,134.0
Useful thermal balance (Σt°u)	2,178.9	2,354.0
July average temperature °C	25,5	26,7
August, average temperature °C	24.4	24.7
September average temperature °C	19.3	22.8
Temperature min. absolute in air °C	-22.0	-10.5
Temp. min. abs. at the soil surface °C	-15.5	-15.0
Annual average temperature °C	13.0	1.8
Maximum air temperature °C	44.0	39.0
Σ of annual rainfall, mm	522.6	270.6
Σ of rainfall, in the vegetation period, mm	324.2	139.6
Σ of the hours of insolation in vegetat. per	1,612.1	1,329.7
Max. average of temperature in August, °C	30.8	33.8
X. temp. in I-st and II-nd decades of June	22.9	26.3
Nr of days with maximum temp. > 30°C	51.0	98.0
Duration of the bioactive period, days	188.4	162.0
Real heliothermic index (IHr)	3.6	3.13
Hydrothermal coefficient (CH)	0.8	0.3
Bioclimatic index (Ibcv) of vine	13.2	24.3
Oenoclimatic index (IAOe)	5,178.6	5,574.1
Heliothermic Index (HI) Huglin	3,130.1	3,063.6
Night Cooling Index (IF)	12.8	14.4
The year characterization	Optimal values for the vine, rich water resources	Rainfall deficit. Rich in heliother-mal resources

Due to excessively high temperatures, associated with prolonged pedological and atmospheric drought, the varieties showed a tendency of entering quickly in ripe (end of July – the beginning of August), a phenomenon caused by very high air temperatures and especially by extreme values exceedingly frequently 30°C. The phenomenon is accentuated when there is a water deficit, as it happened in 2019 (Table 3). According to the table 3, the registered values of 2019 year were close to or higher than the multiannual average for almost all climatic elements, except the sum of annual and vegetation rainfall, where the values are much lower, this year being a deficit in rainfall. According to the value of the Real Heliothermic Index (IHr) of 3.13, the 2019 year registered the optimal value for the vine.

Phenological behaviour of the studied white and black varieties:

The phenological behaviour of the white and black varieties studied during the vegetation period and their calendar delimitation (BBCH Monograph, 2018) is presented in the table 4.

Table 4. Phenological behaviour of the white and black varieties studied during the vegetation period varieties in the Medgidia wine center/2019

Variety	Year	Phenophase/Calendar data						Duration of the vegetation period (days)	
		Sprout	Blooming	Ripe	Full maturity	Harvest	Leaf fall	Sprout-Full maturity	Sprout-Leaf fall
White varieties									
'Chardonnay'	2019	15.04	04.06	13.08	08.09	12.09	23.10	146	191
'Muscat Ottonel'	2019	18.04	04.06	11.08	08.09	12.09	25.10	143	188
Black varieties									
'Cabernet Sauvignon'	2019	18.04	07.06	19.08	21.09	25.09	23.10	156	188
'Merlot'	2019	15.04	05.06	17.08	20.09	25.09	23.10	158	191

The data from the table 4 indicate that: the white and black varieties, sprouted at about the same time first in April 15, 'Chardonnay' and 'Merlot', followed by 'Muscat Ottonel' and 'Cabernet Sauvignon' in April 18. The white varieties bloomed on the same date, June 4, and the black ones at different date (June 5 for 'Merlot' and June 7 for 'Cabernet Sauvignon'. Among the four varieties, 'Muscat Ottonel' reached the ripe earliest August 11, followed by 'Chardonnay' August 13 and then 'Merlot' on August 17 and 'Cabernet Sauvignon' on August 19. Full maturity was reached on September 8 for white varieties and after 12-13 days for the black varieties; harvesting began on September 12 for the both white varieties and after two weeks for the black varieties. Leaf fall began on October 23 for black and white 'Chardonnay' varieties and two days late for 'Muscat Ottonel'. The days number of the vegetation period (from sprout-full maturity, and to sprout-leaf fall) was different for the different varieties (158 days for 'Merlot' and 191 days for 'Merlot' and 'Chardonnay').

Statistics of the fruit elements

Table 5 shows the averages of the data resulting from the evaluation of the fruit elements statistics for the studied varieties.

Table 5. Statistics of the fertility elements and their standard deviation of the black and white varieties in the Medgidia wine center/2019 year

Variety / Average (X)		Total nr. of Shoots	Fertile Shoots	Sterile Shoots	Total no. of inflores-cences	% Fertile Shoots	R.F.C*	A..F.C*	weight of a grape (g)	R.P.I*	A.P.I*
White varieties											
'Chardonnay'	X	24	19	5	28	79	1.2	1.5	91	116	144
STDEV*	σ	0.8485	0.9380	0.9380	0.9164	0.7483	0.1154	0.1252	0.8944	0.9860	0.9120
'Muscat Ottonel'	X	26	22	4	34	84	1.3	1.5	84	109	126
STDEV*	σ	0.8447	0.9759	0.9583	0.9514	0.8904	0.2136	0.1348	0.9730	0.8650	0.9801
Black varieties											
'Cabernet Sauvignon'	X	22	19	3	33	86	1.5	1.7	88	132	150
STDEV*	σ	0.9583	0.9380	1.058	0.9828	0.9174	0.2653	0.5463	0,9514	0.9964	0.9531
'Merlot'	X	24	20	4	26	89	1.1	1.3	94	103	122
STDEV*	σ	0.8484	0.9534	0.9583	0.8325	0.9638	0.2034	0.3142	0.9797	0.7626	0.8990

*R.F.C/A.F.C - Relative /Absolute fertility coefficient; R.P.I /A.P.I - Relative /Absolute productivity indices; STDEV-standard deviation

In the case of white varieties, the highest total number of shoots (26) was at 'Muscat Ottonel' variety and of those, 22 were fertile shoots that had a number of 34 inflorescences, with 6 inflorescences more than the 'Chardonnay' variety, while the 'Cabernet Sauvignon' compared to 'Merlot' had a smaller number of total shoots (22) a smaller number of fertile shoots (19), but had a larger number of inflorescences (33), with 7 inflorescences more than 'Merlot'. The highest percentage of fertile shoots had the black varieties 'Merlot' (89%), then 'Cabernet Sauvignon' (86%) followed by the white varieties, 'Muscat Ottonel' (84%) and 'Chardonnay' (79%). Also from the table 5 we can see that: at the white varieties the absolute fertility coefficient had the same value (1.5), but due to the weight of the grapes the highest absolute productivity index was calculated for Chardonnay and for the black varieties the highest fertility coefficients and productivity indices were founded for 'Cabernet Sauvignon' (150) and the lowest for 'Merlot' (122); all the values calculated for standard deviations were subunit and these data show that the dispersion is close to their average value.

CONCLUSIONS

The registered values of the 2019 year from the climatic point of view were close to or higher than the multiannual average for almost all climatic elements, except the sum of annual and vegetation rainfall, where the values were much lower, this year being a deficit one in rainfall. According to the value of the Real Heliothermic Index (IHr) of 3.13, the 2019 year registered the optimal value for the vine.

In 2019, in the Medgidia wine center, we studied the phenological behaviour of four white and black varieties for wine: 'Chardonnay', 'Muscat Ottonel', 'Cabernet Sauvignon' and 'Merlot'.

The phenophases of sprouting, blooming, ripening and full maturity, were produced at similar data for white varieties and red.

In the same time, we performed the fertility elements statistics of the varieties (total shoots/vine, fertile shoots, sterile shoots, total number of inflorescences, % fertile shoots, relative and absolute fertility coefficients and relative and absolute productivity indices).

The entire study was done in existing plantations

The highest average of fertile shoots was found at the variety 'Muscat Ottonel', 22 fertile shoots/vine. The highest percentage of fertile shoots had the black varieties 'Merlot' (89%), then 'Cabernet Sauvignon' (86%) followed by the white varieties, 'Muscat Ottonel' (84%) and 'Chardonnay' (79%). The highest fertility coefficients and productivity indices were found for 'Cabernet Sauvignon' and the lowest for 'Merlot'.

All the values calculated for standard deviations were subunit and these data show that the dispersion is close to their average value.

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COMPARATIVE RESEARCH ON THE INFLUENCE OF SOME TECHNOLOGICAL SEQUENCES FROM CONVENTIONAL AND ORGANIC VITICULTURE

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Abstract

Although organic viticulture has slowly developed in last decades, especially in Europe, with Italy, France, and Spain as main promoters, in Romania organic viticulture is still at the beginning. The research focused on the main technological sequences (soil management, fertilization, disease, and pest control) in conventional and organic viticulture and the influence on leaf area, yield, grape quality, management costs, and profit in 'Cabernet Sauvignon', 'Feteasca Neagra', 'Pinot Noir', 'Italian Riesling', and 'Chardonnay' wine varieties from Recas-Petrovaselo Vineyards. Variants from organic grapevine registered lower yields compared to the conventional ones, without significant differences in the grapes quality. The management and production costs were higher in the organic viticulture plots, but also the profit was higher due to demand and the higher price of wine. Organic viticulture, through the correlation of the technological sequences with the requirements of the varieties and natural resources from the growing area, has great chances to spread more and more based on the demand and the development of a stable market in Romania.

Key words: *organic viticulture, quality, cost, profit, grapevine.*

INTRODUCTION

Viticulture is an intensive sector of horticulture characterized by a high degree of land use (Roselli et al., 2020). However, in recent years, organic viticulture is beginning to spread on larger areas (Meissner et al., 2019). The organic viticulture is mostly concentrated in Europe (over 85% - OIV, 2019); among the European countries, Spain, Italy, and France encompassed the majority of organic grapevine growing areas (Mann et al., 2012). In Romania, organic viticulture is just at beginning, with very few vineyards converted from conventional viticulture (Dobrei et al. 2018). Therefore, only few studies exist concerning the research results in organic viticulture and grape yield.

Organic viticulture can be differentiated from conventional one by soil management, fertilization, disease and pest control (Merot & Wery, 2017). In addition, vineyard management, have an important share in the grape production costs, and also require the higher inputs with possible polluting effect on the soil, environment and wine–based products (Bindi & Nunes, 2016; Azorín & Garcia, 2020). By optimizing the organic vineyard management result lower pollutant emissions, without significant differences in grape production, quality and economic indicators compared to conventional viticulture (Baumgartner et al., 2007). European research in recent years has provided winegrowers with various soil management solutions with lower fuel consumption and less aggressive with the soil and the environment (Dobrei et al., 2015). Also, there are currently effective alternatives for replacing the conventional fertilizers and classic schemes for diseases and pests control, to can obtain quantitative and qualitative grape production compared to conventional viticulture, in more environmentally friendly conditions (Merot et al., 2020). However, these new solutions must be chosen carefully, according to each variety particularities, the pedo-climatic resources of each grape-growing area and the technical and financial possibilities of each vineyard (Dobrei et al., 2016).

MATERIALS AND METHODS

The research was carried out in the Recas Wine Center, Petrovaselo area, in a full maturity vineyard in expectation for conversion to organic viticulture, during 2016-2018.

The research focused on the main technological sequences which are different from conventional to organic viticulture, namely: soil management system, fertilization, diseases and pest control. Separate experiments were organized for each technological sequence, in order to observe the impact and the differences between the variants for conventional and organic viticulture. The weight and impact of the three technological sequences were also observed separately. The planting distances were 2.2 meters between rows and 1 meter between vines on row resulting 4545 vines density per hectare.

The main varieties cultivated within the vineyard ('Cabernet Sauvignon', 'Feteasca Neagra', 'Pinot Noir', 'Italian Riesling' and 'Chardonnay') were observed for suitability to the requirements of organic viticulture in the soil and climate conditions of the local grapevine growing area. Observations and determinations were made on the influence of experimental variants on physiological indicators (leaf area, photosynthetic efficiency), on grape production and quality, as well as on economic indicators (total costs, production value, production cost and profit). Leaf area was measured by LI- 3100C leaf area meter (LI-COR, Biosciences GmbH, Bad Homburg, Germany). Phothosynthetic efficiency was determined by gas exchange measurements on vine leaves with a portable steady-state gas-exchange system (Li-6200, Licor, USA).

All data were statistically processed by using XLStat statistical software 16.0.6741.2048 version. Pearson correlation was performed to examine the relationship among measurements and yield components. PCA (Principal Component Analysis) illustrate the relationship among different variables (IR/C= 'Italian Riesling' Control; IR/H = 'Italian Riesling' Herbicides; IR/T+H = 'Italian Riesling' Tillage +Herbicides; IR/M= 'Italian Riesling' Mowing; IR/O= 'Italian Riesling' Organic; C/C= 'Chardonnay' Control; C/H = 'Chardonnay' Herbicides; C/T+H = 'Chardonnay' Tillage +Herbicides; C/M= 'Chardonnay' Mowing; C/O= 'Chardonnay' Organic; FN/C= 'Feteasca Neagra' Control; FN/H = 'Feteasca Neagra' Herbicides; FN/T+H = 'Feteasca Neagra' Tillage +Herbicides; FN/M= 'Feteasca Neagra' Mowing; FN/O= 'Feteasca Neagra' Organic; CS/C= 'Cabernet Sauvignon' Control; CS/H = 'Cabernet Sauvignon' Herbicides; CS/T+H = 'Cabernet Sauvignon' Tillage +Herbicides; CS/M= 'Cabernet Sauvignon' Mowing; CS/O= 'Cabernet Sauvignon' Organic; PN/C= 'Pinot Noir' Control; PN/H = 'Pinot Noir' Herbicides; PN/T+H = 'Pinot Noir' Tillage +Herbicides; CS/M = 'Pinot Noir' Mowing; CS/O= 'Pinot Noir' Organic).

RESULTS AND DISCUSSIONS

The PCA model was applied for leaf area and photosynthetic efficiency data for determination the relationship among variables when different soil management for 'Cabernet Sauvignon', 'Feteasca Neagra', 'Pinot Noir', 'Italian Riesling' and 'Chardonnay' varieties was applied.

Leaf area per vine highly influenced (r = 0.7570) the photosynthetic efficiency in leaf area/kg grapes, for 'Cabernet Sauvignon' and 'Feteasca Neagra' variety in all experimental variants, while for 'Italian Riesling', 'Chardonnay' and 'Pinot Noir', the correlation is negative regardless of soil management variant.

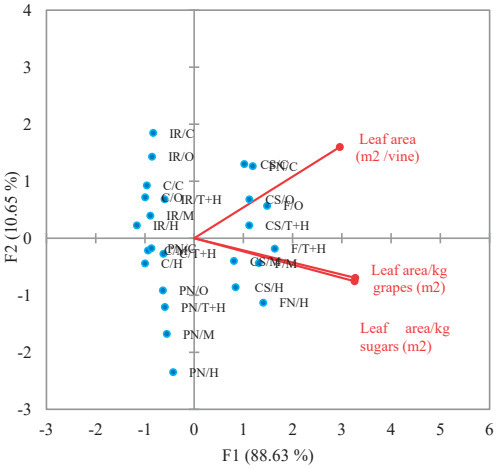

Figure 1. PCA correlation biplot for leaf area variability to soil management methods, during 2016-2018 growing seasons

The link is also strong between leaf area/vine and the leaf area/kg sugars achieved in grape berries (r = 0.7459) in 'Feteasca Neagra' and 'Cabernet Sauvignon' varieties; in 'Pinot Noir', 'Chardonnay' and 'Italian Riesling', the leaf area/vine did not influence the leaf area/sugars ratio. Factor 1 explains 88.63% of the variation of photosynthetic efficiency for both other variables (Figure 1).

The higher correlation between leaf areas per sugars yield in 'Cabernet Sauvignon' and 'Feteasca Neagra', which are more vigorous varieties, can be explained by several studies of Downton et al. (1987), Edsonet al. (1993), Nabi et al. (2000) or Petrie et al. (2000) which found that grapes are a strong sink that have the capacity to stimulates the photosynthetic rate in grapevine leaves maybe due to the demand for assimilates.

In Figure 2 it is presented the influence of different soil management methods on grape production and components during 2016-2018 growing seasons for the same five wine grape varieties. Sugars (kg/ha) content is highly correlated with the grape production (r = 0.905) in 'Cabernet Sauvignon' and especially in 'Italian Riesling' for conventional and organic soil management. Grape production explains 66.25% of data.

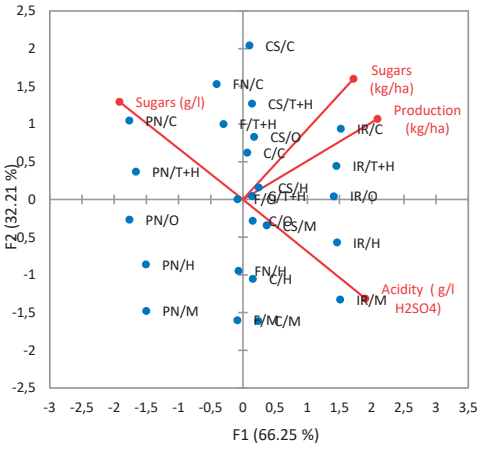

Figure 2. PCA correlation biplot for yield variables (reaction to soil management methods) during 2016-2018 growing seasons

Variables with the highest contribution were sugars content in all grape berries (kg/ha), grape production and sugars in must (g/l). Grape production and sugars yield (kg/ha) in 'Pinot noir' variety were negatively influenced in organic plots and those with herbicides and mowing soil management; the experimental plot with 'Pinot noir' and soil management by vegetation mowing has the lowest score. 'Chardonnay' variety reaction to soil management shows the higher variability. Factor 2 separated the soil management in the experimental plots and explains only 32.21% of the results; sugars yield (kg/ha) contributed the most.

Grape production had positive influence on sugar yield (kg/ha) (r = 0.9605) but the sugars (g/l) in the grape must had negative relationship with grape production and was also highly negative influenced by acidity (r = -0.9397).

Negative results for organic plots are contrary to the findings of Susaj et al. (2013) in Albanian variety 'Kallmet', comparing the organic and conventional soil management, and recorded higher yield in organic practices thanks to the improved soil properties

The behaviour of the wine grape varieties concerning the photosynthetic efficiency when different fertilizers (green manure, Fertilpolina, Humus Vita Stallatico and Fertilpolina + Cropmax) were used during 2016-2018 growing season is presented in Figure 3.

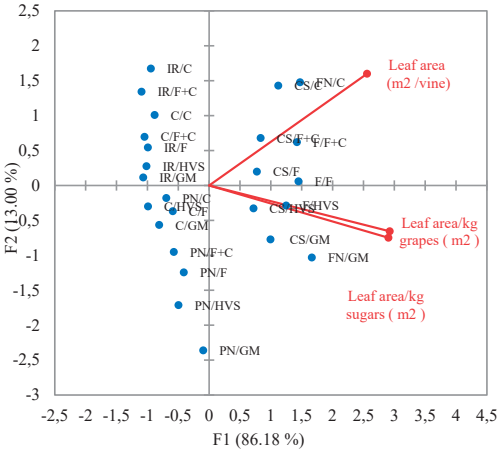

Figure 3. PCA correlation biplot for wine grape varieties (reaction to fertilization) during 2016-2018 growing seasons

PCA diagram show that leaf area per vine influence in acceptable limits the leaf area/kg grapes (0.7062) and leaf area/kg sugars (0.6852), mainly in 'Cabernet Sauvignon' and 'Feteasca Neagra' varieties. 'Chardonnay',

'Italian Riesling' and 'Pinot Noir' varieties had very low or no reaction to different methods of fertilization. There is a very strong correlation between leaf area/kg grapes and leaf area/kg sugars (r = 0.9749) in 'Cabernet Sauvignon' and 'Feteasca Neagra' varieties in all experimental plots (Figure 4).

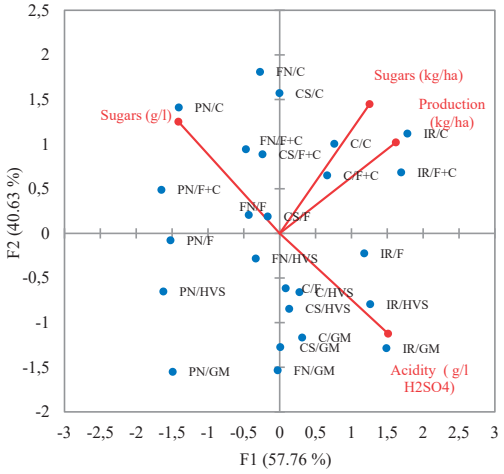

Figure 4. PCA correlation biplot for grape yield quality (reaction to fertilizers) during 2016-2018 growing seasons

Variables variability is explained in 57.76% by F1. The higher acidity was registered in 'Italian Riesling' variety (fertilized with green manure) and the lowest in the 'Pinot Noir' control plot (with highest concentration of sugar in the must). 'Italian Riesling' registered the highest grape production in the control and Fertilpolina+Cropmax plots; the grape production in the same plots was highly correlated with the sugar yield (kg/ha). Very close results for the same variables and fertilizers show the 'Chardonnay' variety. 'Pinot Noir' variety did not register positive reaction for fertilization with Fertilpolina, green manure or Humus Vita Stallatico for none of the variables.

The influence of the diseases and pest control system on the leaf area and the photosynthesis efficiency during 2016-2018 growing seasons is shown in Figure 5.

F1 explained 88.92% of the variability in PCA for the wine grape varieties leaf area after treatments (conventional, organic, and mixed) for diseases and pest control were applied. The highest leaf area per vine was registered in 'Cabernet Sauvignon' and 'Feteasca Neagra' varieties in all three treatments. Moderate leaf area shown 'Chardonnay' and 'Italian Riesling' varieties and the lowest leaf area was observed in 'Pinot Noir' variety.

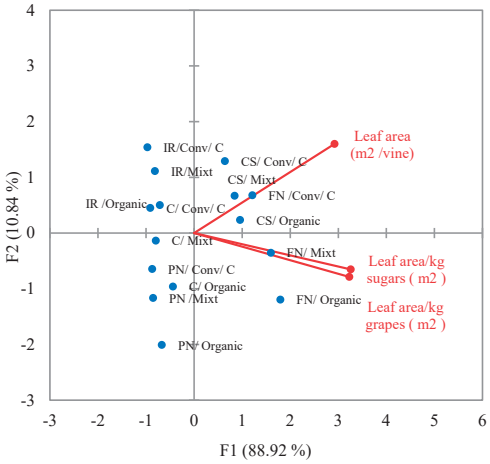

Figure 5. PCA correlation biplot for leaf area performance after diseases and pest control treatments during 2016-2018 growing seasons

The PCA for the grape yield quality variables during 2016-2018 growing season after diseases and pest control treatments accounted for 61.49% of the variability in the dataset (Figure 6).

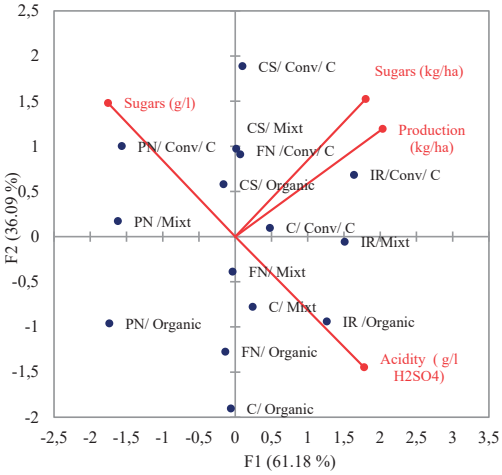

Figure 6. PCA correlation biplot for grape yield quality after diseases and pest control treatments during 2016-2018 growing seasons)

The PCA for the grape yield quality variables during 2016-2018 growing season after

diseases and pest control treatments accounted for 61.49% of the variability in the dataset (Figure 6). 'Cabernet Sauvignon' variety registered the highest results for sugar yield (kg/ha) and grape production (kg/ha), regardless of treatment, but 'Italian Riesling' shown the highest results for the same variables. Except for 'Cabernet Sauvignon' variety, in the other four varieties was found the highest acidity in organic and mixed treatments plots. In the grape must from conventional plot of 'Pinot Noir', it was observed the highest content of sugars (h/l); however, the 'Pinot Noir' grapes from the organic plot do not show any positive influence for quality variables after treatments application.

Organic grapevine growing create the microclimate that positively influence the vine development, grape yield and quality according to Klimenko et al. (2021) research in 'Chardonnay' grapes from Crimea on the background of natural grass strip middle rows.

Soil management systems, besides being expensive, pollute the soil and environment especially if they are not performed with enough caution (Bindi et al., 2016). Until few decades, bare soil middle rows were very common in vineyards, but for economic and environmental performance, many alternative possibilities of soil management have emerged; some, like mowing or cover crops are more environmentally friendly, while other like herbicides require less labour despite being polluting (Dobrei et al., 2015). The influence of soil management on some economic indicators, have different impact (Table 1). The highest production cost of all wine grape varieties was found for bold soil plot and the lowest production cost was found in the organic plot.

Concerning the influence of soil management on the grape production value, the highest value was obtained for the grapes from the organic plots, but this value is decisively influenced by the higher price of organic grapes. The lowest value of production was registered for the grapes harvested from plots maintained by mowing and herbicide application, mainly due to the lower grape yield.

Table 1. The influence of the soil management system on the economic indicators in 2018

Variant Soil management	Total costs (lei/ha)	Varieties	Production costs (lei/ton of grapes)	Production value (lei/ha)	Profit (lei/ha)	Difference to control (lei/ha)
Bare soil (C)	9200	Italian Riesling	1230	22700	13500	-
		Chardonnay	1300	19650	10450	-
		Feteasca Neagra	1270	29340	20140	-
		Cabernet Sauvignon	1310	31575	22375	-
		Pinot Noir	1270	25119	15919	-
Herbicides	8120	Italian Riesling	1070	21080	12960	-540
		Chardonnay	1120	18264	10144	-306
		Feteasca Neagra	1110	26760	18640	-1500
		Cabernet Sauvignon	1150	29535	21415	-960
		Pinot Noir	1110	23175	15055	-864
Tillage + Herbicides	8660	Italian Riesling	1060	22040	13380	-120
		Chardonnay	1100	19240	10580	130
		Feteasca Neagra	1110	28863	20203	63
		Cabernet Sauvignon	1110	30687	22027	-348
		Pinot Noir	1100	24360	15700	-219
Mowing	7800	Italian Riesling	1140	20466	12666	-834
		Chardonnay	1210	17900	10100	-350
		Feteasca Neagra	1180	25950	18150	-1990
		Cabernet Sauvignon	1240	29130	21330	-1045
		Pinot Noir	1180	22350	14550	-1369
Organic	8500	Italian Riesling	910	32340	23840	10340
		Chardonnay	950	28410	19910	9460
		Feteasca Neagra	940	37500	29000	8860
		Cabernet Sauvignon	960	40048	31548	9173
		Pinot Noir	940	31880	23380	7461

The most important indicator, profit, also differs depending on the soil management system; for all varieties, the maximum profit was realised on the organic plots, although, it did not yield the highest grape production, had the lowest production cost per plot and the

highest value of grape production. In the organic plots, the differences from the control vary between 10340 lei per hectare for the 'Italian Riesling' variety and 7461 lei per hectare for the 'Pinot Noir' variety. In the plots maintained by mowing and herbicide application, lower profit values were recorded, compared to the control for all wine grape varieties. Grapevine fertilization has been intensively studied, but due to the climate changes and variability, to the soil and fertilizers diversity, can be further improved, because there is not a perfect solution for universal fertilization recipes. Fertilization must be approached differently from one area to another, from grape variety to variety and from one year to another, because there is a large possibility to find new fertilizers and formulas to combine them to be more efficient. The conventional fertilization, generally based on complex chemical fertilizers, has been replaced in organic viticulture with other types of fertilizers, more environmentally friendly, with a higher recovery rate and with a less harmful impact on wine products and implicitly on humans (Table 2).

Table 2. The influence of the fertilization treatments on the economic indicators in 2018

Variety	Fertilizers	Total costs (lei/ha)	Production costs (lei/ton grapes)	Production value (lei/ha)	Profit (lei/ha)	Difference to control (lei/ha)
Italian Riesling	Conventional (C)	8850	818	21648	12798	-
	Green manure	9400	1039	27147	17747	4949
	Fertilpolina	9200	974	28344	19144	6346
	Humus Vita Stallatico	7950	863	27642	19692	6894
	Fertilpolina+Cropmax	9800	937	31380	21580	8782
Chardonnay	Conventional (C)	8850	908	19486	10636	-
	Green manure	9400	1160	24306	14906	4270
	Fertilpolina	9200	1116	24738	15538	4902
	Humus Vita Stallatico	7950	948	25167	17217	6581
	Fertilpolina+Cropmax	9800	1032	28488	18688	8052
Feteasca Neagra	Conventional (C)	8850	961	27621	18771	-
	Green manure	9400	1235	30444	21044	2273
	Fertilpolina	9200	1117	32936	23736	4965
	Humus Vita Stallatico	7950	995	31964	24014	5243
	Fertilpolina+Cropmax	9800	1139	34432	24632	5861
Cabernet Sauvignon	Conventional (C)	8850	942	28197	19347	-
	Green manure	9400	1197	31408	22008	2661
	Fertilpolina	9200	1081	34024	24824	5477
	Humus Vita Stallatico	7950	966	32920	24970	5623
	Fertilpolina+Cropmax	9800	1112	35260	25460	6113
Pinot Noir	Conventional (C)	8850	1097	24195	15345	-
	Green manure	9400	1485	25316	15916	571
	Fertilpolina	9200	1291	28496	19296	3951
	Humus Vita Stallatico	7950	1177	27024	19074	3729
	Fertilpolina+Cropmax	9800	1332	29428	19628	4283

Regarding the influence of fertilization on economic indicators, issues are different for each indicator analyzed. The impact of fertilization on total expenditure per hectare is different; mixed and organic fertilization options have led to an increase in total costs for all grape varieties. The less expensive fertilization option was when the organic Humus Vita Stallatico was applied. The lowest cost per ton of grapes production was registered in the conventional fertilization, and the highest production cost was found in the plot with green manure fertilization. Among the alternative fertilization options, the closest value to the control for the production cost was in the plots fertilized with Humus Vita Stallatico. Although the organic fertilization plots recorded higher costs per ton of grapes, due to the higher price of organic grapes, the same plots registered the higher values of production and profit per hectare for all wine grape varieties. The highest profit were recorded in organic plots fertilized with the combination of Fertilpolina + Cropmax, ranging between 25460 lei per hectare for 'Cabernet Sauvignon' and 18688 lei per hectare

for 'Chardonnay'. The profit differences registered compared to the control were higher in all experimental plots; the maximum difference was found when fertilization with Fertilpolina + Cropmax was applied. With all of the sustainable advantages of organic viticulture, Weeler and Crisp (2009) mentioned that organic grapevine growing suppose significant increases in input costs driven especially by the labour costs.

Diseases and pests control is another technological sequence that involves a completely different approach between conventional and organic viticulture. Due to climate change and variability, the diseases and pests prevention and control is increasingly difficult and with an increasing impact on the grape production level (Nistor et al., 2018). The necessity for carbon mitigation and pesticides application imposed new strategies and products for diseases and pests control in both conventional and organic viticulture. Therefore, hazardous products with high remnant effect are less and less used in crops. The conventional, organic, and mixed fertilization treatments had different influence on economic indicators. For all grape varieties, the highest total costs were recorded in the plots with conventional diseases and pest control, and the lowest - in the plots with organic control (Table 3). Instead, the cost of production differs from grape variety to another, depending on the amount of production and the grape variety sensitivity to diseases and pests. The highest cost per ton of grapes was recorded in the plots with organic control, except for 'Cabernet Sauvignon' variety, which, being more tolerant to diseases, had similar production costs. Without costs for pesticides, and higher price for grapes, in the organic control plots was recorded higher grape yield and profit than the control plots. The profit was in favour of organic plots, with differences per hectare between 8509 lei in the case of the 'Italian Riesling' variety and 4240 lei per hectare in the case of the 'Pinot Noir' variety. The mixed treatment for diseases and pest control, by alternating organic products with conventional ones, although it ensures higher grape production compared to the organic plots, because grapes are sold at lower price, registered the lowest values of production and profit. Results are confirmed by the opinion of Borca et al. (2019) which found that the treatments for diseases and pest control in the conventional grapevine growing had the highest inputs and impact on the grape production costs, grape yield and quality.

Table 3. The influence of the disease and pest control treatments on the economic indicators in 2018

Variety	Diseases and pest control	Total costs (lei/ha)	Production costs (lei/ton grapes)	Production costs (lei/ha)	Profit (lei/ha)	Difference to control (lei/ha)
Italian Riesling	Conventional (C)	9500	832	22842	13342	-
	Organic	8500	840	30351	21851	8509
	Mixed	8900	818	21750	12850	-492
Chardonnay	Conventional (C)	9500	958	19830	10330	-
	Organic	8500	1033	24669	16169	5839
	Mixed	8900	970	18348	9448	-882
Feteasca Neagra	Conventional (C)	9500	958	29736	20236	-
	Organic	8500	1002	33900	25400	5164
	Mixed	8900	981	27225	18325	1911
Cabernet Sauvignon	Conventional (C)	9500	895	31809	22309	-
	Organic	8500	885	38388	29888	7579
	Mixed	8900	891	29952	21052	-1257
Pinot Noir	Conventional (C)	9500	1129	25236	15736	-
	Organic	8500	1193	28476	19976	4240
	Mixed	8900	1127	23691	14791	-945

CONCLUSIONS

The main technological sequences that require different approach in conventional and organic viticulture are: tillage, fertilization, and disease and pest control. These management practices have major importance in the structure of total expenditures and with a high impact on grape production, quality, and economic indicators. Soil management and fertilization influence the health of plants, with direct influence on photo-synthetic rate. Conventional soil management

offer the best conditions for higher photosynthetic rate, but have the most aggressive impact on the soil and the environment due to much higher carbon emissions compared to the mowing or organic soil management. Although soil management by mowing or organic did not confirm the parameters of the control plot (bold soil), are achievable and necessary alternatives in order to mitigate carbon emissions, soil and water protection, and last but not least to offer the consumer wine products as healthy as possible. The conventional fertilization provides the highest increase in production, while is the most energy-intensive consumer, with the highest degree of soil and water pollution. For many reasons, chemical fertilizers must be increasingly replaced partially or even completely with organic fertilizers or other useful nutrients less harmful for environment and groundwater. Although the alternative options for soil management, fertilization and disease and pest control could not exceed the control plots with conventional viticulture, as regards the production rate, thanks to the availability of many consumers willingness to offer higher prices to benefit from healthy wine products, these variants have led to increased production value and profit rate. 'Cabernet Sauvignon' was the variety with the best results for leaf area, grape production, and sugars yield/ha for all soil management, fertilizers applied and diseases and pest control treatments. With close results ranked 'Feteasca Neagra' variety. 'Italian Riesling' and 'Chardonnay' were versatile registering positive and negative reactions to conventional and organic practices. In most experimental plots regardless organic or conventional practices, recorded negative results, excepting the high sugars concentration in grape must. The main technological sequences that require a totally different approach in conventional and organic viticulture are tillage, fertilization, and disease and pest control, which have a major importance in the structure of total expenditures and with a high impact on grape production, quality and economic indicators.

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LONG-TERM EFFECTS OF ORGANIC FERTILIZERS ON MICROELEMENTS STATUS IN GRAPEVINE LEAF ON CALCAREOUS SOIL

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Abstract

The aim of this study was to determine the effect of different doses of organic fertilizers on the content and dynamics of microelements in vine leaves on carbonate soil during three vegetation. The trial was performed according to randomize complete block design with 6 treatments (unfertilized, farmyard manure 20 t ha^{-1} and 40 t ha^{-1}, peat 20 000 L ha^{-1} and 40 000 L ha^{-1}, NPK (5-20-30) 500 kg ha^{-1}+2x100 kg UREA kg ha^{-1}) in 4 repetitions. Samples of vine leaves were taken three times during the growing period: at the flowering, 2 weeks after flowering and verasion stage. Statistically significant difference in iron leaf content was determined in the first vegetation year (verasion). The highest amount was determined in the treatment with 40 t ha^{-1} of farmyard manure (94 mg kg^{-1} Fe) and the lowest in the treatment with mineral fertilizers (79 mg kg^{-1} Fe). Statistically significant difference in the content of manganese was recorded in the second year of research (flowering). The highest amount was determined in control treatment (30.50 mg Mn kg^{-1}), and the lowest amount in the treatment with NPK fertilizer 500 kg ha^{-1}+2x100 kg UREA (18.50 mg Mn kg^{-1}). In three years of research there were no significant differences between average values of zinc and copper.

Key words*: farmyard manure, peat, microelements, grapevine, alkaline soil.*

INTRODUCTION

Viticulture in Croatia is an important part agriculture and economy. Growing on inadequate rootstock on carbonate soils often reduces the height and quality of yield. On soils with high carbonates content, uptake of iron, zinc, manganese, and copper is significantly lower by the poor solubility of compounds containing the trace elements (Ksouri et al., 2005). Therefore, on such soils, chlorosis often occurs on grapevine, which is a physiological disorder often caused by insufficient or imbalance of essential cations for physiological processes in the plant. This may be due to the high amount of calcium in the soil (carbonate soil) which is usually bound in calcite ($CaCO_3$), a relatively poorly soluble calcium mineral, but in the presence of CO_2 and H_2O turns into a soluble form of calcium bicarbonate ($Ca(HCO_3)_2$), whose dissociated forms of HCO_3^- and Ca^{2+} affect the increase of soil pH (Imas, 2000; Ksouri et al., 2005). The occurrence of grapevine chlorosis in the Plešivica vineyards is most common in June, during flowering or immediately after flowering, and thus has the strongest impact on reducing the yield of grapes due to poor flowering and fertilization. The most common visible symptoms in grapevine are the appearance of pale green to yellow color on the leaves. If the symptoms appear on younger leaves, we talk about the lack of microelements: iron, zinc, manganese, while on older leaves there are symptoms due to magnesium deficiency (Herak Ćustić et al., 2007). Fertilization of vineyards is has a great impact on yield and quality of must and wine. Fertilizer application results in increased yields, however, excessive or unbalanced fertilization can have a negative impact on quality (Delgado, 2004).

The application of organic matter to agricultural soils does not show a significant effect on the total amount of trace elements in the soil, but increases their availability compared to soil fertilized with mineral

fertilizers (Herencia et al., 2008; Moustaoui and Verloo, 1995; Tamoutsidis et al., 2002, cit. according to Petek, 2009).

Furthermore, one-side application of mineral or organic fertilizers cannot achieve sustainable agricultural production. Even with a balanced application of mineral fertilizers, high and quality yields are unsustainable over many years due to the negative effect on the physical and biological properties of the soil (Khan et al., 2008). In addition, one-side application of mineral fertilizers and other chemicals often used in agriculture, in addition to adverse environmental effects, may lead to changes in the composition of fruits, vegetables and reduce the ammount of vitamins, minerals and other nutrients (Masoud, 2012). The main role of microelements in the plant organism refers to their participation in the activity of enzymes (as a component, cofactor or activator). Because of their ability to change valence, they participate in oxido-reduction processes by transporting electrons (Marschner, 1995). Availability of micronutrients is highly dependent on soil characteristics such as pH, $CaCO_3$, organic matter, and available phosphorus (Christensen et al., 1951; Jenne, 1968; Lutz et al., 1997; Olomu et al., 1973; Yuan, 1983; Shuman, 1988, cit. according to Wei, 2005). Interactions with macronutrients also significantly affect micronutrient uptake (Aulakh and Malhi, 2005). It should be noted that temperature and humidity are important factors influencing the availability of micronutrients in the soil. The availability of most micronutrients in the soil decreases at low temperatures and low moisture content due to reduced root activity and reduced dissolution and diffusion (Frageria et al., 2002). Gao et al. (2000) state that organic fertilizer is a better source of available iron, manganese and zinc compared to mineral fertilizers. This is particularly pronounced on carbonate soils because the decomposition of organic fertilizers releases organic acids and CO_2 which leads to lower pH and better nutrient availability (Wei et al., 2005), while Herencia et al. (2008) state that the application of compost does not lead to a significant increase in the total content of micronutrients in the soil, but the available forms increase compared to mineral fertilization. Mann et al. (2006) point out that the highest availability of micronutrients was found in the combination of manure and mineral fertilizer.

Iron is necessary for chlorophyll synthesis, nitrite and sulfate reduction, nitrogen assimilation and electron transport in the process of photosynthesis (Marschner, 1995; Bergman, 1992). Interactions between iron and calcium in soil and plant are very complex. Calcium reducing the availability of iron and lead to chlorosis on plants on carbonate soils (Kabata-Pendias, 2011). In carbonate soils, most iron is found in the form of oxides and other insoluble forms that are not available to plants (Miller et al., 1984). Iron in the form of Fe (III) oxide is hardly soluble in water, but in the presence of various organic compounds it forms chelates, thus becoming available to plants (Kabata-Pendias, 2011). Zinc is part and activator of many enzymes. The great importance of zincis in the biosynthesis of RNA and DNA, proteins, auxins, and in the uptake and transport of phosphorus. It is the basic catalytic component of over 300 enzymes (Marschner, 1995). According to Vukadinović and Lončarić (1998) zinc increases plant resistance to disease (through its effect on protein synthesis), drought (reduces transpiration) and low temperatures. Zinc deficiency is most common in soils with a pH of 6.5-8.0, and in the case of carbonate soils, zinc deficiency is often associated with iron deficiency (Herak Ćustić et al., 2011). Manganese has great importance in water photolysis (Salisbury and Ross, 1992) and in activation of many enzymes (Marschner, 1995; Bergmann, 1992). Factors affecting the availability of Mn^{2+} ions and its deficiency or excess in the plant are: concentration of Mn^{2+} ions and other readily reducing manganese compounds in soil, concentration of other cations in soil, cation exchange capacity (CEC), temperature, organic matter, microbiological activity and redox soil potential. However, the deciding factor is the pH value of the soil and the balance between Mn^{2+} –Mn^{3+} – Mn^{4+} ions (Masoud, 2012). The aim of this study was to determine the effect of different doses of organic fertilizers on the content and dynamics of microelements in vine leaves on carbonate soil during three vegetation.

MATERIALS AND METHODS

Three years fertilization trial was set up on Plešivica wine-growing region, Boričkа location (northwestern Croatia), in a 10-year old vineyard, cv. 'Sauvignon White' grafted on Kobber 5BB rootstock, planted on soil with quite high pH for grapevine growing (pH_{H2O} 8.02), containing 2 mg P_2O_5 100 g^{-1} soil, 14 mg K_2O 100 g^{-1} soil and 13.5% CaO. The trial was set up according to randomize complete block design with 6 treatments: unfertilized (C), farmyard manure 20 t ha^{-1} (FM 1) and 40 t ha^{-1} (FM 2) , peat 20 000 L ha^{-1}(P 1) and 40 000 L ha^{-1} (P 2), NPK (5-20-30 500 kg ha^{-1}+2x100 kg UREA kg ha^{-1}) in 4 repetitions. Samples of grapevine leaves were taken three times during the growing period: at the flowering, two weeks after flowering and at the veraison. Average leaf samples were formed from 80 healthy, fully developed and undamaged leaves, taken opposite to clusters from 40 vinestocks (4 replicates x 10 vinestocks). Dried (105ºC) homogenized grapevine leaf samples were analyzed in triplicate and the results are presented as mean values.

After digestion of dry plant material with aqua regia microelements (iron, manganese, zinc and copper) were determined by atomic absorption spectrometry (HRN ISO 11466:2004).

Statistical data analyses were performed using the SAS 8.2 System (2002-2003).

RESULTS AND DISCUSSIONS

Statistically significant effect of fertilization on iron grapevine leaves content (Table 1) was determined in the first year of the study in third sampling (veraison).

Significantly the highest amount of iron was determined with fertilization of 40 t ha^{-1} of farmyard manure (94 mg Fe kg^{-1}), while the lowest amount was recorded with fertilization of 500 kg ha^{-1} NPK 5-20-30 + 200 kg ha^{-1} UREE (79 mg Fe kg^{-1}).

In all three years (Figure 1), the average values decreased from the first to the third sampling. Statistically significantly the higest values were found in the first sampling - flowering (136.60, 180.88 and 93.27 mg Fe kg^{-1}), and the lowest values in the third sampling - veraison (86.42, 101.21 and 54.56 mg Fe kg^{-1})

Table 1. Iron grapevine leaves content (mg Fe kg^{-1} in DW) in grapevinevine leaves under different fertilization treatments in three year experiment

mg Fe kg^{-1} DW				
	2008			
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	125,25	83,50	84,75 ab	**97,83**
FM 1	131,35	94,75	93,50 ab	**106,53**
FM 2	157,25	87,50	94,00 a	**112,92**
P 1	143,75	101,25	81,00 ab	**108,67**
P 2	135,50	91,25	86,25 ab	**104,33**
NPK	126,50	92,50	79,00 b	**99,33**
Average	**136,60 a**	**91,79 b**	**86,42 b**	
	2009			
C	180,00	103,50	106,00	**129,83**
FM 1	181,25	119,50	96,00	**132,25**
FM 2	178,75	113,00	115,00	**135,58**
P 1	174,00	105,75	96,00	**125,25**
P 2	187,00	109,25	95,75	**130,67**
NPK	184,25	114,25	98,50	**132,33**
Average	**180,88 a**	**110,88 b**	**101,21 c**	
	2010			
C	83,73	70,45	50,90	**68,36 b**
FM 1	102,68	67,63	54,13	**74,81 ab**
FM 2	104,18	80,28	56,68	**80,38 a**
P 1	88,55	70,48	58,23	**72,42 ab**
P 2	92,68	67,60	56,30	**72,19 ab**
NPK	87,83	67,63	51,15	**68,87 b**
Average	**93,27 a**	**70,68 b**	**54,56 c**	

Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Means without any letter indicate no significant differences.

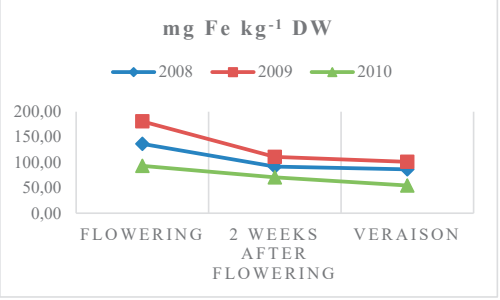

Figure 1. Average iron content (mg Fe kg^{-1} in DW) in grapevine leaves by sampling

Determined values are in accordance with Petek et al. (2008), who state that iron grapevine leaves content of Sauvignom blanc variety ranged between 60.17-161.90 mg kg^{-1} Fe per dry matter, as well as Fregoni (1998) who determined values 65-300 mg kg^{-1} Fe.

Furthermore, according to the average annual values of treatments in all three years of research, the highest values of iron in leaves were determined on the treatment with 40 t ha^{-1} manure with 40 t ha^{-1} (112.92, 135.58 and 80.38 mg Fe kg^{-1}) while in the third year the stated value was statistically significantly the highest.

These results are consistent with research by many authors (McCaslin et al., 1987; Parsa and Wallace, 1979; Prasad, 1981, cit. according to Chen et al., 1998) who conclude that iron deficiency in carbonate soils can be corrected by applying organic fertilizers, and Chen et al. (1988) who state that the application of carbon-rich organic fertilizers (manure, compost) on carbonate soils increases the number of microorganisms that produce Fe-chelators.
Furthermore, authors state that the beneficial effect of organic matter in preventing Fe-chlorosis is not only the result of chelation of iron with humic and fulvo components, but also a stimulating effect on soil microorganisms.
Fertilization had no a significant effect on average zinc values (Table 2) in any year of research by individual sampling (flowering, two weeks after flowering, veraison). However, according to the annual average values, in the first and the second year of the study, relatively the highest values was determined on treatment with 40 t ha^{-1} farmyard manure (22.80 mg Zn kg^{-1}; 11.25 mg Zn kg^{-1}).

Table 2. Zinc grapevine leaves content (mg ZN kg^{-1} in DW) in grapevinevine leaves under different fertilization treatments in three year experiment

mg Zn kg^{-1} DW				
	2008			
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	16,08	17,15	16,25	**16,49**
FM 1	19,19	16,35	18,25	**17,93**
FM 2	16,88	15,53	36,00	**22,80**
P 1	18,04	16,13	17,75	**17,30**
P 2	17,70	16,30	14,68	**16,22**
NPK	16,74	16,00	14,00	**15,58**
Average	**17,44**	**16,24**	**19,49**	
	2009			
C	7,20	5,10	19,73	**10,68**
FM 1	9,15	4,33	12,40	**8,63**
FM 2	8,18	6,05	19,53	**11,25**
P 1	7,00	4,88	14,88	**8,92**
P 2	7,30	8,23	11,70	**9,08**
NPK	7,98	7,20	15,78	**10,32**
Average	**7,80 b**	**5,96 b**	**15,67 a**	
	2010			
C	20,00	8,85	14,68	**14,51**
FM 1	13,88	5,40	12,70	**10,66**
FM 2	18,15	3,68	10,73	**10,85**
P 1	18,40	6,15	12,94	**12,50**
P 2	17,38	10,35	11,53	**13,08**
NPK	21,50	6,13	14,75	**14,13**
Average	**18,22 a**	**6,76 c**	**12,89 b**	

Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Means without any letter indicate no significant differences.

According to average values by individual sampling, the same trend was recorded in all three years (Figure 2). From flowering stage (17.44, 7.8 and 18.22 mg Zn kg^{-1}) to two weeks after flowering (16.24, 5.96 and 6.76 mg Zn kg^{-1}) values were decreased, and than to veraison stage again were raised (19.49, 15.67 and 12.89 mg Zn kg^{-1}).

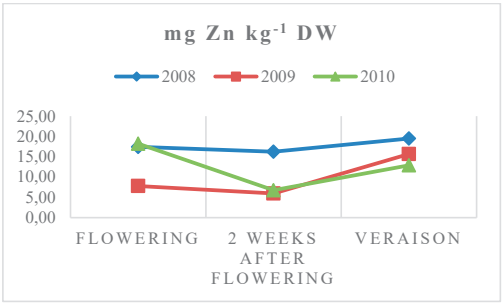

Figure 2. Average zinc content (mg Zn kg^{-1} in DW) in grapevine leaves by sampling

Results are consistent with Čoga et al. (2011) who obtained significantly higher values of zinc in grapevine leaves on pseudogley (18.4 mg Zn kg^{-1}) in flowering stage compared to rendzina (carbonate soil) (13.4 mg Zn kg^{-1}). However, these values are generally low according to Jackson (2000) who stated optimal supply of grapes with zinc is in the range of 25-150 mg Zn kg^{-1} while Fregoni (1998) states values of 20-250 mg kg^{-1} Zn. Furthermore, relatively low values are in agreement with Herak Ćustić et al. (2011) who found a reduced possibility of zinc uptake on soils with a high percentage of physiologically active lime.
The effect of fertilization on the amount of manganese in grapevine leaves (Table 3) was recorded in the second year of research in the first sampling (flowering). The highest amount of manganese was determined on control treatment (30.5 mg Mn kg^{-1}) and treatment with 20 t ha-^{1} manure (27.75 mg kg^{-1} Mn), while the lowest amount was determined on treatment with 500 kg ha^{-1} NPK 5-20-30 + 200 kg ha^{-1} UREE (18.50 mg Mn kg^{-1}).

Table 3. Manganese grapevine leaves content (mg Mn kg^{-1} in DW) in grapevinevine leaves under different fertilization treatments in three year experiment

mg Mn kg^{-1} DW				
	2008			
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	29,75	37,50	37,75	**35,00 a**
FM 1	29,40	33,75	36,25	**33,13 ab**
FM 2	23,98	31,75	31,00	**28,91 b**
P 1	27,92	28,75	34,25	**30,31 ab**
P 2	25,06	32,50	34,00	**30,52 ab**
NPK	26,91	26,75	32,00	**28,55 b**
Average	**27,17 b**	**31,83 a**	**34,21 a**	
	2009			
C	30,50 a	29,75	66,50	**42,3**
FM 1	27,75 a	32,00	65,0	**41,6**
FM 2	23,75 ab	33,50	72,75	**43,3**
P 1	25,50 ab	35,75	73,5	**45,0**
P 2	25,25 ab	30,50	58,3	**38,0**
NPK	18,50 b	35,00	69,0	**41,0**
Average	**25,21 c**	**32,75 b**	**67,5 a**	
	2010			
C	18,13	14,88	24,80	**19,27 a**
FM 1	14,70	12,15	24,43	**17,09 ab**
FM 2	13,90	11,05	20,60	**15,18 b**
P 1	18,08	12,68	24,35	**18,37 ab**
P 2	18,45	13,68	21,40	**17,84 ab**
NPK	18,75	14,00	27,30	**20,02 a**
Average	**17,00 b**	**13,07 c**	**23,81 a**	

Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Means without any letter indicate no significant differences.

According to trend, in the first two years of the study the amount of manganese in grapevine leaves was increase from flowering to veraison, and in the third year it was decrease from the first to the second sampling, and again increase to the third sampling (Figure 3).

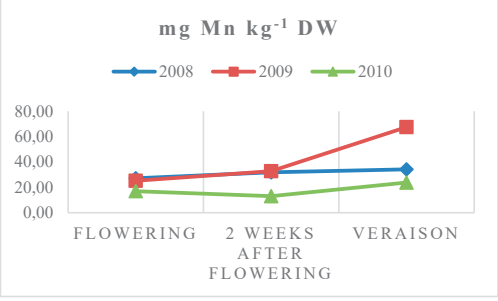

Figure 3. Average manganese content (mg Mn kg^{-1} in DW) in grapevine leaves by sampling

However, these values are lower than optimal (30-100 mg Mn kg^{-1}) as reported by Bergman (1992). Furthermore, in the first two years of the study, the amount of manganese increased from flowering to veraision, what is consistent with Čoga et al. (2008) who states the lowest values in flowering (33 mg Mn kg^{-1}) and the highest in harvest (100 mg Mn kg^{-1}).

Fertilization had no a significant effect on average copper values in any year of research by individual sampling (flowering, two weeks after flowering, veraison), and between average annual values of individual treatments, as well (Table 4).

Table 4. Copper grapevine leaves content (mg Mn kg^{-1} in DW) in grapevinevine leaves under different fertilization treatments in three year experiment

mg Cu kg^{-1} DW				
	2008			
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	11,63	715,00	501,25	**409,29**
FM 1	10,87	717,50	274,48	**334,28**
FM 2	11,22	712,50	266,00	**329,91**
P 1	11,09	715,00	432,50	**386,20**
P 2	11,56	747,50	388,25	**382,44**
NPK	9,79	667,50	452,50	**376,60**
Average	**11,02 c**	**712,50 a**	**385,83 b**	
	2009			
C	7,70	2,78	123,08	**44,5**
FM 1	8,48	3,50	107,23	**39,7**
FM 2	8,80	2,13	105,78	**38,9**
P 1	7,98	2,85	102,35	**37,7**
P 2	9,33	4,20	95,20	**36,2**
NPK	8,73	3,80	100,65	**37,7**
Average	**8,50 b**	**3,21 b**	**105,7 a**	
	2010			
C	397,25	505,25	276,25	**392,92**
FM 1	383,75	526,50	263,50	**391,25**
FM 2	390,25	508,25	252,00	**383,50**
P 1	353,75	566,75	273,25	**397,92**
P 2	394,00	487,50	261,50	**381,00**
NPK	372,50	468,25	269,00	**369,92**
Average	**381,92 b**	**510,42 a**	**265,92 c**	

Determined values in individual samples varied in very wide ranges (2.13 to 747 mg Cu kg^{-1}). According to the average values of individual sampling, the highest values in the first (712.5 mg Cu kg^{-1} and third (510.42 mg kg^{-1} Cu) year were recorded two weeks after flowering, and in the second year of the study in verasion stage (105.70 mg Cu kg^{-1}). Determined lowest values differed significantly depending on the year of the study. Thus, in the first year of the study, the lowest value was recorded in flowering (11.02 mg Cu kg^{-1}), in the second two weeks after flowering (3.21 mg Cu kg^{-1}), and in the third in verasion (265.92 mg kg^{-1} Cu) (Figure 4). These differences can be attributed with use of copper plant protection products.

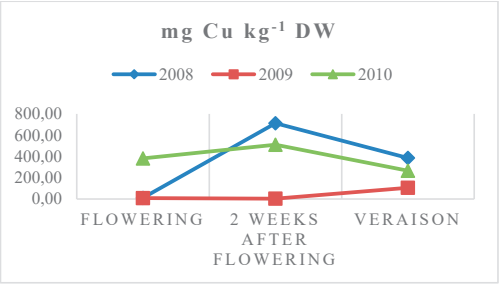

Figure 4. Average copper content (mg Cu kg^{-1} in DW) in grapevine leaves by sampling

CONCLUSIONS

Fertilization with organic fertilizers affected the amount of iron and manganese in grapevine leaves, while the effect of fertilization on the amount of zinc and copper was not determined.

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APPLICATION OF GOMAT ROST AS FOLIAR FERTILIZER UNDER MERLOT VARIETY GROWN WITHOUT IRRIGATION IN RHODOPE COLLAR REGION

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Abstract

The study was conducted at the Department of Viticulture and Fruit Growing. The aim is to find out how Gomat Rost affects the vegetative and reproductive manifestations of a variety 'Merlot'. The results of the experiment showed when applying with doses of 1 to 4 L/Ha, leads to a change in the growth dynamics of the different variants in the experiment. Treated in four different stages of the growing season, it has a positive effect on the vine plants included in the experiment compared to the control variant. The result of the application of higher doses has a positive effect on the growth and shoots maturation and increase the grapes mass. This is found despite the small amount of soil available and 35% atmospheric humidity. This is confirmed by the data on the yield and the sugar and acidity content in the grapes during harvest. In the variants treated with the highest dose of 4 L/Ha sugars and acids remain stable close to those for normal fermentation. This is very important for wine grape varieties in order not to dilute the grape must in order to compensate for the water that has evaporated during veraison. From a technological point of view, vines subjected to water stress causes problems in the fermentation process.

Key words*: Bulgaria, Gomat rost, Merlot variety, foliar feeding.*

INTRODUCTION

It is very difficult to distinguish the soil influence on grape yield and quality of wines obtained, as it is intertwined with the influence of variety, rootstock and the complex climate impact of a country. Another important element in the vine cultivation as a plant is related to nutrition.

Use of natural bio stimulants to improve the quality of grape production (Salvi, 2016). Studies was conducted during the 2015 growing season of *Vitis vinifera* 'Sangiovese' to investigate the effects of foliar fed bio stimulants on the vine, eco-physiological and productive characteristics, to improve quality in a vigorously growing vine. Similar results were obtained from Andraş-Sauca, 2018.

Tillage and foliar fertilizers include control variant, Ca-200 g/L, K-150 g/L, N-20 g/L g, Ca-30 g/L, K-20 g/L and Cu-5 mg/L. The results show that the application of soil and foliar fertilizers increases the quality and quantity of table grapes compared to the control treatment.

The highest berry diameter, weight and size of 100 berries are obtained when vines are treated with Ca 200 g/L. The highest cluster weight was obtained for vines that were treated with K 150 g/L using the soil application method. The highest berry hardness was obtained in vineyards treated with K 20 g/L using foliar application method.

The highest pH and TSS were obtained in vineyards treated with Cu 5 mg/L using also the foliar application method. The highest fruit yield was found in vineyards treated with K 150 g (M.R. Beibulatov, 2018).

It was found that treatment with the preparation "Mars-U" promotes the accumulation of starch 0.4% in variety 'Tempranillo' and up to 0.6% in the variety 'Syrah'. Potassium humate treatment is encouraging the starch accumulation in Tempranillo - from 0.2% up to 0.4% in 'Syrah'. Treatment with the "CryoMix" at a dose of 0.5 l/ha contributed to an increase in the starch content in 'Tempranillo' from 0.2% to 0.15% for 'Syrah'; with a dose of 1.0 l/ha by 0.2% for both varieties; with a dose of 1.5 l/ha from 0.4% for 'Tempranillo' to 0.2% on 'Syrah'.

As the analysis of the agro biological characteristics of the studied varieties showed, under the conditions of equal the foliar treatment with the tested substances had a significant influence on the number of developed shoots and the coefficient of fruiting. The use of Cryoprotectants ("CryoMix" at a dose of 0.5 l/ha and "Mars-U" 1.0 l/ha) before the onset of shoots helps to improve the maturation, which is associated with earlier achievement of optimal shoot length and stopping their growth. This improves the ripening, contributing to increasing the frost resistance of the vine.

The use of tested Cryoprotectants ensures better buds preservation of both grape varieties. Great safety is ensured by the preparations "Mars-U" and "CryoMix" in doses of 0.5 l/ha and 1.0 l/ha. Revealed this foliar treatment using tested Cryoprotectant: Mars-U, Potassium humate.

CryoMix at a dose of 1.5 l/ha contributes to better tissue differentiation, with a high dry matter content in the vines. The Syrah variety was found to have the highest water content, especially when treated with Cryoprotectants: "CryoMix" at a dose of 0.5 l/ha and "Potassium humate" with values of 23.9% and 23.4%. The main source of energy in the soil are plant residues, animal excrements and water-soluble organic compounds, from which "Gomat Rost" originates, it is also mineralized (decomposed) by soil microorganisms. After these processes, energy is released needed to stimulate growth and form quality grapes.

It is accepted that the grape quality is determined by the amount of sugars, organic acids and anthocyanin's contained in red varieties. All other indicators are correlated with the sugar content. Therefore, the quantity of sugars has established itself as one of the main criteria for assessing the grape quality.

MATERIALS AND METHODS

The 'Merlot' variety, planted in 2013 in the Department of Viticulture and Fruit Growing near the village of Brestnik, was used as the object of the study. The experiment was conducted in 2019-2020. The planting distances are 3.0/1.0 m, the vines are formed on a stem bilateral cordon and loaded with spurs. The variety is grafted on the rootstock Berlandieri x Riparia sel. Oppenheim4. The experiment was performed in the following scheme.

Figure 1. V_o - control;
V_1 - treated with "Gomat Rost" - 2 L/Ha

V_2 - treated with "Gomat Rost" - 3 L/Ha; V_3 - treated with "Gomat Rost" - 4 L/Ha

Treatment stages are:

1. Before flowering;
2. After flowering;
3. "Pea" berry size;
4. Two weeks after "Pea" berry size.

Also are included:

- Description of the soil and climate characteristics of the site (terroir).
- Dynamics in growth and shoots maturation.
- Study of the influence of the leaf treatment with "Gomat Rost" through different phenological stages.
- Study on the yield and quality of grapes.
- The indicators were studied in Laboratory complex at AU-Plovdiv. The grapes were picked and processed on 11th September, 2020. The yeast used is Excellence XP 20 g/hl.
- Sugars, %, Titratable acids, g/dm^3

The indicators were examined according to the methods described in the Exercise guide in winemaking (Бамбалов, 2009).

RESULTS AND DISCUSSIONS

Soil characteristic - this study presents the important soil ingredients and necessary factors for making good wine production. The studied area is situated in South Bulgaria, Plovdiv region. Studied area is near the north part of the place, named "Rhodope collar", covering area of 29.7 Ha. The viticulture data is gathering from territories, which are located in training and experimental base in Brestnik village, part of the Department of Viticulture of Agricultural University- Plovdiv. The experimental base is one of the 28th University cellars in the world. Here are located more than 400 different types of grapes. This is the most developed agricultural region for wine production in Bulgaria. The relief is plane to hilly and that makes the studied area most suitable land for different viticulture analyzes. The climate is transcontinental, characterized with a cold winter and a hot summer. The Figure 2 shows the studied area Brestnik, located in Plovdiv region. Blue colors show the water resources and the black lines are the roads in the area.

Figure 2. Studied area

The main soil types in the studied area are Solonchaks and small part of type Chernozems by World Reference Base (2006) Soil Groups. On the next Figure 3 is the soil distribution of these two soil types by pink and yellow colors.
The studied site belongs to the Upper Thracian region of distribution of Cinnamon forest soils and is located in its accumulative part. The profile is very poorly differentiated. The soil is defined as deluvial meadow, clayey - sandy to sandy-clayey. The content of digestible ammonium and nitrate nitrogen in an extract with 1% KCl, absorbable phosphorus according to Egner-Rhyme and digestible potassium with a flame photometer was studied.

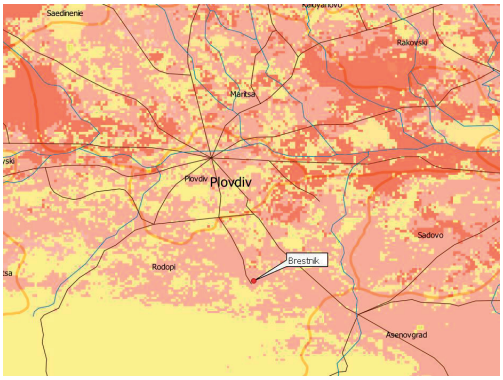

Figure 3. Soil distribution

The content of digestible ammonium nitrogen is relatively low and is a reason for the need to supplement the nitrogen deficiency in these plants. The content of easily digestible phosphorus is also below the norm of 10 m/ 100 g of soil and varies in the range of about 5.46 mg/100 g of soil. The stock of soils with absorbable potassium is good - 26.27 mg/100 g. The soil conditions for the experiment give a clearer picture of the impact of Gomat rost. It is effect on the vegetative and reproductive manifestations in the cultivation of the Merlot variety in a state of N and P_2O_5 deficiency (Table 1).

Table 1. Nitrogen, phosphorus and potassium content in the upper root soil layer

№ of the sample	pH	N mg/1000 g	P_2O_5 mg/100 g	K_2O mg/100 g
30-60 cm	8.0	Общ N- 8.55	5.46	26.27
Common carbonates	g/kg	-	-	62.27
Active carbonates	g/kg	-	-	15.00

Water regime - The average annual amount of precipitation for the period from December 2019 to the end of November 2020 in the area, has a total of 560.6 mm, which is close to the data for the average annual amount of precipitation in Bulgaria.
Research on the physiological condition of the vines include:
Phenological observations - The bud burst of the Merlot variety in the area during the

experimental period begin around middle of the third decade of March and ends in the first decade of April. The "flowering" stage takes place mainly in the first ten days of June, and the “pea” size berries in second half. The veraison starts in the middle of the third decade of July and ends within the third decade of August. Here we must note that the technological maturity of grapes reaches the first ten days of September. This is relatively close to the typical date for this phenological manifestation of this variety, which is around mid-September.

The vines from all variants treated with different concentrations, as well as the control variant, enter and leave the respective phase almost simultaneously. The data from the conducted phenological observations (Table 2) show that the only feature occurred is the reporting of an approximate period of "bud burst" and "flowering" took place for 14 days.

Table 2. Terms of the phenological stages of Merlot variety, in the Brestnik area in 2020

Variant	Bud burst			Appearance on the 1st leaf	Appearance of the1st inflorescense	Flowering			Pea size	Veraison			Technological ripeness
	tart	n mass	end			start	n mass	end		start	n mass	end	
control	24,03	31,03	7,04	18,04	23,04	29,05	6,06	12,06	20,06	25,07	8,08	23,08	11,09
2 L/Ha	24,03	31,03	7,04	18,04	23,04	29,05	6,06	12,06	20,06	25,07	8,08	23,08	11,09
3 L/Ha	24,03	31,03	7,04	18,04	23,04	29,05	6,06	12,06	20,06	25,07	8,08	23,08	11,09
4 L/Ha	24,03	31,03	7,04	18,04	23,04	29,05	6,06	12,06	20,06	25,07	8,08	23,08	11,09

Dynamics in shoot growth - The growth of the shoots begins with the bud burst, which is in 21st March, 2020. It can be seen that the strongest growth distinguishes the shoots from the variant treated with 4 L/Ha “Gomat Rost” in 2020, followed from variant with 3L/Ha, with no significant difference between them (Figure 4). In the next measurement there is a tendency for a gradual increase in the needs of the treated options.As the vegetation progresses, the growth increases, and by the end of May an increase of about 64 - 74 cm is already formed, in the different variants. On average per week it moves within about 15-20 cm. In June, with the increase of the average daily air temperatures, even greater activity in the growth processes is observed.Subsequent measurements made in June show a positive effect on treatment options in terms of "Gomat Rost" as all perform better than control variant. This trend continues in all variants in July.

As this is most clearly seen in the variants treated with 3 to 4 L/Ha. This is evident after the third application of the fertilizer during the “Pea” size stage. The following conclusion can be drawn from the positive result reported when monitoring the growth dynamics in fruit-bearing vineyards. Applied in a young vineyard "Gomat Rost" can have a positive effect on the formation and growth processes, as well as contribute to the faster establishment and entry to fruiting.

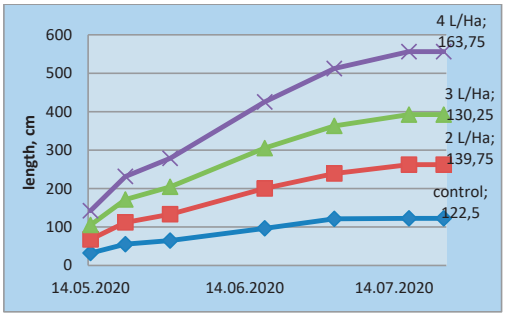

Figure 4. Dynamics in shoot growth

Dynamics of shoots maturation - Maturation is of great practical importance. It is associated with low temperatures resistance, as well as that of buds. The ripening process begins more noticeably in the first ten days of August. This occurs at a relatively high temperature and a minimum rainfall of 46.7 mm/m^2, lasting 4 weeks in all variants. The process is relatively intense, with the individual length of the mature part being about 20-30 cm per week (Figure 5). The dynamics of shoot maturation is directly dependent on climate conditions, as in the growth of shoots.

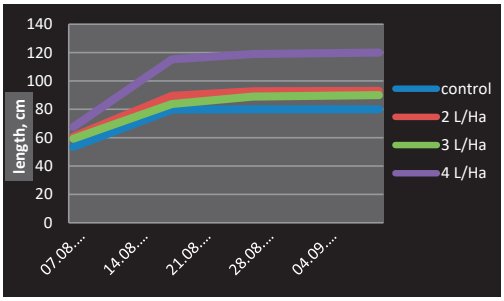

Figure 5. Dynamics in shoot maturation

Research on grape yield - Grape yield is one of the indicators determining the economic

efficiency of the applied agro-technical measures (Table 3).

Table 3. Quantitative changes in the yield of Merlot grapes

Period	Variants	Average yield per vine, kg	Average yield per Ha, kg
2020	контрола	2.950	8850.00
2020	2 L/Ha	2.965	8895.00
2020	3 L/Ha	3.100	9300.00
2020	4 L/Ha	3.600	10800.00

The yield of grapes in variants ranges from 2.950 kg to 3.600 kg per vine. The highest yield was in the variant treated with 4 L/Ha, followed by the variant treated with 3 L/Ha, and in relation to this indicator it can be seen that no significant difference was observed between the control variant and the variant treated with 2 L/Ha. The application of "Gomat Rost", with rates of 3 to 4 L/Ha in the indicated stages in the methodology of the experiment a yield of about 900 to 1000 kg can be established.

The average mass of the clusters changes in the same sequence. The bunches have the highest mass again from the variant treated with 4 L/Ha, followed by the variant treated with 3 L/Ha "Gomat Rost". With regard to this indicator, it can be seen again the formation of a group dynamics between the control and the variant treated with 2 L/Ha (Figure 6).

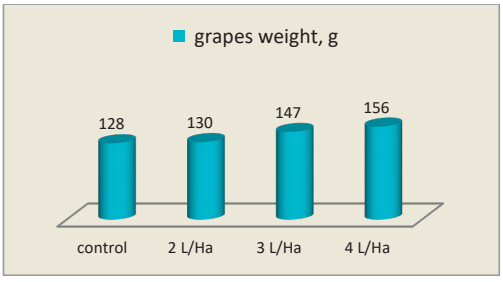

Figure 6. Average bunch mass in Merlot variety

In variants to which higher doses of "Gomat Rost" are applied, a positive trend in the mass of grapes is established. This is because again it has to be underlined thickly that the experiment is under non-irrigated conditions and this cause stress. The length of the bunches is in the range from 16.76 to 18.00 cm, and at the width 8.35 to 9.41 cm. The application of "Gomat Rost" did not have a significant effect on the inflorescences in the different variants. Because according to ampelographic characteristics the average size of a bunch is 17 cm length and 11.1 cm wide (Table 4).

Table 4. Quantitative change in the size of bunches in the Merlot variety treated with different amounts of "Gomat Rost"

Period	Variants	Bunch sizes	
		Length (cm)	Width (cm)
2020	контрола	16.76	8.35
2020	2 L/Ha	17.21	9.41
2020	3 L/Ha	17.80	8.79
2020	4 L/Ha	18.00	8.43

The grapes ripen around mid-September. From a practical point of view, this would lead to earlier planning and implementation of the grape harvest campaign. This is very important because the climate conditions are monitored during the harvest. If rains, that can create conditions for not being able to carry out the grape harvest. Figure 7 presents the results of the application of "Gomat Rost".

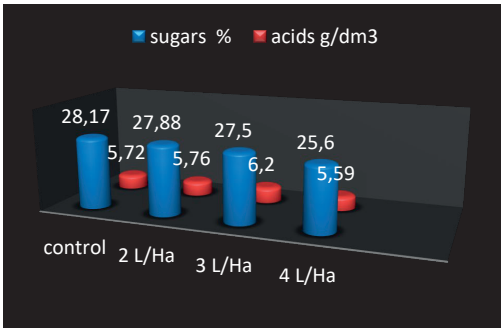

Figure 7. Dynamics of sugars and acidity content

The year 2020 was extreme in terms. The lack of rainfall required for vine growth and grape ripening was significant. It is important for the practice to emphasize that application of "Gomat Rost", in its highest dose, creates a condition for the vine plant to overcome the stress of the low soil and atmospheric humidity and to form yield, mass and shape of grapes typical for the 'Merlot' variety. From a technological point of view, the treated vines with the highest dose used lead to the preservation of relatively balanced sugars and acids.

They are directly correlated between both the rapid and the silent fermentation of the grape must. It can be clearly said from the results obtained so far that "Gomat Rost" has abilities that positively affect the presence of stress caused by drought during the growing season.

CONCLUSIONS

There is a positive effect of "Gomat Rost" on the development of the vine plant, regardless of the nitrogen deficiency. The content of easily digestible phosphorus is also below the norm of 10 mg/100 g of soil and varies in the range of about 5.46 mg/100 g. Here we must also note the fact that precipitation is not evenly distributed regardless of their total amount, which is 560.60 mm close to the national average.

Leaf application of "Gomat Rost" do not have a significant impact on the timing and duration of the individual phenological stages.

The strongest growth is characterized by the shoots from the variant treated with 4 L/Ha " Gomat Rost " followed by the variant treated early with 3 L/Ha. Applied in a young vineyard "Gomat Rost" can have a positive effect on the formation and growth processes, as well as contribute to the faster formation during establishing and entry into fruiting.

Despite the low rainfall during the growing season, ripening of the vines treated with the highest dose of 4 L/Ha is established and the process itself is proceeding at a faster pace.

A yield of about 9000 to 10000 kg/Ha can be formed using the application of "Gomat Rost", with rates of 3 to 4 L/Ha. In variants to which higher doses of "Gomat Rost" are applied, a positive trend in the mass of grapes is established. This is because again it has to be underlined thickly that the experiment is under non-irrigated conditions and this puts stress on the vine. From a technological point of view, the treated vines with the highest dose of fertilizer lead to the preservation of relatively balanced sugars and acids.

Gomat Rost has abilities to positively affect the presence of stress caused by drought during the growing season.

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EFFECT OF SPRAYING PUTRESCINE AND HUMIC ACID SPRAYING ON CHEMICAL PARAMETERS OF TOMATO PLANT *LYCOPERSICON ESCULENTUM*

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Abstract

Field experiment was conducted in An-Najaf governorate during spring of 2016. The aim of this experiment was to study the effect of different concentrations of putrescine 0, 50, 100 mg·L^{-1}. and humic acid 0, 500 and 1000 mg·L^{-1} spraying on chemical parameters of tomato plant. The experiment included nine treatments, i.e. the interactions of three concentrations of putrescine (0, 50, 100 mg·L^{-1}) and three concentrations of humic acid (0, 500 and 1000 mg·L^{-1}). Sprays were applied twice during season at fifteen days interval that was done on forty-five days from cultivation.
Factorial experiment within Randomized Complete Block Design (R. C. B. D.) was used with three replications. Means were compared according to Duncan's Multiple Range Test (D.M.R.T.) at probability of 0.05. Results can be summarized as follows: the use of different concentrations of putrescine with conc. 100 mg·L^{-1} and humic acid with conc. 1000 mg·L^{-1} had significant effects on most of the chemical parameters such as: N, P, K, total soluble carbohydrate and total Chlorophyll in Leaves and N, P, K, T.S.S and amount of vitamin C in fruit compared with control treatment which gave the least values for the above-mentioned parameters. The interactions between two factors (putrescine and humic acid) showed the presence of significant effects on most of the studied chemical parameters.

Key words*: putrescine, humic acid, tomato plant, Lycopersicon esculentum.*

INTRODUCTION

Tomato*, Lycopersicon esculentum* Mill., is in the family Solanaceae and is a widely distributed annual vegetable crop. Tomato was high nutritional quality. It is rich in vitamin C, lycopene and different phenolic compounds (Scalfi et al., 2000). Tomatoes are important not only because of the large amount consumed, but also because of their high health and nutritional contributions to humans. Most important, tomato consumption has been shown to reduce the risks of cardiovascular disease and certain types of cancer, such as cancers of prostate, lung, and stomach (Canene-Adams et al., 2005). United Nations FAO reports that the world production of tomato in 2019 was 180766329.00 tons and in IRAQ was 619543.00 tons (FAO, 2021).

Polyamines (PAs) are organic polycations found in all living organisms. In higher plants putrescine (Put), spermidine (Spd) and spermine (Spm) are the most abundant PAs and are involved in the various developmental processes (Tonon et al., 2004).

Putrescine which having a role in the regulation of plant developmental and physiological processes. (Kusano et al., 2007). A variety of roles have been proposed for PAs, including cell division, root growth, flower and fruit development and apoptosis (Paschalidis & Roubelakis-Angelakis, 2005). PAs were reported to be involved in stabilization of membrane and scavenging of free radicals (Velikova et al., 1998), osmotic adjustment (Aziz et al., 1999), mineral nutrition (Prakash & Prathapasenan, 1988).

Humic substances are a heterogeneous mixture of naturally occurring organic materials those arise from the decay of plant and animal residues. These organic materials contain carbon, which serves as a food source for soil organisms such as bacteria, algae, fungi and earthworms. These soil organisms break the chemical bonds in the residues as they digest the carbon. The remaining by-products serve as

building blocks of humic substances, which are not easily decomposed by soil organisms (Hopkins & Stark, 2003) and this will decrease nutrients leaching with irrigation water, and so increase fertilizers use efficiency (Mikkelsen, 2005). In this concern, Selim et al. (2009) found that application of humic substances through drip irrigation enhanced tubers yield quantity, starch content and total soluble solids and this application associated with the decrease of nutrients leaching, which was reflected on increasing macro- and micronutrients concentration in potato tubers, as well as increasing concentration of these nutrients in soil after tubers harvesting.

The objective of this study was to investigate the effect of foliar application of putrescine and humic acid as well as their interaction on growth improvement, chemical parameters, and determining the best application level of both factors.

MATERIALS AND METHODS

A Field experiment was conducted during the growing season in spring of 2016 at desert region between An Najaf and Karbala provinces. Seeds were sown in agriculture plate on 15/12/2015 and then after 25 days seedlings (3-5 true leafs and 10-15 cm height) were planted in the field.

Then, the experimental plot was divided in to 27 furrows at 4 m length. The distance between each furrow is two, and each furrow was bedding the animal fertilizer as width 35 cm to each furrow about 3 tons·d^{-1}. Seedlings were cultivated at 40 cm apart on furrow. The experiment design was factorial with two factors adopted with Randomized Complete Block Design R.C.B.D. in three replicates with two factors i. e.: 1 - Putrescine with two concentrations 50 and 100 mg·L^{-1} spraying on vegetative part, besides control treatment (spraying with Distilled water only); 2 -Humic acid 500 and 1000 mg·L^{-1} spraying on vegetative parts, besides control treatment (spraying with Distilled water only). Agricultural practices were done equally and when it is considered necessary (cultivation, weeding, etc.) as mentioned in (Matlob et al., 1989). Period between spraying putrescine treatments and humic acid was one weak.

Irrigation was done from well water with E.C. of 5.6 dS·m^{-1} by dripping system. Duncan's Multiple Range Test was used to compare means when it is considered significant at probability of 0.05 (Al-Rawi & Khalaf-Allah, 2000).

Parameters:

Determination of N, P, K in Leaves and Fruits.

Nitrogen Determination in Leaves and Fruits: by using Micro-Kjeldahl according to Jackson (1958).

Phosphorous Determinations in Leaves and Fruits: by using Ammonium molybdenum and Spectrophotometer methods on 882 nm according to Olsen and Sommers (1982).

Potassium Determination in Leaves and Fruits: by using Flame photometer.

Total soluble carbohydrates in Leaves (mg·g^{1}): according to Duboies et al. (1956). Total Chlorophyll in Leaves (mg·100 g^{-1}): By using acetone to extract chlorophyll pigment according to Mackinney (1941), by using the following equation: Ascorbic Acid Determination in Fruits mg·100 g^{-1}: Titration with 2, 6-dichlorophenol indophenol according to (A.O.A.C, 1980).

Total Soluble Solids in fruits (T.S.S.) by using Handel Refractometer according to Al-Ani (1985).

Table 1. Chemical and Physical Characteristics for Field Soil in the Beginning of the Experiment

Characters	Value	Unit
pH	7.1	--
EC	1.4	dS·m^{-1}
Ca^{+2}	16.6	mM·L^{-1}
Mg^{+2}	5.8	
Na^{+}	2.9	
K^{+}	0.6	
SO_4^{-2}	10.8	
Cl^{-}	15.9	
HCO_3^{-}	1.0	
Soluble P	0.16	mg·L^{-1}
O.M.	5.0	g·kg^{-1}
$CaCO_3$	285.5	g·kg^{-1}
Clay	20	g·kg^{-1}
Silt	65	
Sand	915	
Texture	Sandy	

RESULTS AND DISCUSSIONS

With regards to the spraying putrescine, resultd in Table 2 shows that there was a significant difference between treatments of putrescine, particularly treatment of 100 mg·L^{-1} that gave the highest values for chemical parameters of leaves which included N, P, K concentration, total soluble carbohydrate and total chlorophyll in leaves with 2.11%, 0.24%, 1.96%, 2.82 mg·g^{-1} and 36.88 mg·100 g^{-1}, respectively compared with control treatment (sprayed with distilled water) that gave 1.70%, 0.22%, 1.51%, 2.41 mg·g^{-1} and 32.92 mg·100 g^{-1}, respectively.

Moreover, humic acid with concentration 1000 mg·L^{-1} spraying had a significant effect on chemical parameters of leaves which included N, P, K concentration, total soluble carbohydrate and total chlorophyll in leaves were 2.30%, 0.25%, 2.25%, 2.86 mg·g^{-1} and 37.52 mg·100 g^{-1}, respectively. Meanwhile, control treatment gave the lowest values i.e. 1.53%, 0.20%, 1.41%, 2.39 mg·g^{-1} and 32.19 mg·100 g^{-1}, respectively.

The interaction between factors showed significant differences on chemical parameters of leaves, and the treatment of putrescine 100 mg·L^{-1} × humic acid with concentration 1000 mg·L^{-1} gave the highest values the of chemical parameters of leaves were 2.555%, 0.259%, 2.458%, 3.05 mg·g^{-1} and 39.99 mg·100 g^{-1}, respectively compared putrescine 0 × humic acid 0 presented the lowest value of chemical parameters of leaves were 1.452%, 0.178%, 1.236%, 2.31 mg·g^{1} and 31.60 mg·100 g^{-1}, respectively.

Results in Table 3 reveals that there was a significant difference between the treatments of putrescine, the treatment (100 mg·L^{-1}) gave the highest value of chemical parameters of fruits which included N, P, K concentration, T.S.S and amount of vitamin C in fruits were 2.01%, 0.16%, 1.15%, 5.00 and 27.77 mg·100 g^{-1}, respectively compared with the control treatment which gave the lowest means i.e. (1.62%, 0.21%, 1.44%, 4.20 and 23.44 mg·100g^{-1}) respectively.

Spraying humic acid at a concentration 1000 mg·L^{-1} clearly affected on chemical parameters of fruits which included N, P, K concentration, T.S.S and amount of vitamin C in fruits were 2.21%, 0.23%, 1.51%, 5.03 and 26.66 mg·100 g^{-1}, respectively compared with control treatment (without spraying of humic acid) that gained the least values i.e. (1.41%, 0.15%, 0.98%, 4.27 and 23.55 mg·100 g^{-1}) respectively.

The interaction between factors appeared significant differences on all chemical parameters of fruits and the treatment of putrescine 100 mg·L^{-1} × humic acid with concentration 1000 mg·L^{-1} gave the best values were 2.420%, 0.242%, 1.546%, 5.23 and 29.66 mg·100g^{-1}, respectively. While, the treatment of (0 Putrescine ×0 humic acid) which gave the lowest values 1.260%, 0.120%, 0.690%, 3.83 and 22.00 mg·100 g^{-1}, respectively.

Table 2. Effect of putrescine and foliar application of humic acid on chemical parameters of leaves of tomato plant

Treatments		Concentrations	Nitrogen concentration in leaves (%)	Phosphorus concentration in leaves (%).	Potassium concentration in leaves (%).	Total soluble carbohydrate in leaves (mg·g^{-1}),	Total chlorophyll in leaves (mg·100 g^{-1})
Putrescine mg·L^{-1}		0	1.70c	0.22a	1.51b	2.41b	32.92c
		50	1.92b	0.23a	1.83a	2.75a	34.97b
		100	2.11a	0.24a	1.96a	2.82a	36.88a
Humic acid mg·L^{-1}		0	1.53c	0.20b	1.41c	2.39b	32.19c
		500	1.90b	0.24a	1.64b	2.72a	35.05b
		1000	2.30a	0.25a	2.25a	2.86a	37.52a
Putrescine mg·L^{-1}	0	0	1.452ef	0.178c	1.236f	2.31d	31.60e
		500	1.645d	0.233b	1.364e	2.36cd	32.49d
		1000	2.005bc	0.239b	1.940b	2.56c	34.66c
	50	0	1.550e	0.211c	1.475d	2.41c	32.08de
×		500	1.882c	0.238b	1.654c	.286b	34.90c
		1000	2.325ab	0.255a	2.365a	2.98a	37.92b
	100	0	1.592e	0.223bc	1.522c	2.46c	32.90d
Humic acid mg·L^{-1}		500	2.180b	0.244ab	1.895b	2.95ab	37.76b
		1000	2.555a	0.259a	2.458a	3.05a	39.99a

Table 3. Effect of putrescine and foliar application of humic acid on chemical parameters of fruits of tomato plant

Treatments		Concentrations	Nitrogen concentration in fruits (%).	Phosphorus concentration in fruits (%).	Potassium concentration in fruits (%)	T.S.S. in fruits	Vitamin C in fruit, mg.100 g^{-}
Putrescine, mg·L^{-1}		0	1.62c	0.16b	1.15c	4.20c	23.44c
		50	1.84b	0.20a	1.28b	4.64b	24.44b
		100	2.01a	0.21a	1.44a	5.00a	27.77a
Humic acid, mg·L^{-1}		0	1.41c	0.15b	0.98c	4.27c	23.55c
		500	1.86b	0.19a	1.39b	4.54b	25.44b
		1000	2.21a	0.23a	1.51a	5.03a	26.66a
Putrescine, mg·L^{-1}	0	0	1.260e	0.120f	0.690c	3.83c	22.00e
		500	1.605c	0.155e	1.288b	4.00bc	24.00d
		1000	1.992b	0.198c	1.482ab	4.76b	24.33cd
	50	0	1.450d	0.157e	0.984cd	4.20bc	22.33de
		500	1.850bc	0.202c	1.366b	4.63b	25.00d
×		1000	2.225ab	0.235a	1.500a	5.10a	26.00c
	100	0	1.505c	d0.184	1.260b	4.78b	26.33c
		500	2.110b	0.211b	1.522a	5.00ab	27.33b
Humic acid, mg·L^{-1}		1000	2.420a	0.242a	1.546a	5.23a	29.66a

The results show that spraying with the Putrescine in combination with humic acid had a significant effect on the increases in chemical parameters of leaves which included N, P, K concentration, total soluble carbohydrate and total Chlorophyll in leaves and chemical parameters of fruits which included N, P, K Concentration, T.S.S and amount of vitamin C

in fruits (Tables 2 and 3) respectively. That could be attributed to putrescine spraying induction important biological processes such as ionic balance and DNA, RNA and protein stabilization, hence, leading to the enhancement of free amino acids and increasing in leaf sucrose content. Certain PA changes are correlated with changes in the structure and function of the photosynthetic apparatus (Demetriou et al., 2007). Application of Put. lead to improvement in photosynthetic pigment (Zeid, 2004). Ndayiragije and Lutts (2007) reported improvement in net photosynthesis as response to Put treatment in rice, and that Put. induced stomatal closure in wheat which exhibited high water content (Liu et al., 2000) and these all lead to increase all studied chemical parameters which mention above.

Humic acid spraying which promote growth and increased yield and quality in a number of plant species at least partially through increasing nutrient uptake (Karakurt et al., 2009). Humic acid have been reported to enhance mineral nutrient uptake by plants, increasing the permeability of membranes of root cells (Valdrighi et al., 1996). Humic acid is important for chloroplast system through it leads to the increased rate of photosynthesis in plant and consequently, productions of photosynthesis materials have increased in plant when spraying with humic acid also. In general, spraying with humic acid had increased the length of growth period, rate of carbohydrates, amino acids and proteins in plant. In the same direction, rate of retransfer of photosynthesis materials is done to a great extent from growth parts and consequently, weight of plant will be increased (Farnia & Nasrollahi, 2010).

Tahir et al. (2011) pointed that higher leaf chlorophyll associated to humic spraying could be related to increased cell membrane permeability by humic acid and thus promoting greater efficiency in the absorption of nutrients, especially nitrogen a nutrient with direct relation with leaf chlorophyll concentration. Moreover, the effect of humic acid caused good nutritional status in plant and thus, increased the availability of elements such as: N, P, K which had an activation of biotic activity in plant and then conformation of organic acid such vitamin C (Ertan, 2007). David et al. (1994) reported that promoting growth and nutrient uptake of plants due to the addition of humic substances. The plants take more mineral elements due to better-developed root systems. In addition, the stimulation of ions uptake under the applications of humic materials led many investigators to proposing that these materials affect membrane permeability.

CONCLUSIONS

The addition of putrescine and humic acid by spraying on the shoot of tomato had a significant effect in increasing and improving the chemical characteristic. The double interference treatments between putrescine and humic acid had a significant effect on increasing all the studied traits more than single factors.

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THE EFFECTS OF FERTILIZATION WITH ORGANIC SUBSTANCES ON TOMATO (*SOLANUM LYCOPERSICUM* L.)

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Abstract

Among the alternative agricultural systems that have been developed, organic agriculture has deserved increasing interest. This fact is due to the demand of a certain segment of consumers for products with taste, the aspect not being a determining factor for the acquisition. The study aimed to assess whether the application of fertilizers with algae and protein hydrolysate affects the quality of tomato fruits, Siriana cultivar. The applied fertilizers contained substances that are allowed to be used in organic agriculture. The research has been conducted in vegetation pots and quality indicators (chlorophyll pigments, total phenolic content, titratable acidity, soluble solid content, organoleptic characteristics) and production (mean weight per fruit and number of fruits) indicators have been evaluated. The results obtained showed that both organic substances (algae and hydrolyzed proteins) improve the quality of tomato fruits, and production indices were higher by about 5.4% for variant B_AN, respectively by 5.8% for variant B_HP compared to variant control (without organic substances).

Key words*: tomato, biostimulants, Ascophyllum nodosum, hydrolyzed protein, fertilizer.*

INTRODUCTION

The Commission's Farm to Fork and Biodiversity Strategies include the target of reaching 25% of agricultural land under organic farming by 2030 (European Green Deal, 2020).

The EU's harvested production of fresh vegetables (including melons) was 64.8 million tonnes in 2017, a very similar level to that in 2016, of which 17.4 million tonnes were tomatoes, 6.7 million tonnes were onions and 5.8 million tonnes were carrots (EUROSTAT, 2018).

Consumption of tomatoes, like many other plant species that are part of the human diet, is considered to have some positive effects on health.

There are many evidences supporting the anti-inflammatory and anticancer action of tomato fruit bioactive compounds (Raiola et al., 2014*)*.

A regional survey of farmers conducted by Bojacá et al. (2014) in Colombia, found that the fertilization was a cause of abiotic soil depletion.

As a consequence, N losses are dependent on N mineralization, which supplies the excess N in the balance. Assuming a mean N mineralization rate of 0.75 kgN/ ha/day in tunnels during the tomato cropping period, N losses can be equated to 28% of N inputs (Boulard et al., 2011).

The use of biostimulators has played a beneficial role in mitigating the effects due to water shortage and maintaining a level of production (Peripolli et al., 2021).

Ascophyllum nodosum belongs to the class of brown algae and has a wide use in the category of products used in agriculture as a biostimulator with positive effects on some quality indices of crops (Battacharyya et al., 2015; Peripolli et al., 2021).

Protein hydrolysates have great potential to improve crop performance, especially under environmental stress conditions (Colla et al., 2015; 2017).

Seaweed extracts are part of many biostimulators because they have been shown to be factors that promote plant growth and can be applied with good results in conditions of

abiotic stress (Battacharyya et al., 2015; De Saeger et al., 2019).

MATERIALS AND METHODS

Plant materials and treatments

This study is focused on the effect of fertilizers with algae and protein hydrolysate on tomato, Siriana cultivar. The research has been conducted in vegetation pots.

The cultivar Siriana F1 of *Lycopersicon esculentum* was used in experiment. Siriana F1 is an early growing cultivar with indeterminate growth, known to produce its first fruits after ~100 days. The fruits are red, spherical and slightly flattened, with a medium weight of 140g/fruit. One plant can produce 5-5.5 kg of fruits (Inculet et al., 2019).

It has been observed that cultivar Siriana F1 has good resistance to the most important pathogens on tomato crops (Sovarel, 2015).

The applications of all fertilizer were performed by fine atomization on the whole foliage surface, during the vegetation period, using four foliar treatments including 1% concentrated solutions, as follows: first treatment at 21 days after planting and the next three treatments la interval de 7 days after the previous one.

It has been observed that foliar application of seaweed extract is effective in the morning when the stomata leaf is open (Battacharyya et al., 2015).

During the experiment, when fruits were fully ripened, a minimum of three medium fruits were collected for further analyses.

Obtaining and characterizing fertilizers

Fertilizers were obtained in the laboratory using raw materials that are allowed to be used in organic agriculture and were coded thus taking into account the components of each:

Control with code **Bm**: contains secondary nutrients and micronutrients (B, Cu, Fe, Mg, Mn, S, Zn), without organic matter;

Variant 1 with code **B_AN**: contains secondary nutrients and micronutrients (B, Cu, Fe, Mg, Mn, S, Zn) + algae extract (*Ascophyllum nodosum*);

Variant 2 with code **B_HP**: contains secondary nutrients and micronutrients (B, Cu, Fe, Mg, Mn, S, Zn) + hydrolyzed soy protein;

The composition of secondary nutrients and micronutrients (B, Cu, Fe, Mg, Mn, S, Zn) for all 3 samples were in the concentration ranges: B 1.92-2.12 g/L, Cu 0.44-0.55 g/L, Fe 0.44-0.53 g/L, Mg 2.40-2.59 g/L, Mn 0.42-0.54 g/L, S 2.06-2.39 g/L, Zn 0.43-0.51 g/L.

The amounts of algae extract (*Ascophyllum nodosum*) and hydrolyzed soy protein were added so that the organic matter in each of the two products was 30 g/L. The laboratory tests used to determine the composition of the biostimulators were based on the adapted methods described in the Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilisers.

Agrochemical experiments were performed on a soil with the following characteristics:

total nitrogen (Nt) - 0.615%, mobile phosphorus (P_{AL}) - 166 mg/kg, mobile potassium (K_{AL}) - 317 mg/kg, organic carbon ($C_{organic}$) - 9.41%.

Soil samples were analysed by ICPA methodology (Stoica et al., 1986) developed to assess soil properties as follows:

- Organic carbon determined by volumetric method Walkley - Black wet oxidation after the change Gogoasa;
- Total nitrogen (Nt): Kjeldahl method digestion with sulphuric acid at 350^0C, a catalyst of potassium sulfate and copper sulphate;
- Phosphorus accessible (P mobile): method Riehm-Domingo and dosed with colorimetric molybdenum blue method after Murphy-Riley (reduction with ascorbic acid);
- Potassium (K mobile) accessible: after extraction method Egner-Riehm-Domingo and determination by flame photometry.

Statistical analysis

Different lowercase letters mean significantly difference from other treatments at the level of $p<0.05$ according to the least significant difference (LSD) tests.

RESULTS AND DISCUSSIONS

Tomatoes are part of the human diet and there is clear evidence that they contain bioactive compounds with positive effects on human health (Raiola et al., 2014).

This study is focused on the effect of fertilizers with algae and protein hydrolysate on tomato, Siriana cultivar.
Throughout the experiment, specific practices to this crop were performed for all variants.
Bordeaux mixture was used to control diseases and pests. All solutions were prepared before each application. During the experiment, when fruits were fully ripened, a minimum of three medium fruits were collected for further analyses.
The results obtained showed that both organic substances (algae and hydrolyzed proteins) improve the quality of tomato fruits, and production indices were higher by about 5.4% for variant B_AN, respectively by 5.8% for variant B_HP compared to variant control (without organic substances) (Figure 1).

Figure 1. Yield and mean weight for variants treated with bio-fertilizers and control

It has been observed that the application of biostimulants leads to an increase in production yield and to a reduction in fruit ripening times (Mannino et al., 2020*)*.
The differences in production between the variants treated with B_HP and B_AN were insignificant but had a significant increase compared to the control, given in accordance with the studies performed by (Colla et al., 2017).
The rational use of biostimulants for a crop on a soil that contains at least the minimum nutrients needed by the plant is a way to provide plants a possibility of adapting to abiotic stresses. In order to obtain positive results, it is important that the application moments, the concentration of the active elements and the type of components respond to the needs of the tested culture.

The application of fertilizers that can be capitalized superiorly by plants and not to create pollution is a priority in agriculture. This situation is very common for fertilizers containing soluble forms of nitrogen (urea, ammonium nitrate) (Liang et al., 2020).

Table 1. The composition of chlorophyll a and b and carotene

Treatments	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Carotene (mg/g FW)
Bm (control)	0.83 a	0.79 a	0.69 a
B_AN	0.89 b	0.86 b	0.77 b
B_HP	0.90 b	0.87 b	0.77 b

Different lowercase letters mean significantly difference at the $p < 0.05$ level

Regarding the effects of the two fertilizers, it was observed that there are no statistically assured differences between the variants with organic matter content (Table 1). Although the Chlorophyll a and b content was higher for the variant in which the organic source came from the protein hydrolyzate, the difference compared to the variant with algae extract content is not significant, being proven that the application of seaweed leads to the increase of chlorophyll content in plants (Battacharyya et al., 2015; Goñi et al., 2018).
Studies have led to the conclusion that the content of carotenoids is influenced by doses of nitrogen fertilization, the nitrogen source being the variant with protein hydrolysate (Erba et al., 2013).

Table 2. Evolution of total soluble solids, titratable acidity and total phenolic content concentration in tomatoes

Treatments	Total soluble solids, TSS (%)	Titratable acidity, TA, (g/L)	Total phenolic content, mg GAE/g FM
Bm (control)	4.21 a	0.33 a	89.2 a
B_AN	4.49 b	0.37 b	95.1 b
B_HP	4.51 b	0.37 b	95.4 b

Different lowercase letters mean significantly difference at the $p < 0.05$ level.

The increase of the concentration in total soluble solids and titratable acidity in tomatoes leads to the improvement of the taste and the values obtained are in the range of values

obtained by other authors (Table 2) (Sora et al., 2019).
The effect of increasing the weight and content of titratable acidity and skin total phenolics is also manifested for grapevines as a result of treatment with *Ascophyllum nodosum* extract (Frioni et al., 2018).
Tomatoes contain antioxidant compounds that plays an important role in maintaining a normal state of plant nutrition (antibiotics and natural pesticides) and positive effects on human health (natural sources of antioxidant and antimicrobial compounds) (Turhan et al., 2011; Dadáková et al., 2020).
Although both fertilized variants showed increases in the composition of nitrogen, phosphorus and potassium in tomato fruits, the significant increases were for N and P (Figure 2).
Studies performed on tomato crop do not indicate a clear correlation between the composition of these elements and the application of organic fertilizers (Demir et al., 2010; Rouphael et al., 2017).

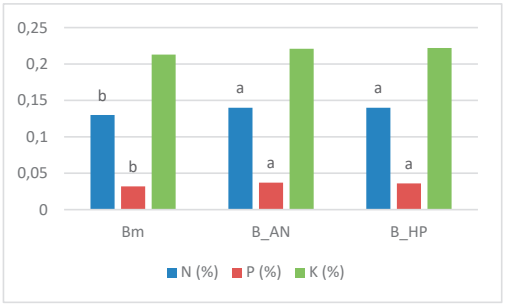

Figure 2. The composition of tomato fruits depending on the variant used for fertilizers.
Different lowercase letters mean significantly difference at the $p < 0.05$ level

Organoleptic determinations were based on 3 criteria: aspect (maximum 15 points), texture (maximum 35 points) and taste (maximum 50 points) (Sora et al., 2019).

Table 3. The results about organoleptic determinations

Variants	Organoleptic evaluation (score)			
	Aspect	Texture	Taste	Total
Bm	11	31	45	87
B_AN	12	33	47	92
B_HP	12	32	48	92

Products treated with fertilizers with organic substances had a better score compared to the variant treated only with the mineral part, due to the ability of biostimulants to produce more marketable tomato fruits (Table 3) (Mannino et al., 2020).

CONCLUSIONS

There is a high demand for the cultivation of quality tomatoes from consumers who are interested in taste and appearance. Because of this there is pressure on farmers to get quality crops with authentic taste. It was observed that the preservation of the taste and quality of the product depends on the treatments performed during the vegetation.
In our study, we obtained 2 products with organic substance with algae extract (*Ascophyllum nodosum*) and hydrolyzed soy proteins. These were applied foliar (4 treatments) to the tomato crop to evaluate the effect on quality and production indicators.
Both products have led to an increase in the content of total soluble solids, titratable acidity and total phenolic content in fruits. Also, a significant increase compared to the control for B_AN and B_HP variants was also for the chlorophyll content in plants.
The improvement of the organoleptic indices is due to a more concentrated composition in nutrients, which leads to the choice of products treated with nutrient solutions that meet the conditions of imputations that can be used in organic agriculture.
Taking into consideration these results, the present work suggests that, the application of the biostimulants represents an efficient method of obtaining quality products for tomato crop in conditions of environmental protection.

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PHYSIOLOGICAL PARAMETERS AND VEGETATIVE BEHAUVIER OF BIOLOGICAL GROWN HEAD LETTUCE TYPE (*LACTUCA SATIVA* L. VAR. *CAPITATA* L.)

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Abstract

The climate change that has occurred in the last three decades is also present for the region of Plovdiv in Bulgaria. Increase of annual average air temperature and temperature values during winter and early spring has been recorded in comparison to the (1961-1990) referent period in Plovdiv region. The lettuce development was researched in a greenhouse in the Agricultural University- Plovdiv. During the lettuce growth six different variants were used: no fertilizer, one chemical, and four organic fertilizers. The effect of the different organic fertilizers was studied through physiological parameters and vegetative behavior of plants. This paper analyzed changes in functional activity of the plant photosynthetic apparatus and productivity of variants with different fertilizers in an unheated greenhouse. The ratio between photosynthetic active radiation (PAR) and quantum yield (qY-Fv/Fm) of PS II was more effective in dark-adapted leaves for the organic fertilizer variants, compared to the no fertilizer variant. No significant difference was observed in the values of the minimal fluorescence Fo in reaction centers of PS II after the dark-adaptation of leaves from the different fertilizer variants. It was estimated the higher value of the chlorophyll content index (CCI) for organic and chemical fertilizers compared to the no fertilizer variant. The main biometric parameters were studied.

Key words: *photosynthetic activity, chlorophyll content index, lettuce, vegetative behauvier, greenhouse.*

INTRODUCTION

In recent years, with the intensification of extreme phenomena of meteorological origin, the advancement of agricultural science and the desire of people to produce qualitative and safe food with minimal risk to the environment, biological farming technologies have aroused scientific interest. Production without mineral fertilizers is important for the environment protection, for the balance and fertility of the soil, as well as for human health. The lettuce is a vegetable, intended only for fresh consumption, which requires good taste and purity of production. Vitamins A, B, C, D, and E can be found in the leaves of the species (Fogg, 1983). The vegetable is one of the main components from the dietary menu and the table in Bulgaria.

The resistance of the species to low temperatures and the duration of the period up to their typical leaf mass reached growth stage make it preferred both for autumn-winter production in unheated facilities and for early spring cultivation. Both the higher temperatures and the changes in the humidification conditions in the country (Marinova et al., 2018; Alexandrov et al., 2004**)**, and the studied area (Georgieva et al., 2017), registered in the recent decades, affect the specific meteorological conditions, the growth and development of the different production **(**Popova et al., 2014**)** and types of lettuces. It is necessary to specify the varieties and the fertilization. Therefore, an experiment in polyethylene greenhouses with a type of lettuce was set (type Head lettuce, variety "Winter butterhead") with six different variants of organic (biological) fertilization, namely: no fertilization; fertilization by means of one chemical; and fertilization by four organic fertilizers.

There are studies on the interaction of different factors on the physiological status of plants (Shopova & Cholakov, 2014). The physiological condition of plants and effect of various stressful factors thereon have been studied using chlorophyll fluorescence properties by many researchers (Mathur et al, 2014; Kalaji et al., 2016). Chlorophyll

fluorescence is a non-invasive measurement of photosystem II (PSII) activity and is a commonly used technique in plant physiology. The sensitivity of PSII activity to abiotic and biotic factors has made this a key technique not only for understanding the photosynthetic mechanisms but also as a broader indicator of how plants respond to environmental change (Murchie & Lawson, 2013). The fluorescence is emitted mainly from chlorophyll *a* of PSII and reflects the primary processes of photosynthesis by light absorption, distribution and transfer of excitation energy and photochemical reactions in PSII. Because of the functional relation of PSII with other components of the photosynthetic apparatus of the chlorophyll fluorescence, it is seen as a proxy for the state of the integral photosynthetic process and the plant organism as a whole (Roháček, 2002). Chlorophyll fluorescence, among others, has been satisfactorily used for monitoring leaf health status in lamb's lettuce (Ferrante & Maggiore, 2007) and storage potential of iceberg lettuce (Schofiled et al., 2005).

The device Chlorophyll Content Meter is useful for improving nitrogen and fertilizer management, and is ideal for crop stress, leaf senescence, plant breeding, health determination, and other studies. Furthermore, the affordability and ease of use make it an exceptional teaching tool for botany and plant science courses (Opti-Sciences 2002; Richardson et al., 2002).

The aim of the present study was to monitor the reaction of the lettuce (type Head lettuce, variety "Winter butterhead") to six different fertilization variants by analyzing the temperature conditions and measuring the main parameters of productivity and photosynthetic activity.

MATERIALS AND METHODS

The experiment was conducted on the experimental field of the Agricultural University of Plovdiv in 2018-2020 in unheated greenhouses on alluvial meadow soil (Mollic fluvisol, FAO 2006). The soil texture is sandy clay loam to clay loam, despite the small amount of total carbonates (2-3%), the soil reaction is slightly alkaline pH $_{(H2O)}$ - 7.7-8.0 (Valcheva et al., 2015). The same authors found a high amount of exchange bases (Ca^{2+}+Mg^{2+} - 20-30 meq/100 g soil) in the composition of the soil sorption complex, and a low content of nitrogen, phosphorus and potassium. The importance of the organic matter of the soil for its fertility is indisputable. However, the nitrogen bound in the organic matter remains hidden in this indicator. The nitrogen in organic form, which is over 95.0% of total soil nitrogen is the basis of soil fertility. Organic nitrogen is the source that supports the plants throughout the growing season and ensures an even supply of nitrogen to the plants. The active fraction of soil nitrogen varies with different soil types and depends on a number of factors - degree of cultivation, field history (previous crops in the crop rotation, fertilization system), biotic and abiotic soil characteristics and some environmental factors, mainly temperature and humidity.

The head lettuce type plants (*Lactuca sativa* L. var. *capitata* L., variety 'Winter butterhead') were planted on 8th of November in polyethylene greenhouses in 4 rows according to the scheme 70+30+30+30/30 cm with a profile of the soil surface a high level bed (100+60cm.) The experiment was based on the block method with four repetitions, using 28 plants per repetition, and a plot size of 3.36 m^2. Organic seeds were provided for seedling production using container technology with 150-hole Styrofoam boards in the following combination - organic seeds - 80.0%, Perlite - 20.0%, Lumbricompost for bioproduction of seedlings (Kostadinov & Filipov, 2013). Several variants were tested: 1. Control (non-fertilization); 2. NPK (mineral fertilization); 3. Italpollina; 4. Arkobaleno; 5. LC (Lumbricompost); 6. Ekoprop NX. The granular fertilizers were introduced as basic fertilization, with soil pre-transplantation at the following norms: N-12.5 kg/da, P_2O_5-1.25 kg/da, + K_2O-4.75 kg/da, Italpollina-25 kg/da, Arkobaleno - 100 kg/da, and Lumbricompost - 400 l/da. The liquid bio fertilizer Ekoprop NX was applied by double treatment in a dose of 100 g/da, before planting - in the 5th leaf seedling phase, and 10 days later on, after the adaptation to the soil. The remaining bio fertilizers are granulated and introduced into the soil before the last tillage and before

planting the seedlings. The biometric measurements were taken three times at one-week intervals in stage-typical leaf mass reached.

Chlorophyll fluorescence imaging. The Chlorophyll fluorescence of the lettuce leaves was measured using a portable device PAR-FluorPen FP 110/D manufactured by Photon Systems Instruments Ltd., Czech Republic. The fluorescence measurement protocol uses short (30 μs) measuring flashes to measure zero level fluorescence (F_0) followed by a strong saturating flash (duration 0.8 s, intensity about 3000 μmol m^{-2} s^{-1}) to measure the maximum fluorescence (Fm). Three strong flashes of saturating light probed the effective quantum yield (Qy) of PSII during the actinic light exposure (Maxwell & Johnson, 2000; Nedbal et al., 2000). Light Meter for direct digital readouts of Photosynthetically Active Radiation (PAR) in the range from 400 to 700 nm, the span in which plants use energy during photosynthesis. PAR is measured as Photosynthetic Photon Flux Density (PPFD), which is indicated by units of quanta (photons) per unit time per unit surface area. The chlorophyll fluorescence transients were measured on the same day in the morning. The periods of measurement were between the end of March and the beginning of April, when the plants were in their typical leaf mass reached growth stage. The nine leaves from each variant were dark adapted for about 30 min by detachable leaf-clips prior each measurement. The numeric value of each parameter (Fv/Fm, F_o, PAR) was determined by integrating it over the measured leaf area.

Physiological estimate of the chlorophyll content index (CCI). The Chlorophyll content index of the leaves was measured using a portable apparatus CCM 200 plus manufactured by Opti-sciences, Inc., NH, USA. The physiological assessment was carried out *in vivo* on the field. The measurements were taken on three dates from a sample of leaves at their typical leaf mass reached growth stage. The periods of the measurements were between the end of March and the beginning of April. 20 leaf measurements in the central part of the leaves were taken for each variant (in each of the repetitions).

Statistical evaluation of the results: The statistic processing of the data was performed by applying the mono-factorial dispersion analysis (Dimova & Marinkov, 1999).

RESULTS AND DISCUSSIONS

Physiological parameters

The mean value of the initial fluorescence (F_0) of the oxidized reaction centers of PSII was highest in the mineral fertilization variant, and lowest in the Italpollina and Arkobaleno organically fertilization variants (Table 1). For this parameter, significant differences in the average values of the different fertilization variants were not calculated. In relation to the studies of Zlatev & Kolev, 2012 and Chen et al., 2018, who believe that a higher value of F_0 is associated with high temperature stress, we can conclude that the temperature conditions in the greenhouse do not lead to a stress response in the plants. For parameter Qy (Fv/Fm), a statistically significant lowest value was registered for the control plants - 0.790 and a highest value was read for the biological fertilization variant Italpollina - 0.812. Higher values were also reported for the all variants compared to the control (Table 1). The comparative characteristic made by dates of measurements shows the largest difference between the unfertilized variant and the variants with organic fertilizers on the second measurement date. The mean value of Qy for all variants in this study indicates the presence of moderate stress in the photosynthetic activity of the plants, most pronounced in the control variant. It was confirmed from the average value of the ratio Fv/Fm or the quantum yield Qy of the different variants, which was close to the normal for healthy leaves - 0.83 (Demmig & Björkman, 1987). The measured photosynthetically active radiation (PAR) is higher on the first two dates, which is associated with the higher daily temperatures and the increased solar radiation compared to the atmospheric conditions during the third reporting date. The higher PAR values on the first two dates are associated with a lower Qy value, and this reduction should not be associated with photoinhibition due to low PAR values. The ratio between photosynthetic active radiation (PAR) and quantum yield (qY-

Fv/Fm) of PS II in dark-adapted leaves was more effective for the organic fertilizer variants Italpollina and Ekoprop, as well as for the variant with mineral fertilization (Table 1).
In parallel with the readings of some indicators of the chlorophyll fluorescence of the leaves, the chlorophyll index - Chlorophyll Content Index (CCI) was measured (Table 2). The lowest mean CCI value was estimated for the non-fertilized control variant, with few exceptions this dependence being maintained for all three dates of measurement. The leaves of the variants fertilized by organic fertilizers Ekoprop have the highest CCI. The significance difference only for organic fertilizer variant Ekoprop compared to the control variant was calculated. The values of the chlorophyll index are in a positive correlation with the values of the quantum yield-Qy (r = 0.648) which proves the inducing effect of the organic fertilizers on the photosynthetic activity of the plants.

Table 1. Chlorophyll fluorescence parameters of the plant leaves for the head lettuce type (*Lactuca sativa* L. var. *capitata* L.) variety 'Winter butterhead') in an unheated greenhouse, averaged for the period 2018-2020 year

	Control	NPK	Italpollina	Arkobaleno	LC	Ekoprop
f_0	4513	3531	3561	4858	4844	5109
f_0	5209	5715	5041	4400	5449	5215
f_0	4626	5226	4882	4848	4335	4149
	4782.6	**4823.9** n.s.	**4494.7** n.s.	**4702.0** n.s.	**4875.9** n.s.	**4824.3** n.s.
Qy=Fv/Fm	0,813	0,810	0,803	0,807	0,810	0,807
Qy=Fv/Fm	0,760	0,787	0,800	0,780	0,792	0,783
Qy=Fv/Fm	0,797	0,825	0,833	0,828	0,827	0,833
	0.790	**0.807***	**0.812 ****	**0.805***	**0.809 ***	**0.808****
PAR	110,0	79,7	97,0	121,0	145,3	135,0
PAR	124,0	135,0	130,0	153,3	183,0	175,3
PAR	145,3	110,0	86,7	83,3	78,0	68,3
	126.4	**108.2** n.s.	**104.6** n.s.	**119.2** n.s.	**135.4** n.s.	**126.2** n.s.

LSD	F_0	Qy	PAR
p = 0.05*	557.1	0.0015	27.6
p = 0.01**	779.3	0.0020	36.9
p = 0.001***	1054.5	0.0022	48.5

n.s. - no significance difference

Table 2. Chlorophyll content index (CCI) of the leaves for the head lettuce type (*Lactuca sativa* L. var. *capitata* L.) variety 'Winter butterhead') in an unheated greenhouse averaged for the period 2018-2020 year

Date of estimate / Variants	Control	NPK	Italpollina	Arkobaleno	LC	Ekoprop
first date	4.85	4.87	5.42	6.68	5.48	6.00
second date	4.36	5.46	4.90	4.60	5.16	5.56
third date	4.90	6.34	4.82	5.02	5.96	5.68
mean value	**4H.70**	**5.55 n.s.**	**5.03 n.s.**	**5.43 n.s.**	**5.53 n.s.**	**5.75***

LSD	
p = 0.05*	0.91
p = 0.01**	1.20
p = 0.001***	1.56

n.s. - no significant difference

Vegetative behavior

Head lettuce type plants are characterized by a faster rate of growth and development and form a bigger vegetative mass, which can also be seen from the readings of the 'Winter butter head' variety. At the moment of the first reading, the fresh mass of the whole plant was relatively high, meeting the market requirements, though (Table 3). The largest mass was formed after mineral fertilization with Italpollina, Arkobaleno and Ekoprop- between 391.25 g and 452.86 g. The yield in fresh mass of the other variant with organic fertilization is 363.62 g.

Table 3. Vegetative behavior of the head lettuce type, variety 'Winter butterhead' in the first biometric measurement, for the experimental period 2018-2020 year

Variant	fresh mass of the whole plant, g			leaves, number			leaf rosette diameter, cm			stem					
										diameter, mm			mass, g		
	year			year			year			year			year		
	13.03. 2019	21.03. 2020	average	13.03. 2019	21.03. 2020	average	13.03. 2019	21.03. 2020	average	13.03. 2019	21.03. 2020	average	13.03. 2019	21.03. 2020	average
1. Control	298.42$^{n.s}$	202.16$^{n.s}$	250.29	30.12$^{n.s}$	28.91$^{n.s}$	29.52	34.06$^{n.s}$	32.08$^{n.s}$	33.07	21.57$^{n.s}$	20.00$^{n.s}$	20.78	21.50$^{n.s}$	19.24$^{n.s}$	20.37
2. NPK	412.25	354.08	383.17	37.17	35.74	36.45	38.17	35.66	36.91	25.55	23.20	24.38	28.50	26.33	27.41
3. Italpollina	426.17	356.33	391.25	37.25	36.83	37.04	38.50	35.99	37.24	24.58	23.98	24.28	31.50	26.91	29.21
4. Arkobaleno	447.33	369.58	408.46	37.50	37.66	37.58	39.23	36.24	37.73	24.74	25.29	25.02	32.33	28.66	30.50
5. LC	391.17$^{n.s.}$	336.08$^{n.s.}$	363.62	36.92	35.58	36.25	37.84	35.49	36.66	25.28	23.07	24.17	27.09	24.99	26.04
6. Ekoprop NX	480.39	425.33	452.86	42.33	39.91	41.12	39.30	38.91	39.11	25.60	25.48	25.54	35.28	31.49	33.38
GD 95% = (+); (-)	**100.25**	**92.23**		**6.35**	**3.24**		**1.5**	**2.49**		**2.59**	**2.41**		**6.38**	**7.48**	**6.93**
GD 99% = (++); (--)	**138.85**	**127.74**		**8.79**	**4.50**		**2.09**	**3.45**		**3.59**	**3.34**		**8.63**	**10.36**	**9.495**
GD 99.9% = (+++); (---)	**191.57**	**176.24**		**12.14**	**6.20**		**2.88**	**4.76**		**4.96**	**4.61**		**12.19**	**14.3**	**13.245**

n.s. - no significance difference

Relatively small differences were reported in the diameter of their rosette. The largest diameter is reported for for Ekoprop and Arcobaleno - 39.11 and 37.73 cm, respectively. The variants with organic fertilization have formed a rosette with a diameter between 36.62 and 39.11 cm.

The plants had formed a relatively smaller number of leaves. The plants, fertilized with Ekoprop had the highest number of leaves - 41.12, followed by Arcobaleno with 37.58. The other variants formed between 29.52 and 41.12 leaves.

Larger differences were observed with the indicators of the stem. The differences in diameter were significant. It was the largest with Ekoprop - fertilization - 25.54 mm and with Arkobaleno- 25.02 mm. The highest mass value had the stem with the Ekoprop variant of fertilization - 33.38 g, followed by the resulting stem in the case of using Arkobaleno - 30.50 g. With the smallest mass of the stem were the unfertilized plants and Lumbricompost, 20.37 and 26.04 g, respectively.

During the two experimental years, with the exception of the fresh mass of plants fertilized with Lumbricompost, the statistical difference between the tested variants was proved.

The second reading revealed that the plants had continued to grow, albeit slowly (Table 4). The fresh mass of the whole plant had increased compared to the previous moment of measurement. The largest were the plants, fertilized with Ekoprop - 524.14 g. The options with organic fertilization - Italpolina, Lumbricompost and Arkobaleno - were close in value to conventional fertilization and ranged from 476.96 g to 481.62 g.

There were small differences between the variants of fertilization in the diameter of the plant rosette. It ranged from 34.92 mm for the control variant (no fertilization) to 39.56 mm for the variant of fertilization with Ekoprop.

The values of the stem indicators were equalized. The diameter of the stem ranged from 23.06 mm for the control variant (no fertilization) to30.53 mm for the variant of fertilization with Ekoprop.

Table 4. Vegetative behavior of the head lettuce type, variety 'Winter butterhead' in the second biometric measurement, for the experimental period 2018-2020 YEAR

Variant	fresh mass of the whole plant, g			leaves, number			leaf rosette diameter, cm			stem					
										diameter, mm			mass, g		
	year			year			year			year			year		
	20.03. 2019	21.03. 2020	average	20.03. 2019	21.03. 2020	average	20.03. 2019	21.03. 2020	average	20.03. 2019	21.03. 2020	average	20.03. 2019	21.03. 2020	average
1. Control	358.50 n.s.	205.75 n.s.	282.13	40.09 n.s.	30.58 n.s.	35.33	36 n.s.	33.83 n.s.	34.92	24.03 n.s.	22.08 n.s.	23.06	33.59 n.s.	20.83 n.s.	27.21
2. NPK	505.58	387.41	446.50	46.50	39.66	43.08	38.83	37.49	38.16	27.74	25.75	26.75	39.50	36.66	38.08
3. Italpollina	539.84	414.08	476.96	46.58	41.66	44.12	38.92	38.08	38.50	31.39	27.24	29.32	39.75	39.75	39.75
4. Arkobaleno	542.42	420.83	481.62	49.61	41.91	45.76	40.34	38.33	39.33	32.35	28.36	30.36	40.50	41.33	40.91
5. LC	496.08	365.99	431.04	46.42	39.24	42.83	38.69	37.49	38.09	27.57	25.66	26.61	39.50	36.49	38.00
6. Ekoprop NX	607.45	440.83	524.14	49.83	42.91	46.37	40.61	38.50	39.56	32.49	28.58	30.53	40.61	41.74	41.17
GD 95% = (+); (-)	**121.74**	**129.33**		**5.37**	**6.67**		**2.72**	**2.89**		**2.58**	**3.35**		**2.32**	**15.65**	
GD99% = (++); (--)	**168.61**	**179.12**		**7.44**	**9.24**		**3.77**	**4.01**		**3.58**	**4.65**		**3.21**	**21.68**	
GD 99.9% = (+++); (---)	**232.62**	**247.12**		**10.26**	**12.75**		**5.20**	**5.53**		**4.94**	**6.41**		**4.43**	**29.91**	

n.s. - no significance difference

The mass of the stem showed comparatively small differences between the variants. It was slightly higher for Ekoprop, Arkobaleno and Italpolina fertilization, namely: 41.17 g, 40.91 g and 39.75 g respectively.

The statistical difference between the tested variants by experimental years has been proven.

Plant growth was registered at the moment of the third harvest (Table 5). The fresh mass of the whole plant had increased slightly. The largest were the plants, fertilized with Ekoprop - 531.47 g, exceeding the mineral fertilized ones by 52.26 g. The other variants with organic fertilization were similar in size to conventional fertilization.

New leaves had been formed in all variants of fertilization, ranging from 1.42 after Italpolina to 3.90 after mineral fertilization. The total number of leaves was between 38.27 for the unfertilized plants to 48.72 for the ones, fertilized with Ekoprop.

Leaf growth was also registered, which caused the formation of a larger leaf rosette, with a diameter, ranging from 33.66 mm to 39.67 mm. In most variants, the mass had increased significantly, while its diameter of the stem had grown less. The plants fertilized with Ekoprop had the largest mass - 50.85 g, while the ones, fertilized with mineral fertilization and Italpolina were with a mass of 48.95 and 48.12 g. This trend is valid for the diameter as well, with Ekoprop and Arkobaleno fertilized plants having the largest diameters. The diameter had increased by 2-3 mm, but the

differences between the variants were significant - 4-9 mm. The statistical difference between the tested variants was proved for the two experimental years.

Table 5. Vegetative behavior of the head lettuce type, variety 'Winter butterhead' in the third biometric measurement, for the experimental period 2018-2020 YEAR

Variant	fresh mass of the whole plant, g			leaves, number			leaf rosette diameter, cm			stem					
										diameter, mm			mass, g		
	year			year			year			year			year		
	28.03. 2019	21.03. 2020	average	28.03. 2019	21.03. 2020	average	28.03. 2019	21.03. 2020	average	28.03. 2019	21.03. 2020	average	28.03. 2019	21.03. 2020	average
1. Control	409.07 n.s.	207.49 n.s.	308.28	40.25 n.s.	36.29 n.s.	38.27	36.59 n.s.	30.74 n.s.	33.66	25.54 n.s.	24.03 n.s.	24.79	34.58 n.s.	25.83 n.s.	30.21
2. NPK	559.08	399.33	479.21	49.42	44.54	46.98	39.50	35.58	37.54	30.49	27.74	29.12	56.50	41.40	48.95
3. Italpollina	578.00	424.74	501.37	46.84	44.25	45.54	39.75	35.49	37.62	31.50	31.39	31.45	53.92	42.33	48.12
4. Arkobaleno	580.09	437.25	508.67	51.17	46.54	48.85	40.50	37.66	39.08	32.36	32.35	32.36	54.34	42.57	48.45
5. LC	555.92	366.16	461.04	46.50	42.87	44.69	39.50	36.08	37.79	30.08	27.57	28.82	50.17	38.31	44.24
6. Ekoprop NX	616.86	446.08	531.47	50.50	46.95	48.72	41.25	38.08	39.67	35.28	32.49	33.88	57.56	44.14	50.85
GD 95% = (+); (-)	**138.15**	**156.67**		**5.99**	**6.74**		**2.32**	**2.97**		**3.39**	**2.58**		**14.06**	**10.98**	
GD 99% = (++); (--)	**191.33**	**216.99**		**8.30**	**9.34**		**3.21**	**4.11**		**4.70**	**3.58**		**19.47**	**15.21**	
GD 99.9% = (+++); (---)	**263.97**	**299.38**		**11.46**	**12.88**		**4.43**	**5.67**		**6.49**	**4.94**		**26.83**	**20.99**	

n.s. - no significance difference

CONCLUSIONS

The ratio between photosynthetic active radiation (PAR) and quantum yield (qY-Fv/Fm) of PS II in dark-adapted leaves was more effective for the organic fertilizer variants Italpollina and Ekoprop,

The leaves of the variants fertilized by organic fertilizers Ekoprop have the highest CCI.

The values of the chlorophyll index are in a positive correlation with the values of the quantum yield-Qy, (r = 0.648) which proves the inducing effect of the organic fertilizers on the photosynthetic activity of the plants.

Organic fertilizers are able to meet the need for essential nutrients when growing lettuce in polyethylene greenhouses. All variants with organic fertilization comply with the quality standard from the beginning to the end of harvesting. The variant with organic fertilization with Lumbricompost form plants close in their average mass to those, grown after conventional fertilization with mineral fertilizers, and the plants grown after organic fertilization with Italpollina, Arkobaleno, and Ekoprop surpass it.

Under the meteorological conditions of the experiment, biological fertilization does not require photosynthetic activity of plants compared to those found after mineral fertilization.

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ASSESSMENT OF THE GROWTH POTENTIAL IN LIQUID CULTURES OF SOME EDIBLE AND MEDICINAL MUSHROOMS

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Abstract

The purpose of this study was to assess the growth potential in liquid cultures conditions of ten edible and medicinal mushrooms named: Flammulina velutipes, Lentinus edodes, Pleurotus eryngii 2600, Pleurotus ostreatus var. Florida, Trametes versicolor, Hericium coralloides, Ganoderma lucidum, Ganoderma applanatum, Agaricus campestris and Laetiporus sulphureus. The evaluation of the mushroom growth potential was carried out using six types of culture media with different chemical composition. After 21 days from the cultures initiation, it was found that Ganoderma species had the greatest potential for the production of biomass in liquid culture conditions on all six media; also, the pH values of culture media have undergone important changes from baseline.

Key words*: fungal biomass, growth conditions, liquid culture, mushrooms.*

INTRODUCTION

Growing mushroom in submerged culture, a procedure applied in the last two decades, may result in a fast production of abundant mycelial biomass. Therefore, this method may present an important advantage over cultures practiced on solid media (Zhong & Tang, 2004; Tang et al., 2007). In addition, submerged cultivation is considered industrially efficient because many bioactive compounds found in liquid culture. Some of these compounds, such as: polysaccharides, proteins and their complexes, phenolic compounds, triterpenoids, steroids, alkaloids, have important medicinal properties (cholesterol-lowering,anti-diabetic, antioxidant, antitumor, immune-modulating, antimicrobial, and antiviral activities, etc.) (Nandi et al., 2019). Since the production of these compounds is usually associated with specific environmental conditions, *in vitro* imitation of these conditions is an effective strategy to influence secondary metabolic pathways.
Most fungi have different growth requirements. The biomass development, as well as bioactive compounds production, are strongly affected by several environmental parameters, including the composition of the culture medium (in particular the nature and concentration of the carbon source) (Lee et al., 2004; Papinutti, 2010). For bioprocessing, not much research has been carried out regarding the effects of the chemical composition of the culture media on the biomass production made by different species of edible/medicinal mushrooms grown under submerged conditions.
In this context, the aim of this study was to investigate the growth potential of some edible/medicinal mushroom species in different liquid culture media conditions for the production of mycelial biomass.

MATERIALS AND METHODS

Mushroom samples. Ten mushroom species, named: *Flammulina velutipes*, *Lentinus edodes*, *Pleurotus eryngii* 2600, *Pleurotus ostreatus* var. *Florida*, *Trametes versicolor*, *Hericium coralloides, Ganoderma lucidum*, *Ganoderma applanatum*, *Agaricus campestris* and *Laetiporus sulphureus* were used for experiments. The mushrooms strains were kept in the collection of Faculty of Biotechnology of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. Stock

cultures were maintained in test tubes on 2% malt extract agar, at 4^0C.

Culture media and performed assays. To investigate the growth potential of mushroom mycelia in submerged culture, the following liquid media were used: malt extract broth (ME) (Merck), potato dextrose broth (PD) (Merck), potato-malt-peptone broth (PMP) (Kim et al., 2002), mushroom complete medium broth (MCM) (Kim et al., 2002), glucose - malt extract - yeast broth (GMY) (Pickard et al., 1999) and yeast – malt extract broth (YM) (Kim et al., 2002). The synthetic media composition are presented in Table 1.

Table 1. Composition of liquid media

Composition of culture media	Culture media and nutrient concentrations (g/l)					
	1 PD	2 ME	3 PMP	4 MCM	5 GMY	6 YM
Potato infusion	**4**	-	-	-	-	-
Dextrose	**20**	-	**24**	-	-	-
Peptone	-	-	**1**	**2**	-	**5**
Glucose	-	-	-	**20**	**10**	**10**
Malt extract	-	**20**	**10**	-	**3,5**	**3**
Yeast extract	-	-	-	**2**	**2,5**	**3**
KH_2PO_4	-	-	-	**0.46**	**2**	-
K_2HPO_4	-	-	-	**1**	-	-
$MgSO_4x7H_2O$	-	-	-	**0.5**	**0.5**	-
pH control	**5.0**	**5.4**	**4.6**	**5.9**	**5.2**	**5.9**

Note: PD: Potato Dextrose Broth; ME: Malt Extract Broth; PMP: Potato- Malt-Peptone medium; MCM: Mushroom complete medium; GMY: Glucose - Malt - Yeast extract medium; YM: Yeast Malt extract.

The inocula were prepared by adding actively growing mycelia from a newly prepared culture (mycelial agar discs with 0.5 cm of diameter) into 100 mL in 250 mL Erlenmeyer flask. For the submerged culture, 100 mL of the each type of liquid medium (see Table 1) were prepared in a 250 mL flask. The cultures were incubated for 3 weeks, with stirring at 110 rpm and 25°C. During the incubation period, once a week, the pH variation in the culture media was measured with a electronic pH meter WTW, inoLab® 730 and compared with the initial pH of inoculated media (pH control). At the end of the experiment, the mycelial mass was recovered from the each liquid medium by filtration and weighed in a wet state. Then, the wet biomass was dried at 70^0C, for 4 hours, and weighed again. Thus, it was established which of the culture medium was most suitable for obtaining an increased amount of fungal biomass.

Statistical analysis. Measurements on the pH value were performed in triplicate and compared with the initial pH values. The data obtained were statistically processed in Excel, by analysis of the variance (ANOVA) using the Student t-test.

RESULTS AND DISCUSSIONS

The investigation of mushrooms growth potential under submerged conditions for biomass production was performed using six variants of liquid culture media, different in terms of chemical composition. After 21 days from fungal mycelium inoculation the developed biomass was filtered, weighed and quantified as wet biomass (Figure 1).

Depending on the composition of the culture medium and the cultivated mushroom species, it can be observed that are differences in the accumulation of mycelial biomass. Also, mushroom mycelium appearance, in terms of color and consistency varies depending on the culture medium. For example, the mycelium of *L. sulphureus,* developed on the MCM and PMP media, was more compact and with spherical agglomerations on the culture medium surface. Contrariwise, the biomass developed on the PD medium had a fluffy appearance with a specific orange color of the mushroom fruiting body. On the GMY and YM culture media, the fungus retained the specific orange color, but the surface developed mycelium has a folded appearance (Figure 2).

The non-isoprenoid polyene laetiporic acid A, recently described from fruit-bodies of the fungus *Laetiporus sulphureus*, was found to be the major orange pigment also in mycelium grown in liquid culture (Davoli et al., 2005).

Between the 10 types of fungi studied, *Ganoderma* species showed the highest potential for biomass production under submerged conditions on all six variants of the tested culture media (Figure 3).

Among *Ganoderma* species, *G. applanatum* developed a significant amount of wet biomass, obtained especially on PD (30.57%), GMY (20.56%) and YM (16.28%) (See Figure 1).

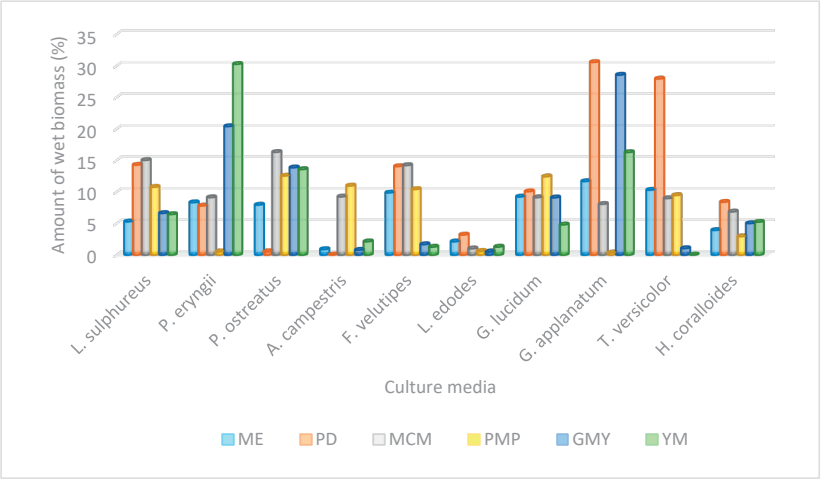

Figure 1. Amount of wet biomass harvested from the six types of liquid media
Abbreviations: ME: Malt Extract Broth; PD: Potato Dextrose Broth; MCM: Mushroom Complete Medium; PMP: Potato- Malt- Peptone medium; GMY: Glucose - Malt - Yeast extract medium; YM: Yeast Malt extract.

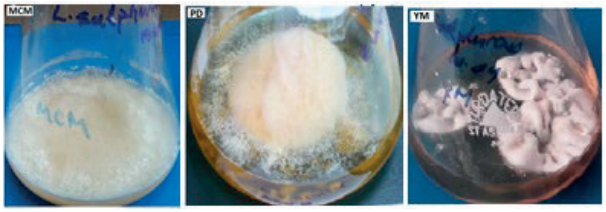

Figure 2. Appearance of *L. sulphureus* mycelia on MCM, PD and YM liquid media

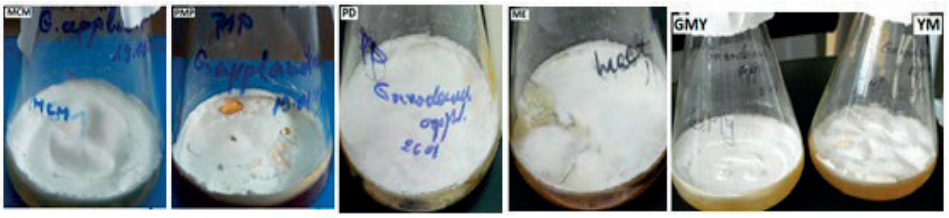

Figure 3. *G. applanatum* - abundant biomass on all types of culture media

After filtering and quantifying, the wet biomass harvested from each variant of culture medium was dried and weighed. Contrary to expectations, it has been found that the dry biomass obtained from a large amount of wet biomass is quite low. For example, *G. applanatum* which had the highest amount of wet biomass on PD (30.58%), GMY (28.56%) and YM (16.28%) media, only 5.33 g, 4.36 g and 6.04 g of dry biomass were obtained after drying. The highest amount of *P. eryngii* wet biomass was obtained on the YM medium (30.27%) and only 6.29 g of dry biomass were recorded after drying (Figure 4).

These differences could be explained due to the particularities of the resulting fungal mycelium (consistency, morphology, etc.), to the media composition and the cultural conditions, and not least of the cultivated species. Therefore, the effect of culture media on mycelium growth varies with the species of fungi.

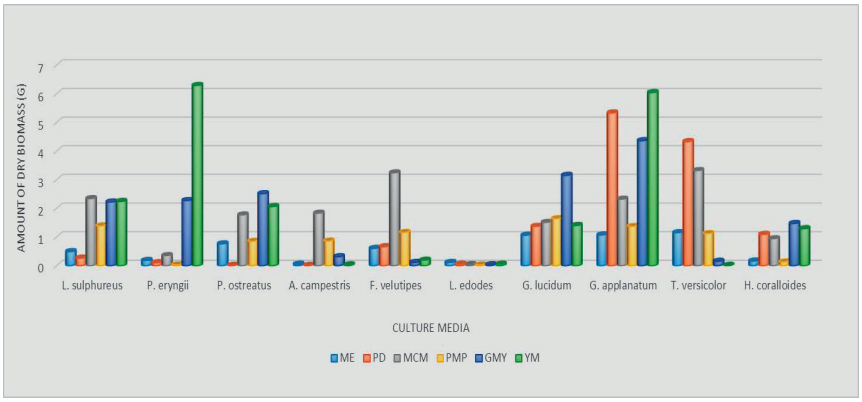

Figure 4. The amount of fungal dry biomass (g) obtained from the six types of liquid media

One of the main factors affecting the biosynthetic potential of fungi in submerged culture is the pH of the medium. This can affect cell membrane functions, uptake of different nutrients from the extracellular environment, solubility of mineral salts, ionic potential of substrates, enzymatic activity and biosynthesis products (Elisashvili, 2012). Environmental pH is an important parameter that has been shown to affect fungal morphology (Gibbs et al., 2000). For basidiomycetes, it has been reported that an optimal pH is around pH 6.0 (Kim et al., 2002). With regard to mushroom species tested, our results shown that the mycelial mass increased quite well at a pH between 4.6 and 7. At the end of the 21 days of culture, performed at the room temperature, study on pH variation in all culture media showed that pH values underwent major changes compared to the initial values. The most drastic decrease was recorded at *Laetiporus sulphureus* grown on the ME (from pH 5.4 to pH 2.1) and PD (from pH 5.0 to pH 2.0) (Figure 5). The values between the initial pH and the final pH in culture media were statistically calculated. As data shown, there are no significant differences for the MCM, PMP, GMY and YM culture media. On the contrary, in the case of ME and PD media, there are significant differences between the initial and final pH (Figure 5). The maximum biomass of *L. sulphureus* (15.04% fresh weight) was harvested from MCM medium at a pH between 5 and 6.9. Acidification of the ME and PD media could be due to the gradual accumulation in the extracellular environment of some acids released by the organism as a result of some metabolic processes which are induced by the composition of culture media (ME or PD). Regarding *P. eryngii*, it was observed that obtaining a maximum quantity of fresh biomass (30.27% on YM and 20.40% on GMY respectively) was produced at a final pH of 6.2; this mean that the fungus has adjusted the medium pH during incubation. As can be seen from the figure below, significant differences between the initial and final pH values exist only in the GMY medium (Figure 5). The strain of *P. ostreatus* var. Florida recorded a significant amount of biomass (16.31%) on MCM medium, with a final pH around 6.9. During the incubation period, the fungus adjusted the initial pH of the culture medium, releasing some alkaline compounds in the extracellular environment. The pH variation was negligible only in the MCM medium (Figure 5). Acidification of the culture medium (pH 3.27) resulted in a reduced amount of biomass (0.57% fresh mass). The maximum amount of biomass obtained from *A. campestris* was recorded on PMP medium (10.97%), at a final pH of 6.8. Although the initial pH of the PMP medium was 4.6, the fungus gradually increased the initial pH during incubation.

The only liquid media in which the pH variation registered insignificant values were: MCM, GMY and YM. *F. velutipes* recorded mycelial biomass values at a final pH between 4 and 6.1, which suggests that this fungus prefers an acidic pH. The pH variation towards neutral values (pH 7.0) resulted in very low quantities of biomass (in the GMY and YM media). The increase of the pH value with the accumulation of biomass may be due to the

elaboration in the culture medium of some basic compounds (e.g. proteins). The difference between the initial pH and the final pH was significant in all tested culture media (Figure 5). In *L. edodes* culture, a well-developed biomass was obtained on PD (3.2%) and ME (2.13%), respectively, at a pH between 3.9 and 4.3. In the case of *G. lucidum*, the pH variation (between 4.2 and 6) during the incubation period does not seem to significantly influence the amount of biomass (Figure 5).

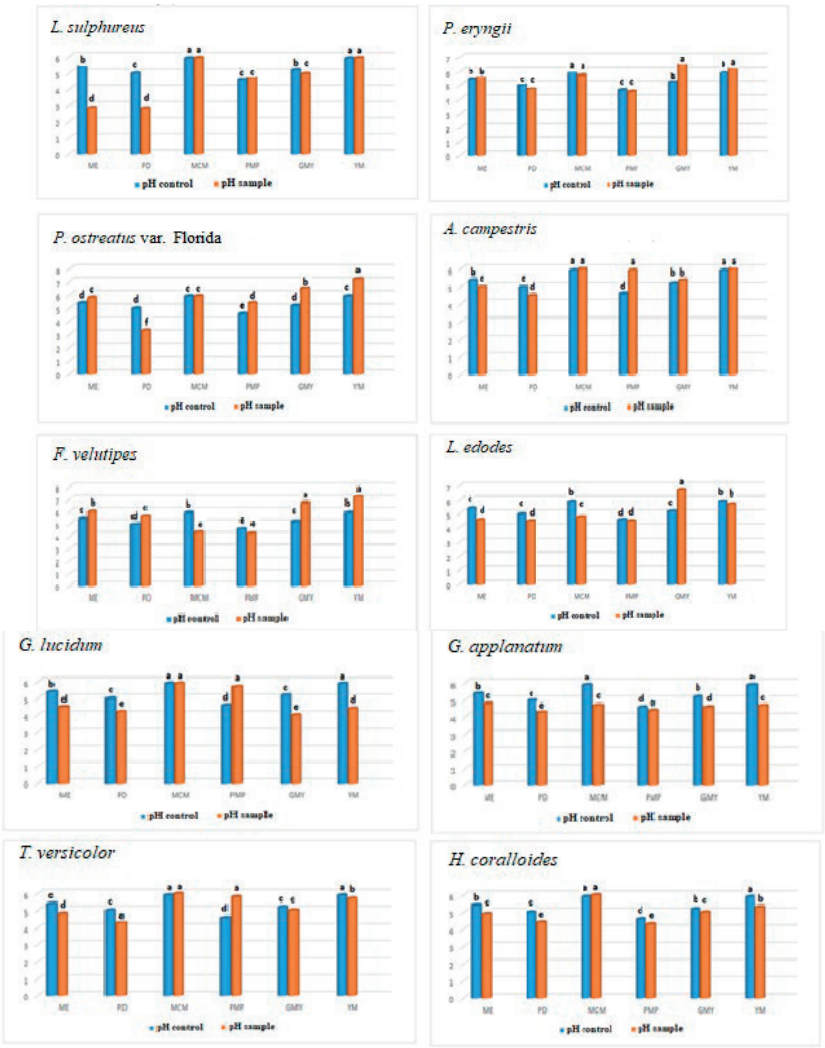

Figure 5. PH variations in submerged culture media during 21 days of incubation, at 25^{0}C

Note: The pH values were calculated based on three measurements to determine if there are significant differences from the initial pH values (pH control). Values with the same letter are not significantly different for $p < 0.05$ (Student t Test)

Insignificant values of pH variation were observed only in the MCM medium. On the other hand, the highest values of *G. applanatum* mycelial biomass were obtained when the pH value was around 4.3. During the culture period, the pH variations were around 4.3-4.7. Significant differences were observed for all culture media. Within the combinations performed for *T. versicolor* and *H. coralloides*, the maximum amount of fresh biomass was obtained on PD medium (27.94% and 8.4%

respectively). The pH variations were around 4.3 (Figure 5).

Many studies developed in recent years have shown that the mycelium growth rate, but also the production of secondary metabolites in submerged culture, depend on the chemical composition of the liquid medium and the conditions of incubation (Zhong & Tang, 2004; Lin & Sung, 2006; Fidler et al., 2013; Popa et al., 2014).

Following these results, it was possible to identify the optimal culture medium for each tested mushroom species, which led to a high production of mycelium biomass. Also, at the beginning and at the end of the experiment, the pH values of the culture media were recorded (Table 2).

Table 2. Optimal liquid media and pH values for maximum fungal biomass yield

Species	Culture media	Initial pH	Final pH	Fresh biomass (g / 100 ml)
Laetiporus sulphureus	MCM	5.9	6.9	15.04
Pleurotus eryngii 2600	YM	5.9	6.2	30.27
P.ostreatus Florida	MCM	5.9	6.9	16.31
Agaricus campestris	PMP	4.6	6.8	10.97
Flammulina velutipes	MCM	5.9	4.1	14.22
Lentinus edodes	PD	5.0	4.3	3.20
Ganoderma lucidum	PMP	4.6	6.2	12.43
Ganoderma applanatum	PD	5.0	4.2	30.57
Trametes versicolor	PD	5.0	4.1	27.94
Hericium coralloides	PD	5.0	4.1	8.40

PD: Potato Dextrose; PMP: Potato- Malt –Peptone; MCM: Mushroom Complete Medium; YM: Yeast-Malt.

CONCLUSIONS

The results showed that the optimal liquid media for maximum fungal biomass yield were: PD for *G. applanatum, T. versicolor, H. coralloides and L. edodes* species; MCM for *P. ostreatus* "Florida", *L. sulphureus* and *F. velutipes*; PMP for *G. lucidum* and *A. campestris* and YM culture medium only for *P. eryngii* 2600 mushroom. Study of the pH variation in the culture media during the incubation period showed that the pH values underwent major changes compared to the initial values. In general, it was found that acidification of the culture medium resulted in a reduced amount of biomass.

Liquid culture of edible and medical mushrooms is viewed as a promising alternative for efficient production of valuable metabolites for the research and development of new pharmaceutical products from mushrooms.

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THE INFLUENCE OF RADIATION REFLECTED BY EMCOPAD DOCTOR TECH DEVICES ON TOMATOES ON A WELL-AERATED PERLITE SUBSTRATE

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Abstract

The study was conducted in the Hortinvest greenhouses of the University of Agronomic Sciences and Veterinary Medicine-Bucharest and refers to the use of a device that is the subject of a patent, published internationally and which was used experimentally in a tomato crop, in the system unconventional, on perlite substrate. We noticed a faster growth of tomato fruits following the application of EMCOPAD-Doctor Tech devices compared to the variant not exposed to the reflected electromagnetic field. The differences in physiological maturation compared to the untreated variant were 4-5 days when the device was placed directly on the immature fruit and 4 days when it was placed directly on the stem of the plant near the insertion of the inflorescence. The aim of the study was to identify new methods to shorten the fruit ripening period, non-aggressive.

Key words: *EMCOPAD-Doctor Tech, tomatoes, fruit, perlite substrate*

INTRODUCTION

Environmental conditions, such as climate change and pollution, as well as the population explosion, directly affect plant crops. Remedying these issues through industrial treatments and solutions has deepened these problems. For these reasons, researchers in the field have had to find innovative solutions to improve crop quality.

Magnetotherapy or electromagnetic field therapy is known as a healing method used since ancient times, it is even mentioned in Indian Vedas.

In the case of plants, the application of magnetotherapy is just beginning. However, there are enough studies to show the positive effects of using electromagnetic fields on plants at different stages of their cultivation.

Through this paper, we aim to analyse the effect of electromagnetic fields on the cultivation of tomatoes, by reducing the period of development and ripening of the fruit.

Research on the influence of seed exposure to different intensities of the magnetic field has shown its beneficial effect on pea seeds (Dobrescu et al., 2000), *Zea mays*, barley or other fruit species (Rochalska, 2005; Rochalska & Orzeszko-Rywka, 2005).

Maffei (2014) mentions that plants feel different wavelengths of light and react to electrical signaling, but can not escape the effect of the geomagnetic field.

Recently, based on the beneficial effect of the magnetic field on plants, there has been a special interest from many researchers (Occhipinti et al., 2014).

Jedlička et al. (2015) demonstrated the impact of extremely low frequency electromagnetic fields on the germination of tomato seeds (*Solanum lycopersicum* L.) as well as plant growth.

EMCOPAD DOCTOR TECH/PEM - PASSIVE DEVICES The original devices called EMCOPAD Doctor Tech/PEM-Coherent ElectroMagnetic Patches, made in accordance

with the patent published under number PCT-WO/2018/037379, were used.
In medicine they are activated by the energy imbalance manifested by a high electrical potential from the acupuncture points above which they are located.
The devices start to act when they are placed on the acupuncture points in imbalance and cease to function when the energy balance is achieved. If they remain on the body, they will resume their action when another imbalance occurs.
Between the periods of activity, a waiting state is installed, which is manifested by the lack of any electromagnetic effect.
The devices are used in medicine but, in 2020, they were also tested in tomato cultivation on the 'Cheramy' cultivar.
The advantage of field interaction allows an approximate positioning of the devices on the plant. The device is maintenance-free, does not wear out and has an indefinite duration of use for normal use.
The operation of the device does not require materials, batteries or charging electricity from the mains.
The use of the devices does not oblige the expenditure on consumables, the simple positioning above the points being sufficient.

MATERIALS AND METHODS

The preliminary study was carried out in Hortinvest greenhouses, in the cultivation of tomatoes on perlite substrate with granulation of 5mm diametre, well aerated, during October-December 2020. We chose the inflorescences with the same number of fruits. We used the EMCOPAD-Doctor Tech divais that was placed when the fruits were formed, according to the experimental variants: V1 Witness; V2-EMCOPAD-Doctor Tech placed on the first fruit of the first inflorescence; V3-EMCOPAD-Doctor Tech placed on the sixth fruit of the first inflorescence; V4 EMCOPAD-Doctor Tech located at the base of the plant stem; V5-EMCOPAD-Doctor Tech located next to inflorescence 1; V6 EMCOPAD-Doctor Tech located next to inflorescence 2.
We followed the location of the technical maturation after its placement of fruits until their physiological maturation, fruit size, fruit mass as well as nitrate content.All data were interpreted statistically as well as the correlation between experimental variants and fruit size. We used in the experiment the 'Cheramy' cultivar, with undetermined growth, with fruits of about 16-20 g, and can be harvested in bunches.

RESULTS AND DISCUSSIONS

Analysing the obtained data, we could see that in the case of placing EMCOPAD-Doctor Tech on the fruit, its mass increased compared to the rest of the fruits in the inflorescence but also compared to the mass of the first fruit in the case of the control variant. Its mass was 20.18 g at V2 compared to V1 - control of 15.2 g. If we look at the average mass of fruits in the inflorescence we could see that there were differences compared to the control variant, most fruits having higher average masses (Figure 1).

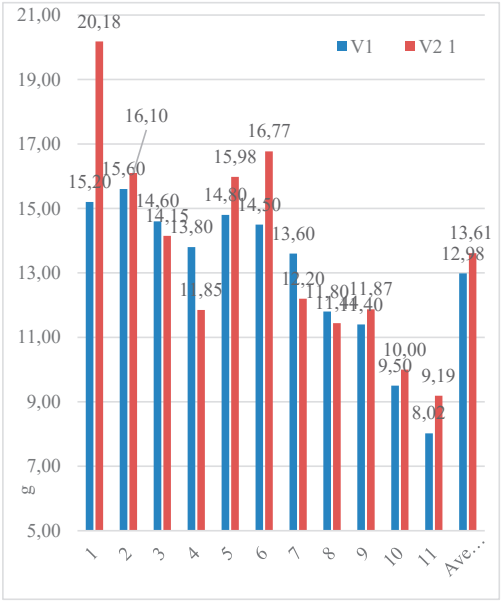

Figure 1. Influence of EMCOPAD application (V2) to control variant (V1)

In the case of V3- EMCOPAD-Doctor Tech placed on the sixth fruit in the first inflorescence we found that the fruit in the inflorescence had an average weight of 18.23 g, higher than the control variant of 14.5 g. We

found that the average mass of all fruits was higher than the control variant (Figure 2).

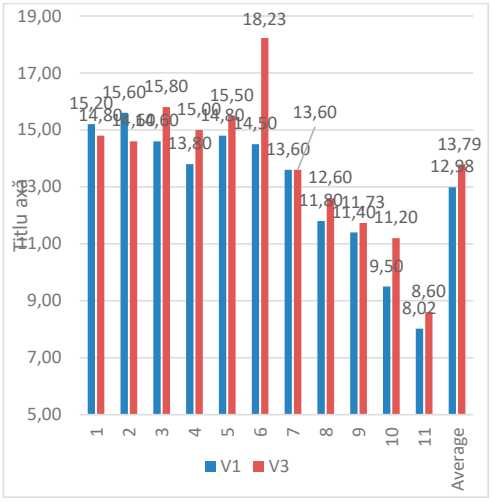

Figure 2. Influence of EMCOPAD-Doctor Tech application (V3) to control variant (V1)

If we placed EMCOPAD-Doctor Tech (V4) at the base of the plant stem, we also found an increase in the mass of fruit on the plant, which is 8.7 g for fruit 11 compared to 8.02 g for V1. On average, in the case of variant 4, the average mass of the fruit was 13.74 g compared to the control V1 of 12.98 g (Figure 3).

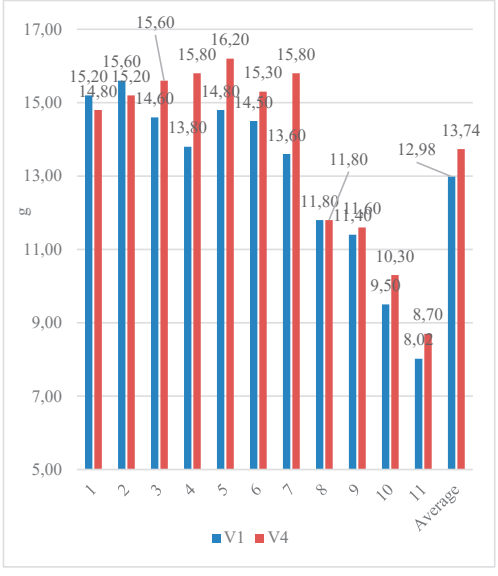

Figure 3. Influence of EMCOPAD application (V4) to control variant (V1)

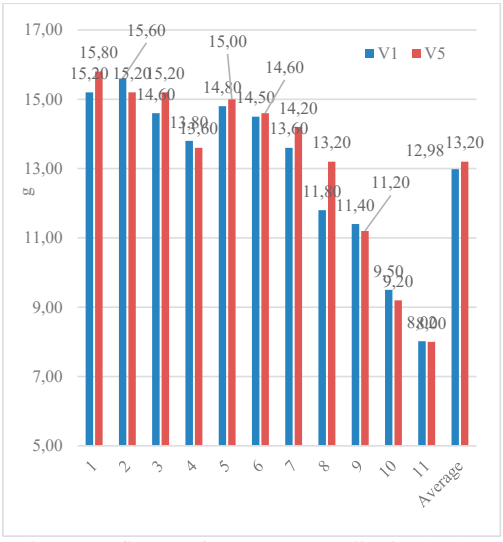

Figure 4. Influence of EMCOPAD application (V5) to control variant (V1)

In the case of the EMCOPAD-Doctor Tech V6 variant located next to inflorescence 2, we found the same tendency to increase the mass of fruits in the inflorescence. We found that, on average, the mass of the fruit was higher, of 13.45 g/fruit in the variant to which we applied EMCOPAD-Doctor Tech near the inflorescence 2 (V6) compared to the control variant of 12.98 g/fruit (Figure 5).

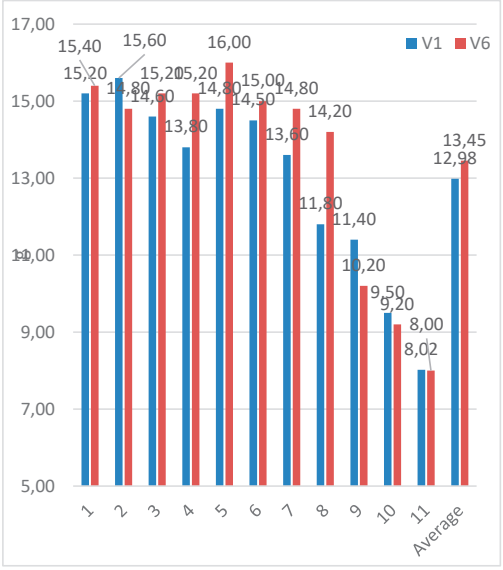

Figure 5. Influence of EMCOPAD- application (V6) on control variant (V1)

Analysing the data on the difference between the values obtained on average from the three plants observed to which we applied EMCOPAD-Doctor Tech to fruit 1 we found that compared to the control variant the difference was distinctly very significant. The weight of the fruit was on average 20.18 g with 4.98 g more than the control. We also found that the fruit was 32.76% higher than the control variant (Table 1).

Table 1. The influence of EMCOPAD-Doctor Tech applied to the first fruit

Variant	Mass (g)	Difference (g)		Significance (%)
V(0) Average	16.03	0.83	105.46	**
V(1)	15.20	0.00	100.00	Ct
V(2)	20.18	4.98	132.76	***
V(3)	14.80	-0.40	97.37	N
V(4)	14.80	-0.40	97.37	N
V(5)	15.80	0.60	103.95	*
V(6)	15.40	0.20	101.32	N

DL5% = 0.540 DL5% in % = 3.5526
DL1% = 0.780 DL1% in % = 5.1316
DL0.1% = 1.130 DL0.1% in %= 7.4342

If we applied EMCOPAD-Doctor Tech to fruit no. 6, we also noticed an increase in weight. It was 18.3 g at V3 with 3.73 g over the control variant. The difference was 25.72% over the control variant. From a statistical point of view, we found that the difference was distinctly very positive (Table 2).

Table 2. The influence of EMCOPAD-Doctor Tech applied to the sixth fruit

Variant	Mass (g)	Difference (g)	(%)	Significance
V(0) Average	15.74	1.24	108.52	**
V(1)	14.50	0.00	100.00	Ct
V(2)	16.78	2.28	115.72	***
V(3)	18.23	3.73	125.72	***
V(4)	15.30	0.80	105.52	*
V(5)	14.60	0.10	100.69	N
V(6)	15.00	0.50	103.45	N

DL5% = 0.620 DL5% in % = 4.2759
DL1% = 0.890 DL1% in % = 6.1379
DL0.1% = 1.290 DL0.1% in % = 8.8966

Analysing, on average, the average mass of fruits in the inflorescences, we found that in the case of V1 Mt the average mass of fruits was the lowest, 12.98 g, and in the case of all variants to which we applied EMCOPAD-Doctor Tech the average mass of fruit was higher by 6.19% over the control in the case of V3 and by 1.67% in the case of V5 (Table 3).

Table 3. Average mass of tomato fruits on experimental variants

Variant	Mass (g)	Difference (g)	(%)	Significance
V(0) Average	13.46	0.47	103.64	N
V(1)	12.98	0.00	100.00	Ct
V(2)	13.58	0.59	104.57	*
V(3)	13.79	0.80	106.19	**
V(4)	13.74	0.75	105.80	**
V(5)	13.20	0.22	101.67	N
V(6)	13.45	0.47	103.62	N

DL5% = 0.490 DL5% in % = 3.7741
DL1% = 0.700 DL1% in % = 5.3915
DL0.1% = 1.020 DL0.1% in % = 7.8562

Analysing the average total mass of the inflorescences we found that the control V1 presented inflorescences with an average mass of 142 g. It was noted that all the variants we used EMCOPAD-Doctor Tech the total mass of the inflorescence was higher, statistically positive, very significant view (Table 4).

Table 4. Total mass of the inflorescences

Variant	Fruit Mass (g)	Difference (g)		SEMF (%)
V(0) Average	148.08	5.26	103.69	***
V(1)	142.82	0.00	100.00	Ct
V(2)	149.73	6.91	104.84	***
V(3)	151.66	8.84	106.19	***
V(4)	151.10	8.28	105.80	***
V(5)	145.20	2.38	101.67	***
V(6)	148.00	5.18	103.63	***

DL5% = 0.620 DL5% in % = 0.4341
DL1% = 0.880 DL1% in % = 0.6162
DL0.1% = 1.280 DL0.1% in % = 0.8962

Figure 6. Aspects of tomato plants

Figure 7. Section through tomato fruit

CONCLUSIONS

The aim of this study was to identify new non-aggressive methods capable to shorten the fruit ripening period of the tomato fruits

During the study, we noticed a faster growth of tomato fruits following the application of EMCOPAD-Doctor Tech devices compared to the variant not exposed to the reflected electromagnetic field. The differences in physiological maturation between the two abovementioned tomato variants were the followings: a reducing of the period with 4-5 days when the device was placed directly on the immature fruit and 4 days reducing when it was placed directly on the stem of the plant near the insertion of the inflorescence. The conclusion is that the use of the electromagnetic field created by the EMCOPAD-Doctor Tech device has positive impact on the tomato culture by reducing the physiological maturation period as mentioned above.

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SYMPTOMS OF PEPINO MOSAIC VIRUS IN GREENHOUSE TOMATOES OF BELARUS AND REACTIONS OF TEST PLANTS

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Abstract

This study was carried out to detect the Pepino mosaic virus in various tomato hybrids grown in greenhouses. Total of 194 plant sample were collected from the greenhouse during 2019-2020 years. As a results of DAS-ELISA was found 54 of samples with PepMV, which was identified both in monoinfection and in the complex with Cucumber mosaic virus, Tobacco mosaic virus and Tomato mosaic virus. The possible symptoms of PepMV during the growing season of tomatoes include interveinal chlorosis, deformations, mosaic and yellow spots on leaves and also blotchy ripening fruits. The reaction of 10 plant species to the inoculation of PepMV was established. The results showed the greatest susceptibility of Nicotiana rustica and Datura stramonium, where the maximum concentration of viral particles was detected 4 weeks after infection (OD 405 nm: 0.952-1.013).

Key words*: PepMV, tomato, test plants, DAS-ELISA, symptom.*

INTRODUCTION

In the Republic of Belarus, tomato (*Lycopersicon esculentum*) is grown in greenhouses under conditions of long-term crop rotation. The grown assortment of tomato hybrids allows satisfying the demand in the consumer market segment in the country and increasing the volume of exports.

Due to the absence of breeding centers in the country, vegetable growers buy seeds from international vegetable-breeding companies (De Ruiter, Rijk Zwaan, Syngenta et al.).

It is known that many pathogens persist in seeds which contributes to their introduction into new regions (Hanssen et al., 2010). This is the main way for the spread of such dangerous viruses as Tomato ringspot virus (ToRSV) or Tomato brown rugose fruit virus (ToBRFV) that infects tomato culture (EPPO, 2017; EPPO, 2021).

Previously, Cucumber mosaic virus (CMV), Tobacco mosaic virus (TMV), Tomato mosaic virus (ToMV), TAV (Tomato aspermy virus), Potato virus X (PVX) and also Potato virus Y (PVY) were detected in greenhouse tomato plantings, the level of development of which ranged from 5.6 to 37.5%. (Vabishchevich, 2012; Vabishchevich et al., 2020). Pepino mosaic virus (PepMV) periodically in tomato plant samples was noted.

PepMV is a Potexvirus (family *Alfaflexiviridae*) which infected tomato crops worldwide (EPPO, 2013). For example, the occurrence of PepMV on tomato crops was noted in Germany, Italy, the Netherlands, Poland, Romania and others. It is also known that the main host plants of PepMV are pepino (*Solanum muricatum*), potato (*Solanum tuberosum*) and some weed species (Cordoba et al., 2004; Blystard et al., 2015).

The main source of the virus is tomato seeds, where the pathogen remains in the coat (Ling, 2007). The infection of tomato seeds can vary from 0.005 to 0.057% (Hanssen et al., 2010).

As for most potexviruses PepMV mainly spreads mechanically from plant to plant without the involvement of an obvious vector (King et al., 2012). There is evidence that bumble bees (Shipp et al., 2008) and the soil-borne fungus *Olpidium virulentus* (A. Br.) Schroet. (Alfaro-Fernández et al., 2010) can function as vectors for PepMV. Also, recent studies suggest that tomatoes pests (e.g. *Trialeurodes vaporariorum* Wetw.), as well as some types of entomophagous (e.g. *Aphidius colemani* Viereck) can act as vectors too (Noäl et al., 2014; 2016).

The damage from PepMV is associated with a decrease in the commercial quality of tomato fruits and their quantity, which can vary depending on the hybrid, time, conditions, the

way the virus enters the plant, as well as its strain composition and the presence of other viral pathogens (mixed infection) (Spense et al., 2016). Soler-Aleixandre et al. (2005) reported high losses with the collapse of up to 90% of plants; others describe low yield losses of up to 15% (Verhoeven et al., 2003) or no quantitative yield losses, but significant reduction up to in fruit quality (up to 40%).

PepMV was first detected in greenhouse tomato plantings in Belarus in 2012, but no further targeted research has been carried out (Blotskaya & Vabishchevich, 2013). The objectives of this work was to identify the *Pepino mosaic virus* in tomato plants and to study the symptoms of the disease on various test plants.

MATERIALS AND METHODS

Phytosanitary monitoring of tomato plantings was performed in 11 greenhouse complexes of the republic during 2019–2020 years. Inspection and sampling were made according to the recommendations presented in the EPPO diagnostic protocol for PepMV (PM 7/113 (1), 2013).

The samplings were made from tomato plants with a wide range of virus-like symptoms: various types of mosaics on leaves and fruits, lightening of veins, chlorosis, reduction, wrinkling of leaves, etc. The samples were placed inside polyethylene bags and brought to the laboratory.

Identification was performed using the DAS-ELISA method (double antibody enzyme-linked immunosorbent assay) for PepMV. (commercial kits BIOREBA AG, Switzerland). Each ELISA test included two positive and two negative controls. Samples were rated positive if the mean optical density at 405 nm (OD) of the sample exceeded three times the mean of two wells containing extract from healthy plants (Samson et al., 1993). In the same way, the samples were tested for the presence of pathogens such as CMV, TMV, ToMV, PVX, *Tomato spotted wilt virus* (TSWV).

Plants *Nicotiana tabacum* L., *N. glutinosa* L., *N. rustica* L., *Datura stramonium* L., *Capsicum annuum* L., *Lycopersicon esclentum* Mill., *Physalis pruinosa* L., *Phaseolus vulgaris* L., *Cucumis sativus* L. and *Cucurbita pepo* Mill. were tested for their susceptibility to PepMV.

The indicator plants were grown under laboratory conditions in pots with a peat substrate. When 5-6 true leaves were formed, the plants were transplanted into 5-liter pots for further keeping in the greenhouse. The distance between the pots did not allow contact between plants. Watering was carried out daily in accordance with the needs of the plants.

Individual equipment was used to care for the plants, and the necessary measures were taken to prevent the development of pests.

As an inoculants, the juice of the leaves of tomato (*Prunus* hybrid) infected with PepMV was used. Virus was inoculated locally by standard procedure (Jeffries, 1998). Five plants of each cultivar were inoculated with the isolates used and as control 5 plants was inoculated with water.

The plants were inoculated by PepMV at the stage of 3-4 full-grown leaves. The inoculated plants were observed regulary in a long period post inoculation. DAS-ELISA testing was performed 4 and 20 weeks after inoculation to confirm viral infection in the test plants and to determine the accumulation of viral particles.

RESULTS AND DISCUCCIONS

Plants with virus-like symptoms on differents tomato hybrids were noted in greenhouses. Also the symptoms that charactered by PepMV on tomato plants were noted. Symptoms such as interveinal chlorosis, leaf deformation, mosaic and yellow spot on the leaves, shoots and even pedicels of tomato have been observed. In addition, yellowish stripes covering the entire stem, up to the point of growth and inflorescences tomato were noted. Various types of mosaic, cracking or deformation were observed in fruits, in particular on cherry tomato hybrids (Figure 1).

DAS-ELISA tests were carried out on the leaf samples collected from 194 plants with virus infections symptoms in order to determine the existence of PepMV. The results showed that 54 samples of 6 tomato hybrids grown in different greenhouses were infected with PepMV. Thus, the incidence of PepMV infection for 194 samples was 27.84% of which 11.34% of the samples contained

monoinfection. In 16.5% of the studied samples, a complex defeat of PepMV with other viruses was established. At the same time, the species composition of viruses involved in pathogenesis and the level of their accumulation in tomato plants varied in the same hybrids.

The possibility of PepMV development in tomato plants together with other viral pathogens is noted in the works of many authors. Thus, PepMV was detected with CMV, *Tomato chlorosis virus* (TCV), *Tomato torrado virus* (ToTV), etc. (Gómez et al., 2010). In our studies PepMV detected together with CMV, TMV, ToMV or PVX in different combinations (Table 1).

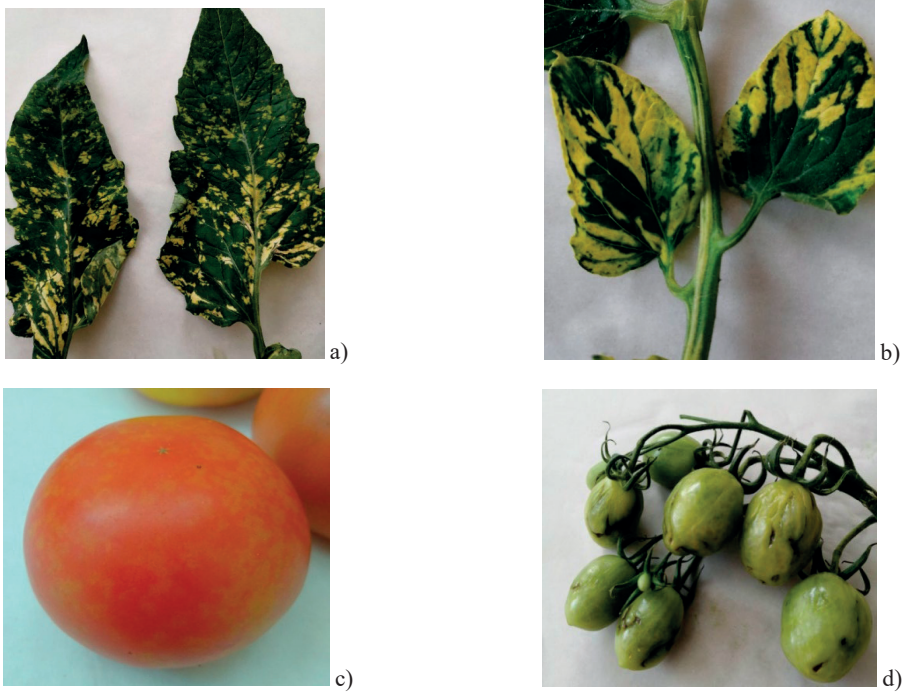

Figure 1. Symptoms Pepino mosaic virus on tomato plants:
a - yellow mottling on the leaves, b - yellow leaf spot and streakiness on the shoots,
c, d - spotting and deformation of fruits

Table 1. Species composition of viruses co-occurring with Pepino mosaic virus in tomato plants (determined by DAS-ELISA method, 2019-2020)

Complex infections		
2-component	3-component	4-component
PepMV + TMV	PepMV + TMV + CMV	PepMV + ToMV + TMV + CMV
PepMV + CMV	PepMV + TMV + PVX	–
PepMV + ToMV	PepMV + ToMV + CMV	–

In most cases, the presence of a complex infection in a plant leads to a change in the nature of the phenotypic manifestation of the disease: an increase in symptoms or a weak development of external signs. Co-infection of tomato with PepMV and TMV showed symptoms of venous chlorosis (a) and reduction of leaf blades (b) (Figure 2).

a)

b)

Figure 2. Symptoms on tomato leaves at complex infection Pepino mosaic virus with Tobacco mosaic virus

On susceptible tomato, plants were infected with PepMV together with CMV manifested interveinal chlorosis and mosaic (a), and on the fruit – deformation and blackening (b) (Figure 3).

a)

b)

Figure 3. Symptoms on leaves (a) and fruits (b) of tomato at complex infection Pepino mosaic virus with Cucumber mosaic virus

The presence of PepMV in combination with ToMV was manifested in the form of a pale green leaf spot of the upper layer of tomato or on young shoots.

This wide variation in symptoms observed with viral infections in tomato suggests that both positive and negative interference can occur between species. It is known that under conditions of mixed infections, the pathological effect of viruses is due to the nature of the interaction of pathogens with the host plant and the relationship with each other.

In this regard, the fact of establishing a high frequency of occurrence of PepMV in combination with other viruses requires a more detailed study of the specificity of accumulation and translocation of the pathogen, depending on the composition of the infection.

To determine the response to infection and assess the level of its accumulation, we inoculated a number of test plants with PepMV isolate under laboratory conditions. The test results showed that 8 out of 10 species tested were susceptible to the virus.

It should be noted that *D. stramonium* plants showed the fastest and brightest response to inoculation with PepMV isolate. On the 7th day after infection, a yellow mosaic was observed on the inoculated leaves plant. Local chlorotic lesions, leaf deformities, or systemic yellow vein streak were then noted (Figure 4).


Figure 4. Reaction of *Datura stramonium* L. to infection with Pepino mosaic virus

It is known that the reaction of plants of the genus *Nicotiana* to infection with PepMV is variable and strongly depends on the strain composition of the pathogen, the type and even the variety of tobacco. For example, the reaction to mechanical inoculation with the polish isolate of *N. tabacum* cv. 'White Burley' plants manifested itself as vein chlorosis and mosaic. *N. tabacum* cv. 'Xanthi' reacted in the same way (Pospieszny et al., 2003). In other studies, *N. tabacum* cv. 'Xanthi' plants did not respond to inoculation by such strains PepMV as EU-tom, Ch2 or US1 (Verhoeven et al., 2003, Gomez et al., 2009). Fakhro et al. (2011) unrecorded any symptoms on *N. tabacum* L. cv. 'Samsun' after mechanical inoculation by european isolate of PepMV. However, in our experiments, a positive reaction of *N. tabacum* cv. 'Samsun' to the virus was noted, which was noted already on the 7th day after inoculation in the form of a chlorotic mosaic. Among other *Nicotiana* species, *N. rustica* was also susceptible to the PepMV isolate, where a systemic mosaic was observed. The reaction of *N. glutinosa* plants to PepMV inoculation was asymptomatic.

The results of laboratory experiments by some researchers showed that pepper plants of various varieties were not infected with PepMV or the manifestation of symptoms was local (Salamone & Roggero, 2002., Blystard et al., 2015). Overall, the scientists concluded that pepper is not a systemic host for the three viral strains (EU-tom 1066, Ch2 PCH06/104, US1-PRI) used in the study, and it is likely that *Capsicum annuum* L. is not an important host in the epidemiology of PepMV.

In our studies, to assess response to infection of PepMV was used *C. annuum* cv. 'Alesya' (belarusian selection). Despite the same conditions of infection and maintenance of pepper plants, mixed results were obtained. So, 10 days after inoculation of the plants, local symptoms in the form of a light yellow mosaic 2 out of 5 test pepper plants appeared. Other plants were asymptomatic even 4 weeks after inoculation with PepMV. After 4 months, the response of susceptible pepper plants was divided into soft mosaic and marginal chlorosis (Figure 5).


Figure 5. Symptom development of *Pepino mosaic virus* isolate in *Capsicum annuum* cv. 'Alesya'

It should be noted that tomato plants are highly susceptible to virus infection. One week after inoculation and throughout the entire study period on *L. esculentum* cv. 'Lyana' noted systemic symptoms of lesion: yellow or light green spotting, chlorotic lesion and leaf deformation (Figure 6a). Plants of *Physalis* genus normally is not infected by PepMV. Cases of local and systemic reactions of *P. floridana* to the Polish isolate of the PepMV-SW virus are known (Pospiezny et al., 2007). In our studies used *P. pruinosa* cv. 'Yantar'. As a result, the reaction in the form of deformation and swelling of the leaf blade manifested itself only in 2 plants on the 30th day after inoculation (Figure 6b).

a)

a)

Figure 6. Symptom development of PepMV in *Lycopersicon esculentum* cv. 'Lyana' (a) and *Physalis pruinosa* cv. 'Yantar' (b)

When infected with different PepMV isolates, symptoms on *P. vulgaris* plants may be absent or appear as small-spotted spots (Jorda et al., 2001; Pospieszny et al., 2003). The same spotting was observed in *P. vulgaris* cv. 'Motolskaya White' in our experiments.

During the experiment, the visual signs of infection PepMV plant cucumber (*Cucumis sativus* cv. 'Verasen') and pumpkin (*Cucurbita pepo* var. *clypeata* cv. 'Malyshka') was absent. The results of enzyme-linked immunosorbent assay of test-plant samples also confirmed the absence of PepMV virus particles.

PepMV is mainly accumulated in *D. stramonium* and *N. rustica* plants, where the content of viral particles 4 weeks after infection reached 1.013 and 0.952 units OD (optical density), after 20 weeks – more than 2,400 OD. In plants *L. esclentum* and *C. annuum* high virus concentration only 20 weeks after inoculation was observed (Figure 7).

CONCLUSIONS

As a result of the ELISA-test of 194 tomato plant samples in 54 samples PepMV was detected. The virus was identified both mono-infection and in combination with other viruses from *Bromoviridae, Virgaviridae* and *Alphaflexiviridae* families. The most characteristic symptoms of PepMV on tomato plants are yellow spot on the leaves, shoots and pedicels; spots on fruits and their deformation. In conditions of complex damage to tomato plants, chlorosis, reduction of leaf blades and mosaic were noted. An asymptomatic course of the disease is also possible.

During artificial infection of 10 species of indicator plants, 8 showed various kinds of mosaic lesions. The highest susceptibility to PepMV of plants by *D. stramonium* and *N. rustica* was established. On these plants also after a long time of cultivation the maximum concentration of viral particles detected.

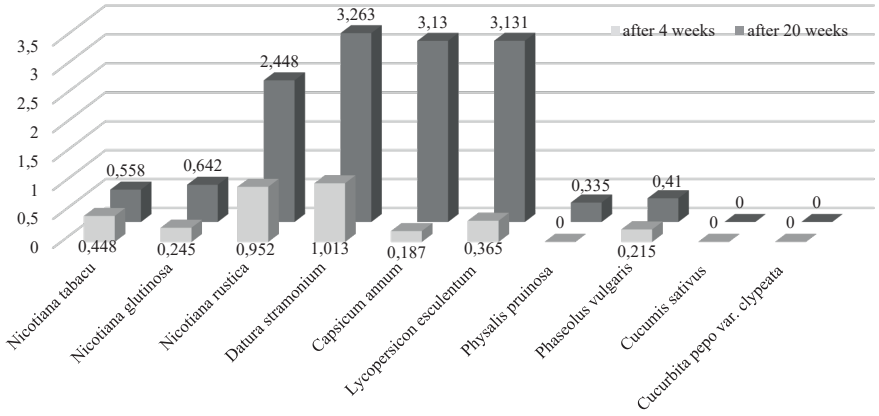

Figure 7. The content of Pepino mosaic virus in the test-plants («0» – results OD ≤ negative control)

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FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARCHITECTURE


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ORNAMENTAL, PHYSIOLOGICAL AND ENZYMATIC EVALUATION OF SOME GLADIOLUS SPECIES

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Abstract

The genus Gladiolus (Iridaceae family), with about 270 species, is native to different regions of South Africa and the Mediterranean area, in the first region having a much larger distribution with a larger number of species (more than 114 species). In the Mediterranean area, the ten to twelve species of gladiolus offered taxonomic confusion due to their similarities and morphological variations due to localized evolution. The study effectuated during the period 2018-2020 at two Gladiolus species (G. byzantinus and G. imbricatus), existing in the collection of the Iasi University of Life Sciences, had as purpose the analysis of some ornamental characters and also the content of photosynthetic pigments and the enzymatic activity of the leaves. G. byzantinus formed denser shrubs (higher number of shoots per plant), but in G. imbricatus there were more leaves/ shoot, longer flower stalks, denser inflorescences (shorter distance between flowers). G. byzantinus bloomed about two weeks earlier, but flowering time was seven days longer in G. imbricatus. The content of assimilative pigments was higher in G. imbricatus and was correlated with a lower enzymatic activity.

Key words*: assimilative pigments, enzymatic activity, gladiolus, ornamental characters.*

INTRODUCTION

The genus *Gladiolus* belongs to the family Iridaceae and includes about 270 species. This genus is native to different regions of South Africa, tropical Africa and the Mediterranean area, in the first region having a much larger distribution with a larger number of species (more than 114 species). In the Mediterranean area, the ten to twelve species of gladiolus offered taxonomic confusion due to their similarities and morphological variations due to localized evolution. This led to several descriptions of Mediterranean species, which were later treated by many authors as synonymous or placed in lower taxonomic ranks (Hamilton, 1980, quote de Mifsud et al., 2013).

The European species were cultivated at least 500 years ago and were first identified as a species in New Forest, Great Britain, in 1855. Trade in these species evolved in a very short time and occupied the top places. in the international trade in flowers (Cantor & Tolety, 2011).

As an underground organ, the *Gladiolus* species have a corm, that is renewed with each cycle of vegetation and flowering. Under conditions of humidity and appropriate temperature one can form up to 4-5 new corms, at the base of which are formed small cormlets (3-8 mm diameter), capable of flowering after 2-3 years (Șelaru, 2007). The stems are generally unbranched and the flower spikes are one-sided, with bisexual flowers, each subtended by two green bracts. Tepals are united at their base into a tube-shaped structure.

In 2010 a study was conducted in the Floriculture experimental field ("Ion Ionescu de la Brad" University of Agriculture Sciences and Veterinary Medicine of Iași) shows that the species *G. imbricatus* has a good adaptability to environmental conditions in this area, noting insignificant differences from the specimens in the natural habitat (decrease in plant height, a low delay in flowering by about 3-7 days), the species covering almost the same decorative period as in the natural habitat, decorating by flowers from May to June (Chelariu & Draghia, 2011).

When the temperatures are high and rainfall is reduced quantitatively, the number of days required for corms formation is about 45 to 50 (Tomiozzo et al., 2019). The crop success (the vegetative growth, development and, finally, the height of the flower stem, the number of flowers and the production of tubers) is strongly

influenced by the size of the corms (Bose et al., 2003 and Uddin et al., 2002, quoted by Sarkar et al., 2014).

The size of the corms and the planting distance directly influence the vegetation start of the plants, their growth, as well as the formation of the stems and the number of flowers on the stem (Toporaş, 2008). Plant height, number of leaves/ plant, time required for flowering, flower stem length, number and size of new corms and cormlets were significantly improved by the use of larger parent corms (Sharma & Gupta, 2003).

Determining the assimilative pigments content in the process of plant growth and development provides useful information on the photosynthetic state during the phenophases of vegetation. Ecological conditions of culture and especially water stress reduce the photosynthetic rate per unit area of leaves (Graca et al., 2010). In addition, Abo El-Kheir (2007) indicated that the reduction of the available moisture content in the soil leads to both a significant decrease in the concentration of Chl. *a*, Chl. *b*; the total content of chlorophyll pigments (a + b), as well as the content of carotenoid pigments. At gladiolus, the increase in photosynthetic pigments content is highlighted as a response of plants to foliar application of antioxidants and is due to their role in strengthening the photosynthetic activity and the chlorophyll biosynthesis, or protecting the chloroplast from oxidative damage resulted from oxidative stress (Munne-Bosch et al., 2001).

The content of assimilative pigments in the leaves of *G. grandiflorus* is significantly influenced not only by ecological conditions, vegetation phenophase but also by organic fertilizer (Hassan et. al., 2020; Abdou et al., 2013; Khalil, 2015; Abdou et al., 2018, 2019). Also, ascorbate peroxidase (APX) and catalase (CAT) are enzymes that metabolize H_2O_2 caused by stress and control the potential impact to maintain the cellular concentration of H_2O_2 at a level necessary for the normal growth and development of plants (Gill & Tuteja, 2010; Ray et al., 2012; Anjum et al., 2014b).

This paper presents an analysis of the ornamental characters and also the content of photosynthetic pigments and the enzymatic activity of two *Gladiolus* species (*G. byzantinus* and *G. imbricatus*), growing in conditions of the North East region of Romania (Iasi city).

MATERIALS AND METHODS

The observations and measurements were performed during 2018-2020, in the experimental field of the Floriculture discipline, "Ion Ionescu de la Brad" University of Life Sciences from Iaşi. The laboratory analyzes were performed within the Horticultural Research Center of the Faculty of Horticulture from Iaşi.

The experimental plot is located in temperate-continental climat with excessive nuances. During the analyzed period, the average annual temperatures registered a slight increase, starting from an average of 10.5°C in 2018 and reaching the value of 12.0°C in 2020. The rainfall regime varied a lot during the experiment. The highest amount of precipitation was recorded in 2020 and the driest year was 2019.

Soil is a chernozem cambic with sandy-loam texture, with pH 7.8

The establishment of the crops was made on October-November, with corms having a mass between 5.06 and 3.5 g from the species *G. byzantinus* and *G. imbricatus* from the own collection of the Floriculture discipline. The corms were planted in rows, at a depth of 10 cm in a very well prepared substrate. The planting distances are 25-30 cm between rows and 10-15 cm distance between plants per row (Cantor & Pop, 2008).

The *G. byzantinus* (Figure 1a) presents as an underground organ an ovoid corm, covered with fibrous tunics. The leaves, 4-5 number, are ensiform and can reach a height of 70 cm, 1-2.5 cm wide. The floral stem is erect and reaches 0.5-1 m in height; presents 5-15 flowers, brightly colored in red-purple, very beautiful, arranged unilaterally, less bilaterally, often unbranched, blooming in May-June (Kerguélen & Lonchamp 1999).

The *G. imbricatus* (Figure 1b) is a rare, legally protected perennial species protected at European level by Directive 92/43 / EC (Annex I) of 21 May 1992 (on the conservation of natural habitats and of wild fauna and flora) (https: / /ro.wikipedia.org/wiki/F%C3%A2nea%C8%9Ba_Izvoarelor_Cri%C8%99ul_Pietros).

As an underground organ it has globular corms. The stem is erect, unbranched and cylindrical. It is 30-60 cm tall and usually has three lanceolate leaves.

The inflorescence is a unilateral racemose crown and has 4 to 12 zygomorphic flowers, 2.5 cm long, with purple-violet tepals (Frantsuzenok & Nikonovich, 2005). The fruit is a short, obovate, obtuse, trimmed capsule (Brickell & Cathez, 2004). It grows on wet meadows and swamps and blooms from May to June. In Romania it is found in the meadows of the sub-Carpathian regions (Păun et al., 1980) and in the spontaneous flora of Maramureș, Transylvania, Banat, Maramureș, Oltenia, Muntenia, Moldova. In the Iasi area it was reported in the Bârnova forest (Oprea, 2005).

a. b.

Figure 1. *G. byzantinus* (a) și *G. imbricatus* (b) (original photo)

The experience included two variants (each species representing a variant) distributed in randomized blocks with three repetitions (20 plants/repetition).

The examination of the morphological characters and phenology was made on the plants from the collection.

The results were compared to the average of the variants (considered control), and the interpretation was made using the analysis of the variance, with the LSD test (Saulescu and Saulescu, 1967).

The symbols used to indicate the significance of the differences from the control are: ns = non-significant; o/x = negative/positive significant difference; oo/xx = negative/positive distinct significant difference; ooo/xxx = negative/positive very significant difference.

The assimilative pigments were tested by the spectrophotometric method according to Lichtenthaler (2001). The extraction of photosynthetic pigments was performed from the leaves of the studied species according to the Current Protocols in Food Analytical Chemistry (Hartmut et al., 2001). To extract the photosynthetic pigments, fresh material was weighed (between 0.03-0.05 g). After weighing, the samples were placed in a grinding mortar and then quartz sand and Ca CO_3 (in powder form) were added to prevent the conversion of chlorophylls to porphyrins. To grind the tissue, 2-3 ml of pure acetone is added to the mortar in several stages until the vegetable material has been well ground, after which the liquid has been passed in a graduated cylinder. The process was repeated until the acetone was no longer colored, the volume of the filtrate was finally brought to 10 ml and then centrifuged for 10 minutes at 10,000 rpm. After extraction, the extracted samples were read using the UV-VIS spectrophotometer at E661.6 for a chlorophyll, E644.8 for b chlorophyll and E470 for carotenoid pigments. Sampling was performed using the T70 UV/VIS Spectrophotometer PG.

To determine the activity of catalase (CAT) and ascorbate peroxidase (APX), 0.5 g of plant material was ground on ice in K phosphate buffer with a pH of 7.0. To obtain the supernatant from which the enzymatic activity was analyzed, the extract obtained from milling was centrifuged at 12,000 rpm for 20 minutes, at 4°C. The APX activity was determined according to the method of Chen & Asada (1989) by monitoring the decrease in absorbance at 290 nm. The reaction mixture (3 ml) consisted of 1.5 ml of phosphate buffer (pH 7.0), 300 µl of ascorbic acid, 600 µl of H_2O_2 and 600 µl of enzyme extract. One unit of enzyme activity was calculated as the amount of enzyme required for the oxidation of 1.0 mM ascorbate/min/g fresh substance. The total activity of CAT was tested by measuring the initial rate of H_2O_2 disappearance according to the method of Aebi (1984). The reaction mixture (1 ml) contained 1.5 ml of phosphate buffer (pH 7), 1.2 ml of H_2O_2 and 300 µl of enzyme extract. The decrease in absorbance was monitored over time, at 240 nm. One unit of enzymatic activity (units/min/g fresh

substance) is calculated as the amount of enzyme needed to release half of the oxygen peroxide from H_2O_2. Experiments were performed in triplicate and data for APX and CAT were presented as mean values with standard deviations.

RESULTS AND DISCUSSIONS

In the Figure 2 are presents data on the share of corms started in vegetation. In the species *G. byzantinus* the percentage of corms started in vegetation is 89.0%, with 2.5% lower than in *G. imbricatus*, in which the share of starting corms in vegetation is 91.5%. Regarding the number of plants that formed floral stems, the observations showed that the plants of *G. imbricatus* formed floral stems in proportion of 97,2% and in *G. byzantinus* only 59.6% of the plants started in vegetation formed stems.

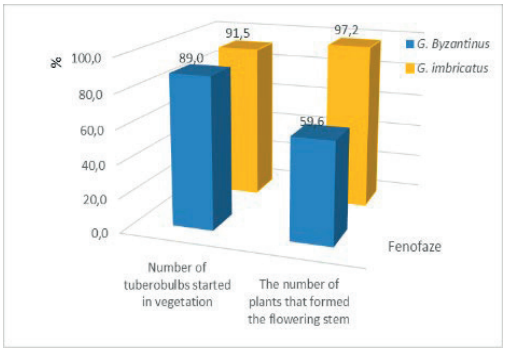

Figure 2. The share of corms in the vegetation and of the plants that formed floral stems

According to the calendar data that marked the main phenophases: the start of vegetation, the appearance of flower stalks, the onset of flowering and the end of flowering, the time required for each of them was calculated. From Figure 3 it can be seen that the number of days from planting to the start of vegetation is 132 for *G. byzantinus* and 151 for *G. imbricatus* (Figure 3).

It was found that the number of days from the onset of vegetation to the appearance of the flower stem is equal between the two species, however the number of days calculated from the appearance of the stem to the opening of the first flower in the inflorescence varied from 14 days at *G. byzantinus* and seven days at *G. imbricatus* (Figure 3).

The duration of flowering, expressed by the number of days from the opening of the first flower to the complete passage of flowers is longer for the species *G. imbricatus*, totaling a number of 16 days compared to 13 days for the species *G. byzantinus* (Figure 3).

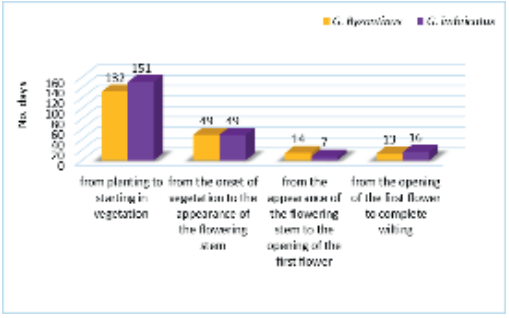

Figure 3. Duration of the main phenophases (no. of days)

The aspects related to the phenology of the two species are also shown in the diagram, in Figure 4, from which it can be seen that *G. byzantinus* starts in vegetation and blooms two weeks earlier. but has a shorter flowering time by approx. seven days compared to *G. imbricatus.*

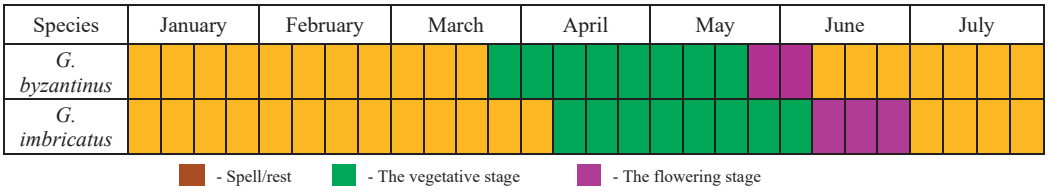

Figure 4. Phenological diagram

In the Tables 2, 3 and 4 were present data on some morphological properties of plants from the two species of *Gladiolus*. The length of the leaves varied from 35.5 cm (*G. byzantinus*) to 56.3 cm (*G. imbricatus*). Very significant positive differences were recorded in the species

G. imbricatus, in which the leaf length exceeded by 22.3% the average of the experiment and by 6.8% *G. byzantinus* (Table 2). The width of the leaves had similar values for the two species (approx. 2 cm) and the differences were non significant. Regarding the number of shoots per plant (Table 1), higher values were at *G. byzantinus* (1.6 shoots per plant, compared to *G. imbricatus* which formed only one shoot per plant).

Table 1. Leaves and shoots characteristics

Species	Leaf length			Number of shoots / plant		
	Absolute values (cm)	Relative values (%)	Difference/ Significance	Absolute values (no.)	Relative values (%)	Difference/ Significance
G. byzantinus	35.5	77.34	-10.4^{000}	1.6	123.08	0.3^{x}
G. imbricatus	56.3	122.66	10.4^{xxx}	1	76.92	-0.3^{0}
$\bar{x}$	45.9	100.0	0	1.3	100.0	0
			$LSD_{5\%} = 2.4$ $LSD_{1\%} = 4.1$ $LSD_{0,1\%} = 7.6$			$LSD_{5\%} = 0.3$ $LSD_{1\%} = 0.5$ $LSD_{0.1\%} = 1.0$

The height of the floral stem is a very appreciated character, especially in the case of capitalizing the plants as cut flowers. From the statistical analysis of the data on the height of the floral stems, the longer stems were highlighted in *G. imbricatus,* which exceeded the average of the experience by 11,65%, respectively with significant positive differences (Table 2).

Table 2. The biometric features of floral stems and inflorescences

Species	Floral stem height			Height to the first flower			Inflorescence length		
	Absolute values (cm)	Relative values (%)	Diff./ signif.	Absolute values (cm)	Relative values (%)	Diff./ Signif.	Absolute values (cm)	Relative values (%)	Diff./ signif.
G. byzantinus	61.4	88.35	-8.1^{00}	38.8	83.44	-7.8^{00}	22.7	98.69	-0.3^{ns}
G. imbricatus	77.6	111.65	8.1^{xx}	54.3	116.77	7.8^{xx}	23.3	101.75	0.3^{ns}
$\bar{x}$	69.5	100.0	0	46.55	100.0	0	23.0	100.0	0
			$LSD_{5\%} = 4.4$ $LSD_{1\%} = 7.2$ $LSD_{0.1\%} = 13.5$			$LSD_{5\%} = 2.7$ $LSD_{1\%} = 4.4$ $LSD_{0,1\%} = 8.3$			$LSD_{5\%} = 1.4$ $LSD_{1\%} = 2.3$ $LSD_{0.1\%} = 4.3$

The first basal flower in the inflorescence was located at a height of approx. 39 cm above ground level in *G. byzantinus* and over 54 cm in *G. imbricatus* (Table 2).

Although the values indicating the length of the inflorescences are not statistically assured (Table 2), the differences between the two species being very small, it should be noted the share of the inflorescence in the total floral stem (37% in *G. byzantinus* and almost 30% in *G. imbricatus*) (Figure 5).

An important element for the both use of these species in landscaping and for their use as cut flowers is the number of flowers in the inflorescence.

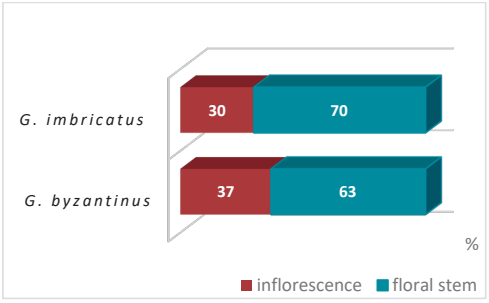

Figure 5. The length of the inflorescence in the floral stem (%)

Also in this character, *G. imbricatus* had better results, respectively 12 flowers/inflorescence (with

21.21% above the average of the experience), compared to 7.8 flowers/ inflorescence in *G. byzantinus*. The differences from the mean were significant (positive in *G. imbricatus* and negative in *G. byzantinus*).

From the data presented above (Table 3) it was found that the length of the inflorescences was relatively close to the species analyzed. However, the difference was in the density of the inflorescences, determined by the distance between the flowers (Table 3). Thus, *G. byzantinus* was characterized by looser inflorescences, with a distance between flowers of 3.7 cm, unlike *G. imbricatus*, with denser inflorescences, the distance between flowers being 1.7 cm, with over 50% lower (Table 3).

Table 3. Flowers and inflorescences characteristics

Species	No. flowers/inflorescence			The distance between the flowers in the inflorescence		
	Absolute values (no.)	Relative values (%)	Difference/ Significance	Absolute values (cm)	Relative values (%)	Difference/ Significance
G. byzantinus	7.8	78.79	-2.1^{00}	3.7	137.04	1^{xx}
G. imbricatus	12	121.21	2.1^{xx}	1.7	62.96	-1^{00}
$\bar{x}$	9.9	100.0	0	2.7	100.0	0
			$LSD_{5\%} = 1.3$ $LSD_{1\%} = 2.1$ $LSD_{0,1\%} = 3.9$			$LSD_{5\%} = 0.5$ $LSD_{1\%} = 0.8$ $LSD_{0.1\%} = 1.5$

It is known that photosynthetic activity is related to the content of photosynthetic pigments (Macintyre et al., 2002). Chlorophyll, which captures light and transfers energy in order to conduct photochemical reactions, is one of the most active photochemical compounds in photosynthesis. Photosynthetic efficiency and cell growth are associated with the quantify-cation of chlorophyll and have been highlighted on algae cultures's systems (Masojidek et al., 2000; Tremblin et al., 2000).

Conventionally, chlorophyll content is measured using spectroscopic and chromatographic methods (Lichtenthaler & Wellburn, 1983; Gilmore & Yamamoto, 1991).

Photosynthetic pigments represented by chlorophylls and carotenoids are those that provide valuable information on the installation of physiological stress in plants in different culture conditions. The values of chlorophyll content in leaves are closely correlated with the ecological conditions, the stress caused by high temperatures and drought, inducing a decrease in the total content of photosynthetic pigments. In addition to decreasing of the total content of photosynthetic pigments, this stress also induces an increase in the content of carotenoid pigments in the leaves.

In order to highlight the growth and development of the *G. imbricatus* and *G. byzantinus* species under the culture conditions of Iași during research period 2018-2020, the content of photosynthetic pigments was analyzed by the spectrophotometric method. In order to obtain conclusive results, the harvesting of the vegetal material was carried out during the flowering period of the plants and after the end of the flowering. The samples were collected at the same time of the day these being represented by leaves that have reached maximum maturity. After determinations were made, in both phenophases of vegetation, was observed a higher content of assimilating pigments in *G. imbricatus* species compared to G. *byzantinus* species (Table 4). The results regarding the total content of photosynthetic pigments showed higher values during the flowering period of the plants at both species. The total content of photosynthetic pigments increased in the flowering period of the plants compared with the post-flowering period by 0.29 mg/g s.p. at *G. imbricatus* and by 0.71 mg/g s.p. at the *G. byzantinus*. Studies showed that under normal ecophysiological conditions the ratio of chlorophyll *a*/chlorophyll *b* is around 3:1 (Lichtenthaler et al., 1981). The results of chlorophyll pigments content as well as the values of the Chl. *a*/ Chl. *b* ratio can provide useful information on the interaction between plants and the environment (Richardson et al., 2002). The values of this ratio were within the theoretical limits, ranging

between 2.93 at *G. imbricatus* and 2.58 at *G. byzantinus*. Was observed, in case of both species *G. imbricatus* and *G. byzantinus*, an increase in the total content of carotenoid pigments after the flowering of the plants. Within this phenophase, by correlating the decrease of the values of the photosynthetic pigments content with the increase of the carotenoid pigments content, it is highlighted that the development of physiological processes in plants is manifested normally, the data confirming the results according to which the content of chlorophyll pigments generally decreases during the installation of senescence (Fang et al., 1998). The ratio of chlorophyll pigments/ carotenoids was higher for the both species during the flowering period of the plants (3.75 in *G. imbricatus* and 4.11 in *G. byzantinus*). The increase in the content of carotenoid pigments in the period after the flowering of the plants determined a decrease of the chlorophyll / carotenoid pigments ratio, the values obtained by the two species being of 3.66 for *G. imbricatus* and 3.88 for *G. byzantinus*.

Table 4. Average content of photosynthetic pigments (mg g^{-1} F.W)

Species	Phenophase	Chl. *a* mg/g F.W.	Chl. *b* mg/g F.W.	x+c mg/g F.W.	Σ	Chl.*a*/ Chl.*b*	Chl./Car.
G. imbricatus	flowering	2.94±0.03	1.06±0.02	1.07±0.05	5.06	2.77	3.75
	after flowering	2.79±0.02	0.95±0.04	1.02±0.02	4.77	2.93	3.66
G. byzantinus	flowering	2.77±0.04	0.94±0.02	0.90±0.03	4.62	2.94	4.11
	after flowering	2.24±0.03	0.87±0.05	0.80±0.04	3.91	2.58	3.88

Studies have shown that there was an increase in ascorbate peroxidase (APX) activity along with the activity of other enzymes in the case of plants where have been subjected to stress conditions (Shigeoka et al., 2002). APX can also regulate redox signaling pathways involved in plant development (Caverzan et al., 2012), and transcriptional expression of APX is dependent on the stage of plant or tissue development (Agrawal et al., 2003; Teixeira et al., 2006). Changes in the activity of antioxidant enzymes such as APX, superoxide dismutase (SOD) and catalase (CAT) have been described during senescence period of the plant and are related to cleansing processes (Alaey et al. 2011, Gerailool & Ghasemnezhad, 2011). There are several reports of antioxidant defense systems at ornamental plants, such as chrysanthemum (Bartoli et al., 1997), daylilies (Panavas & Rubinstein, 1998) and gladiolus (Ezhilmathi et al., 2007; Sairam et al., 2011).

In order to study the species *G. imbricatus* and *G. byzantinus* in the ecological conditions of Iași from 2018-2020, biochemical determinations were performed regarding the enzymatic activity of the leaves. During the flowering and after flowering, determinations were made regarding the enzymatic activity of ascorbate peroxidase (APX) and catalase (CAT).

The results obtained regarding APX at the two species for the two developmental phenophases were compared both with each other and with the average. The difference regarding the increase of ascorbate peroxidase's activity can be considered a practical measure for assessing the level of stress caused by ecological conditions or by the development of physiological processes which are specific to the phenophase of vegetation.

By comparing the results, the highest increases of APX activity was observed at *G. byzantinus* species (the average content being 567.59 UP/g/min FW during flowering and 629.86 UP/g/min FW after flowering). Lower values of APX were registred on *G. imbricatus* species (average content being 525.93 UP/g/min FW during flowering and 567.28 UP/g/min FW after flowering) (Figure 6).

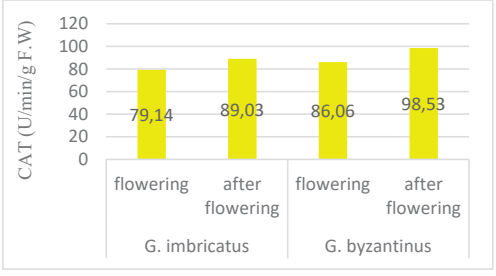

Figure 6. Ascorbate peroxidase activity determined in leaves of *G. imbricatus* and *G. byzantinus* [(U/g/min) fresh substance]

The determinations performed during after flowering period showned the APX activity

increased with 41.35 UP/g/min F.W. at *G. imbricatus,* and with 62.27 UP/g/min F.W. *G. byzantinus* species in comparation with the values obtained during flowerin period. The results obtained for the two species in the two vegetation periods were compared with the average value of the results. The results obtained showed againste the average an increase in APX only in the case of *G. byzantinus* species after flowering when the percentage was 9.98%.

The increase of APX activity in the post-flowering period does not indicate an abiotic stress caused by the culture conditions but highlights the development of physiological processes depending on the vegetation phenophase.

The biochemical results obtained in the case of the two species were correlated with the biometric measurements and the phenological determinations, highlighting a good development of these species at the climatic conditions in Iași from 2018-2020.

Regarding the results of catalase activity (CAT) by comparing the results, the same trend was highlighted as in the case of APX (Figure 7). The highest increases in CAT activity were highlighted in the species *G. byzantinus* in both vegetation phenophases. Within the two species, the lowest values of CAT activity were obtained in *G. imbricatus*, the average content being 79.14 UP/g/min F.W. at flowering and 89.03 UP/g/min F.W. (Figure 7).

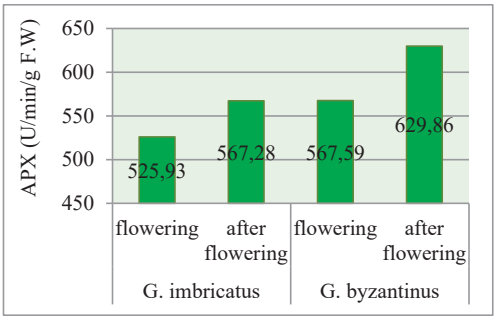

Figure 7. Catalase activity determined in leaves of *G. imbricatus* and *G. byzantinus* [(U/g/min) fresh substance]

Compared to the average value of CAT obtained by the two species, during the flowering period, the decrease was 10.26% in *G. imbricatus* and 2.41% *G. byzantinus*. Within the two species, significant increases of CAT compared to the average were registred only by the species *G. byzantinus* in the post-flowering period, the values presenting increases compared to the average by 11.72%.

CONCLUSIONS

In the ecological conditions of Iasi, the two species of gladiolus studied have distinctive ornamental characters and can be used in various ways.

G. byzantinus formed denser shrubs (higher number of shoots per plant).

G. imbricatus there were more leaves/ shoot, longer flower stalks, denser inflorescences (shorter distance between flowers).

G. byzantinus bloomed about two weeks earlier, but flowering time was seven days longer in *G. imbricatus.*

The content of assimilating pigments was higher in *G. imbricatus* and lower in *G. byzantinus*.

In both species, the content of photosynthetic pigments was higher in the case of determinations made during the flowering period.

The chlorophyll *a* / chlorophyll *b* ratio had values above 2.5, which suggests that the plants showed a normal growth and development from a physiological point of view. The increase of the content in carotenoid pigments from the period after flowering caused the decrease of the chlorophyll pigments/ carotenoids ratio, the values obtained by the two species were of 3.66 for *G. imbricatus* and 3.88 for *G. byzantinus*.

The enzymatic activity of both ascorbate peroxidase (APX) and catalase (CAT) recorded higher values especially in the samples colected in the post-flowering period. The results obtained in *G. byzantinus* showed increases compared to the average by 9.98% of APX and 11.72% of CAT.

The results regarding the enzymatic activity are correlated with those of the photosynthetic pigments content, the specie that showed the increase of the enzymatic activity (*G. byzantinus*) also registered the decrease of the chlorophyll pigments content.

G. byzantinus and *G. imbricatus* can be used both as cut flowers and in landscaping.

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GENUS *OPHRYS* L., 1753 IN ROMANIA – TAXONOMY, MORPHOLOGY AND POLLINATION BY SEXUAL DECEPTION (MIMICRY)

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Abstract

Pollination by sexual deception is thought to be the most remarkable mechanism of pollination, mainly characteristic to Orchidaceae, which has independently evolved throughout Europe, Australia, Asia, South Africa and Central- and South-America. Sexual deceptive mimicry is a complex deceptive mechanism in which the orchid flowers went through remarkable evolutionary morphological changes in their structure and function, especially that of the labellum, in order to achieve visual (shape and colour), tactile (texture and pilosity) and olfactory (sex-hormones or *pheromones) mimicry of the signals used by various insect female species in breeding condition. Because insect mating signals are usually very specific, pollinator attraction by sexual deception is also very specific, each orchid only attracting one or two insect species. In Romania, Ophrys genus is represented by four species only: Ophrys insectifera, Ophrys sphegodes and Ophrys oestrifera (all cross-pollinated by insects) and Ophrys apifera (self-pollinated). This article describes and illustrates for the first time in Romania, the phenomenon of pollination by pseudocopulation of Ophrys insectifera and its unique pollinator, the solitary male wasp Argogorytes mystaceus, and Ophrys oestrifera and its male bee pollinator Eucera longicornis. In the same time, it is described in detail the self-pollination mechanism employed by Ophrys apifera. Using advanced techniques of ultra-macro photography, details of floral organs are illustrated in order to show the spectacular morphological modifications of the bee-like flower labellum.*

Key words: *Orchidaceae; pollination; pollinaria; reward; sexual deceptive mimicry; pseudo-copulation; functional morphology.*

INTRODUCTION

"Whenever, […] *people are deceived* […] *it is clear that the error slid into their minds through the medium of certain resemblances to the truth"* - **Socrates (470-399 BCE)**

Distribution: *Ophrys* is a monophyletic genus of orchids. Following the lead of Kew Botanical Garden's, the World Checklist of Selected Plant Families, the genus is represented by ***148 species*** and ***nothospecies*** (species of hybrid origin). They are widespread across much of Europe, from the Canary Islands to the Caspian Sea, from southern Scandinavia to North Africa, east to Western Asia (North West Iran), with the greatest diversity in the Mediterranean Basin (Pridgeon *et al*., 2001; Delforge, 2016). The genus is close to the *Serapias-Orchis-Himantoglossum* assemblage, from which it derives. However, this genus is genetically very isolated, therefore, it does not form intergeneric (bigeneric hybrids) with any other orchid genus. (Anghelescu *et al*., 2021a; Delforge, 2006).

Generic name: The official, accepted name of the *Ophrys* genus was established and published in **1753**, by the Swedish botanist **Carl Linnaeus (1707-1778)**, author abbreviated as **L**. The original publication details are: *Sp. Pl*.: 948. **1753**. **Etymology:** The ***generic name***, ***Ophrys***, originates in the ancient Greek word ***ophrys*** (eyebrow, eyelid) and was first used by Roman naturalist and philosopher, **Gaius Plinius Secundus (23-79 BCE)**, known as **Pliny the Elder**, for a plant of uncertain origin. ***Ophrys*** (eyebrow) is a reference to the ***hairy rim*** or the ***hairy margin***, a reference to

the ***hairy margins of the labellum*** that ***mimic the pilosity of the female bees***, hence the generic vernacular name of this genus, the **Bee Orchids**. *Ophis* was also a name given by the Swiss botanist **Gaspard Bauhin (1560-1623)** to the genus *Neottia* (previously *Listera*), which flowers have a green hood and forked labellum, resembling the *head* and *tongue of a snake*.

Evolution & diversification

Hymenopterans (bees and wasps) diversified in the Cretaceous **110-140 million years ago** (mya), in parallel with the diversification of **Angiosperms**, about **150 mya** (Danforth *et al*., 2013). **Genus *Ophrys*** only appeared about **4.9-5 million years ago** (Breitkopf *et al.,* 2015) but soon after, it experienced episodes of massive diversification through pollinator transitions, allowing both the exploitation of new ecological niches and the establishment of reproductive isolation between lineages. The explosive speciation rate of the genus *Ophrys* (bee orchids) is among the highest reported in Angiosperms, with diversification rates peaking at between 4 and 8 lineages per million years in some clades (Breitkopf *et al*., 2015). This spectacular radiation has given rise to several hundred species in the Mediterranean region of the western Palaearctic (Delforge, 2016). According to Baguette *et al*. (2020), this spectacular adaptive radiation is due to the particular coevolutionary dynamics between these plants and their pollinators. Many plant species are generalists, they attract and can be pollinated by many different pollinator species. But some families have evolved highly specialised plant-pollinator interactions. One of those families is the Orchidaceae, a very diverse flowering plant family with approximately 27,500-29,000 species (Chase *et al*., 2015). About ***one-third*** of these has evolved a ***food-*** or a ***sexually deceptive pollination strategy***.

Sexual Deceptive Mimicry

Ophrys are ***non-rewarding*** species with ***nectarless flowers*** that ***do not offer any recompense*** to the pollinating insects. As a consequence, their pollination is based exclusively on ***deceit*** and ***mimicry***, which in this case becomes one of the most refined, sophisticated and fascinating strategies in the Plant Kingdom, known as the ***sexual deceptive mimicry***. ***Sexual deception*** in orchids occurs when the plant sends a sexual signal to the pollinator, tricking it into thinking the flower is a female of its own kind available for sex. ***Sexual deceptive mimicry*** is a complex deceptive mechanism in which the orchid flowers undergo remarkable evolutionary, morphological changes in their structure and function, especially in that of the labellum, in order to achieve visual and olfactory mimicry of the signals used by insect females in breeding condition. It involves a wide array of pollinator attractants and stimulants including ***olfactory***, ***visual*** and ***tactile*** floral signals. ***Sexually deceptive orchids*** mimic the ***pheromones, shape, size, colour, texture and pilosity of a large variety of female insects***, mainly of the orders **Hymenoptera** Linnaeus, 1758 (bees, wasps) and **Diptera** Linnaeus, 1758 (flies). The resulting convergent resemblance among two unrelated kingdoms, ***plants*** and ***animals*** - ***orchids*** and ***insects***, is staggering even to human perception (De Angelli & Anghelescu, 2020).

First mentions of *Sexual Deceptive Mimicry*

Charles Robert Darwin

"*I never was more interested in my life in any subject than this of orchids*", wrote Darwin, **1861** (Darwin, 1861) in a letter addressed to his friend **Sir Joseph Dalton Hooker (1817-1911)**, director of The Royal Botanic Gardens, Kew from 1865 until 1885.

Although earlier botanists including French naturalist, **Bernard de Jussieu (1699-1777)**, the younger brother of **Antoine Laurent de Jussieu (1748-1836)** [who fully established the **Orchidaceae** family, in **1789**, (Anghelescu *et al*., 2020a) and Scottish botanist **Robert Brown (1773–1858)**, had described the structure of orchid flowers and observed flowers explored by insects, the nature and variations of pollination mechanisms in orchids were firstly fully studied by the English naturalist, explorer and geologist **Charles Robert Darwin (1809-1882)**. In **1862**, Darwin published the first edition of his book named ***Fertilisation of Orchids***, with the subtitle ***On the Various Contrivances by which British and Foreign Orchids Are Fertilised by Insects and on the Good Effects of Intercrossing***. This

was his first essential contribution to the understanding of the pollination strategies employed by orchids to attract/deceive pollinators and accomplish successful cross-fertilization, explaining in the same time, how such complex ecological relationships could result in the ***co-evolution of orchids and insects*** (Darwin, 1862). Among the various deceptive British orchid species investigated by Darwin were the intriguing *Ophrys* orchids, to which he devoted a chapter description in his book, ***Fertilisation of Orchids***. On page 56, he included a short footnote mentioning a description by Church of England cleric and botanist **Mr. Gerard Edwards Smith (1804-1881)**, of some interesting observations by Mr. Price, perhaps the **Reverend Ralph Price**, Rector of Lyminge and Paddlesworth, Kent, to whom Smith dedicated his book, ***A Catalogue Of Rare Or Remarkable Phaenogamous Plants, Collected In South Kent*** **(1829)** (Vereecken & Francisco, 2014), admitting that he was not able to fully understand the insect's behaviour: "*Mr. Price has frequently witnessed attacks made upon the Bee Orchis by a bee, similar to those of the troublesome Apis* [*Bombus*] *muscorum*." (Smith, 1829; p. 25), "*What this sentence means I cannot conjecture*" (Darwin, 1862; p. 56). Darwin was obviously bewildered by these results, but failed to provide a rational, scientific explanation in accordance with his ***theory of natural selection*** for the insect-like flowers, a theory which did not tolerate mutual errors or waisted resources (deceiving pollinators or the lack of nectar). He incorrectly rejected the idea that plants attract their pollinators through an '*organized system of deception*' (Darwin, 1862; p. 45) on the grounds that insects, particularly bees, would be too intelligent to fall for '*so gigantic an imposture'* (Yam & Arditti, 2009). In his understating, the (co)adaptations between plants and insects had to be based on ***mutual benefits***, at least to some extent, and regarded the ***deceiving orchids*** as impossible to further proliferate/reproduce, being already engaged in an evolutionary dead end. However, Mr. Price's intriguing '*attacks*' of a bee on an *Ophrys* flower (Vereecken & Francisco, 2014), were probably the very first references to the *pseudocopulation* attempts by pollinating male insects, which remained largely unknown until the beginning of the 20th century.

Henry Correvon & Alexandre Pouyanne

By the early 20th century, Darwin's orchid book, ***Fertilisation of Orchids*** had already been edited several times and translated in several languages, inspiring various amateur and professional botanists to further investigate in much greater detail, their native orchid flora and orchid pollination mechanisms. The eminent Swiss botanist **Henry Correvon (1854-1939)**, creator of the first alpine botanical garden of Switzerland and author of the ***Album des orchidées d'Europe centrale et septentrionale*** **(1899)**, was particularly interested in the fertilization of orchids, especially in the pollination of *Ophrys apifera* and *Ophrys insectifera* (Vereecken & Francisco, 2014). During the preparation of a new edition of his book, Correvon contacted one of his collaborators, Maurice-Alexandre Pouyanne, to gather more facts on the biology of the *Ophrys* orchids, which were not native to Switzerland. **Maurice-Alexandre Pouyanne (1867-mid 20th century)** was an amateur botanist and President of the Court of Appeal in Sidi-Bel-Abbès, Algeria, who performed detailed observations on the fertilization of several *Ophrys* orchids in his home region of North Africa. In **1916**, Correvon introduced Pouyanne's findings to the famous **National Botanical Society of France**, (today known as the **F.F.O. - Fédération France Orchidées**), in a threefold, co-authored paper (Correvon & Pouyanne, 1916). In this pioneering, ground-breaking, first report, Pouyanne describes with astonishing, accurate detail how the flowers of ***Ophrys speculum***, the **Mirror Orchid (Fig. 1)** are pollinated by the orchid's only known pollinator, the male scoliid wasp, ***Dasyscolia ciliata*** Fabricius, 1787 (Family Scoliidae Latreille, 1802; Order Hymenoptera Linnaeus, 1758). He noticed that the solitary ***male*** insects often attempt ***copulation*** with the ***pseudo-female flowers***, a phenomenon later termed as ***precopulatory courtship behavior***, ***pseudocopulation*** or ***Pouyannian mimicry*** **(Fig. 2)**. Stimulated by the striking resemblance to a wasp female, whose ***shape, size, color, texture*** and ***pilosity*** are perfectly mimicked by the orchid, the male reaches high levels of

sexual stimulation on the orchid labella (Correvon & Pouyanne, 1923).

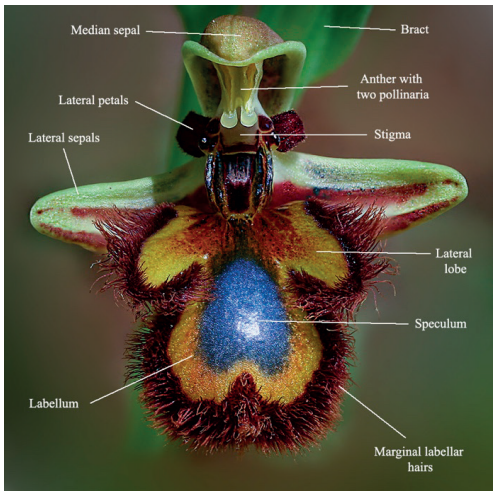


Fig. 1. *Ophrys speculum* - detail of the flower and the central area of the labellum, the ***speculum***. The ***intense brilliant blue colour of the speculum***, which has fascinated scientists and naturalists for many years, and the ***glossiness of the labellum***, have been hypothesized to improve the *sexual mimicry* of the flower by resembling the *sheen* on the *folded wings of an insect at rest* (Viniolini *et al*., 2012). Photo courtesy of Helmut Presser (Greece)

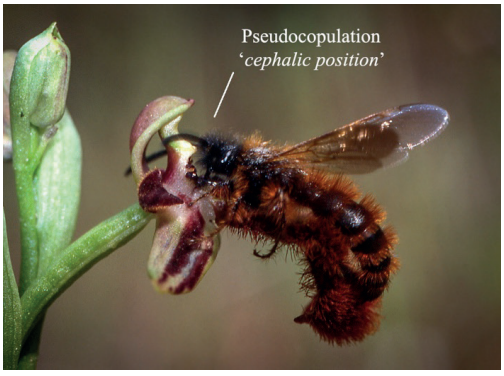


Fig. 2. A male scoliid wasp, *Dasyscolia ciliata*, pseudocopulating the flower of the Mirror Orchid, in ***cephalic position*** on the labellum. Guided by '*the reddish-brown colour of the hairs of the labellum [that] matches almost miraculously the colour of the body pubescence of the female wasp'* (Paulus, 2006), the male is positioned with the head toward the centre of the flower, thus collecting the pollinaria on its head. Photo courtesy of Helmut Presser (Greece)

After several unsuccessful attempts to mate, the ignored, naïve male will take flight, often with the ***pollinia*** firmly attached to its body, usually the head or abdomen. During a successive visit, the pollinator will transfer the pollinia to the stigma of another flower, during pseudocopulation. Since mating signals are usually specific to a certain species of insects, pollinator attraction by sexual deception is also ***very specific***, with each orchid species only attracting one or two insect species (Stökl *et al.*, 2009). In this report, Pouyanne also provides the first adaptive explanation for the *Ophrys* insect-like flowers, which he suggests were devoid of nectar but instead they were able to attract the insects by a ***complex sexual mimicry strategy***, involving ***visual***, ***tactile*** and ***chemical (olfactive - pseudo-pheromones) signals***.

John Godfery & Bertil Kullenberg

The pioneering findings of Correvon and Pouyanne were further confirmed and extended on other representatives of the genus *Ophrys* from southern France, by British naturalist **Masters John Godfery (1856-1945)**. Godfery's classification of the genus *Ophrys* introduced the section *Pseudophrys* (Godfery, 1928), which encompasses species that deliver the pollinaria on their ***pollinators' abdomen*** tip during pseudocopulation, hence the term ***abdominal position*** **(Fig. 3)**. This pollination strategy is used by sympatric orchid species which have the same pollinator, in order to achieve reproductive isolation (and prevent hybridization), by a mechanical barrier (*abdominal* vs. *cephalic* pseudocopulation), provided by a different micromorphological pattern of the labellum (Schlüter & Schiestl, 2008; Cortis *et al.,* 2009). The rest of the species, characterized by ***cephalic position***, were included in section *Euophrys* (syn. *Ophrys*, Godfery, 1928). However, both sections, *Pseudophrys* (Godfery, 1928) and *Euophrys* (syn. *Ophrys*, Godfery, 1928) are no longer in use today.

Edith Coleman & Bertil Kullenberg

It was not long before the extraordinary findings on sexual deception mimicry, which were first met with disbelief by their contemporaries since they stroke as ***highly anthropomorphic***, were confirmed on other continents as well. During the late 1940s, the Swedish entomologist **Bertil Kullenberg (1913-2007)** performed a series of

studies on *Ophrys* pollination, both in Sweden but also, in Morocco. He reconfirmed Pouyanne's and Godfery's findings/results on the deceiving nature of the nectarless/rewardless

Fig. 3. Copulation attempts of a male chaffer of the genus *Blitopertha* Reitter, 1903, (Superfamily Scarabaeoidea Latreille, 1802; Order Coleoptera Linnaeus, 1758) in the ***abdominal position*** on the flower labella of *Ophrys urteae* Paulus, with ***pollinaria*** on its abdomen. Photo courtesy of Helmut Presser (Greece)

Ophrys species and on the emission of their almost imperceptible scent. Kullenberg also published the first photograph of a male scoliid wasp *Dasyscolia ciliata* pseudocopulating on a flower of *Ophrys speculum* (Kullenberg, 1949; Vereecken & Francisco, 2014). Kullenberg was among the first to start using modern techniques and technologies in his multidisciplinary approaches in the study orchid pollination, such as a spectrophotometer to measure the relative reflectance curves of the colours and gas chromatography (GC) to analyse the of volatiles emitted by the flowers and insects. He was particularly interested in insect chemical ecology and behaviour. Based on Pouyannes' observations/studies, Kullenberg was also the first to establish the hierarchical importance of the different floral stimuli (olfactory, visual, and tactile), demonstrating that the specific floral scent emitted by each orchid species is crucial in long distance attraction of specific pollinators.

Once landed, the pollinators are further stimulated by the micromorphological pattern of the labellum, which tactilely stimulates the male insects (***thigmotaxis***) and guides them into the right positioning for the most efficient uptake of the orchid's pollinaria. Following Kullenberg observations/studies, Borg-Karlson (1990), used electrophysiological tests [gas chromatography coupled with electro-antennographic detection (GC-EAD) and mass spectrometry (GC-MS)] to identify organic compounds emitted by *Ophrys* flowers or by females of their pollinators, paved the way for a new generation of studies (Vereecken & Francisco, 2014).

Aims of the present study are: (1) to briefly summarize the history of orchid pollination by sexual deceit from Darwin to the present day; (2) to give a detailed taxonomical overview of *Ophrys* genus in Romania; (3) to describe in detail the main morphological characteristics of Romanian *Ophrys* taxons, by using advanced techniques of ultra-macro photography; (4) to illustrate this exceptional ***deceptive pollination strategy*** on the particular Romanian representative species included in this study.

MATERIALS AND METHODS

1. Study species: All four representatives of the Romanian orchid flora are the subject of this study **(Table 1).**

Table 1. Detailed list and taxonomical classification of the Romanian taxons, which belong to four separate clades or metaspecies (Bateman, Sramkó, & Paun, 2018)

***Ophrys* Clades**				
Clade *Insectifera*	**Clade *Apifera***		**Clade *Scolopax***	**Clade *Sphegodes***
Ophrys insectifera L., 1753	*Ophrys apifera* Huds., 1762	*Ophrys apifera* Huds., var. *aurita* (Moggr.) Gremli, 1887	*Ophrys oestrifera* (Steven) K.Richt., 1890	*Ophrys sphegodes* Mill., 1768

2. Study sites, populations counts and time frames: Studies of several populations of the four mentioned taxons were conducted between 2017-2021, in various locations across Romania, where they occurred in more or less stable populations, each year **(Table 2).**

Table 2. Describes in detail the locations (names), altitudes (a.s.l.- above the sea level), substrates, types of habitats, number of individuals, year(s) and the number of hours of pollination monitoring

Ophrys insectifera						
Location(s)	**Altitude (a.s.l.)**	**Substrate**	**Type of habitat**	**No. of individuals**	**Year**	**No. of hours**
Bucegi Natural Park	1020 m	Alkaline/ calcareous	Mixed forest Partial shade	20-25	2017 2018 2019 2020 2021	5 4 3 5 4
Nistorești, Prahova County	520 m	Alkaline/ calcareous	Deciduous forest Partial shade	2-3	2017 2018 2019 2020 2021	3 4 4 3 5
Breaza, Prahova County	530 m	Alkaline/ calcareous	Mixed forest Partial shade	1-2	2017 2018 2019 2020 2021	3 5 3 4 5
Ophrys apifera						
Iron Gates Natural Park	80-85 m	Alkaline/ calcareous	Grassy meadow Full-sun	12	2017 2018 2019 2021	3 4 3 5
Râmnicu Sărat, Buzău County	150-158 m	Alkaline/ calcareous	Grassy meadow Full-sun	2	2021	6
Ophrys apifera* var. *aurita						
Bozioru, Buzău County	410-415 m	Alkaline/ calcareous	Grassy meadow Full-sun	2	2018	1
Râmnicu Sărat, Buzău County	150-158 m	Alkaline/ calcareous	Grassy meadow Full-sun	2-3	2021	6
Ophrys oestrifera						
Bucegi Natural Park	990-1010 m	Alkaline/ calcareous	Grassy meadow Full-sun	2-3	2017 2018 2019 2020 2021	5 4 4 3 6
Nistorești, Prahova County	528 m	Alkaline/ calcareous	Deciduous forest Partial shade	4-6	2017 2018 2019 2020 2021	4 4 3 4 5
Breaza, Prahova County	545 m	Alkaline/ calcareous	Grassy meadow Full-sun	3-4	2017 2018 2019 2020 2021	5 4 3 5 4
Tărlung River Brașov County	518-522 m	Alkaline/ calcareous	Grassy, swampy meadow Full-sun	14-18	2018 2019 2020 2021	5 4 5 5
Râmnicu Sărat, Buzău County	150-158 m	Alkaline/ calcareous	Grassy meadow Full-sun	3-4	2021	6
Ophrys sphegodes						
Tinăud, Cluj County	240-250 m	Alkaline/ calcareous	Grassy meadow Full-sun	100-120	2017 2018 2020 2021	4 8 6 8

3. Pollination monitoring: The inflorescences were studied from the beginning to just after the peak of anthesis, when the flowers are freshly opened and most attractive to the pollinators. The observer (NA) was initially located approximately 6-8 meters from the subjects. Once the insects were observed to have landed on the labellum, the observer (NA) recorded in writing the frequency of the visiting insects, the time spent on the flower/ inflorescence and the pollinia removal. On several occasions, the observer (NA) approached the flowers and the behaviour of visitors was recorded on digital photographs, from the moment they landed on the labella, until they left flowers/inflorescence.

4. Digital photographic equipment: Individual plants were photographed with body cameras: Canon 5D Mark III, Nikon D3 and Nikon D850. lenses: Nikon Micro NIKKOR 60mm, Venus Optics Laowa 100mm 2X Ultra Macro and Canon MP-E 65mm 1-5x Macro Lens. Additional equipment: Manfrotto Tripod, Litra Torches 2.0s. Adapted Helion FB tube was used for Automated Focus Bracketing. Images were analysed using Adobe Photoshop CC 2021, Helicon Focus and Zerene Stacker Software (previously used by Anghelescu *et al*., 2021b).

RESULTS

1. General description: *Ophrys* are long-lived herbaceous, terrestrial, perennial, geophytes.

2. Habitats: Most *Ophrys* species occur in mesic to dry grasslands, shrublands, verges, open pine woods and wooded meadows, where the cover and height of the herbaceous layer are limited, hence the competition with other herbaceous species is rather low (Eber, 2011; Jacquemyn & Hutchings, 2015). In Romania, *Ophrys* grow on nutrient-poor, warm and dry to fairly moist, alkaline/calcareous substrates (chalk and limestone), sometimes (slightly) acidic bogs (*Ophrys insectifera*, *Ophrys oestrifera*), in full sunlight, on grassy meadows, short grasslands or open woodland (pine, oak, beech). They can also be found growing adjacent to deciduous/mixed forest margins, in partial to heavy shade (*Ophrys insectifera*). The altitude preferred by Romanian *Ophrys* species is from 150-1200 meters a.s.l.

3. Morphology: Non-reproductive organs: They may present two to three ovoid-ellipsoid, entire ***root-tubers***. ***Stems*** are flexuous, spindly (*Ophrys insectifera* **- Fig. 4a**) or thicker (*Ophrys apifera*), fleshy, glabrous (hairless), yellowish-green (*Ophrys insectifera*, *Ophrys sphegodes*) to vivid green. ***Leaves*** are green, entire, sometimes yellowish-green (*Ophrys insectifera*) or glaucous, silvery or greyish-blue (*Ophrys oestrifera* **- Fig. 4b**), near erect, round to linear-oblong, unspotted and usually form a basal rosette (except *Ophrys insectifera*). ***Bracts*** are concave, leaf-like, smaller. ***Inflorescences*** are lax, few flowered (*Ophrys sphegodes* - bearing up to 3-4 flowers) to floriferous (*Ophrys insectifera*, *Ophrys oestrifera* - bearing up to 16-18 flowers). ***Flowers*** are usually showy, vividly coloured, insect-shaped (insectiform). ***Sepals*** are large, greenish, rose-pinkish to whitish, with the large median sepal bending over the ***gynostemium***.

Fig. 4a. *Ophrys insectifera* & **4b.** *Ophrys oestrifera* – full plants in their natural habitats © NA

Petals are smaller, triangular (*Ophrys apifera*, *Ophrys oestrifera*) or filiform/antenna-shaped (*Ophrys insectifera*), glabrous or hirsute, rose-pinkish to whitish. Their margins are fimbriate (having a fringe or border of hairlike or finger-like projections), sometimes with wavy edges (*Ophrys sphegodes*). ***Labellum*** is insect-shaped, flattened or arcuate to convex, sometimes with two conical protuberances at the base. The surface is brownish coloured, velutinous and short haired (downy). The labellum is three lobed, with two smaller

lateral lobes (with longer haired margins) and a large ***median lobe***, which serves as a landing platform for pollinators. ***Median lobe*** may be bilobed, the lobes being separated by a ***notch*** (incision, groove). The central area of the median lobe is usually adorned with lines and markings or with a glabrous, metallic blue-silvery area, known as the ***speculum*** (*mirror*). The shiny speculum mimics the iridescent folded wings on the back of a female insect. Above the speculum, there is a shield-shaped, vividly coloured area, marked by a line, known as the ***basal field***. On other side of the basal field, there are two small, shiny, dark bulges, known as ***pseudo-eyes***. They were interpreted as mimics of the eyes of insects or the tegulae of their wings (Paulus 2006). Darwin was among the first to notice these shining bulges with '*an almost metallic luster, appearing like two drops of fluid*' (Darwin, 1862, p. 57), near the base of the *Ophrys* labellum and regarded them as a likely example of '*sham nectaries'* (Sprengel, 1793). However, their location close to the stigmatic surface of the flower led him to suppose that these shining protuberances, were the attractive feature that would draw the insects' attention (Vereecken & Francisco, 2014). The labellum has ***no spur*** and ***no nectar*** is secreted. It ends into a distinct, deflexed, horizontal or upturned (apical) green, fleshy ***appendage***/***appendix***, which presumably provides a tactile stimulation to the male insects during pseudocopulation. The ***appendage***, which shows great species-specific variability in size and shape, is considered a putative ***osmophore*** believed to be responsible for much of the highly volatile long-range attractants (***pseudopheromones***) production in the flower.

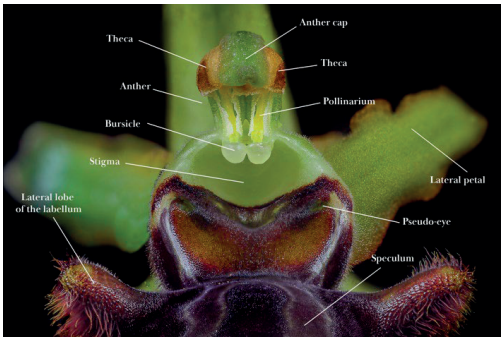


Fig. 5. *Ophrys sphegodes* - flower close up. © NA

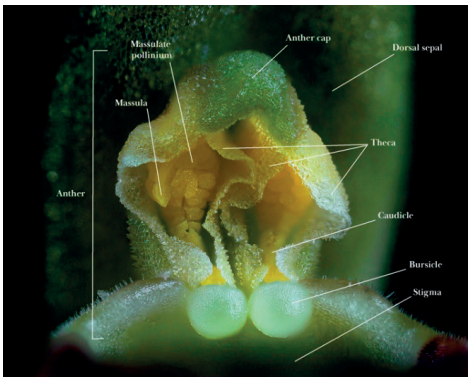


Fig. 6. *Ophrys sphegodes* - dehiscent anther frontal view (pollinarium & anther cap); inside the globular ***bursicle***, the ***viscidium*** is protected from drying © NA

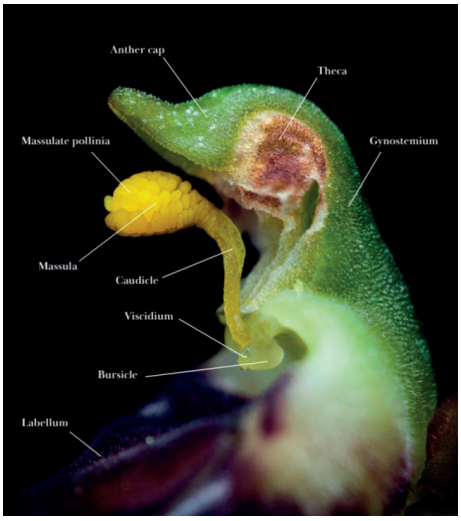


Fig. 7. *Ophrys sphegodes* - ***dehiscent anther*** side view; the ***thecae*** are dehiscent, each containing a ***pollinarium*** that terminates in a globular ***bursicle*** which contains the adhesive ***viscidium***. © NA

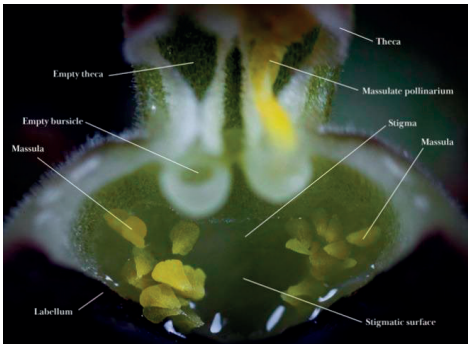


Fig. 8. *Ophrys sphegodes* - ***stigmatic cavity*** containing several ***massulae*** that started to germinate on its wet, sticky surface. © NA

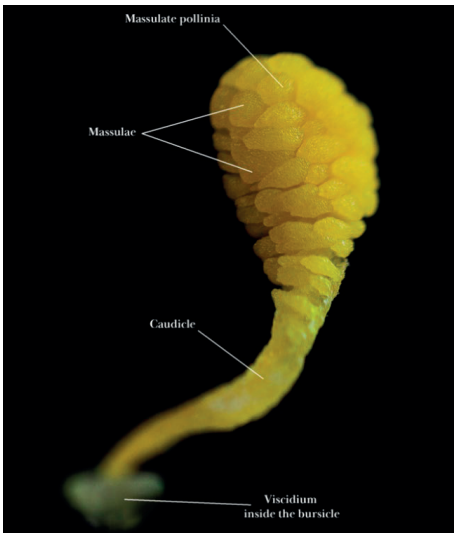


Fig. 9. *Ophrys sphegodes* - entire ***massulate pollinarium*** composed of several ***compacted blocks of pollen grains***, the ***massulae*** that form the ***pollinium***, followed by a ***caudicle*** and the adherent disc, the ***viscidium*** © NA

Reproductive organs. ***Gynoecium*** (represented by the ***stigma***, placed at the base base) and ***androecium*** (represented by the ***unique anther***, placed on the top of the stigmatic cavity) are fused into an elongated, central organ, known as the ***gynostemium***. ***Anther*** is bithecal/dithecal (bi-chambered). Each ***theca*** contains a yellow, club-shaped ***pollinarium*** (two parallel pollinaria are thus present per *Ophrys* flower). Each ***pollinarium*** is composed of a flat, translucent, adhesive disc - the ***viscidium***, followed by an elongated stalk - the ***caudicle*** and ending with a swollen, apical part - the ***pollinium***. In *Ophrys* genus, the pollinium is termed as ***massulate pollinium***, being composed of numerous, compacted blocks of pollen grains, which cohere well and form the wedge-shaped ***massulae***. The only other plant group which also has developed pollinia also is the milk weeds,
Asclepiadiaceae. Presumably for the same reasons, they have also developed a large number of deceptive flowers (Ollerton & Liede, 1997). There are ***no auricles*** and ***no staminodia*** present (Claessens & Kleynen, 2011; 2016). ***Stigma*** is roundish, elliptic, concave, three-lobed, with a large median lobe, the ***rostellum***. ***Rostellum*** is three lobed and roof-like, placed above the stigma, preventing the contact of the pollinia with the stigma (preventing self-pollination). ***Median rostellar lobe*** is small and the ***lateral rostellar lobes*** carry a ***globular bursicle***, each. Each ***bursicle*** contains an individual, round, flat, adhesive and translucent ***viscidium***. ***Ovary*** is sessile, not or slightly twisted, glabrous, bent over, bringing the lip into a downwards position. (Woods, in Pridgeon *et al*., 2001; Delforge 2005; 2006).
4. Chromosome numbers: 2n = 2x = 36. However, tetraploidy was shown in the case of *Ophrys fusca*: **2n = 4x = 72** (D'Emerico, in Pridgeon *et al*., 2001).
5. Flowering time: *Ophrys* species flower once a year, from April to June, depending on the altitude (De Angelli & Anghelescu, 2020). It is common that individual plants do not necessarily flower every year (Hutchings, 2010) and the proportion of plants that flower within a population greatly varies from year to year. For example, during long-term studies in southern England, it has been observed that the average frequency of plants flowering from one year to another was 27.4% in *Ophrys apifera* and 83.7% in *Ophrys sphegodes* (Wells & Cox, 1989; Hutchings, 2010). Successful flowering usually depends on tuber size, leaf number and the amount of rains during autumn, when the leaves start to appear above ground (Wells & Cox, 1989). ***Drought*** represents a very impotant limiting factor, as it prevents inflorescence formation and also causes premature sensecence of the flowers, thus reducing the chances of pollination and seed production (Neiland, 1994).
6. Pollinators: In Romania, the most currently known pollinators of *Ophrys* species are male bees and wasps of the **Order Hymenoptera** Linnaeus, 1758. They belong to the widely distributed families: **Apidae -** *Eucera longicornis* (Linnaeus, 1758), unique pollinator of *Ophrys oestrifera*, **Andrenidae** - *Andrena nigroaenaea* (Kirby, 1802), unique pollinator of *Ophrys sphegodes* and **Crabronidae** Latreille, 1802 - *Argogorytes mystaceus* (Linnaeus, 1761), unique pollinator of *Ophrys insectifera*. In the Mediterranean Basin, *Ophrys apifera* is accidentally visited *by Eucera longicornis.*
7. Pollination strategies: All *Ophrys* species are self-compatible (able to self-pollinate). However, except *Ophrys apifera* (self-pollinated), all other species employ pollination by ***sexual deception*** to attract their highly specific male pollinators. A single pollination

event implies the removal of the pollinium and the deposition of the pollinia on a second flower.

***Ophrys insectifera* L., 1753 - Fly Orchid**

The ***specific epithet***, ***insectifera***, originates in the Latin ***insectum*** (insect) and ***phór(os)*** (to carry) meaning ***carrying an insect***, a reference to ***insect-shaped labellum*** that mimics the shape, size, color, pilosity, texture and smell (***pseudo-pheromones***) of a female insect.

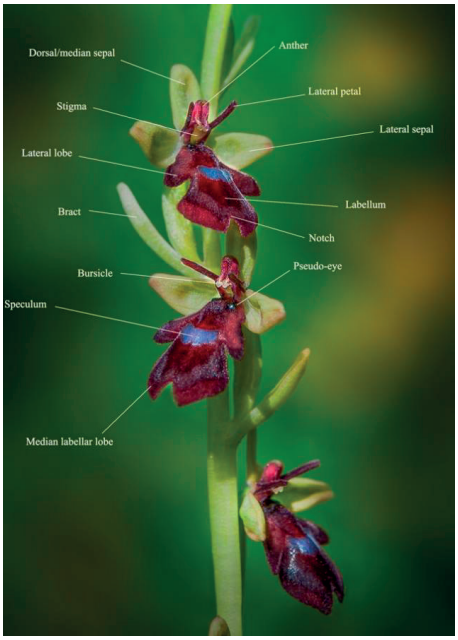


Fig. 10. *Ophrys insectifera* - inflorescence detail. The brown-reddish flowers resemble bees perching on a blade of grass. The ***lateral petals*** are long and filiform, ***mimicking insect antennae***. © NA

Fig. 11. *Ophrys insectifera* - ***speculum*** detail. It is metallic-blue colored (sometimes grayish) and shiny, mimicking the ***folded wings of a female insect.*** © NA

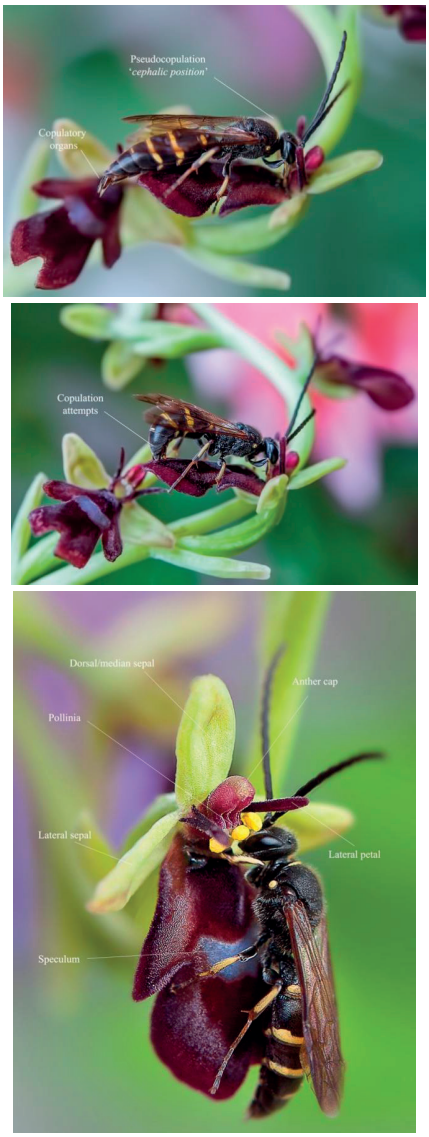


Fig. 12-14. *Ophrys insectifera* - pollinated by pseudocopulation by the solitary wasp, *Argogorytes mystaceus.* Attracted by the ***orchid's pheromones***, it is seduced by the visual stimuli offered by the labellum that resemble a female wasp. Aroused by the ***sexually-stimulating hairs on the labellum***, the male aligns with the labellum and ***attempts to copulate*** with the orchid flower. After several unsuccessful attempts to mate and frustrated by the lack of interest from the insensible (***pseudo***) ***female,*** the male flies off with the ***pollinia*** (yellow pollen sacs) firmly attached to its body, in frantic search of more authentic female companionship. The process of **pseudocopulation** took about **7-8 minutes**, during which the males wasp pseudocopulated with all freshly opened flowers, spending up to **72 seconds on each flower**. © NA

***Ophrys oestrifera* M.Bieb., 1808 - Botfly Orchid**

The ***specific epithet***, ***oestrifera***, comes from the family of botflies, **Oestridae** Leach, 1815, and the Latin ***phór(os)*** (to carry) meaning ***carrying a (furry) fly***, a reference to ***insect-like furry labellum*** that mimics the botflies/maggot flies.

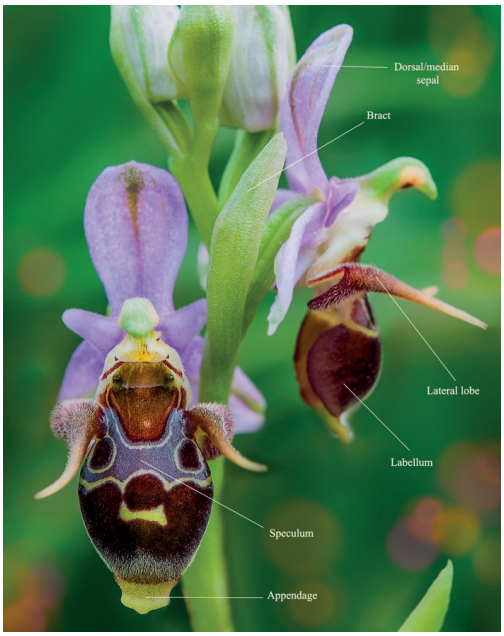


Fig. 15. *Ophrys oestrifera* – ***inflorescence*** detail. The flowers are large, with pinkish-violet lateral petals and sepals. The ***labellum*** is brounish, furry and has a shiny, metallic-blue, edged yellowish, ***speculum***, in the form of ***H or X***; the ***green appendage*** is prominent. © NA

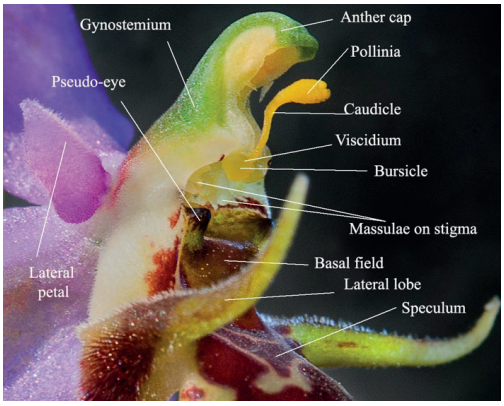


Fig. 16. *Ophrys oestrifera* – ***stigmatic cavity*** entirely filled with ***massulae***, as a result of successful pollination. © NA

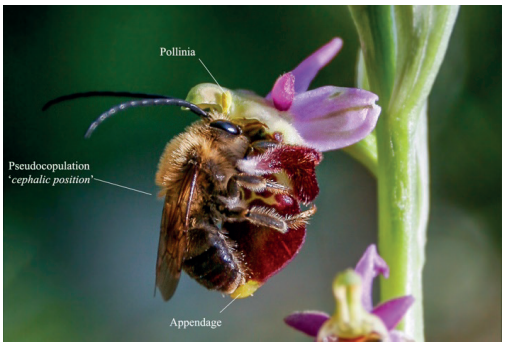


Fig. 17. *Ophrys oestrifera* pollinated by pseudocopulation by a ***male Eucera longicornis***. A **Long-horned** male bee embraces the labellum in a ***pseudocopulation attempt***, touching the ***green appendage*** (which mimics ***female reproductory organs***) with its copulatory organs. Trying to find the female's head, the male sticks its head in the center of the flower, touching the stigma and thus removing the ***pollinia***. The ***pseudocopulation*** process took **6 minutes**, with approximately **68 seconds** per flower.

Fig. 18. *Ophrys oestrifera* – new individual plant in process of getting pollinated by a pollinia-carrier, male *Eucera longicornis.* The **long-horned male bee**, with the ***pair of pollinia*** stuck on its head (from a previous ***pseudocopulation attempt***) is approaching a new, unpollinated inflorescence. **Photos 17-18** courtesy of **Dan Anghelescu & George Avanu**.

***Ophrys sphegodes* Mill., 1768 - Spider Orchid**

The ***specific epithet***, ***sphegodes***, originates in the ancient Greek word ***sphêx***, ***sphec*** (wasp), *ad litteram* meaning ***carrying a wasp***, a reference to the ***wasp-like labellum***. However, *Ophrys sphegodes* is not pollinated by wasps,

but by ***bees only***. Due to a distinctive, highly variable **H** markings on the labellum (even among the flowers of the same inflorescence), which resemble a ***spider***, and its early-spring flowering time, *Ophrys sphegodes* is also known as the **Early Spider Orchid**.

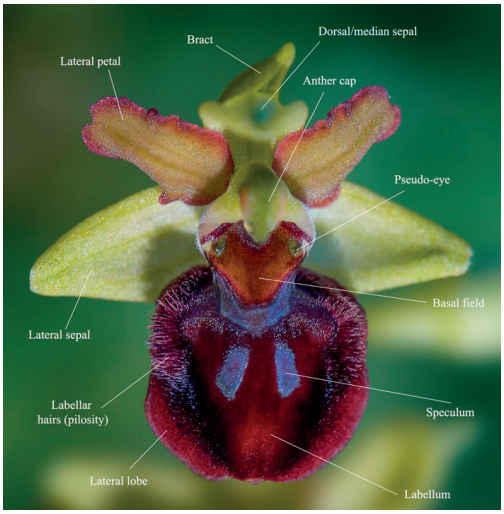


Fig. 19. *Ophrys sphegodes* – flower detail. The ***flowers*** are medium-sized. The ***sepals*** are greenish yellow. The ***lateral petals*** are auriculate, greenish, washed red. The ***labellum*** is brownish red to dark brown, with furry margins and a velvety surface, trilobed. The ***median lobe*** has a central H-shaped bluish-grey to reddish-brown speculum. © NA

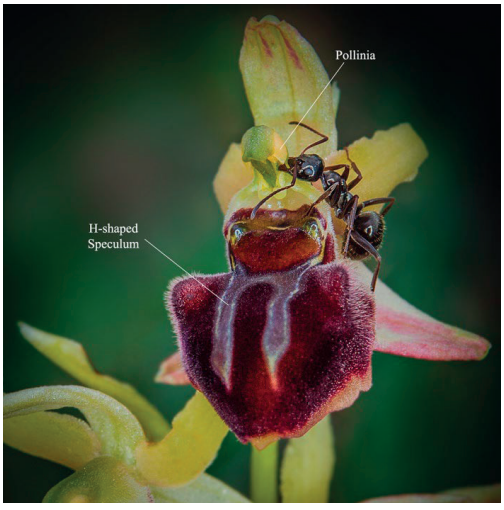
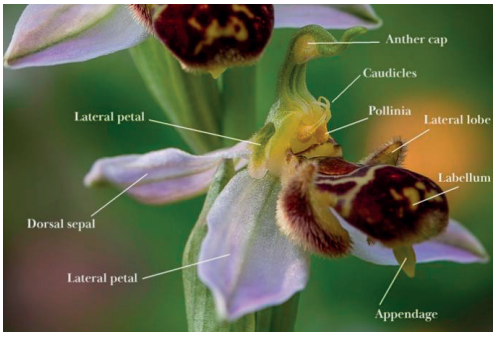


Fig. 20. *Ophrys sphegodes* – insect visitors. Its usual pollinator is the mining bee, *Andrena nigroaenaea*; the flowers are often visited by numerous insects, e.g., **wood ants**, *Formica (Serviformica) cinerea* Mayr 1853, which rob the orchid's pollen by chewing the pollinium. © NA

***Ophrys apifera* Huds., 1762 - Bee Orchid**

The ***specific epithet***, ***apifera***, originates in the Latin ***apis*** (bee) and the Greek ***phór(os)*** (to carry), meaning ***carrying a bee***, a reference to the ***bee-like labellum***. Despite its name, *Ophrys apifera* is never pollinated by bees, this orchid being an ***exclusively autogamous orchid*** that ***self-pollinates***.



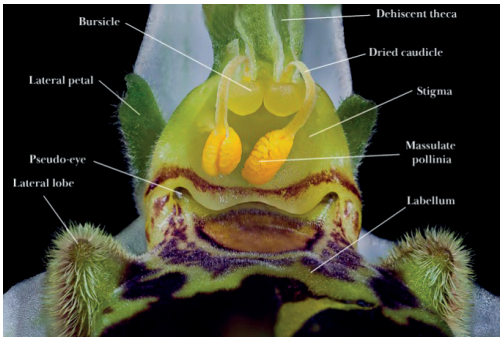


Fig. 21-22. *Ophrys apifera* – ***self-pollination***. Shortly after anther dehiscence (within mimutes), the long ***caudicles*** wither and bend forwards and downwards, until the ***massulate pollinia*** reach their own ***stigma***, under below. A gentle breeze is often enough to blow them onto the sticky stigmatic surface. © NA

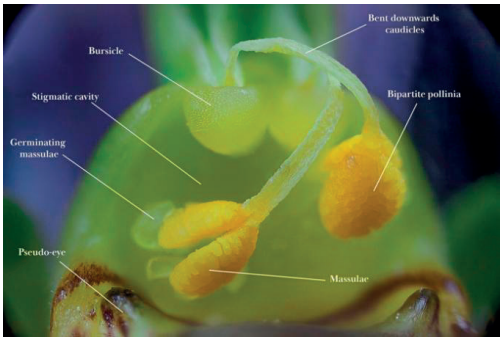


Fig. 23. *Ophrys apifera* – ***stigmatic cavity*** filled with ***massulae***, which adhered to the ***stigmatic surface*** and started to germinate (self-pollination). © NA

Fig. 24. ***Ophrys apifera*** **var.** ***aurita*** **(Moggr.) Gremli, 1887**. The ***infraspecific epithet*** (***variety epithet***), ***aurita***, originates in the Latin ***aurīta*** (long ears), meaning ***ear-shaped***, a reference to the ***filiform, elongated lateral petals***, which resemble ***two pricked up ears***. © NA

8. Fruit: Many *Ophrys* species can flower and fruit for several years in succession (Hutchings, 1989; Wells & Cox, 1991). Therefore, although the species are capable of producing fruit more than once, all orchid plants are effectively ***monocarpic*** because they may flower only once before dying. Each fertilised *Ophrys* flower produces a single, elongated, ovoid, erect, ***greenish fruit pod***. ***Fruit set*** varies from year to year, greatly depending on the presence of the pollinators and on the exposure to sunlight. Our observations/counts show relatively high fruit set, e.g., *Ophrys insectifera* (20-35%), *Ophrys oestrifera* (30-32%), *Ophrys sphegodes* (17-26%) and *Ophrys apifera* (35-45%).

9. Seeds: The ***seeds*** usually mature by July-August/September, depending on the species. The greenish ***fruit pod*** matures and transforms into a dehiscent, brownish ***seed capsule***, closed at both ends. *Ophrys* seed capsules are fairly large and may contain in average 5000-20.000 tiny seeds per capsule (Arditti & Ghani, 2000), which is more than any other European orchid genera (Neiland & Wilcock, 1995).

DISCUSSIONS

The use of DNA analyses for *Ophrys* systematics led to major controversies regarding the number of species, ranging from **251 species** (Delforge, 2006) to **9-11 macrospecies** or ***clades*** (***molecularly cohesive monophyletic groups***) (Bateman *et al*., 2018): *Insectifera*, *Apifera*, *Umbilicata*, *Fuciflora* (incl *Scolopax*), *Sphegodes*, *Speculum*, *Bombyflora*, *Tenthredinifera* and *Fusca*. The species richness is mostly due to a high degree of hybridisation, with parents and hybrids (partially) sharing the pollinator communities, a phenomenon common to other genera (Anghelescu *et al*., 2020b). Although most researchers accept that some taxonomic exaggeration exists, ***intrageneric hybridization*** is considered a source of evolutionary novelties that could ultimately lead to ***pollinator shifts*** and ***reproductive isolation*** (***speciation***) (Baguette *et al*., 2020).

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PRELIMINARY STUDIES ON THE CURRENT STAGE OF RESEARCH ON THE PRODUCTION OF PLANTING MATERIAL AND GERBERA CUT FLOWERS IN DIFFERENT VARIANTS OF HYDROPONIC CULTURE

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Abstract

The study was carried out in the doctoral thesis entitled, Research on the production of seedlings and flowers cut by gerbera in various variants of hydroponic cultivation, and refers to the identification of the best way to obtain as many flowers per plant as well as recommendation of the best type of substrate for obtaining cut flowers. In the study we used three varieties of gerbera, 'Dune', 'Balance' and 'Blind Date''. The substrate variants used in the experiments were: V1 - peat with a pH of 4; V2 - Perlite; V3 - 50% Perlite + 50% peat with pH-4; V4 - 50% Perlite + 50% peat with pH 5.5; and V5 - Peat with pH 5.5 (Control Variant). We followed the response of plants grown in different types of substrate on the growth, development and yield of gerbera flowers in the unconventional system. Analyzes and correlations were performed with environmental factors to determine their influence on the number of flowers and their quality.

Key words*: gerbera, cultivar, substrate, soilless.*

INTRODUCTION

Gerbera (*Gerbera jamesonii*), family *Asteraceae*, order *Asterales,* is one of the flowering species that has a diversity of shapes being attractive through the colors of the inflorescences.

Toma (2009) mentions that at the basis for obtaining the horticultural species is the genus *Gerbera* which includes about 40 herbaceous species. He states that the most frequently cultivated forms are the species from which the cross resulted from the horticultural species *Gerbera hybrida* Hort. namely the species *Gerbera jamesonii* Bolus et Hook and *Gerbera viridifolia* Sch.

Yuniarto et al. (2018) appreciate that the gerbera is a species of great importance for some growers and is also highly valued as a flower in the pot.

Gerbera is a species sensitive to low temperatures (Mansito and Aballero, 1989; Singh and Mandhar, 2002; Gelder, 2014), diseases and pests, nutrition regime (Savvas, 2001). Pettersen and Gislerød (2003) estimate based on research conducted in the greenhouse that the highest yield on the growth of gerbera plants as well as the formation of the number of flowers per plant was obtained at a lighting time of 20 hours. Panter et al. (2016) emphasize that additional LED lighting contributes to an increase in production.

As for the gerbera culture substrate, it needs to be very well aerated, light, well supplied with nutrients. All types of substrate used in unconventional crops, vegetables and flowers can be used with very good results (Drăghici, 2017. Gerbera (*Gerbera jamesonii* L.) is one of the flowering species that can be grown in different substrates perlite, mineral wool, vermiculite, sand, coconut fiber (cocopeat), clay, organic substrates, compost, zeolite, pumice stone, sand and so on (Khalaj, 2007; Fakhri et al., 1995).

Different substrate variants were tested such as some volcanic rocks (Barrios-Díaz et al., 2012) zeolite, perlite Maloupa et al. (1993). Guerrer et al. (2017) and William et al. (2010) mention that the granulation of the substrate is very important. In some areas but also in the period with very high temperatures, gerbera plants grown only on the perlite substrate were less developed but those grown on perlite mixed with coconut fibers positively influenced the growth (Paradiso and Pascale, 2008).

In some areas the substrate of rice husks, coconut peat, castor residue and vermicompost in equal proportions (Chauhan et al., 2014) or only peat or vermicompost Arunesh et al., (2020) can also be used.

Growing in containers, pots, polyethylene bags or plastic baskets involves the individual planting of plants in containers and has the same advantages as the culture on tanks. Gerbera grown in pots has recently become more common in interior decoration but dwarf varieties are used.
Sujatha et al. (2002) mention that for a proper vegetative growth of gerbera plants, it is necessary to apply a nutrient solution with an appropriate pH. Enache et al. (2019) based on some studies, he appreciates that the use of structured water in the preparation of the nutrient solution leads to obtaining vegetative growths and production increases.
The application of biostimulators has led to a much better growth and flowering of gerbera plants (Petre et al., 2018; Ahmed et al., 2020).
Carrying out foliar treatments with amino acids (glycine, arginine, asparagine, alanine, tryptophan) led to an increase in the number of leaves per plant, the leaf area, the total mass of plants as well as the number of flowers per plant Abd-Elkader et al. (2020) and Petra et al. (2020).
The aim of the study was to identify the best culture substrate to obtain as many flowers as possible on the plant.

MATERIALS AND METHODS

The study was conducted in the greenhouses of the Hortinvest Research Center between May 2019 - August 2021 The biological material used in the experiments was represented by 3 varieties of gerbera, 'Dune', 'Balance' and 'Blind Date' characterized as follows: 'Dune' is a variety with orange flower, semi-double, with a floral stem of about 60 cm and a flower diameter of 11-12 cm. It is a productive variety, the first flowers appear 12-14 weeks (85-90 days) after planting. 'Balance' has standard semi-double white flowers, with floral stems of 40-70 cm), flower diameter of 10-13 cm and a shelf life of 13-15 days. 'Blind Date' has a red flower, has a diameter of 10-12 cm, the floral stem is about 45 cm.
The substrate variants used in the experiments were: V1- peat with a pH of 4; V2 - Perlite; V3 - 50% Perlite + 50% peat with pH-4; V4 - 50% Perlite + 50% peat with pH 5.5; and V5 - Peat with pH 5.5 (Control Variant). Gerbera seedlings were planted in pots with a capacity of 3 l filled with substrate according to experimental variants. In each variant I used 5 repetitions. In culture, in the unconventional system, the pots were placed on mattresses filled with perlite with a granulation of perlite 5 mm in diameter.
The culture was pursued in the period 2019-2021. The nutrient solution had a pH of 5.5 and an EC of 2.8.
We aimed to ensure the environmental factors in the greenhouse, temperature, light, atmospheric humidity and we recorded the growth of plants, the number of leaves and flowers per plant.
All data were statistically processed.

RESULTS AND DISCUSSIONS

In the 'Dune' variety, in 2019, the first year of cultivation, starting with May, until December, we obtained a total of 13.3 flowers per plant on acid peat substrate with a pH of 4.0. The highest number of flowers per plant was obtained at V 5- Peat with pH 5.5 of 58.31 flowers followed by V3 -50% Perlite + 50% acid peat with 58.23 flowers. Between 2020 and 2021 we obtained the fewest flowers / plant at V1- acid peat pH 4 of 17.5 flowers (2020) and 6.25 flowers / plant (2021). The highest number of flowers per plant was registered in 2020 of 58.75 flowers / plant at V 4- 50% Perlite + 50% peat and in 2021 of only 12.5 flowers / plant until August. At this cultivar, in the period 2019-2021, 37.05 flowers were obtained at V1- acid peat pH 4 and 112.06 flowers at V 5- Peat with pH 5.5, Figure 1).

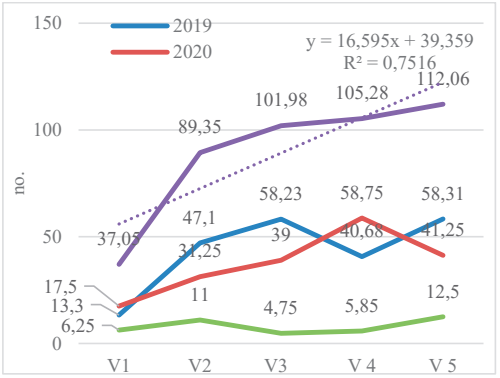

Figure 1. The total number of flowers obtained per plant in the period 2019-2021 'Dune' variety

In the 2019-2021 cultivation period, for the 'Balance' variety, we obtained the highest number of flowers per plant at V5-peat with pH 5.5 of 108.00 flowers/plant compared to V1-peat with pH 4 where we obtained only 83, 10 flowers per plant. We found that the culture substrate had a very large influence R^2 = 0.7458. In 2019, for the 'Balance' variety, we obtained the highest flower production per plant of 53.86 flowers/plant at V 4 - 50% Perlite + 50% peat with 5.5 pH and 43.10 flowers/plant at V2 - Perlite. In 2020 of the number of flowers per plant, the highest number of 43.45 flowers/plant was obtained at V 5 - peat with pH 5.5 and at V1 - peat with pH 4 of only 25.50 flowers/plant. In 2021am we obtained a total of only 10.75 flowers/plant at V 5- peat with pH 5.5 and 18.25 flowers/plant at V2 on Perlite substrate which shows that the fertilizers on this substrate were much better assimilated by to the plant. In August the plants came to rest (Figure 2).

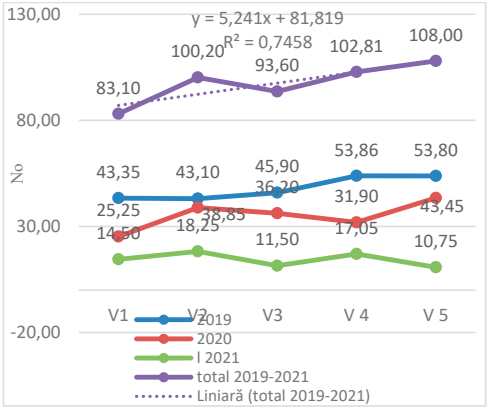

Figure 2. The total number of flowers obtained per plant in the period 2019-2021 'Balance' variety

In the case of the 'Blind Date' variety, a total of 57.00 flowers per plant were obtained at V1 - peat with pH 4 and 102.80 flowers/plant at V3 - 50% Perlite + 50% peat with 4. We found that the type of substrate had an average influence R^2 = 0.5108. In this variety we obtained in year 1 (2019) the highest number of flowers per plant, their number being between 31.5 flowers/plant at V1 - peat with pH 4 and 50.54 flowers/plant at V4 - 50 % Perlite + 50% peat with 5.5 pH. Starting with 2020, the number of flowers per plant began to decrease, obtaining an average of 43.00 flowers/plant at V5 - peat with pH 5.5 and 16 flowers/plant at V1 - peat with pH 4. In the year 2021 we obtained a number of 9.5 flowers / plant at V1 - peat with pH 4 and 20.00 flowers per plant at V2 – Perlite (Figure 3).

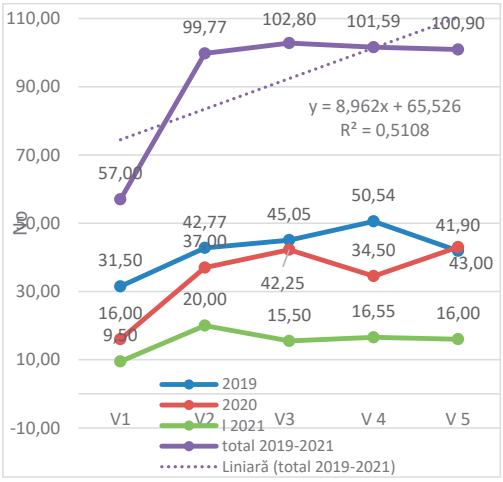

Figure 3. Total number of flowers obtained per plant per plant in the period 2019-2021 variety 'Blind Date'

Analyzing the data obtained for the 'Dune' cultivar, on every month, we could see that on the substrate where we used acid peat (V1-peat with pH 4) we obtained flowers much later after planting, starting with September. We also found that at V 4- 50% Perlite + 50% peat with 5.5 pH by May no flowers were formed on plants compared to V2, V3 and V5 where 8 flowers / plant 9 were obtained, 5 flowers / plant respectively 9.66 flowers / plant (Figure 4.).

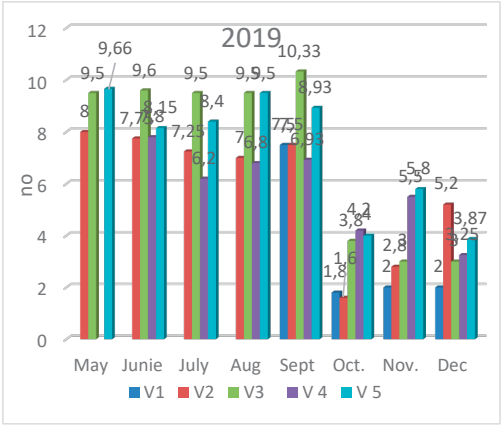

Figure 4. Number of flowers per plant obtained in 2019 from May to December in the 'Dune' variety

In 2020, in the culture of gerbera, during January-December we found that variants 1, 4 and 5 showed a continuous flowering, with a short period of rest in August when the temperatures were higher. Most flowers formed between February-June and September-December for all substrate variants (Figure 5).

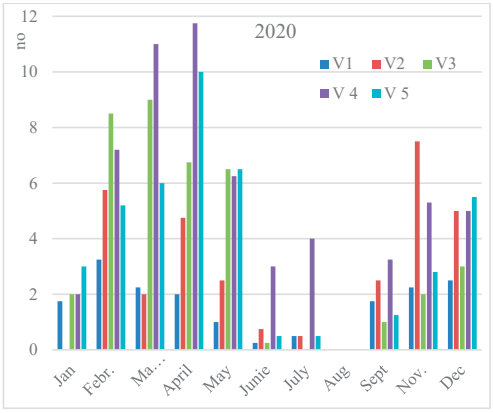

Figure 5. Number of flowers per plant obtained in 2020 from January to December in the 'Dune' cultivar

In 2021, the 'Dune' variety formed a smaller number of flowers per plant compared to the 2019 crop year (Figure 6).

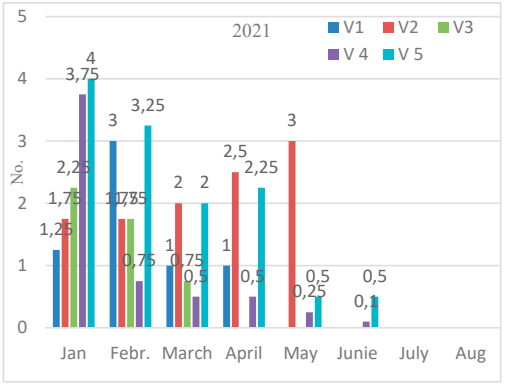

Figure 6. Number of flowers per plant obtained in 2021 from January to December in the 'Dune' variety

In the case of the *Balance* variety, in 2019 we noticed that the plants formed a higher number of flowers per plant in the period May-September followed by a period starting with October in which the number of flowers decreased following as in the following period, by December 2019 it should grow slightly (Figure 7).

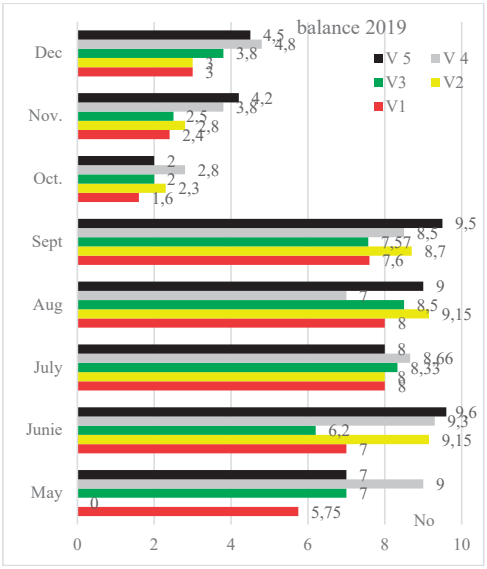

Figure 7. The number of flowers per plant obtained in 2019 for the 'Balance' variety

In 2020, for the 'Balance' variety, we noticed a period of abundant flowering from January to July, followed by a period of vegetative rest starting with August and lasting until November (Figure 8).

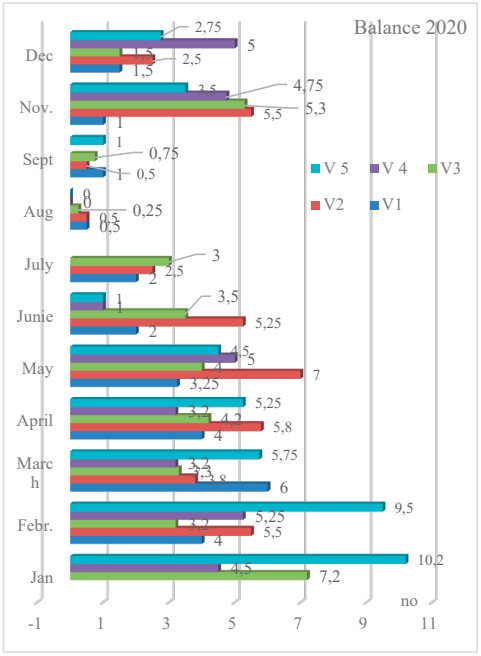

Figure 8. The number of flowers per plant obtained in 2020 for the 'Balance' variety

In 2021 we found an increase in the number of flowers per plant, but smaller compared to 2019 and 2021 (Figure 9).

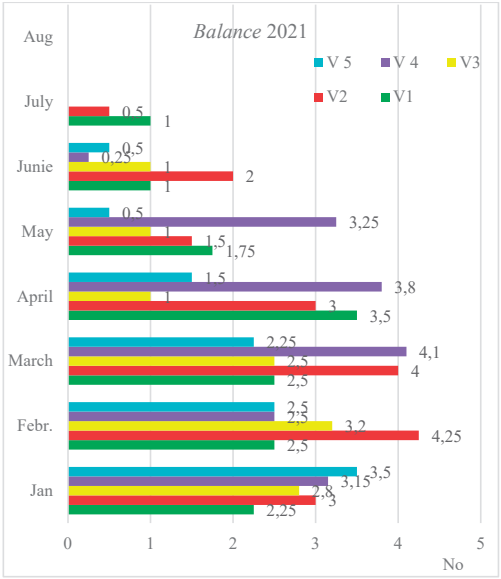

Figure 9. The number of flowers per plant obtained in 2021 in the 'Balance' variety

Figure 10. Aspect from culture in 2019

Figure 11. Aspect from culture in 2020, 'Balance' variety

Figure 12. Aspect of cultivation in 2020 varieties 'Dune' and 'Blind Date'

Analyzing the data on the number of flowers formed per plant in 2019 for the 'Blind Date' variety, we found that the highest number of flowers was obtained in June-September, for all substrate variants. I also noticed that in October the number of flowers per plant decreased, for all types of substrates it will increase by December (Figure 13).

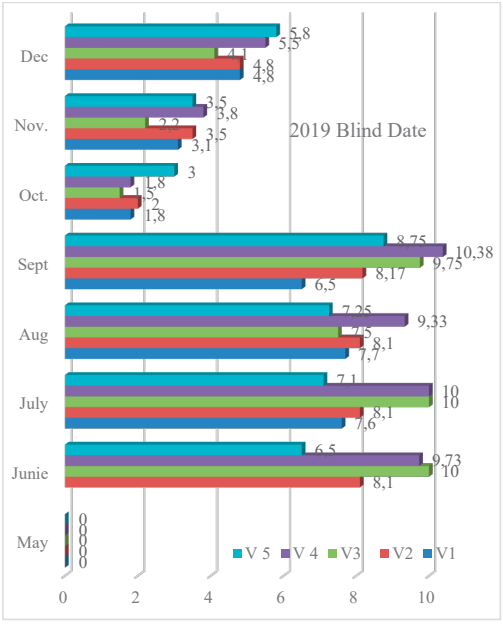

Figure 13. Number of flowers per plant obtained in 2019 in the 'Blind Date' variety

From the Figure 14 we notice the distribution of the number of flowers harvested at the 'Blind Date' variety in the period 2020-2021. We noticed that in 2020 between January and April the number of flowers harvested was higher on the perlite substrate. The number of

harvested flowers started to decrease from July, the plants coming to rest and from November 2020 to increase until May 2021. Starting with June, the plants showed signs of exhaustion.

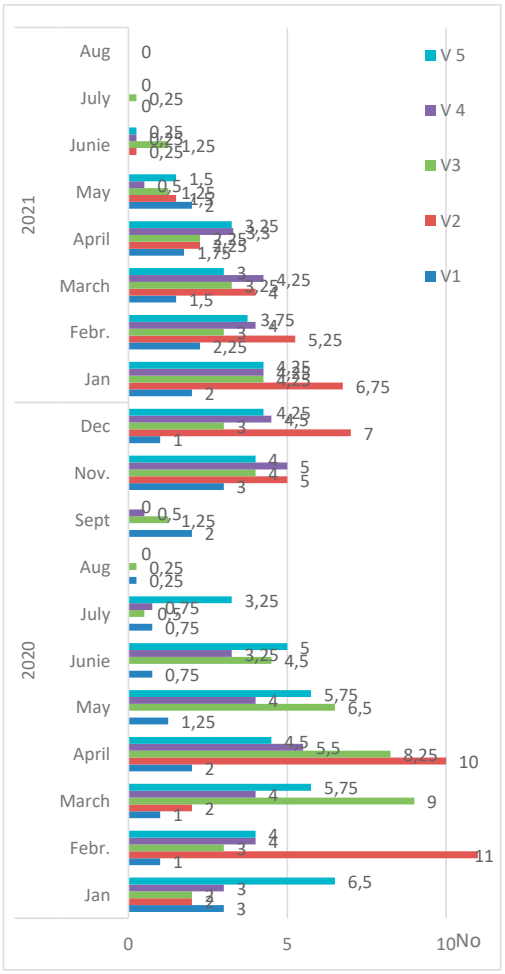

Figure 14. Number of flowers per plant obtained in 2020-2021 in the 'Blind Date' variety

CONCLUSIONS

Cultivation of gerbera varieties on substrates led to high yields of cut flowers. Thus, in the case of the 'Dune' variety, cultivated on a peat substrate with pH 5.5 (V5), a total of 112.06 flowers per plant were obtained compared to the use of a peat substrate with a pH of 4 where they were obtained in the three years of cultivation a number of only 37.05 flowers/ plant. The mixture of peat perlite led to a higher number of flowers per plant.

In the case of the 'Balance' variety in the study period studied, we obtained the highest number of flowers per plant at V5-peat with pH 5.5 of 108.00 flowers/plant compared to V1-peat with pH 4 where we obtained only 83.10 flowers on the plant. The culture medium had an important influence.

In the 'Blind Date' variety, the lowest number of flowers per plant (57.00 flowers) was obtained at V1 - peat with pH 4 and the highest at V3 - 50% Perlite + 50% peat with 4 of 102.80 flowers / the plant.

In the case of all varieties, the highest production of flowers was achieved in the first and second year of cultivation.

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PHYSIOLOGICAL PARAMETERS CHANGES IN HYACINTH BULBS DURING COLD STORAGE

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Abstract

Although the bulbs are produced in many countries, the Netherlands is the center of the world flower bulb industry. Bulbs are specialized underground organs that undergo a series of physiological and biochemical changes during the cold season. The main purpose of this paper was to to compare the correlations between physiological parameters and biochemical changes. The storage conditions for hyacinth bulbs were: T:1°C and RH: 90%, and the five cultivars of hyacinths chosen for analysis (Gipsy Queen, Jan Boss, Miss Saigon, Pink Pearl, White Pearl), these being analyzed at two different moments (before and after the storage period), and the analyzes performed were: respiration and transpiration rates, mass loss, glucose, fructose and soluble solids contents. From obtained results it was observed decreases of the mass loss between 6% (for Jan Boss and Pink Pearl cultivars) and 10% (for White Pearl cultivar), between the initial and the final moment of analyses. For respiration rate were observed increases between 4 times (for Jan Boss cv.) up to 6 times (for Pink Pearl cv), between the initial and final moments. The soluble solids content recorded an increase between 10% for Miss Saigon cv. up to 20% for Gipsy Queen cv., between the initial and final moments.

Key words*: hyacinth, mass loss, respiration rate, transpiration rate.*

INTRODUCTION

Although the bulbs are produced in many countries, the Netherlands is the center of the world flower bulb industry (De Hertogh, 1974). Bulbs are specialized underground organs that undergo a series of physiological and biochemical changes during the cold season (Khodorova, 2013).

The common hyacinth, *Hyacinthus orietalis,* belong to the *Hyacinthaceae* family (Addai, 2011), and is a perennial plant with high resistance to cold (Saniewski, 1977). The height at maturity reaches up to 25-30 cm, depending on the cultivar. It blooms very well both in the sun and in partial shade and lasts 2-3 weeks.

The factors that differentiate vegetative buds into flowering buds are temperature and storage time (Delian, 2013).

Low temperatures during storage cause the end of dormancy and the growth of flowering buds (Burzo, 2016). Hyacinth bulbs, like tulip bulbs, go through rest period during the summer (Burzo, 2016). The geophytes have stored reserves in their bulb (Addai, 2011), this is why the floricultural greenhouse industry use only large bulbs for forcing (De Hertogh, 1974).

Mitochondrial respiration increases when flower formation is induced (Kanneworf, 1994).

The correlation between the reserves of the bulb and the flower production, is important for the flower production industry and it depends on the bulb size (Addai, 2011).

Koksal (2010) suggests that knowledge about biochemical changes during bulb storage is largely related to onion bulbs, rather than ornamental ones.

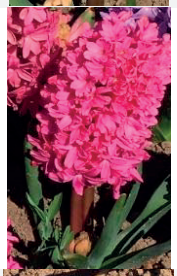
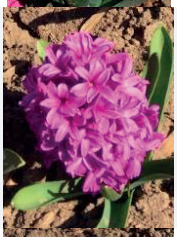
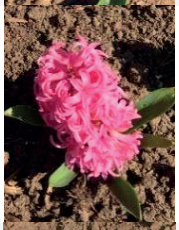
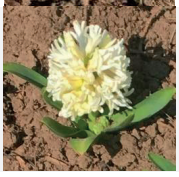
The most important biochemical changes during storage period is the transformation of starch into sugars (Koksal, 2010).

The main purpose of this paper was to to compare the correlations between physiological parameters and biochemical changes of five hyacinth cultivars, before and after the storage period, in cold room conditions.

MATERIALS AND METHODS

The hyacinth bulbs with about 75-85 g, in uniform size, for each cultivar, were stored in perforated paper bag.
The hyacinth cultivars studied were: 'Gipsy Queen' (orange flowers), 'Jan Boss' (red flowers), 'Miss Saigon' (blue-purple flowers), 'Pink Pearl' (intense pink color flowers), 'White Pearl' (white flowers) (Figure 1, Table 1).

Table 1. Features hyacinth cultivars studied

Cultivar	Flowering period	
Gipsy Queen	April – May	
Jan Boss	April – May	
Miss Saigon	April – May	
Pink Pearl	April – May	
White Pearl	March - April	

The bulbs were stored and monitored in cold room (Figure 2), under following conditions: T: 1°C and RH: 90%, for 160 days (Burzo, 2005; Burzo, 2017), in the Postharvest Technologies Laboratory of the Research Center for Studies of Food Quality and Agricultural Products, of the USAMV Bucharest.

Figure 1. Hyacinth cultivars used in this study, in experimental field

The main purpose of this study is to determine the correlations between physiological parameters like: respiration and transpiration rates and biochemical changes like: mass loss, total soluble solids (TSS), and the contents of glucose and fructose, after 160 days of cold storage.

Figure 2. Hyacinth bulbs after 160 days stored in cold storage

Respiration rate was determined with a static, closed system, in containers with hermetic closure with a volume of 1180 ml (Figure 3A, 3B). With the Lambda T NDIR Monitor, ADC BioScientific Ltd., the respiration rate was measured and the results were expressed in mg CO_2/kg/hour (Enciu, 2020; Stan, 2020).
The transpiration rate was measured through gravimetric analysis (Figure 3 C) (Fante, 2014; Enciu, 2020; Stan, 2020) and the results were expressed in g water/100 g f.w./hour.

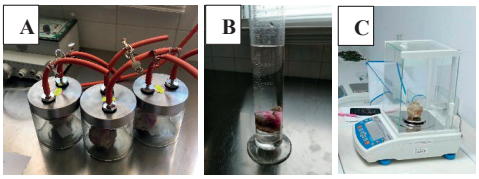


Figure 3. Physiological parameters determination

The water and dry matter contents were determined using the Memmert UN110 oven, for 24 hours at 105ºC (Figure 4), method also used by Delian (2011) and Popa et al. (2019).

Figure 4. Determination of dry matter content by oven

The contents of total soluble solids, glucose and fructose were determined from 3 bulbs for each sample (Figure 5): with refractive device Kruss DR301-95 (% Brix) for total soluble solids, with refractive device Milwaukee MA873 (%) for glucose and with refractive device Milwaukee MA872 (%) for fructose.

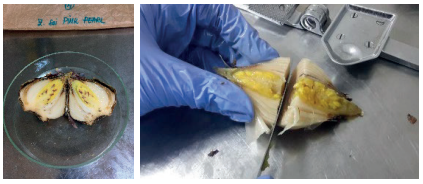


Figure 5. Determination of total soluble solids, glucose and fructose of hyacinth cultivars

Statistical analyses were performed using Excel, like: mean, standard deviation, ANOVA single factor, T Test and correlations (Pomohaci, 2017) and also applying Tukey HSD and Bonferroni and Holm (https://astatsa.com/OneWay_Anova_with_TukeyHSD/_get_data/).

RESULTS AND DISCUSSIONS

For hyacinth bulbs, the respiration rate (Figure 6) registered increases between 4 times (for 'Jan Boss' cv.) up to 6 times (for 'Pink Pearl' cv), between the initial and final moments. Kanneworff W. (1994) suggest that this behavior is an adaptation to low temperatures, being high energy users, due to number increased of mitochondria according to Khodorova (2013).

Koksal (2010) suggest that for long-term storage is not convenient due to high mass loss (Table 2).

At respiration rate, for all cultivars, significant differences ($P<0.05$) were registered, for the initial moment, using ANOVA single factor.

For 'Gipsy Queen' bulbs, between respiration rate (Figure 6) and TSS (Table 2) was registered a significant positive correlations $R^2 = 0.7924$, with linear regression equation $y = 0.0459x + 20.23$ and between transpiration rate (Figure 7) and water content (Table 2), was registered a strong significant negative correlations $R^2 = 0.828$, with linear regression equation $y = - 287.43x + 73.575$.

Respiration rate showed significant differences ($P<0.05$) for initial moment, between 'Gipsy Queen' cv. and 'White Pearl' cv., and between 'Miss Saigon' and 'White Pearl' cv., applying Tukey HSD and between 'Gipsy Queen' cv. and 'White Pearl' cv., applying Bonferroni and Holm (https://astatsa.com/OneWay_Anova_with_TukeyHSD/_get_data/). No significant differences appeared for respiration rate between cultivars., for the final moment.

For 'Jan Boss' bulbs, between respiration rate (Figure 6) and TSS (Table 2) was registered a positive correlation $R^2 = 0.3994$, with linear regression equation $y = 0.0432x + 20.946$ and between transpiration rate (Figure 7) and water content (Table 1) was registered a very significant negative correlations $R^2 = 0.9419$, with linear regression equation $y = - 224.98x + 72.504$.

For'Miss Saigon' bulbs, between respiration rate (Figure 6) and TSS (Table 2) was registered a positive correlations $R^2 = 0.3706$, with linear regression equation $y = 0.0222x + 22.723$ and between transpiration rate (Figure 7) and water content (Table 2) was registered a very significant negative correlations $R^2 = 0.9745$, with linear regression equation $y = -239.52x + 73.763$.

For 'Pink Pearl' bulbs, between transpiration rate (Figure 7) and water content (Table 2) was registered a semnificant negative correlations $R^2 = 0.8231$, with linear regression equation $y = -101.79x + 66.729$.

For 'White Pearl' bulbs, between transpiration rate (Figure 7) and water content (Table 2) was

registered a negative correlations $R^2 = 0.5543$, with linear regression equation y = -73.878x + 67.173.

The transpiration rate for hyacinth bulbs, (Figure 7) during storage registered increases with 2 times (for 'Gipsy Queen' and 'Jan Boss' cvs.) up to 3 times more (for 'White Pearl'cv.) between the initial and final moments. From obtained results it was observed decreases of the mass loss between 6% (for 'Jan Boss' and 'Pink Pearl' cultivars) and 10% (for 'White Pearl' cultivar), between the initial and the final moment of analyses.

For transpiration rate, no significant differences (P<0.05) were registered, between all five cultivars, for each moment, applying Tukey HSD and Bonferroni and Holm (https://astatsa.com/OneWay_Anova_with_TukeyHSD/_get_data/).

Between the initial moment and the final moment significant differences (P<0.05) were registered, for transpiration rate, for 'Gipsy Queen', 'Jan Boss', 'Miss Saigon', and 'Pink Pearl' cvs., applying Tukey HSD and Bonferroni and Holm0(https://astatsa.com/OneWay_Anova_with_TukeyHSD/_get_data/).

For transpiration rate, no significant differences (P<0.05) were registered, between the initial moment and the final moment, for 'White Pearl' cultivar, applying Tukey HSD and Bonferroniand Holm (https://astatsa.com/OneWay_Anova_with_TukeyHSD/_get_data/).

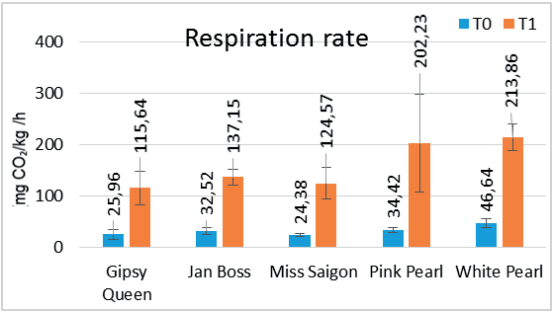


Figure 6. Respiration rate during storage in cold room

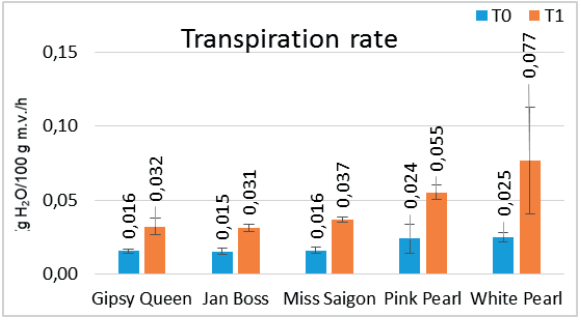


Figure 7. Transpiration rate during storage in cold room

Table 2. Variation of water content (%), TSS(%), glucose(%) and fructose(%) during storage in cold room

	Water content (%)		TSS (%)				Glucose (%)				Fructose (%)			
	T0	T1	T0		T1		T0		T1		T0		T1	
Samples			Average	Std	Average	Std	Average	Std	Average	Std	Average	Std	Average	Std
Gipsy Queen	69,6	63,9	20,9	0,4	26,0	0,2	22,0	0,4	27,5	0,4	22,1	0,3	27,7	1,2
Jan Boss	69,2	65,3	22,3	4,0	26,9	1,5	23,4	4,9	28,0	1,2	24,1	4,2	28,3	0,7
Miss Saigon	70,0	64,9	23,1	1,6	25,7	1,5	24,2	2,2	26,5	1,2	23,4	2,5	26,4	1,3
Pink Pearl	64,6	60,8	29,0	1,6	26,7	1,6	29,8	1,6	28,9	1,9	29,7	1,4	29,4	2,2
White Pearl	66,9	60,0	21,9	3,7	25,2	4,2	23,0	3,9	26,9	4,1	23,0	3,5	27,6	4,5

The soluble solids content for hyacinth bulbs, (Table 2) during storage registered increases between 10% (for Miss Saigon cv.) and 20% (for Gipsy Queen cv.) between the initial and final moment. Only for Pink Pearl cv., from the obtained results it was observed a decrease of the soluble solids content with 8.5% between the initial and final moment.

The glucose content for hyacinth bulbs (Table 2) during storage registered increases between 9.5% (for Miss Saigon cv.) and 20% (for Gipsy Queen cv.) between the initial and final moment. Also fructose content registered increases between 11% (for Miss Saigon cv.) and 20% (for Gipsy Queen cv.), between the initial and final moment.

Only for Pink Pearl cv., from the obtained results it was observed decreases of the glucose content with 3% and fructose content with 1%, between the initial and final moment, mostly due to the respiration rate increase.

Between all cultivars, the contents of total soluble solids, glucose and fructose registered significant differences ($P<0.05$) for the initial moment, using ANOVA single factor.

After storage, for all cultivars, the contents of total soluble solids, glucose and fructose registered no significant differences ($P>0.05$) using ANOVA single factor.

No significant differences ($P>0.05$) were registered between the initial moment and the final moment, for soluble solids content (White Pearl cv.), for glucose content (Pink Pearl and White Pearl cvs.) and for fructose content (Pink Pearl cv.), applying T Test.

According to the results obtained in this study, shown in Table 2, similar to Burzo (2005), it is concluded that exposure of bulbs to low temperatures leads to increased glucose, fructose and sugar levels (Koksal, 2010).

CONCLUSIONS

'Gipsy Queen', 'Jan Boss' and 'Miss Saigon' cultivars presented positive correlations between respiration rate and TSS content during storage period.

'Gipsy Queen', 'Jan Boss', 'Miss Saigon' and 'Pink Pearl' presented significant negative correlations between transpiration rate and water content.

The 'Pink Pearl' cultivar showed a different behavior compared to the others, the values registering decreases in soluble solids content by 8.5%, glucose content by 3% and fructose content by 1%, while the rest of the cultivars registered increases in values, between the initial and final moment. 'Pink Pearl' and 'White Pearl' bulbs started the vegetation period during their storage in the cold room, according to the physiological processes results.

However, it requires further research for the changes appeared on the physiological and biochemical processes, to highlight the influence of temperature on bulbs.

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PHOTOPERIODISM, AN IMPORTANT ELEMENT FOR THE GROWTH AND FLOWERING OF *CHRYSANTHEMUM*S

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Abstract

The durations of the light phase and that of the dark phase (photoperiod) varies with the season, so their succession as well as their development time influences the plant response. Therefore, physiological parameters such as: height of main stems, number of leaves after planting, leaf area (cm^2), time to flowering, flowering time and the number of flowers, and diameter of buds and flowers (mm), may be studied in order to optimize the cut flower production for chrysanthemums grown in protected areas. Depending on the necessary environmental conditions, the floral induction and flowering of Chrysanthemum morifolium may be adjusted using camouflage (artificially shortening the length of the day by covering the plants). The purpose of this paper is to review the evolution of research on the behaviour of chrysanthemum plants exposed to a certain photoperiod.

Key words*: Chrysanthemum morifolium, photoperiod, photoperiodism, growth, flowering.*

INTRODUCTION

Chrysanthemums (*Chrysanthemum indicum* L. and *Chrysanthemum morifolium* Ramat.) are among the oldest, most spectacular, and most important ornamental herbaceous plants. They belong to the family Asteraceae (Compositae), genus *Chrysanthemum*. At first, chrysanthemums flowers were small, with a golden-yellow color, similar to wild plants in nature, which are found today in China and Japan (Dowrick, 1953). They have evolved over time following successive selections, using various techniques, reaching today the great diversity in terms of inflorescence shape, flower color and plant vigor.

Some experts point to the genetic resources of modern chrysanthemums as East Asia (Fukai, 2003; Zhao et al., 2009), others say chrysanthemums are native to the northern hemisphere, both Asia and Northeast Europe (Jeong et al., 2012; Singh & Chettri, 2013; Wang et al., 2019).

Today, over 30,000 chrysanthemum varieties are known globally, with about 3,000 varieties grown in China alone (Dong et al., 2020). Over 15,000 varieties are listed in Japan alone, and in the UK, accordingly to the National Society of Chrysanthemums, over 6000 varieties are listed (Datta, 2013).

Due to the existing relationships between environmental factors and the growth and development of chrysanthemum plants, the researchers paid special attention to the duration of the light and dark period during their vegetation period. Over time, these aspects have been researched through various approaches, namely: the behavior of plants at different latitudes; by studying species at a certain latitude but in different seasons; by prolonging the daylight with artificial light and by dimming the light for a certain period of time during the day. Thus, in 1920, in the writings of Garner and Allard, it is stated that, after 1918, when the darkroom was first used during experiments, the term "length of day" appeared, which referred to the length lighting of the period for every 24 hours. The terms "long day" (exposure to light for more than 12 hours) and "short day" (exposure to light for 12 hours or less) also appeared. Therefore, the relationship between the length of the day and the time of flowering acquires a great significance in crop yields. Subsequent studies classified chrysanthemums, as being short-day plants (SD), which respond to the decrease in

the day length by their transition from the stage of vegetative growth to the stage of reproduction (Thomas & Vince-Prue, 1997; Oda et al., 2017; Yang et al., 2018). Their flowering can be obtained with the help of the photoperiod, being a plant sensitive to this factor (Șelaru, 1995).
The duration of the light phase and the dark phase (photoperiod) varies depending on the season, so that their succession, as well as their development time, influences the plant's response. Therefore, physiological parameters, such as: height of main stems, number of leaves after planting, leaf area (cm^2), time to flowering, flowering time and number of flowers and diameter of buds and flowers (mm), can be studied to optimize the production of cut flowers for chrysanthemums grown in protected areas.

MATERIALS AND METHODS

In order to describe the evolution of the research on the behavior of chrysanthemum plants exposed to a certain photoperiod, the documentation on this subject was revised. Data and information were collected from the field of scientific research and these data were grouped so that they could lead to a deeper interpretation in this field.

PHOTOPERIODICITY

The etymology of the word "photoperiodism" derives from the Greek words "light" and "duration" and can be defined as day-long responses that allow living organisms to adapt to seasonal changes in their environment (Thomas & Vince-Prue, 1997; Thomas, 2017).
The duration of light (photoperiod), rather than the intensity of light, influences the earlier flowering of chrysanthemums. And low light intensity affects plant vigor (Laurie & Poesch, 1932).
The American researchers Wightman Garner and Henry Allard (1920) had a special contribution in the field, conducting the first experiments on the behavior of plants in the photoperiod. They found that plants bloomed in response to changing day lengths and made it clear for the first time that flowering in plants could be accelerated by either short days (SD) or long days (LD). They also introduced the terms "photoperiod" and "photoperiodism" (photoperiod response capacity), as well as the classification of plants into groups, according to their reaction to the photoperiod. In their writings, "photoperiod" is indicated for the favorable length of day for each organism, and "photoperiodism" is suggested for the organism's response to the relative length of day and night (Garner & Allard, 1920). Nowadays, photoperiodism can be defined as the response of plants through growth, development or metabolism, depending on the day light duration (Delian, 2019), and chrysanthemum, being a plant that responds to the photoperiod (period of exposure to light during 24 hours) is called photoperiodic sensitive. The light phase or photoperiod is also called the lumen period, and the dark phase is known as the nictiperiod (Burzo et al., 1999; Bădulescu, 2009; Burzo, 2016). In 1997, researchers Thomas and Vince-Prue grouped plants into three broad categories, according to their photoperiod flowering responses, namely: short-day plants (SDP); long day plants (LDP) and neutral plants. Chrysanthemums (*Chrysanthemum indicum* L. and *Chrysanthemum morifolium* Ramat.) belonging to the first category.
After the discovery of photoperiodism, various methods were found to control the flowering of chrysanthemums based on the photoperiod (Laurie 1930; Laurie & Poesch, 1932; Poesch, 1936).
Laurie and Poesch (1930-1932) experimented and demonstrated that the natural length of the day can be changed in the greenhouse protected space. They used this technique of covering chrysanthemum plants with black satin cloth (opaque), so they obtained a shorter photoperiod than the natural one. Thus, chrysanthemums bloomed 22 to 56 days earlier, with the same floral diameter, with slightly lower stem height, but in compliance with marketing standards. Until the 1930s and 1950s, photoperiodism was not applied in the commercial floriculture industry (Erwin, 2006). After that, the technique was taken widely used to increase the flowering season of photoperiod-sensitive plants.
Adams and colleagues (2001) argued that it is advisable to use photoperiod sensitivity, which

allows the acceleration of flowering, thus reducing lighting costs, but at the same time preserving the quality of plants. The easiest way to provide short days is to pull an opaque reflective cloth over the plants at the end of the day and remove it in the morning (Erwin et al., 2002). However, after 1950, to meet the demand for chrysanthemums throughout the year, growers adjusted the flowering time using artificial lighting and interruptions or night break (NB) (Fukai, 2014; Higuchi, 2018). In general, as light sources, for artificial lighting in the cultivation of plants in greenhouses, in addition to incandescent lamps, are also used: fluorescent lamps, metal halides and sodium at high pressure (Jeong et al., 2012).

One effect observed in the production of chrysanthemum flowers by treating them with short artificial days, was the lighter color of these flowers compared to those induced by the natural photoperiod. Following shading, the development of chrysanthemums was significantly influenced, especially by the sudden decrease of anthocyanins in the petals of rays from calatidium (Hong et al., 2015). The color of the flowers is an important feature that influences the commercial value of chrysanthemum varieties (Ohmiya, 2018). Thus, transcriptome analyzes of the molecular mechanism of chrysanthemum flowers color change in short-day photoperiods were performed. The results showed that the anthocyanin synthesis is strictly regulated by the photoperiod, which can be useful in the molecular growth of chrysanthemums (Dong et al., 2020).

THE ROLE OF THE CIRCADIAN CLOCK

The sensitivity of the chrysanthemum to light changes throughout the day is regulated by the circadian clock. It measures the length of the day and influences some basic plant activities, such as growing and reproducing. Circadian rhythms in plants have led to the discovery of an internal biological clock consisting of a molecular oscillator and an input path that allows the clock to be reset according to external indicators, such as the photoperiod. So, the clock allows an estimate of the time that synchronizes the process of flower initiation with the photoperiod (Micallef, 2011).

Imaizumi and Kay (2006) say that in short-day plants, the clock-regulated factor functions as a flowering suppressant. They also claim that the photoperiodic flowering path can be separated into two functional areas: a circadian clock and a regulated circadian mechanism for measuring the length of the day.

The German biologist Erwin Bünning made a great contribution by proposing in 1936 "the daily endogenous rhythm as the basis of the photoperiodic reaction", also called the "Bünning hypothesis", which later became the "external coincidence model". Thus, plants can track the length of the day, the photoperiod, with the help of an endogenous timer. This clock is synchronized with physiological and molecular processes up to the day-night cycle, allowing plants to anticipate future conditions (Green et al., 2002; Dodd et al., 2005; Johansson & Staiger, 2014).

PHOTORECEPTORS

Light signals are received by plants through a wide range of photoreceptors, including phytochromes and cryptochromes, which absorb light at specific wavelengths (Lin, 2000).

The main photoreceptors that regulate photoperiodic flowering in many plants are phytochromes (Song et al., 2015).

The light reception, as well as the inductive photoperiod duration are determined at the leaves level by means of phytochromes (Burzo et al., 2000; Bădulescu, 2009; Burzo, 2016). The photoperiod duration is received at the level of leaves with a medium maturity. They react best under the influence of the photoperiod compared to senescent leaves (Bădulescu, 2009).

Photochromes are best known in two forms: “stable” or “inactive” form (storage), which absorbs red light radiation (r - 660 nm); and the “unstable” or “active” form that absorbs distant red light radiation (fr - 730 nm) (Delian, 2019).

Flowering of *Chrysanthemum morifolium* Ramat. is inhibited when the required long night phase is interrupted by a short period of exposure to red light (night break; NB). But to obtain the reverse effect of this inhibition, the

plants are subsequently exposed to distant red light (FR), thus involving phytochromes in the flowering response (Higuchi et al., 2012).

FLORAL INDUCTION AND FLORIGEN

Flowering is an important phenomenon found in flowering plants and not only in them. It can be influenced by several factors, but especially by the photoperiod (Garner & Allard, 1920; Chailakhyan, 1968; Pearson et al., 1993; Mattson & Erwin, 2005; Song et al., 2015; Thomas, 2017; Higuchi, 2018; Torabi et al., 2020).

The oldest concept of the physiological nature of flowering plants dates back to 1880 and was published by the German botanist Julius von Sachs, who claimed that a specific organ-forming substance was involved in the formation of each plant organ. His hypothesis referred both to plant formation and especially to flower-forming substances.

Mikhail Kh. Chailakhyan, following numerous photoperiodic experiments, including chrysanthemums, proposed in 1936 the existence of a universal plant hormone that he called "florigen". He claimed that it is produced by the leaves and it is involved in flowering. He also found in his experiments (1968) that plant species, regardless of the nature of their photoperiodic reaction, contain more sugars and starch in long day conditions and more nitrogenous compounds and proteins in short day conditions. He observed that the nitrogen deficiency in the chrysanthemum culture substrate inhibits its flowering, while in other long-day species (barley, oats, mustard) it stimulates it. Chailakhyan also demonstrated that floral induction can be transmitted from an induced plant to an uninduced plant by grafting. Zeevaart (1958) states in his paper that grafting is used more recently in transmitting the inducing stimulus of flowering from a flowering plant (donor) to one that does not bloom (recipient), but according to the literature, this is possible only if the receiver is defoliated. Chrysanthemum flowering can be grouped into two phases: induction of flower initiation and development of flower buds to anthesis, both processes being promoted for short days. But the initiation of chrysanthemum flowers on long days is inevitable, depending on the variety and the aging process in the apical meristems (Cockshull, 1976).

Floral induction can be defined as a process that forms, under the influence of inductive factors, a complex biochemical messenger, who can lead to a change in the expression of flowering genes (Bădulescu, 2009). It can be classified as a transition phase, according to which the plant passes from vegetative to reproductive growth, being able to produce flowers (Delian, 2019).

Floral induction of plants can be influenced by several external inductive factors, such as: photoperiodism (duration of the photoperiod), thermoperiodism (high temperatures), vernalization (low positive temperatures), as well as some internal factors, such as: hormones, autonomous cycle, nutrition (Dobrescu et al., 2018). Depending on the species, a single factor or several factors that act simultaneously can induce the flowering of plants (Bădulescu, 2009; Burzo et al., 1999).

Florigen is a hypothetical signal produced in the leaves that induces floral initiation at the top of the shoots (Zeevaart, 2008), but it can last for several days or even weeks. The change in the leaf can be seen as an "induction", while the peak response (initiation of flowering) can be called "evocation".

In the case of chrysanthemums, the floral induction is conditioned by the short days necessary for the transition from the vegetative to the reproductive cycle.

These short days must be consecutive, and their number varies according to the species and variety. In the case of standard chrysanthemum, it needs 21-28 consecutive short days for floral induction, while twig type chrysanthemum needs more time, namely 42 consecutive short days (Toma, 2013).

In chrysanthemum there are two types of buds, namely: crown buds and terminal buds. "Wreath buds" are those flower buds which, when they appear, differ in being surrounded by leaves, while the terminal buds are flower buds which are surrounded by other flower buds. In the case of shaded chrysanthemums, only terminal buds appear (Laurie and Poesch, 1932).

Plant growth, being an irreversible change over time, produces changes in size, shape and number (Hunt, 2003). For example,

chrysanthemums do not bloom unless they have a minimum number of leaves reached.
The duration of the photoperiod is fixed by the photosensitive pigments (phytochromes, cryptochromes), and they pass from the inactive phase to the active phase. The circadian biological clock helps determine the number of inducible photoperiods. In nictiperiodic plants, floral induction can be canceled if a short day is followed by a long day. However, if a plant has gone through the number of inductive photoperiods, it will flourish even if it is not subsequently exposed to the inductive photoperiod (Burzo, 2016).

RESULTS AND DISCUSSIONS

Depending on the necessary environmental conditions (in our case, the photoperiod), floral induction and chrysanthemum flowering can be adjusted using both camouflage (artificially shortening the length of day covering the plants) and artificial lighting with night break (NB).

APPLICATIONS OF PHOTOPERIODISM

Chrysanthemum being a sensitive plant throughout the day, allows the selection of varieties and facilitates their maintenance in a vegetative state or bringing them in the reproductive period (at flowering), by the grower in accordance with market requirements.

CONCLUSIONS

The aim of this paper is to review the evolution of research on the behavior of chrysanthemum plants exposed to a certain photoperiod. Successive stages of development can be seen in the progress of models that have been adapted in the production of chrysanthemum cut flowers. Looking ahead, research into the contribution of photoperiodism to regulating chrysanthemum flowering remains open.

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A RESEARCH ON GREENHOUSE HEATING WITH SOLAR ENERGY USING VACUUM TUBULAR COLLECTORS AND NANOFLUID

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Abstract

In this research, vacuum tube collectors with high efficiency in converting solar energy to heat energy will be used for heating greenhouse with nano-fluid as working fluid. Since the fluid exiting in such collectors is at a higher temperature (100-120°C.) than plane collectors, they have a wider use than plane collectors. The use of vacuum tube solar collectors increases the usability for storing heat for greenhouse heating in early spring and late autumn seasons. Solar energy and greenhouse heating system will be designed as open system. However, the system will be used as a nano-fluid heat storage and transport fluid (working fluid). The nanofluid water will be mixed with aluminum(Al_2O_3) nanoparticles. In the system, mixing ratios of water and aluminum oxide (Al_2O_3) and appropriate flow rate will be determined for the first time depending on the heat requirement of the research greenhouse. Heat sum, thermal efficiency of storage and greenhouse units energy and exergy analyzes will be determined. In addition, environmental sustainability of the system will be evaluated by exergetic life cycle analysis.

Key words:*vacuum tube collector, greenhouse, heating, nano-fluid.*

INTRODUCTION

Controlling the ambient temperature is an important process in greenhouse technique affecting plant growth and development, yield and quality. All plants need temperatures between certain lower and upper limit values to develop. The lowest air temperature depends on the continuously occurring low temperatures and the difference in temperature between day and night. On the other hand, the highest air temperature depends on the rate of change of relative humidity. In the greenhouse air-conditioning technique, the most suitable air temperature is defined as the highest temperature required for a physiological process that can be maintained continuously without decreasing its speed. The optimum temperature for the plant depends on the physiological process that the plant performs. The air temperature; The effects of air temperature on the physiological processes of plants such as photosynthesis, respiration and water intake are different. If the air temperature in the greenhouse environment is lower than the freezing point of the plant, it can directly cause physical damage to the plant cells. The tolerance of plant cells to the highest and lowest temperatures varies depending on the plant species. The negative effects of low temperatures on plants occur in the form of cold and frost damage. Cold and frost events occurring in plant growing environments can cause plants to die by being damaged (Ozturk, 2017). For the reasons mentioned above, the main purpose in greenhouse cultivation is to maintain the indoor temperature at the optimum level. In order to achieve the highest efficiency expected from the crop production in greenhouses, the greenhouse should be heated in periods when the outdoor temperature is lower than optimal conditions.

MATERIALS AND METHODS

In this study, it was aimed to store solar energy for short term (day to night) using sensible heat storage method using nanoparticle (Al_2O_3) mixed water (nanofluid) as heat storage material in order to be used in heating the

plastic greenhouse with 280 m^2 floor area under Adana climate conditions. The solar energy storage application in the greenhouse aims to reduce the heating energy requirement of the greenhouse. Due to the reduction of the amount of energy that should be used for greenhouse heating, heating expenses will be reduced and energy saving will be provided. As an important result of saving energy, CO_2 consumption, which is one of the main gases creating greenhouse effect in the atmosphere, will also decrease significantly, as fossil fuel consumption will decrease for greenhouse heating applications. So; Important contributions will be made in terms of greenhouse producer, national economy, human health and environmental protection. In solar energy greenhouse heating applications, high heat transfer between the heat collection and storage units and the greenhouse is desired. In addition, studies are carried out to provide solar energy systems, which are a renewable energy source, for high heat transfer applications. However, improving heat transfer in solar energy systems is one of the critical issues in energy saving and compact designs. Water is generally preferred as heat transfer fluid in solar energy systems. Although the heat transfer coefficient of the water between the fluids is high, mixing the nanoparticles with high heat transfer coefficient increases the total heat transfer coefficient. The fluid formed by mixing water and nanoparticles is called nanofluid. Nanofluids improve thermophysical properties, such as thermal diffusion and thermal conductivity, and also create a slight increase in pressure drop and pumping power in the base fluid. (Hong et al., 2006; Hwan et al., 2008; Jang et al., 2007). For this reason, many researchers have pointed out the use of nanofluids as an innovative technique to increase heat transfer efficiency in solar energy systems (Khattak et al., 2016; Azwadi et al., 2016; Azwadi et al., 2014; Lee 2014; Zainal et al., 2016; Mohammed et al. ., 2016). Recently, studies on the suspension of nanoparticles with water as heat storage and transfer fluid, and the use of traditional heat transfer fluids in place (Wen et al., 2009; Ny et al., 2016; Abubakar et al., 2016). As a result, the total efficiency of the system can be increased by using Al_2O_3/water mixture for solar heating and greenhouse heating applications.

The project, which is designed for daily storage of greenhouse heating and solar energy using sensible heat storage method, will be carried out in Adana Agricultural Extension and Training Center Directorate. The schematic view of the solar storage and greenhouse heating system is given in Figure 1. The system mainly consists of the following 4 units: 1) Heat collection unit consisting of vacuum tube solar collectors; 2) Heat storage unit where the collected heat is stored; 3) Plastic greenhouse heated with the stored heat; 4) Heat transfer unit between the heat collection-storage units and the greenhouse.

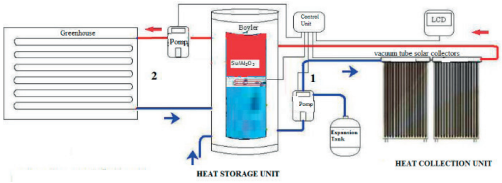

Figure 1. Solar Energy Storage and Greenhouse Heating System

During the daytime, with the operation of the pump unit 1 indicated in Figure 1, the heat energy collected by the solar collectors with vacuum pipes will be stored in the heat storage unit. Depending on the plastic greenhouse indoor temperature, the pump unit 2 will be operated to recover heat from the heat storage unit. In this case, nanofluid circulation will be provided between the heat storage unit and the heating pipes in the plastic greenhouse unit. When the temperature of the nanofluid heated in the solar active heating system is higher than the temperature required to be sent to the greenhouse, the nanofluid heated in the system can be used directly to heat the greenhouse. In cases the operating temperature of the system is low, a higher rate of solar collectors is used.

Plastic Greenhouses

In the research, it will be established in 280 m^2 floor area under Adana climate conditions.

For polyethylene (PE) plastic greenhouses, design variables of solar greenhouse heating system are determined. One of the two plastic greenhouses to be installed in the same design will be used for control purposes. The

dimensions of the plastic greenhouses to be installed are: 40 m (length) × 7 m (width) × 7 m (height). Cucumber will be produced in research and control greenhouses.

Heat Distribution System in Greenhouse

For the heat distribution in the plastic greenhouse, 50 mm diameter polyethylene (PE) plastic heating pipes will be placed between the plant rows. The operating temperature of plastic pipe heating systems is 70/50°C. In the technical design of the system, the inlet temperature of the heating fluid is 70°C and the return temperature is 50°C.

Heat Collection Unit

Vacuum Tube Solar Collectors It will be used for collecting solar energy that will be stored as heat energy in the heat storage unit. Since the outputs of vacuum tube collectors are at higher temperatures (100-120°C), they have a wider usage area than plane collectors.

Heat Storage Unit

As heat storage unit will be used, cylindrical storage tank to be designed from corrosion-resistant metal material.

Measurements

In the greenhouse in teriors, sensors will be placed in three different areas, namely the entrance-middle-exitsections. Sensors will be measured at two different heights, air temperature and relative humidity. The temperature of the nanofluid circulating between the heat collection and storage unit sand the plastic greenhouse will be measured. In the external environment; air temperature, air relative humidity, solar radiation intensity and wind speed values will be measured.

Determination of Greenhouse Heat Demand

The amount of heat required per floor area for the heating of the plastic greenhouse to be installed is determined from the following equation (Ozturk, 2008b).

$$q_s = \frac{A_{\ddot{o}}}{A_t} \cdot u(T_i - T_d) - I.\tau.\gamma \quad \text{.....(1)}$$

Where:

q_s = Heat requirement per floor area (W/m^2);
$A_{\ddot{o}}$ = Greenhouse cover surface area (m^2);
At = Greenhouse floor area (m^2);
U = Total heat loss coefficient ($W/m^2{}^{\circ}C$);
T_i = Greenhouse indoor air temperature ($^{\circ}C$);
T_d = Outdoor air temperature ($^{\circ}C$);
I = Total solar heat (W/m^2);
t = Total radiation permeability of the greenhouse;
γ = Total radiation at indoor temperature effective is the rate of conversion to irradiation.

$$Q_s = q_s \times A_s \quad \text{.....(2)}$$

Where:

Q_s = Greenhouse total heating requirement (W);
q_s = Heat requirement per floor area (W/m^2);
A_s = Floor area of greenhouse (m^2).

Overall Heat Loss Coefficient

The overall heat loss coefficient (u, W/m^2, °C); for the 1°C difference between sera in door temperature and outdoor temperature, it indicates the total heat loss of each m^2 of greenhouse surface area (Ozturk, 2003a). In the Mediterranean Region climate conditions. For plastic greenhouses covered with UV+IR added PE, the overall heat loss coefficient can be determined from the following equation (Ozturk, 2003a).

$$u = 2.83+0.10\ v_r \quad \text{.....(3)}$$

Where, v_r = the wind speed (m/s).

Indoor Air Temperature

In order to grow different types of plants in greenhouses, the indoor air temperature should be adjusted in the range of 10-28°C. The heat requirement of the plastic greenhouse to be installed will be calculated depending on the indoor temperature of 12°C.

Outdoor Air Temperature

In determining the outdoor temperature, the average of the lowest temperatures occurring at the coldest time of the year depending on the climatic conditions of the region where the greenhouse is located is taken in to account. For the design of heating systems, the heat requirement of the plastic greenhouse to be installed will be calculated depending on the outdoor temperature of 0°C

Sizing of the Greenhouse Heating System

Determination of Heating Pipe Length

Total heating pipe length to be used in hot water heating systems in greenhouses, total heat requirement of the greenhouse and the heat gained from the unit length of the heating pipe intended to be used. Depending on the amount, it is calculated as follows (Ozturk, 2012).

$L_b = Q_s/Q_b$…….(4)

Where:

L_b = Length of the heating pipe (m);
Q_s = Greenhouse total heat requirement (W);
Q_b = Amount of heat gained from the pipe (W/m).

Determination of Heat Transfer from the Heating Tube

The total amount of heat transferred from the heating pipe to the greenhouse environment is calculated from the following equation. (Ozturk, 2012). For the heating system in the plastic greenhouse, 50 mm diameter polyethylene (PE) pipe will be used as the heat exchanger.

$$Q_b = \frac{4\pi L_b \Delta T_b}{\frac{1}{\alpha_i d_i} + \frac{\ln(d_d/d_i)}{\lambda_b} + \frac{1}{\alpha_d d_d}} + Q_r \text{……..…..(5)}$$

Where:

L_b = Length of the heating pipe(m);
Q_b = Heat gained from the pipe (W/m);
ΔT_b = Temperature difference (°C);
α_i = Internal surface heat transfer coefficient (W/m^2°C);
α_d = External surface heat transfer coefficient (W/m^2°C);
d_d = Tube outer diameter (m);
d_i = Tube inner diameter (m);
Q_r = Amount of thermal power passing by radiation (W);
λ_b = Heat transmission coefficient (W/m°C).

Determination of the Amount of Thermal Power Transmitted from the Heating Pipe to the Greenhouse Environment by Radiation

The amount of thermal power (Q_r, W) transmitted from the heating pipes in the plastic greenhouse to the greenhouse environment by radiation is calculated as follows (Ozturk, 2012).

$$Q_r = \varepsilon \cdot \sigma \cdot A_b(T_b^4 - T_s^4) \text{……...(6)}$$

Where:

ε = Radiation emission value,
σ = *Stefan-Boltzmann* constant (2.6697×10^{-8}W/m^2 K^4),
A_b = Pipe surface area (m^2),
T_b = Absolute temperature of the heating pipe (K),
T_s = Absolute temperature of greenhouse ambient air (K).

Sizing of the Heat Storage Unit

Determining the Amount of Heat Storage Material

The amount of heat storage material that should be used as sensible heat storage material in the heat storage unit will be calculated from the equation below (Ozturk, 2012).

$$m = \frac{Q_s}{c_p \times \Delta T} \text{…….....(7)}$$

Where:

m = Amount of heat storage material (kg);
Q_s = Greenhouse heat requirement foreseen to encounter solar energy (kJ/day);
c_p = Specific heat of water (kJ/kg ˚C);
ΔT = Temperature increase in water(˚C).

Determination of Heat Storage Unit Volume

In determining the volume of the heat storage unit (heat tank), the maximum amount of heat that can be stored daily is taken into account. The volume of the heat storage unit will be determined from the equation below (Ozturk, 2012).

$$V = \frac{Q_s}{p_s \times c_{ps} \times \Delta T} \text{…….(8)}$$

Where:

V = Heat storage unit volume (m^3);
Q_s = Greenhouse heat requirement foreseen to encounter solar energy (kJ/day);
p_s = Water density (kg/m^3);
c_{ps} = Specific heat of water (kJ/kg K);
ΔT = Average heat of the heat storage material for the heat storage phase (˚C).

Determination of the Heat Collection Unit Surface Area

After determining the amount of heat energy required meeting a certain ratio of annual heat requirement for greenhouse heating, the required collector area for this energy collection is calculated. The efficiency of the

vacuum tube solar collectors to be used in the heat collection unit to be designed will be considered as ç = 60%.

The collector area to be used in the heat collection unit will be calculated from the following equation (Ozturk, 2012).

$$A_t = \frac{Q_s}{I \times \eta_t} \quad \text{.....(9)}$$

Where:

A_t = Collector surface area (m^2);
Q_s = Greenhouse heat requirement foreseen to encounter solar energy (kJ/day);
I = The amount of solar energy coming to the collector surface (kJ/m^2);
η_t Collecting efficiency (%).

Determination of Circulating Pump Flow

After determining the amount of heat energy required to meet a certain ratio of annual heat requirement for greenhouse heating, the required collector area for calculating this energy is calculated.

The efficiency of the vacuum tube solar collectors to be used in the heat collection unit to be designed will be considered as n = 60%.

The collector area to be used in the heat collection unit will be calculated from the equation below (Ozturk, 2012).

$$V_p = \frac{Q_s}{c_p \cdot \rho \cdot (T_g - T_ç)} \quad \text{..... (10)}$$

Where:

Vp = Flow rate of circulating pump (m3/s);
Qs = Required heat quantity (kW);
ρ = Density of the fluid (kg/m3);
cp = Specific heat of fluid (kJ/kg°C);
Tg = Fluid inlet temperature (°C);
Tç = Fluid outlet temperature (°C).

Determining the Amount of Fuel to Be Saved and the Fuel Expense

In case the plastic greenhouse to be installed is heated by solar energy, the thermal values and cycle efficiencies related to fuels and fuel unit prices will be taken into consideration in order to determine the fuel quantity values to be saved.

Determination of the Amount of Reduction in Carbon Dioxide Emission

Along with the energy conservation efficiency, the environmental impacts of solar energy storage application will be evaluated as well. The reduction in the emission of carbon dioxide (CO_2) gas, which is one of the main gases that create a greenhouse effect in the atmosphere, will be determined.

Al_2O_3Preparation of Water Nanofluid

In this study, Al_2O_3-water nanofluid was chosen as heat transfer fluid in solar-tube solar collectors for greenhouse heating. In experiments, Al_2O_3 will be used as nanoparticle and pure water will be used as basic fluid. Two-step method will be used to calculate Al_2O_3-water nanofluid. An ultrasonic homogenizer will be used to prevent lumps that may occur while preparing nanofluids and to increase the stability of the nanofluid. While preparing the nanofluid, a 4 L capacity reactor will be used to prevent its heating. The properties of Al_2O_3 nanoparticles are shown in Table 1.

Table 1. Properties of Nanoparticles Used in the Study (Ghaderian and Sidik, 2017)

Formula	Molecule mass	Phase	Particle size	Surface area	Melting	Thermal conductivity
Al_2O_3	101.96	Gama	< 50nm	35-43	2040°C	37.14
	g/mol			m^2/g		W/mK

While preparing the nanofluid, the Al_2O_3 nanoparticle will be added to the water in a different volumetric ratio and the Al_2O_3-Water nanofluid will be prepared using the two-step method. The volume concentration of the Al_2O_3 particle to be added to the water is expressed by the following equation (Sharafeldin and Grof, 2019).

$$\phi\ (\%) = \frac{\frac{m_{np}}{p_{np}}}{\frac{m_{np}}{p_{np}} + \frac{m_{bf}}{p_{bf}}} \text{ x } 100. \quad \text{.....(11)}$$

Equality ϕ refers to volumetric ratio (%), m mass (kg), p density kg / m^3, bf basic fluid (water) and np nanoparticle.

Thermal conductivity of Al_2O_3-Water nanofluid will be measured with the hot wire method commonly used in this project. Measurement results will be compared with the results in the literature. Specific heat plays an important role in the entropy production and thermal efficiency of a solar thermal application. The specific heat of nanofluids is determined by the equation below (Palm, et al., 2006).

$$C_{p,nf} = \phi C_{p,np} + (1-\phi) C_{p,bf} \text{.....(12)}$$

In the equation above, *Cp*, *nf*, *Cp*, *bf* and *Cp*, *np* represent the specific heat values of the nanofluidic, basicfluid (water) andnanoparticle, respectively. At the end of the study, how much the conductivity of water increases with Al_2O_3 nanoparticle will be numerically revealed. The Al_2O_3-water nanofluidto be prepared will be put in the heat storage tank for heating purposes in the experimental system and experiments will be carried out.

CONCLUSIONS

In case of using solar energy in greenhouse heating; The greenhouse producer will contribute to the country's economy, human health and environmental protection. In case solar energy is stored for the purpose of greenhouse heating with sensible heat storage method, heating costs, which have a large place in the total production expenses of greenhouse agriculture, will decrease. As a result of thecalculations, it has beendeterminedthat 60-70% savingwill be achievedfromtheamount of coalrequiredforgreenhouseheatingapplicationwi th solar heatingsystem. Due to the decrease in heating costs, the production cost of the products grown in greenhouses will decrease. Decreasing the production costs of the products grown in the greenhouse will facilitate the marketing of these products in the foreign and domestic markets. Depending on the reduction of the amount of energy that should be used for greenhouse heating, significant energy savings will be achieved. As an important result of saving energy, CO_2 consumption, which is one of the main gases that create a greenhouse effect in the atmosphere, will decrease significantly, as fossil fuel consumption will also decrease for greenhouse heating applications. Thus, contribution will be made to environmental protection.

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CHEMICAL COMPOSITION OF ESSENTIAL OIL OF *ARTEMISIA SCOPARIA* (ASTERACEAE) FROM ROMANIA

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Abstract

This paper presents the composition of the essential oil extracted from Artemisia scoparia in different phenophases. The essential oil has been extracted by hydro distillation and analysed by gas chromatography coupled with mass spectrometry (GC-MS). The obtained results emphasized the presence of some major chemical compounds in the maturity period, such as beta pinene (2.92%), methyl eugenol (18.63%), capillene (33.33%), spathulenol (8.45%) and ent-spathulenol (14.84%), while during the flowering phase beta-pinene (9.30%), limonene (2.58%), gamma-terpinene (3.05 %), capillene (66.20%). During the growing season, beta-pinene (9.74%), limonene (3.52%), gamma-terpinene (3.93%) and capillene (71.22%) were determined. In the three phenophases the main common chemicals were β-pinene and capillene.

Key words: *Artemisia, essential oil, capillene, phenophase.*

INTRODUCTION

Artemisia scoparia belongs to the genus *Artemisia*, family Asteraceae (Boakye et al., 2017).

This genus contains almost 500 species spread all over the world (Bora & Sharma, 2011). The varied composition of volatile oils in *Artemisia* species presents different therapeutical effects like: antimalarial, antitumor, antihepatitis, antioxidant, antipyretic, antispasmodic (Tan et al., 1998), anti-infertility or anti-nervous disorders (Joshi, 2013). It is also used in agriculture and food industries, with an antifungal, antiparasitic, anticancer (Bayala et al., 2014), antimicrobial and insecticidal effects (Pandey & Singh, 2017), as gastronomic herbs (Hayat et al., 2009), or cosmetics like perfumes, soaps and detergents (Boakye et al., 2017). The strong and aromatic scent of some species of *Artemisia* genus is due mainly to high concentrations of volatile terpenes, constituents of their essential oils, especially in leaves and flowers (Abad et al., 2012).

A. scoparia is considered a medicinal plant, being found in countries such as: Iran, India, Saudi Arabia, China, Korea, Japan, Pakistan, and Central Europe. It has been used to treat inflammation, fever, jaundice (Ding et al., 2021), hepatitis, and to cure ear aches (Boakye et al., 2017). *A. scoparia* oil has bioherbicidal properties, as it causes severe phytotoxicity and interferes with the growth and physiological processes of some weed species (Kaur et al., 2010). It has a strong scent, and its flowering period is July-October (Boakye et al., 2017). The major chemical compounds which are found in *A. scoparia* were β-myrcene, γ-terpinene, neral and cis-p-mentha-2-en-1- ol, β-caryophyllene, p-cymene and p- cymene-8-ol (Kapoor et al., 2004), γ-terpinene, eugenol, eugenyl valerate, limonene, p-cymene, eugenyl isovalerate and eugenyl butyrate (Ali et al., 2000), myrcene as the major constituent followed by (+)-limonene, (Z)-beta-ocimene, gamma-terpinene and acenaphthene (Singh et al., 2009), camphor, 1,8-cineole, and beta-caryophyllene (Cha et al., 2005).

The objective of this study was to analyze the composition of volatile oil in different phenophase of *A. scoparia* species.

MATERIALS AND METHODS

The plants of *A. scoparia* come from the spontaneous flora of Romania (Mahmudia - Tulcea County). The biological material was analyzed fresh in various phenophases. The

extraction and analysis of the volatile oil were done within the Faculty of Horticulture, Bucharest. Fresh herbal parts of the collected plants were subjected to hydro distillation for 3h using a Singer-Nickerson equipment to produce oil. The separation and identification of components has been carried out using an Agilent gas chromatograph, equipped with quadruple mass spectrometer detector. A capillary column DB-5 452 (25 m length x 0.25 mm i.d. and 0.25 µm film thickness) and helium as carrier gas were used (Bădulescu et al., 2010). The initial oven temperature was 60°C, then rising to 280°C at a rate of 4°C/min. The NIST spectra bank was used for to identify the essential compounds, which were verified with the Kovats indices.

RESULTS AND DISCUSSIONS

Following the analyse performed on the volatile oil extracted from *A. scoparia*, it was found that the number of identified substances was 18 during the growth period, 16 during the flowering period and 14 during the maturity period. During the growth period, there was noted as the majority of chemical compounds have been represented by β-pinene (9.74%), limonene (3.52%), trans-β-ocimene (2.74%), γ-terpinene (3.93%) and capillene (71.22%) (Figure 1).

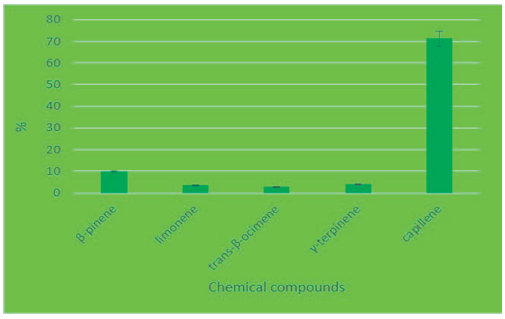

Figure 1. Major chemical compounds in the volatile oil of *Artemisia scoparia* in the growth phenophase

The minor chemical compounds found in *A. scoparia*'s oil during the growth period were α-pinene, myrcene, α-terpinene, p-cymene, cis-β-ocimene, terpinen-4-ol, phenyl cyclohexadiene, lavandulol, pentadienyl benzene, β-caryophyllene, β-cubebene, β-himachalene and spathulenol (Figure 2). From the category of minority chemical compounds analysed in the growth phase, six compounds are not found in the other two phenophases (α-terpinen, p-cymene, terpinen-4-ol, phenyl cyclohexadiene, lavandulol, and pentadienyl benzene).

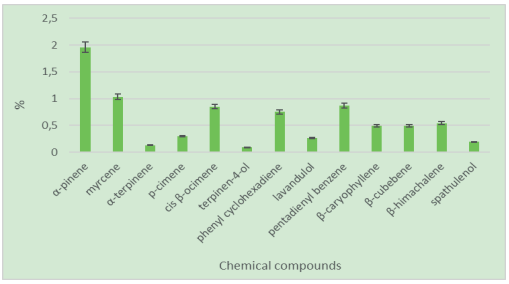

Figure 2. Minor chemical compounds in the volatile oil of *Artemisia scoparia* in the growth phenophase

During the flowering period, the majority chemical compounds were β-pinene (9.30%), limonene (2.58%), trans-β-ocimene (3.17%), γ-terpinene (3.05%), methyl-eugenol (5.49%) and capillene (66.20%) (Figure 3). The major compound named capillene was also found in a chemotype of *Artemisia dracunculus* L. (Tarragon), collected at flowering stage from naturally growing population of Shansha (Himachal Pradesh), North-West Himalaya, India (Chauhan et al., 2010), as well as in a chemotype of *A. scoparia* (full-blooming stage) from Serbia (Ickovski et al., 2020). The minor chemical compounds in the same period were α-pinene, myrcene, cis-β-ocimene, eugenol, cyclohexadiene-1-ol benzene, β-caryophyllene, β-himachalene, spathulenol, β-eudesmol and isoeugenol acetate (Figure 4). Also, among the minor chemical compounds identified in the flowering phenophase, 4 of them (eugenol, β-eudesmol, cyclohexadiene-1-ol benzene and isoeugenol acetate) were not found in the growth and maturation phenophases.

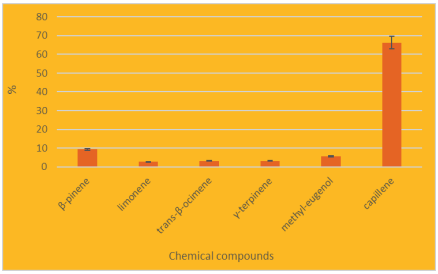

Figure 3. Major chemical compounds in the volatile oil of *Artemisia scoparia* in the flowering phenophase

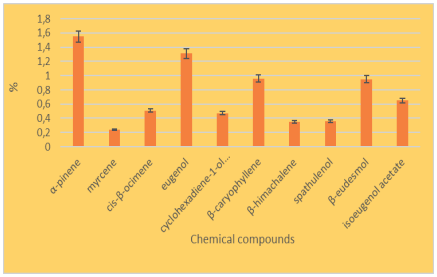

Figure 4. Minor chemical compounds in the volatile oil of *Artemisia scoparia* in the flowering phenophase

Following the analysis of volatile oil in *A. scoparia* plants (during the flowering period) in Iran, the main chemical compounds that were found there were 1-phenyl-penta-2,4-diyne, β-pinene, limonene and (E)-β-ocimen (Safaei-Ghomi et al., 2005). Another study showed that β-ocimene and β-pinene at the vegetative stage, and β-pinene and 1-phenyl-penta 2,4-diyne at the budding and flowering stages were the most abundant constituents (Ranjbar et al., 2020). According to Danesch et al. (2010), the main chemical compounds (during flowering stages) were methyl eugenol, caryophyllene oxide, spathulenol and sabinene. During the maturity period, the following chemical compounds were highlighted β-pinene (2.92%), eucalyptol (2.04%), artemisia ketone (3.61%), camphor (2.36%), methyl eugenol (18.63%), β-caryophyllene (5.11%), capillene (33.33%), spathulenol (8.45%), and ent-spathulenol (14.84%) (Figure 5).

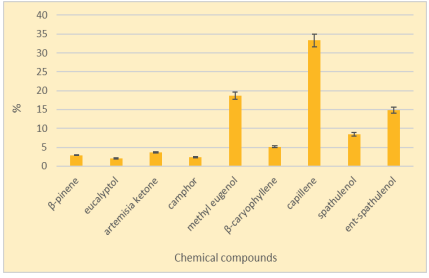

Figure 5. Major chemical compounds in the volatile oil of *Artemisia scoparia* in the maturity phenophase

The major chemical compound ent-spathulenol was only found during the maturity period, whereas some minor chemical compounds were considered the following: α-pinene, cimen, chrysanthenone, β-cubebene, caryophyllene oxide (Figure 6). In the maturity phenophase, from the category of the minor chemical compounds, the following substances were not found in the growth and flowering phenophases: cimen, artemisia ketone, chrysantenone, camphor and caryophyllene oxide.

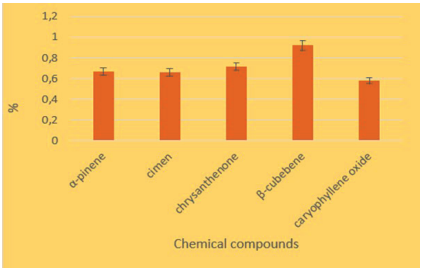

Figure 6. Minor chemical compounds in the volatile oil of *Artemisia scoparia* in the maturity phenophase

The chemical compound methyl-eugenol was present only in the flowering and maturity phenophase, registering a higher value in the maturity phenophase (18.63%), compared to the flowering stage (5.49%). Methyl eugenol has antimicrobial and antifungal activity (Joshi, 2013).

The chemical compound β-caryophyllene showed a higher value in the maturity phenophase (5.11%) compared to the other two phases, growth and flowering (0.49 and 0.96% respectively) of the same compound. β-caryophyllene has been used for therapeutic agent in traditional medicine and has antimicrobial activity (Yoo & Jwa, 2018). The major chemical compounds common to the three phenophases were β-pinene and capillene. In the three phenophases, for capillene there were registered high values: 71.22% (growth phenophase), 66.20% (flowering phenophase) and 33.33% (maturity phenophase). The chemical compounds limonene, trans-β-ocimene and γ-terpinene, which were found in great quantities in the growth and flowering phenophases were not found in the maturity phenophase. Also, the chemical compounds, eucalyptol, artemisia ketone, camphor and spathulenol present in greater amounts during the maturation period, were not found in the other two phenophases (growth and flowering).

The analysis of leaf and root oils of *Artemisia capillaris* Thunb. syn. *Artemisia scoparia* Waldst. & Kit. (Asteraceae family) showed the dominant presence of phenyl alkynes (61.2%,

85.5%), viz. capillene 60.2% and 82.9%, respectively (Joshi et al., 2010). In the case of the chemical compound β-pinene, higher values were obtained in the growth phenophase (9.74%), respectively flowering (9.30%) compared to the maturing phenophase (2.92%). β-pinene has an antibacterial role (Rivas da Silva et al., 2012), antiproliferative activity against the cancer cells (Li et al., 2009), showing antidepressant-like and sedative-like activity (Guzmán-Gutiérrez et al., 2012). The analysis of the volatile oil extracted from plants in the Tajikistan region indicates the presence of the main chemical compounds: diacetylenes 1-phenyl-2,4-pentadiyne, capillene, β-pinene, methyl eugenol, α-pinene, myrcene, limonene, and (E) -β-ocimene (Sharopov & Setzer, 2011). The major constituents of the oil of *A. scoparia*, collected in Khorasan province, from Iran, were: β-pinene, carvacrol, limonene, cis-ocimene, methyl eugenol, and trans-ocimene (Khayyat & Karimi, 2004). The main identified constituents of the oil obtained from the vegetative stage were as follows: α-thujone, β-thujone, camphor and 1,8-cineole, while for the oils of floral budding and flowering stages, the major ones were α-thujone and β-thujone respectively (Mirjalili et al., 2007). The research conducted by Kaur et al. in 2010 showed that the main chemical compounds from the leaves of *A. scoparia* plants growing in wastelands around Chandigarh were: p-cymene, myrcene and (+) - limonene, respectively.

The essential oil of *A. scoparia* (full-blooming stage) from Serbia was rich in capillene (63.8%), β-pinene (26.1%), (Z)-β ocimene (23.8%) and limonene 10.7%) as the major compounds according to Ickovski et al. (2020). The major components of the essential oil from Turkey determined by Demirci et al. (2005) were capillene (53.0%), β-pinene (20.8%), β-caryophyllene (16.4%), (Z)-β ocimene (16.4%), mircene (12.8 %) and limonene (11.0%). Our results are similar to those obtained by Ickovski et al. (2020) and Demirci et al. (2005), capillene being the main chemical compounds.

CONCLUSIONS

The species of the genus *Artemisia* are plants with a wide use due to the variation of the content in chemical compounds. The main chemical compound was capillene which can be useful in various industries as well as in chemotaxonomic determination. The composition of the volatile oil according to the obtained results and the existing data in the specialised writings, varies depending on the phenophase, the parts of the plant and the ecotype.

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THE PRESENCE OF CHROMIUM IN AGRICULTURAL SYSTEMS. A COMPREHENSIVE REVIEW

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Abstract

Currently, the entire world is facing major challenges related to agricultural practices and heavy metals contaminations of agricultural systems and food production. On the other hand, the structure, texture, and properties of the soil have deteriorated as a result of intensive conventional agriculture based on the addition of different inputs. Along with these, toxic metals affect agricultural soils, crops, food chain, becoming a major threat to living systems. Among these is chromium (Cr), an element naturally occurring in rocky soils and volcanic dust. The increased use of chromium in several multiple activities causes soil and water contamination. Differently from other heavy metals like lead, cadmium, and copper, chromium presents different degrees of toxicity depending on its chemical form. In the present review, we present data regarding chromium abundance in agricultural systems, factors favouring the absorption in the plant and bioaccumulation in different organs and tissues, bioaccumulation and translocation factors, its toxicity in plants, animals, and human through the food chain, and how it can be quantified using different types of analysis.

Key words*: chromium toxicity, chromium uptake and transport, environmental pollutant.*

INTRODUCTION

Environmental pollution with toxic metals became one of the most important issues that undermines global environmental sustainability efforts. In the last decades, the contamination, especially with hexavalent chromium [Cr (VI)] form in both terrestrial and aquatic ecosystems increased rapidly with industrialization, severely impacting our environment and natural resources, especially water and soil (Usman et al., 2020). If the crops are cultivated on a polluted agricultural soil, the toxic elements are translocated in crops and then through the entire food chain.

Contamination of water and soil with chromium can be made by fertilizers, fungicides, plastic film, sewage irrigation and industrial activities (mining, chemical industry, tannery wastes) and also from exhaust smoke of vehicles (Reijonen & Hartikainen, 2016; Li et al., 2020; Ahmad et al., 2021).

Having in view that chromium has a lot of environmental implications and negative effects on human and animal health, the aims of this paper were to review the literature data concerning chromium and to present its influence in agricultural systems, the bioaccumulation in different organs and tissues, its toxicity in plants, animals, and human through the food chain.

For the present study, several scientific journal articles, proceedings papers, reports and official methods were selected and reviewed. The literature search was carried out using the most important and commonly practical databases, such as Web of Science, Science Direct, Scopus, PubMed and Google Scholar.

Firstly, the following specific words were chosen: chromium in health, chromium in soil, chromium abundance in agricultural soil, chromium accumulation in plant organs, and toxicity of chromium, methods of extraction and analysis.
The publications were chosen, collected and reviewed based on the criteria of this paper which is correlated to the objective.

1. Chromium in soil

Governmental authorities and international organizations have set maximum permissible limits for chromium in soil.
The Word Health Organization (WHO) recommended safe limits for Cr (VI) in wastewater and soils used for agriculture are 0.05 $mg \cdot L^{-1}$ and 0.1 $mg \cdot kg^{-1}$ respectively (Kinuthia et al., 2020). In the agricultural soil, limit values for chromium are: in US 26 $mg \cdot kg^{-1}$, Spain 80 $mg \cdot kg^{-1}$, Croatia 80-120 $mg \cdot kg^{-1}$, Czech Republic 200 $mg \cdot kg^{-1}$and 64 $mg \cdot kg^{-1}$ in Canada (Broomandi et al., 2020; Christou et al., 2021). In Romania the safe limits for Cr 30 $mg \cdot kg^{-1}$, and for Cr (VI) 1 $mg \cdot kg^{-1}$, according to Environmental protection law no.137/1995. The use of irrigation water can have harmful effects on health because it can contain trace elements like chromium (Cr), nickel (Ni), cadmium (Cd). The maximum limits for irrigation water according to the policies of Netherlands and Spain and Belgium are 2 $mg \cdot L^{-1}$ for Cr (VI) and 5 $mg \cdot L^{-1}$ for total chromium in EU Member States (Tumolo et al., 2020).
The presence of high concentrations of Cd and Cr salts produce negative effects on chlorophyll and photosynthetic activities in plants (Del Bubba et al., 2013; Nawaz et al., 2021).
Cr mobility and bioavailability in soil is influenced by physiological and chemical properties of the soil such as: pH, electrical conductivity (EC), organic carbon content, cation exchange capacity (CEC). Soil properties are interconnected with plant growth and development.
Depending on the pH values, chromium can be found as trivalent chromium [Cr (III)] or hexavalent chromium [Cr (VI)]. Cr (VI) can be reduced to Cr (III) by Fe (II), phosphate, organic matter. Cr (III) it is almost precipitated in acidic media (pH 5.5). For plants, toxicity of Cr is dependent on oxidation state, Cr (VI) being more toxic than Cr (III). Cr (VI) as chromate (CrO_4^{2-}), it is water soluble and it is more mobile and toxic than Cr (III). Cr (VI) can be found in the soils that have a high pH (>7.5), low organic matter and a high Mn content. If it is found in the soil of a high-valent Mn as electron acceptor, it causes the oxidation of Cr (III) to (VI). In soil, redox reactions of Cr and Mn are strongly influenced by microbial activity as well as by physico-chemical soil properties (Del Bubba et al., 2013; Ding et al., 2014; Reijonen & Hartikainen, 2016; Hamilton et al., 2020; Tumolo et al., 2020). Some researchers (Zhang et al., 2021) demonstrated in a study that distribution factor values in silt-clay for both total Cr were 1.27 and 2.29 for Cr (IV) times higher than those in coarse sand samples. It can be said that fine soil particles have a high environmental risk because of their stronger accumulation ability and mobility. High levels of Cr in soil can affect microbial diversity and enzyme activities like cellulase (Li et al., 2021).
The ratio of soluble and stable organic C and Fe, Al, and Mn concentrations found in organic matter are involved in the process of Cr adsorption/desorption. Soils that have a high level of organic matter can stimulate the reduction of hexavalent chromium. Cr (VI) could stay in the soil for years, especially in sandy soils and those with a low level of organic matter (Del Bubba et al., 2013).

2. Chromium in crops

Crops can take and accumulate Cr (VI) in their edible tissues, from polluted soils. This is a concern related to the food chain, people and animal health. In the plants, Cr toxicity have a negative impact on physiological and biochemical processes such as: photosynthesis, transpiration, pigment biosynthesis, root growth and nutrient uptake, leading to perturbations in redox homeostasis and signalling, damages in membrane lipids, DNA, proteins and enzymes, affect also enzymatic activities linked to starch synthesis and N-metabolism, seed germination, flowering, fruit setting, crop yield loss, and deteriorate food quality (Del Bubba et al., 2013; Singh et al., 2013; Stambulska et al., 2018; Sharma et al., 2020; Christou et al., 2021).

The reduction of sugar content in chromium stressed plants was also observed, this probably being related with the photosynthetic inhibition or stimulation of respiration for higher energy requirements. Soluble sugar content can be an important parameter for assessing the effects of Cr on plants (Kakkalameli et al., 2021; Sharma et al., 2020). Very low concentration (<1mg·kg^{-1}) of Cr (VI) in soils can produce ecotoxic effects.

High content in chromium can influence the uptake of plant nutrients like: iron (Fe), magnesium (Mg), phosphorus (P) and calcium (Ca). In rice, an excessive chromium exposure leads to a decrease in the uptake of: N, P, K, Zn, Cu and Fe (Singh et al., 2013; Sharma et al., 2020). A study conducted by Ding et al. (2014) regarding the influence of Cr on the carrots cultivated in soils with pH below 7.5 evidenced that its application generated the decrease of carrot fresh weight and yield.

Nawaz et al. (2021) in their study showed that Cd had the maximum translocation from soil to wheat (*Triticum aestivum* L.) shoot, followed by N, Cr and Ni.

In wheat, maize, peas, rice, beans, sunflower, canola and sorghum, Cr have toxic effect on: photosynthetic pigments, photosynthetic apparatus, inhibit the electron transport, inactivate the Calvin cycle enzymes, decreased CO_2 fixation, decrease the activity of Ribulose-1,5-bis-phosphatecarboxylase/oxygenase (RuBisCO), change the morphology of leaves, reduced root length, lamellar structure, the ultrastructure of chloroplasts, enhance hydrogen peroxide (H_2O_2) production and lipid peroxidation. Cr (VI) decrease leaf water potential, which can develop water stress in plants (Del Bubba et al., 2013; Singh et al., 2013).

In bush beans, sunflower, mung bean, chromium reduced water potential, increased transpiration rate, reduced diffusive resistance, and wilting, reduced diameter of the tracheal vessels.

In various cereals and legumes chromium affects the uptake of nutritive elements, inhibiting assimilatory enzymes. In beet, a high concentration of Cr inhibits electron transport sites in photosynthesis in isolated chloroplasts, affecting the photosynthesis. In Chinese cabbage, Cr inhibits seed germination and development and subsequently reduces the dry matter production and yield (Del Bubba et al., 2013).

In tomato plants was observed an increased accumulation of chromium due to the presence of citrate, aspartate and oxalate, which converted inorganic chromium into organic complexes, which are available for the plant to uptake. In *Brassica rapa* and *Spinacia oleracea*, Cr uptake and translocation to aerial parts is made through Fe channels (Sharma et al., 2020).

Cr (VI) unlike other metals it directly reacts with DNA, forming DNA–protein and DNA–DNA cross-links, being very mutagenic in plants like: *Brassica napus*, *Arabidopsis thaliana*, *Trifoliumrepens*. It can also induce DNA mutation; chromosomal aberrations and mitotic aberrations in: *A. cepa* and *V. faba*. In roots of *P. sativum* it was observed that Cr (VI) alter cell cycle dynamics and ploidy levels (Singh et al., 2013).

There are plants that can accumulate chromium (1000 mg·kg^{-1}) and are categorized as chromium hyperaccumulators. For example, *Helianthus annuus* it is a moderate chromium accumulator in shoots and *Brassica napus* is a low accumulator in whole plant (Singh et al., 2013).

Cr (VI) being very soluble than Cr (III) (which is immobile in ambient environments, being found bound to organic matter) can lead to more toxic effects on animals and human's health.

Soil and crops contamination with chromium it is reflected in water, beverages and food, throughout the whole food chain.

Maximum permissible limits set for foodstuff by FAO 2002 are 1 mg·kg^{-1}fresh weight (F.W.), and for fruits and vegetables of 2.3 mg·kg^{-1} F.W. (FAO 2011; Christou et al., 2021). According to EFSA's tolerable daily intake (TDI) of 0.3 mg·kg^{-1} body-weight/day is for Cr (III).

Contamination of drinking water and food with high levels of Cr (VI) has a 60-fold increase in the rate of gastric cancer, which leads to loss of life expectancy (Del Bubba et al., 2013).

The main form of Cr found in food is Cr (III), the food and beverages consumed by humans should be examined for this priority toxic element (Yaman, 2020). The United States

Environmental Protection (USEPA) listed Cr (VI) as one of the seventeen metals that are dangerous to human health (Usman et al., 2020).

3. Chromium effects on human health

Cr (VI) is classified as belonging to group 1 carcinogen element by the International Agency for Research on Cancer (IARC) and it is very common in polluted environments and industrial places (Wang et al., 2017). Absorption of Cr depends on particle size, oxidation state and its solubility and health effects depend on dose, exposure level and duration (Shekhawat et al., 2015; Tumolo et al., 2020). Absorption and metabolism of chromium species in the human body can be realized through oral, dermal and inhalation pathway Cr (III) it is less absorbed than Cr (VI), so the transport to cells will be different. Cr (III) enters in the cell by passive diffusion or phagocytosis (Shekhawat et al., 2015; Wang et al., 2017).

Cr (VI) enters into the cell via a non-specific anion channel and in the cell is reduced by glutathione to Cr (V) and after that converted to Cr (III).

In red blood cells Cr (III) binds to the cellular components and then it is unable to leave the cells. As a result of this process, hydrogen peroxide and free radical species are produced, which generate high levels of oxidative stress that lead to lipids, protein and DNA modification. These modifications limit the DNA repair capacity of the cells. Cr also can induce tumor suppressor gene p53 (Shrivastava et al., 2002; Shekhawat et al., 2015; Wang et al., 2017; DesMarais& Costa, 2019; Engwa et al., 2019; Tumolo et al., 2020; Yaman, 2020). The main reduction of Cr (VI) to Cr (III) takes place in tissue of lungs (Shekhawat et al., 2015).

It was found also that chromium competes for one of the binding sites on transferrin, in this way there were several studies that investigated possible interactions between iron and chromium (Yaman, 2020).

The Cr (VI) is considered the most toxic, causing acute and chronic toxicity. The most important heath reactions after contact, inhalation, or ingestion of Cr (VI) are the following: dermatitis, allergic and eczematous skin reactions, skin and mucous membrane ulcerations, perforation of the nasal septum, allergic asthmatic reactions, bronchial carcinomas, damage to the lower respiratory tract, gastro-enteritis, hepatocellular deficiency, renal oligo-anuric deficiency, anaemia and possibly death (Baruthio, 1992; Wilbur et al., 2012; Wang et al., 2017; Engwa et al., 2019; Li et al., 2020).

In the liver, kidney, spleen and bone was found a higher concentration of chromium, than in other organs (Shekhawat et al., 2015; Yaman, 2020). The main routes for the excretion of chromium are via kidney/urine and the bile/faeces (Shrivastava et al., 2002).

Cr (VI) is considered to be reduced in the stomach to Cr (III), which presents low ability to enter cells (Yaman, 2020).

Drinking chromium contaminated water can lead to mouth ulcers, indigestion, acute tubular necrosis, vomiting, abdominal pain, kidney failure and even death (Shekhawat et al., 2015). Lee et al. (2019) found that is a correlation between soils contaminated with high levels of Cr and Sjogren's syndrome, which is an autoimmune disorder with symptoms like dry eyes and dry mouth, patients with this syndrome can have also lymphocytic infiltration into extra glandular tissues, not only in lacrimal and salivary glands (Fox, 2011).

4. Chromium effects on animal health

Studies on rats and mice showed that if Cr (VI) is given in drinking water then it shows the ability to cross the placenta and reach to fetal tissue. Also, different health effects that includes cellular infiltration in the liver, pancreatic and small intestine was observed and carcinogenic activity (intestines and oral carcinogenicity) in both the sexes of rats and mice if they drink contaminated water (Shekhawat et al., 2015). In the studies on hamsters and mice parenteral administration of Cr (III) or Cr (VI) results in embryotoxicity or fetotoxicity and teratogenicity (WHO 1996). We can say that chromium is responsible for the toxic effects in humans and in animals also. Cr (VI) is mainly responsible for all carcinogenic activity in comparison to trivalent chromium (Shekhawat et al., 2015).

5. Chromium remediation

There are some soil remediation techniques such as: chemical stabilization, reduction, transformation using adsorption, desorption, precipitation and oxidation-reduction reactions, solidification, soil washing, membrane filtration, photocatalysis, bioremediation using microorganisms, nano-particles and phytoremediation using terrestrial and aquatic plants (Sathya et al., 2020; Azeez et al., 2021; Li et al., 2021; Prasad et al., 2021; Sharma et al., 2021; Wang et al., 2021). Soil phytoremediation can be performed by: phytoextraction, rhizofiltration, phytostabilization, phytovolatilization, phytodegradation and rhizodegradation (Aparicio et al., 2021).

Yang et al. (2021) in their study mentioned the reductants such as: iron-based reductants (elemental Fe and Fe (III)), sulfur compounds (sodium sulfite and sodium thiosulfate) and organic amendments (humic, tartaric acids, isopropyl alcohol) that can be used for soil remediation.

6. Extraction methods of chromium from different matrices

Strictly analytically, various extraction procedures have been studied in order to determine Cr species in soil and plant samples. The sample extractions have to be chosen according to what chromium species we need to identify and quantify.

Sample preparation before analysis

Before conducting each analysis, the several different soil samples were dried in air (~ 25ºC) and then passed through a 2 x 2 mm stainless steel sieve (Franco et al., 2011).

As a rule, the decomposition of the matrix is a mandatory step before their elemental analysis (Szymczycha-Madeja et al., 2014).

For soil sample preparation there are official methods like:

- EPA Method 3050B, for acid digestion of sediments, sludges, and soils;
- EPA Method 200.2. Sample Preparation Procedure for Spectrochemical Determination of Total Recoverable Elements.

Extraction solvents

Extraction solvents that can be used for the identification of Cr (VI) in soil and plant samples are: oxidants (H_2O_2), acids at various concentrations (e.g., acetic acid, nitric acid, hydrofluoric acid), buffered salts (e.g., ammonium acetate), neutral salts ($CaCl_2$, Na_3PO_4 or Na_2CO_3), chelating agents (e.g., EDTA, DTPA, EDDS) (Leśniewska et al., 2017).

Methods of extraction

For soil and plants, there are several **conventional methods**, as:

a) open vessel hot-plate wet digestion;

b) closed vessel microwave-assisted wet digestion. The conventional extraction methods have *advantages* as:

- large sample amounts;
- complete destruction of organic matter;
- simplification to a variety sample;
- application to a variety sample;

Also, the conventional extraction methods have *disadvantages* as:

- like slow, laborious, time-consuming;
- loss of volatile analytes;
- possible contaminations of samples;
- high temperature treatments, lower precision.

a) Open vessel hot-plate wet digestion method has a series of *advantages*: - large sample amounts;

- lower temperatures as compared to dry ash;
- application to a variety sample.

Also, the method has *disadvantages* like:

- the slow, time-consuming;
- laborious;
- loss of volatile analytes;
- contaminations of samples;
- use of large amounts of strong oxidizing reagents;
- pre-concentration of reagent impurities, incomplete solubilisation of sample constituents;
- high temperature treatments.

According to literature, quantification by flame atomic absorption spectrometry (FAAS) could be achieved by using:

- dry ash 0.25 g, 450°C, 8 h, 5 mL HNO_3 (25%), final volume 10 mL (Narin et al., 2004);
- 5.0 g, 500°C, 6 h ash: 2 mL HCl (6 $mol \cdot L^{-1}$), final volume 25 mL (Chen et al., 2009).

For the samples that will be analysed by inductively coupled plasma - optical emission spectrometry (ICP-OES) and inductively

coupled plasma mass spectrometry (ICP-MS), it can be used:
- a mixture of HNO_3 (10 mL), 3h, (0.25 g + 10 mL), brought to a final volume of 25 mL (Kara 2009);
- a mixture of HNO_3 (10 mL), 1h, + $HClO_4$ (1 mL), 1h, (0.5g + 11 mL), brought to a final volume of final volume 100 mL (Ashraf and Mian, 2008).

b) Closed vessel microwave-assisted wet digestion

The method has a series of *advantages:*
- closed system;
- reduce volume of aggressive reagents;
- minimal contamination;
- lack of loss volatile analytes;
- minimal contaminations of samples;
- ➢ good precision and accuracy. Also, the method has *disadvantages* such as:
- high cost of equipment;
- ➢ small sample amounts;
- time required for cooling;
- cleaning vessel;
- control of process;
- the matrix may require a different microwave program.

For this type of digestion, there are reported the following protocols:
- HNO_3 (20 mL), extraction time 70 min, (2.0g + 20 mL), to a final volume of 50 mL, (Shen and Chen, 2008); - HNO_3 (3 mL), 30 min, + H_2O_2 (0.5 mL), 30 min (0.25 g + 3.5 mL), to a final volume of 25 mL for ICP-OES and AAS detection (Dash et al., 2008).

7. Quantification methods for chromium

a) *solid matrices* - US EPA method 3060A.

Generally, the Cr in *soil samples* is quantified by using the following techniques:
- flame atomic absorption spectrometry (FAAS) (Aksuner et al., 2012);
- graphite furnace atomic absorption spectrometry (GF AAS) (Dash et al., 2008);
- inductively coupled plasma optical emission spectrometry (ICP-OES) (McKenzie et al., 2010);
- inductively coupled plasma mass spectrometry (ICP-MS) (Kara, 2009);
- X-ray fluorescence spectrometry (XRFS) (Salvador et al., 2002).

b) *water samples*

Generally, the Cr in water samples is achieved by using:
- ISO 11885, ISO 17294-2, ISO 15586, ISO 18412 (Cr VI), ISO 23913 (Cr VI), ISO 9174 (Section 4), EPA 200.8 (using ICP-MS), recommended by Codex Alimentarius;
- EPA 218.7 for determination of hexavalent chromium in drinking water by ion chromatography with post-column derivatization and UV-VIS spectroscopic detection;
- EPA 200.9 for Determination of Trace Elements by Stabilized Temperature Graphic Furnace Atomic Absorption.

c) *other matrices*
- EN 13804:2002, EN 13805:2002, EN 13806:200, EN 14082:2003, EN 14083:2003, CEN/TS 15506:2007 and AOAC 974.27 (using AA spectrophotometer), recommended by European Commission in 2012 regarding food;
- EN 14082, EN 14083, AOAC 2006.03, AOAC 2011.19 (using ICP-MS) / ISO 20649 |IDF 235, according to the FAO/WHO Food standards programme codex from 2015, regarding infant formula.

CONCLUSIONS

Our review study revealed that Cr is present in water, soil, plants, and food in various concentrations. Its availability depends on different agricultural systems factors and it can have both positive and negative influence on soil, plants, human and animal health depending on the concentration and oxidation state. Cr (VI) species are toxic and carcinogenic, meanwhile Cr (III) plays an important role in molecular mechanisms of biological systems.

From agricultural point of view, there are technologies for remedying contaminated soils and irrigation water that can be used.

There are currently advanced techniques for chromium quantification even at trace levels from various samples (soil, plant, water, etc).

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CASSIS FRUITS - NATURAL SOURCE OF FOOD AND ANTIOXIDANTS THROUGHOUT THE MATURATION PERIOD

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Abstract

The paper presents the biological variation in biochemical compounds of fruits belonging to Ribes nigrum L. species during the maturation process. The fruits from two varieties were collected in the following phenological stages: early first fruits (SIII), advanced first fruit (SIV), harvesting maturity (SV), and consumption maturity (SVI). The extracts were subjected to analysis. Total phenolic content (TPC) expressed as gallic acid equivalent (GAE), total flavonoid content (TFC) expressed as rutin equivalent (RE), and free radical scavenging activity (FRSA) expressed as mg/mL ascorbic acid equivalent (AAE), and gas-chromatographic profile were determined. The phenolic content differed considerably during the maturation process. Thus, the maximum value of TPC was achieved by 'Kzvana' fruits in the SV stage with 7.36 mM GAE/ml extract. The flavonoid content was highlighted in 'Roxia' fruits in the SVI stage with 1.24 mM RE/mL extract. With regard to FRSA, 'Kzvana' fruits have better activity. Also, the aromatic profile was characterized using gas chromatographic analysis.

Key words*: blackcurrant, maturation phenophases, phenolics, gas-chromatography.*

INTRODUCTION

Currently, there is an increasing trend of consumer acceptance for *Ribes nigrum* L. fruits, largely due to the variety of potential health benefits of active compounds such as natural antioxidants (Tabart et al., 2006; Raudsepp et al., 2010; Tabart et al., 2012). These compounds are mainly represented by flavonoids, phenolic acids, and tannins, and because of their high content the blackcurrants have come to be regarded as superfood (Ruiz del Castillio et al., 2004). There is a need also to analyse the function and structure of such compounds especially in fruits, so pleasant to us all (Milivojevic et al., 2009).

Currants are one of those fruits that have a boost intake of both pleasant-like sugars and aromatic compounds and nutraceuticals like myricetin, quercetin and isorhamnetin with neuroprotective activity and polysaccharides with immunostimulatory, antitumor, antimicrobial and antiinflammatory effects. Also, currants around the world are important for the food industry mainly because of their colour and organoleptic properties, which makes them suitable material for diverse food applications (Xu et al., 2018; Eksi Karaagac et al., 2020; Rodrigues et al., 2020).

Black currants are perennial bush plants, economically important, growing in temperate zones of Europe, Russia, northern Asia, and New Zealand (Woznicki et al., 2015).

Black currant berries, are favoured for their organoleptic properties such as pleasant color and intense taste, which are due to phenolic compounds like anthocyanins, and the presence of sugars, acids, and volatile compounds (Varming C. et al., 2004; Tarko et al., 2020).

Many morphological and biochemical changes are occurring during growth and development. This depends on the physiological state of the plant, agriculture technologies (Vagiri et al., 2013), and other factors such as genotype, climate, degree of ripening, harvesting time, and storage conditions (Rubinskiene et al.,

2006). For example, the genetic background determines the sugar accumulation of black currants and the response to weather conditions (Zheng et al., 2009). Also, accumulating evidence suggests that genotype may have a profound influence on the content of bioactive compounds in berries (Pantelidis et al., 2007). Other compounds that are accumulating are the phenolic compounds, some of the secondary metabolites occurring in plants. Their biosynthesis depends on numerous enzymes, and their metabolism is combined with morphological and biochemical regulatory patterns of plants (Thitilertdechaa and Rakariyatham, 2011). Although many studies have investigated the fruit quality and antioxidant properties of berries (Gipson et al., 2013; Ferreira Zielinski et al., 2015), little is known about variation in fruit quality, levels of phenolic compounds, and antioxidant activities of different currants varieties in relation to fruit maturation stages for varieties grown in Romania (Diaconeasa et al., 2015).

The aim of this paper was to investigate the natural variation in biochemical compounds of two varieties of berries belonging to *Ribes nigrum* L. species during maturity process. These two varieties of blackcurrant are part of a group of 12 varieties studied. All were analyzed according to the same criteria, in order to highlight the best of them, for their cultivation on large areas. The correlations between the compounds and maturation stages were also investigated in order to see the connection between the state of maturation and biosynthesized compounds.

MATERIALS AND METHODS

Biological material

The biological material consists of fruits in different maturation stages from 'Roxia' and 'Kzvana' varieties, harvested from the experimental field of the research bio-base of the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Figure 1. *Whole fruit fistfruits*

Figure 2. *Advanced firstfruits*

Figure 3. *Harvesting maturity*

Figure 4. *Consumption maturity*

Fruit extracts preparation

The fruit extracts were performed using the ratio of 1:6 g L^{-1}. The fresh fruits were smashed, over which an ethanol solution was added (Saúl Olivares-Galván et al., 2020). The resulting mixture was subjected to ultrasound extraction on ice bath for the epicarp patency, for 30 minutes. Afterwards, the extracts were filtered, followed by centrifugation. The supernatant was used for the further analyses.

Determination of total phenolic content (TPC)

Total phenolic content in fruit extracts was performed spectrophotometrically using Folin-Ciocâlteu method from the literature (Sariburn et al., 2010). The total phenolic content was expressed as gallic acid equivalent (GAE) per ml of extract.

Determination of total flavonoids content (TFC)

Total phenol content in fruit extracts was determined spectrophotometrically using an adapted method (Sushant Arya et al., 2019). The total flavonoid content was expressed as rutin equivalent (RE) per ml of extract.

DPPH•free radical scavenging activity (FRSA)

Free radical scavenging activity of black currant extracts was determined using the stable radical 2,2 diphenyl-1-picrylhydrazyl (DPPH•, Sigma), according to the method described in the literature (Suleria et al., 2020). Free radical scavenging activity was expressed as mg/mL ascorbic acid equivalent (AAE).

Gas-chromatographic profile

Qualitative determination of volatile biologically active compounds was performed using a Thermo Trace 1310 gas chromatograph coupled with an ISQ system mass spectrometer, according to an adapted method (Vulic et al., 2012). The system is equipped with a chromatographic column (Thermo Scientific Trace GOLD GC Column), having a length of 30 m, an inner diameter of 0.25 mm, and a stationary phase 5% phenyl methylpolysiloxane, with a thickness of 0.25 µm. The mobile phase was helium, with a flow rate of 1 ml/min. The initial temperature was 40℃, with a ramp of 10℃/minute, reaching up to 250℃. The volume of sample introduced was 1 ml, its vaporization taking place in about 30 minutes.

Statistical analysis

A general linear model, Duncan test was used for the comparison of means for the content of bio compounds between groups, using Statistical Package for Social Science (SPSS version 21.0). The statistical significance was considered for the probability value of difference $p < 0.05$. The obtained results were expressed as mean values ± standard deviation (SD). Microcal Origin version 6.0 software was used for the charts design and Pearson correlation coefficient.

RESULTS AND DISCUSSIONS

Total phenolic content

The results of the quantitative determination of total phenolic content are shown in Figure 5. The graphs were made in the form of bars to highlight the difference between the varieties on the same maturation stage. Concerning the concentration of total phenolic accumulated during fruit maturation stage III, the upper limit is found in 'Kzvana' genotype with a concentration of 5.87 mM GAE / ml extract.

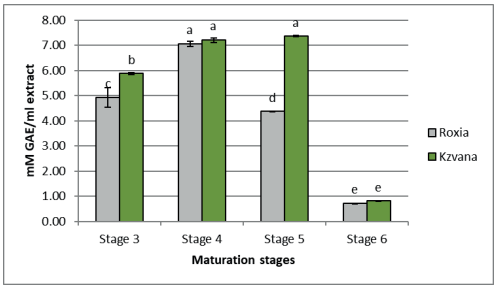

Figure 5. Total phenolic contents values for 'Roxia' and 'Kzvana' fruit genotypes

In stage IV of fruit maturation also it can be observed an accumulation of polyphenol content for both Roxia and Kzvana genotypes with the concentrations of 7.2 mM, respectively 7.06 GAE/ml extract. In stage V of fruit maturation, the phenolic content increases, reaching a highest point of 7.36 mM GAE / ml extract in the 'Kzvana' genotype case. In the final stage of maturation, both genotypes have recorded a sharp decline of phenolic content. A possible explanation can be drawn from their role in plant growth and development. Thus, with defense function, phenols are found in larger quantities in the early stages of fruit maturation when the attacks are susceptible to pests and diseases, decreasing when they reach harvest and consumption maturity.

According to the obtained results, apparently as the fruit matures, the phenolic content decreases. On one hand this may be due to the fact that during fruit maturation the plant does not need a high level of phenols, instead the amount of flavonoids may increase, with a higher ratio, both groups of active principles having the same genetic precursors.

Total flavonoid content

As regards the quantitative determination of total flavonoid content, the results are shown in Figure 6. From the quantitative analysis of fruits extracts of *Ribes nigrum* L. one can notice that the value of flavonoid content for the genotypes studied is directly proportional to the progress of fruit maturation process, except for the Roxia genotype in stage IV of maturation. For maturation stage III it can be observed that between the genotypes studied in terms of flavonoid content there is no statistically significant difference. With regard to the maturation stage IV, the highest concentration of flavonoid was recorded in the 'Roxia' genotype case with a value of 1.34 mM RE / mL extract.

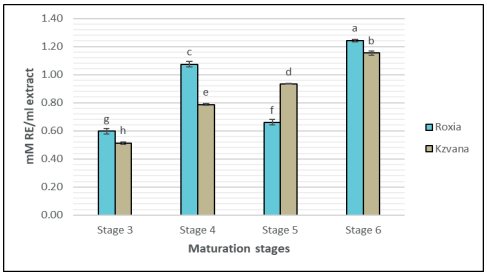

Figure 6. Total flavonoid content values for 'Roxia' and 'Kzvana' fruits

Concerning the quantity of flavonoid in the case of fruits in the maturation stage V, it can be observed that compared with stage IV, the values are decreased, the maximum concentration being reached by the 'Kzvana' genotype. The influence of maturation stage (IV) on the accumulation of flavonoid content can be concretized in a maximum value of these compounds available for both genotypes, here emphasizing the variety 'Roxia'.

Evaluation of free radical scavenging activity

The free radical scavenging activity is one of the modalities of expressing the therapeutic, biological and nutritional value of fruits and vegetables. Therefore, in Figure 7 is presented free radical scavenging activity of black currant fruit of 'Roxia' and 'Kzvana' genotypes.

In the maturation stage III, there are no significant differences of free radical scavenging activity values between the fruits of 'Roxia' and 'Kzvana' genotypes. During the fourth maturation stage analyzed, free radical scavenging activity of 'Roxia' and 'Kzvana' fruits registered increases throughout maturation, subsequently reaching a maximum activity in the maturation stage VI.

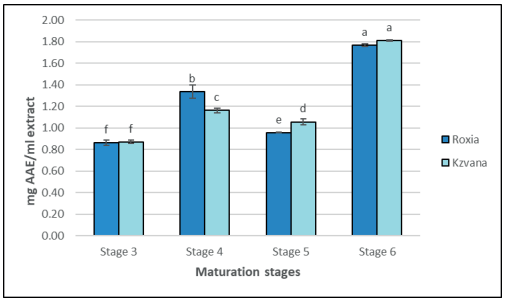

Figure 7. Free radical scavenging activity of *Ribes nigrum* L. genotypes

Thus, the values of free radical scavenging activity expressed as mg AAE / ml extract is between 0.86 - 1.77 for 'Roxia' genotype, and 0.87 - 1.81 for 'Kzvana' genotype.

Correlations between free radical scavenging activity and TPC

Correlations between free radical scavenging activity and phenolic content were tested in different stages of *Ribes nigrum* L. fruits maturation. Also, by testing the correlation between the value of free radical scavenging activity and the amount of phenolic content, it was observed that there is no direct interdependence, the correlation of those two characteristics showed that the free radical scavenging activity was not influenced by the total phenolic content, the correlation being distinct insignificant with the correlation coefficient R^2 = 0.277 for the probability p=0.08 for 'Roxia' (Figure 8), and R^2 = 0.299, p=0.078 for 'Kzvana' (Figure 9).

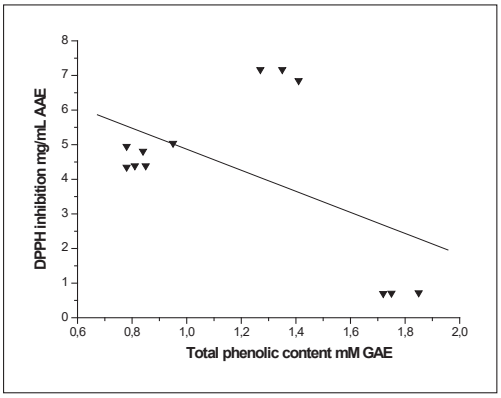

Figure 8. Correlations between the free radical scavenging activity and the total phenolic content for 'Roxia' fruits

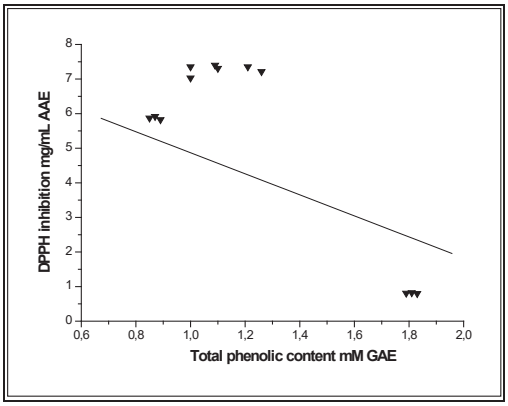

Figure 9. Correlations between the free radical scavenging activity and the total phenolic content for 'Kzvana' fruits

Correlations between free radical scavenging activity and TFC

As it is well known, the phenolic compounds have a very high antioxidant activity. Since the extracts obtained from the fruits of genotypes of the *Ribes nigrum* L. species are very complex in terms of the biochemical composition, it is very difficult to distinguish the compounds with the most antioxidant activity. Black currants are rich in active compounds that possess antiradical properties (Burdulis et al., 2009). These properties are also influenced by genotype (Nour et al., 2013). Today, in the literature, there are researches that show the link between free radical scavenging activity and flavonoids (Olajire et al., 2011; Muniyandi et al., 2019). This was

also demonstrated within this study. Thus, regarding the correlation between free radical scavenging activity and flavonoid content for the genotypes studied, it can be observed that there is a linear correlation strongly positive, Pearson correlation coefficient (R^2) exceeding the value of 0.900 for the probability $p < 0.05$, for all the maturation stages of the fruit. The correlation between free radical scavenging activity and flavonoid content for the variety 'Roxia' is presented in Figure 10. As it can be seen, it was evidenced a strong positive correlation, with the value of $R^2 = 0.960$ for the probability $p < 0.001$.

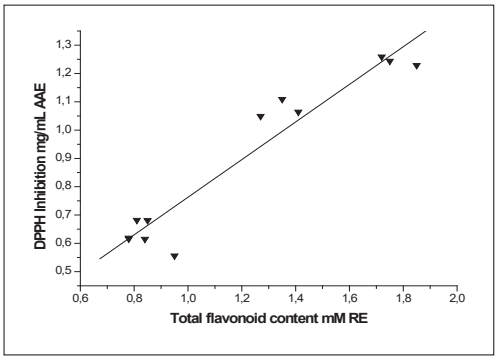

Figure 10. Correlation between the free radical scavenging activity and the total flavonoid content for 'Roxia' fruits

According to the graphical representation (Figure 11) for 'Kzvana' genotype, free radical scavenging activity has a positive linear correlation with the flavonoid content, this time more moderate, with a correlation coefficient $R^2 = 0.861$ for the probability of $p < 0.05$.

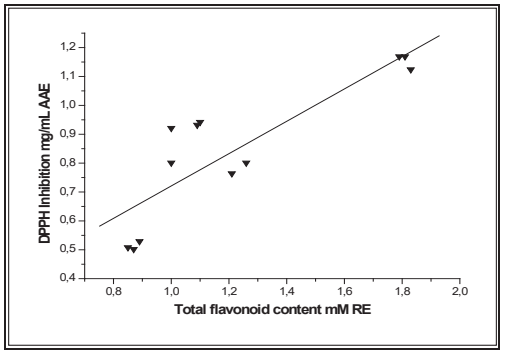

Figure 11. Correlation between the free radical scavenging activity and the total flavonoid content for 'Kzvana' fruits

However, the content of phenolics may correspond or not to the free radical scavenging activity of a fruit. This is due to the presence of other biologically active compounds with low molecular weight, such as glutathione (thiol), that participate in the redox reactions (Sochor et al., 2010).

The profile of the aroma compounds of the black and red currant (Table 1) is similar to that of most berries. According to the literature, berries may contain the following classes of compounds: aliphatic alcohols, aliphatic esters, monoterpenes, oxygenated monoterpenes, sesquiterpenes, phenols (Turemis et al., 2003). In the case of currants, over 150 volatile compounds have been identified, the most important classes being monoterpenes, sesquiterpenes, esters and alcohols (Varming et al., 2004; Liu et al., 2018)).

The most important aroma compounds for blackcurrant include esters such as 2-methylbutyl acetate, methyl butanoate, ethyl butanoate and ethyl hexanoate with sweet fruit flavor, nonanal, β-damascenone (result of carotenoid degradation), some monoterpenes (α-pinene, 1,8-cineole, linalool, terpinen-4-ol and α-terpineol), aliphatic ketones (1-octen-3-one), as well as sulfur compounds 4-methoxy-2-methyl-butanethiol, which give the specificity of smell for black currant (Vulic et al., 2012; Varming et al., 2004).

Regarding the volatile compounds identified for the varieties of *Ribes nigrum* L., alkanes and their isomers (n-Decane, n-Nonane and 2,2-Dimethoxybutane, 1,3,3-trimethoxybutane) were identified, highlighting the variety Roxia; other classes of compounds were: monoterpenes (D-Limonen), Kzvana variety with the highest content, oxygenated monoterpenes (Dihydro-Citronelol), and monoglycerides of fatty acids with antimicrobial role (Altieri et al., 2007), such as 2-mono-stearin in the case of the 'Kzvana' variety.

Also, following the evaluation of fruit extracts, components of essential oils with antimicrobial effect were determined, such as estragol (Bagamboula et al., 2004), in this case highlighting the 'Roxia' variety. The content in sugars is highlighted by the presence of sucrose in a high percentage in the case of 'Kzvana' variety.

Table 1. Profile of volatile compounds for black and red currant

Identified compounds	*Kzvana*	*Roxia*
	Aria %	
2,2-Dimethoxybutane	9.73	19.8
2-methyl, methyl ester butanoic acid	0.82	1.48
2,4-dimethyl-1-heptene	3.05	-
1,3,3-trimethoxybutane	-	-
3,3-dimethoxy-2-butanone	0.86	1.29
2-ethyl-1,3-Dioxolan-4-methanol	2.94	6.34
n-Nonane	0.71	1.36
Itaconic anhydride	1.8	0.54
3,6-Dimethyloctane	0.98	0.54
3-Methylnonane	-	-
Phenol	0.58	0.34
n-Dean	1.46	2.06
3,3-Dimethyloctane	0.59	1.31
4-methyldecane	0.75	1.55
d-Limonene	0.59	0.28
Dihydro-Citronelol	0.9	1.47
5-ethyl-1-nonene	1	1.49
3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	1.3	-
Estragole	1.24	1.73
3,5-dimethyl-Benzaldehyde	0.74	1.21
5-Hydroxymethylfurfural	1.81	-
4,6-Dimethyldodecan	0.82	1.01
4-Methyldecane	0.71	0.52
Sucrose	17.66	1.19
Fructose		30.74
d-Mannose	13.04	-
L-Sorbose	23.97	-
D-Galactose	-	-
2-methylhexadecan-1-ol	-	-
3-Icosene	1.06	-
(3E) –Isotridecanol	1.77	2.14
7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	1.26	-
2-mono-stearin	3.7	-
2-mono-palmitin	-	-

Aldohexoses (mannose, fructose), phenols, monoterpenes (D-Limonen) were also found in the identification of poultry compounds of blackcurrant varieties.

There were identified monosaccharides, in nature found as hexoses, like D-mannose (in 'Kzvana'), which it seems that they may facilitate the improvement of the human diet and health (Sharma et al., 2018; Shintani, 2019; Wu et al., 2020; Lin et al., 2021).

The retention times for each identified compound are shown in Figures 12 and 13.

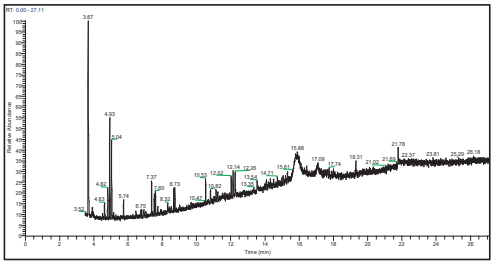

Figure 12. Gas chromatogram for the 'Roxia' variety

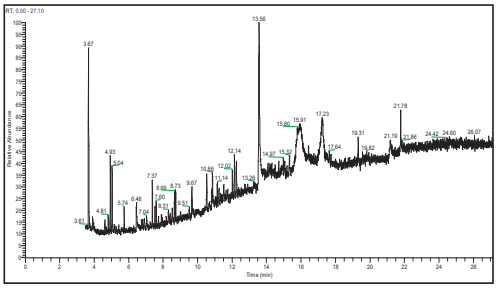

Figure 13. Gas chromatogram for the 'Kzvana' variety

CONCLUSIONS

With regard to the obtained results from the characterization of black currant extracts we may say that total phenolic and flavonoid content in black currant fruits is influenced both by the genotype and maturation stage. The influence of maturation stage on total phenolic content, the maximum values were recorded in maturation stage IV for 'Roxia' genotype, and stage V for 'Kzvana' genotype. The maximum accumulations of total flavonoid content, were recorded in the last stage analyzed for both genotypes, but emphasizing 'Roxia' genotype. Concerning free radical scavenging activity, 'Kzvana' genotype was highlighted in the last stage of maturation process.

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THE B.E.V.A. METHOD IN THE ASSESSMENT OF THE WOODY VEGETATION OF THE MARGHILOMAN BUZAU PARK

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Abstract

Relaxing in the shade of a tree, enjoying its fragrance, the oxygen offered as a gift, the refuge it offers, gives all the measure of the wealth of trees. An individual good, a collective wealth, the tree has a value attributable to the functions it performs. Whether the tree is young or centuries-old, it is directly or indirectly influenced by people, which is why it is important to appreciate its value. This is why it is necessary to assess the financial value of ornamental trees and estimate the amount of compensation in the event of damage. The chosen research topic is carried out in Buzău, on the domain of Alexandru Marghiloman. He built for his wife a wonderful tree park, which has become public today, on an area of 16,000 square meters. To date, there is no single reference assessment scale unanimously recognized by communities, experts, insurance companies and the judiciary. The B.E.V.A. method - the Tree Value Assessment Scale created in France (Barème d`Evaluation de la Valeur d`un Arbre) (or the method of large cities in France) - was used to evaluate the trees in Marghiloman Park. B.E.V.A. is used to calculate the value of a tree in an urban landscape or road alignment based on 4 criteria (species, girth, health, aesthetic value). The results have been registered and are part of the green inventory of this park.

Key words: *B.E.V.A., Marghiloman Park, woody vegetation.*

INTRODUCTION

Alexandru Marghiloman built for his wife a wonderful tree park, which became public nowadays. Today, it covers an area of 16,000 m^2, out of the initial 22,500 m^2, surrounding the 'Albatros' villa and its outbuildings, a park famous in the past due to its English style and the multitude of exotic plants acclimatized here.

The ensemble was built in several stages in the second half of the nineteenth century (ca. 1884) and consists of 'Villa Albatros', the work of French architect Paul Gottereau, a secondary body and stables, in a huge tree park. The buildings were abandoned in 1985 and are in poor condition. At present, only 'Villa Albatros' has been restored, the rest being severely damaged.

MATERIALS AND METHODS

B.E.V.A. - Tree Value Assessment Scale (Barème d'Evaluation de la Valeur d'un Arbre) (or the method of the large cities in France) - this method consists of the product of certain indices that represent the variety, aesthetics and health, location as well as size.

These scales are accredited through decisions of the deliberative assemblies of the communities and integrated in contractual documents, such as tree mapping, road regulations and general clauses/rules applicable to all public works contracts.

Depending on their specificity, local authorities have sometimes adapted this scale by changing the indices.

The arrangement or the value of the arrangement of the tree is obtained by multiplying the following 4 indicators between them:

1. species and variety index;
2. size index (circumference);
3. health index;
4. location and aesthetic value index.

Species and variety index

It is based on the average retail price (price including VAT, rounded) of a tree with a 10/12 trunk, according to the compilation of offers on the market. The reference price is the one in force in the year of the damage, an update being made every year. The value of the index to be taken into account is one tenth of the unit

reference price. This index makes it possible to express the species' rarity, the multiplication and cultivation difficulties, the growth time and the adaptation to the region. It also allows the introduction of a monetary value from the beginning in calculating the value of the arrangements.

Size index (circumference)

The index is obtained by measuring the circumference of the trunk, measured one meter from the ground, and expresses the increase in value depending on the age of the tree and its size (Figure 1).

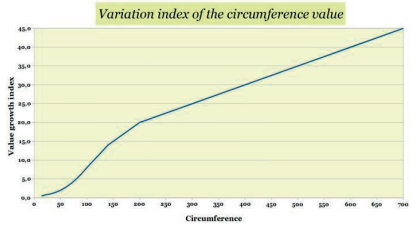

Figure 1. Variation index of the circumference volume

Health index

The health is estimated based on the general condition of the aerial parts: poorly healed lesions, injured trunk, parasites, the tree's development stage, its vigor. The reference health state is that of the tree before the injury. The index value can vary from 2 to 8.

Mark 8: Class A: very good condition (healthy, vigorous);

Mark 6: Class B: slightly affected (healthy, medium strength, minor lesions and alterations in the process of healing);

Mark 4: Class C: altered (low strength, unhealed, unscarred wounds);

Mark 2: Class D: irreversible (in the process of irreversible decline. Significant damage, confirmed presence of woody fungi, predominantly dead wood).

Location and aesthetic value index

The value of the index can vary from 3 to 8. It corresponds to the sum of 3 criteria (Marks):

Impact on the landscape

Mark 4: Presence remarkable by status;

Mark 3: Very strong impact;

Mark 2: Significant impact;

Mark 1: Insignificant impact.

Group homogeneity

Mark 2: Homogeneous grouping;

Mark 1: Heterogeneous grouping.

Patrimonial interest

Mark 2: Protected by laws or regulations;

Mark 1: It is not specifically protected

Scale for assessing the extent of damage caused by tree injury.

Damage to a tree is estimated in relation to the landscape value of that tree.

The amount of compensation will depend on the extent of the injury and will be calculated according to the scale shown below.

Damage to the trunk, flaking or peeling bark

Large wounds heal very slowly or not at all. They are often the site of outbreaks of infection, reducing the strength, vitality and value of the tree. In the case of lesions, percentage of the maximum width (horizontal measurement) of the injury expressed in centimeters, in relation to the circumference of the trunk at the height of the injury, is established.

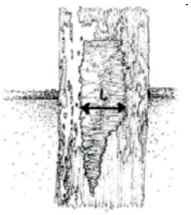

Figure 2. Measuring the wound on the trunk of a tree

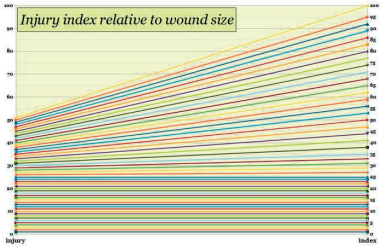

Figure 3. Injury index relative to wound size

If more than 50% of the sap tissue is destroyed, i.e. when the wound is more than half the circumference of the shaft, the shaft will be considered lost. Compensation is a percentage of the arrangement value proportional to the extent of the damage. This ratio is set by the scale presented below.

Broken, torn or dried branches

Assessing the degree of damage to a tree's crown is a function dependent on the volume of the destroyed crown. The volume before mutilation is taken as reference (Figure 4).

If intervention is required on the entire crown to rebalance the shaft, the damage rate is a function of this reduction.
If half of the branches are broken, depreciated, the tree is considered lost.

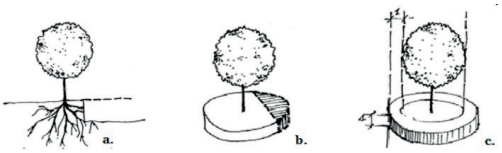

Figure 4: a. Affected tree; b. Affected root volume; c. The volume of the root taken as reference

When the damage requires sanitary or reforming dimensions, the compensation to be paid must take into account the cost of the interventions. The compensation is a percentage of the value of the arrangement in proportion to the size of the damage. This ratio is set by the scale.

Affected root system

Cut roots: the damage assessment is calculated as described above, taking into account the proportion of cut or broken roots in relation to the entire root system. This total volume is assimilated to the volume of the soil around the tree corresponding to a cylinder 1 m deep and 2 m in diameter larger than the crown's projection on the ground.

The cost of replacing a tree

In assessing the cost of compensation claimed for any damage to the departmental patrimony of trees, the landscape value of the tree may be increased, as appropriate, by the cost of auxiliary services: cutting and excavation works, supplying with new trees, replanting works.

RESULTS AND DISCUSSIONS

Results obtained regarding the value of trees

When carrying out this evaluation, regarding the inventory value of the ornamental trees located on the Marghiloman Domain, there were preparatory, organizational, logistical, data collection and processing activities.

All this has led to the design, testing and implementation of a calculation program that can give a real picture of the inventory value of ornamental trees on the analyzed surfaces with applicability in any landscaping.

Figure 5: Marghiloman Park: 2011 (Buzău Municipal Plan)

Placement and mapping of trees

The surface of the Marghiloman Domain is covered by plots with vegetation, a pond, constructions and alleys destined for pedestrian traffic and horse walking.

For rigor and to give scientific value to the study, we first proceeded to the topographic marking of all the trees on the analyzed surface. The result is the maps used in locating and listing each tree.

Following the field work, it was found that the accuracy of the maps was good enough, and, in case of inconsistencies, the errors were corrected and any omissions or changes in the positioning of the arboreal vegetation suffered over time were corrected on the map.

Preparation and fieldwork

The first step was to divide the area of interest into lawns that received an identification code, a code that was later used in the work and in filling in the fieldwork information on the observation sheets.

The next step was to complete the observation sheets with the information resulting from the measurement and observation of each tree. This after each identified tree has received a unique highlight code.

Switching to digital format and calculating the value of ornamental trees

Because the calculations are laborious, we drew up a calculation program through which data can be obtained that can be analyzed, exploited according to needs and that highlights the monetary value of the trees on the domain.

Below, we present the structure of the spreadsheet of a plot that includes the transcription of the information collected in the field and its processing (Figure 6).

It has the following fields:
Current number of the evaluated tree
Species
Tree/specimen code
Impact on the landscape (**R**emarkable, **P**owerful, **W**eak (S), **I**nsignificant
Landscape impact index (calculated)
Patrimonial interest (**P**rotected, **N**ot protected)
Solitary, **G**roup (layout)
Solitary, group (**H**omogenous, **He**terogeneous)
Homogeneity index (calculated)
General state
Very good (F), **S**lightly affected (**U**), **A**ltered, **I**rretrievable
Health index (calculated)
Trunk observations
Circumference at 1,0 m from the soil
Size index (calculated)
The circumference at the maximum width of the wound
Maximum width of the wound
Injury percentage (calculated)
Trunk damage index (calculated)
Crown observations
Diameter [m]
Damage percentage [%]
Crown damage index (calculated)
Roots observations
Root damage index
Damage percentage (calculat)
PLOT VALUE BALANCE SHEET Lei
The price of a specimen in the nursery [Lei]
TREE EVALUATION Lei
Tree value (calculated)
Devaluation from trunk injury (calculated)
Devaluation from crown injury (calculated)
Devaluation from root injury (calculated)
Current value (calculated)

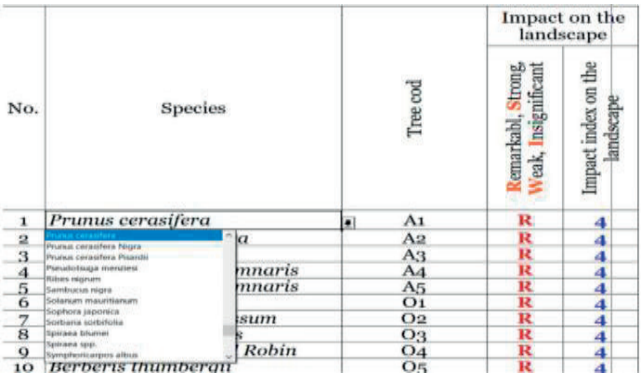

Figure 6. Detail of entering the data collected in the field in the spreadsheet

Number of copies made	
worked	identified
21	21

BALANCE SHEET VALUE PLOT [Lei]					
3330	9324	0	0	0	9324

No.	Species	Tree cod	Impact on the landscape: Remarkabl, Strong, Weak, Insignificant	Impact index on the landscape	Property interest: Protected, Unprotected	Index of patrimonial interest	Solitary, Group: Solitary, group (H=Homogenous, H=heterogeneous)	Homogeneity index	General state: Very good, Slightly damaged, Altered, Irrecoverable	Health index	Trunk remarks: Circumference, 1.0 m from the package (cm)	Size index	The circumference at the maximum width of the wound	Maximum wound width	Injury percentage	Trunk damage index	Crown remarks: The diameter [m]	Percentage of damage %	Index of decrease a crown	Roots remarks: Percentage of damage %	Root damage index	The price of a specimen in the nursery [Lei]	TREE EVALUATION [Lei]: Tree value	Devaluation from torso injury	Devaluation from crown injury	Devaluation from root injury	Current value
1	*Prunus cerasifera*	A1	R	4	U	1	S	2	V	8	10	0,5			0	0	0,6		0		0	90	252	0	0	0	252
2	*Cedrus deodora aurea*	A2	R	4	U	1	S	2	V	8	10	0,5			0	0	1		0		0	3240	9072	0	0	0	9072
3	*Cedrus deodara*	A3	R	4	U	1	S	2	V	8	7	0			0	0	2		0		0	400	0	0	0	0	0
4	*Chamenyparis columnaris*	A4	R	4	U	1	S	2	V	8	10	0,5			0	0	1,5		0		0	160	448	0	0	0	448
5	*Chamenyparis columnaris*	A5	R	4	U	1	S	2	V	8	12	0,5			0	0	0,8		0		0	160	448	0	0	0	448
6	*Juniperus chinensis*	O1	R	4	U	1	S	2	V	8	10	0,5			0	0	0,6		0		0	55	154	0	0	0	154
7	*Vaccinium corymbosum*	O2	R	4	U	1	S	2	V	8	0,9	0			0	0	0,8		0		0	120	0	0	0	0	0
8	*Euonymus europaeus*	O3	R	4	U	1	S	2	V	8		0			0	0	0,7		0		0	45	0	0	0	0	0
9	*Photinia fraseri Red Robin*	O4	R	4	U	1	S	2	V	8		0			0	0	1,5		0		0	36	0	0	0	0	0
10	*Berberis thumbergii*	O5	R	4	U	1	S	2	V	8		0			0	0	15		0		0	35	0	0	0	0	0
11	*Berberis thumbergii*	O6	R	4	U	1	S	2	V	8		0			0	0			0		0	35	0	0	0	0	0
12	*Weigela florida*	O7	R	4	U	1	S	2	V	8		0			0	0	1,5		0		0	32	0	0	0	0	0
13	*Aesculus carnea*	A6	R	4	U	1	S	2	V	8	38	1,4			0	0	2		0		0	280	2195	0	0	0	2195
14	*Juniperus chinensis*	O8	R	4	U	1	S	2	V	8	0,2	0			0	0	0,7		0		0	55	0	0	0	0	0
15	*Juniperus chinensis*	O9	R	4	U	1	S	2	V	8	0,2	0			0	0	0,7		0		0	55	0	0	0	0	0
16	*Pinus mugo*	A7	R	4	U	1	S	2	V	8		0			0	0			0		0	113	0	0	0	0	0
17	*Juniperus chinensis*	O10	R	4	U	1	S	2	V	8		0			0	0			0		0	55	0	0	0	0	0
18	*Liriodendron tulipifera*	A8	R	4	U	1	S	2	V	8	13	0,5			0	0	2		0		0	105	294	0	0	0	294
19	*Juniperus chinensis*	O11	R	4	U	1	S	2	V	8		0			0	0			0		0	55	0	0	0	0	0
20	*Juniperus chinensis*	O12	R	4	U	1	S	2	V	8		0			0	0			0		0	55	0	0	0	0	0
21	*Cedru*	A9	R	4	U	1	S	2	V	8		0			0	0			0		0	210	0	0	0	0	0

Figure 7. Spreadsheet with data for a plot and tree evaluation

Number of copies made	
worked	identified
21	21

BALANCE SHEET VALUE PLOT [Lei]					
3 330	9 324	0	0	0	9 324

The price of a specimen in the nursery [Lei]	TREE EVALUATION [Lei]				
	Tree value	Devaluation from torso injury	Devaluation from crown injury	Devaluation from root injury	Current value
90	252	0	0	0	252
3 240	9 072	0	0	0	9 072
400	0	0	0	0	0
160	448	0	0	0	448

Figure 8. Detail of the balance sheet calculation for the data entry sheet

To these is added the box that highlights the number of specimens inventoried on the plot and those that have been identified and evaluated (worked on), as well as the value balance for the worked plot.
The spreadsheet subsequently has a sheet with the value of the seedlings in the nursery (Figure 9).

		Dendrological balance [pieces]					Surface		Pieces		
		Deciduous	Nuts	Coniferous	Ornamental	Pieces	[m²]	[ha]	Identified	Remained	% worked
	SUM	1119	37	136	399	1691	82960	8,296	277	1427	16,38
AREA	57180	25	0	1	21	47	1984	0,1984	41	6	87,23
	59962	1063	37	135	376	1611	68130	6,813	207	1417	12,85
	63924	31	0	0	2	33	12846	1,2846	29	4	87,88

Figure 9. Domain tree balance

A comprehensive picture of the value of the trees, as well as the distribution on each area (cadastral number) and the plots is given by spreadsheets that include information on tree and shrub species as well as nursery prices for seedlings.

		Dendrological representation [%]				
		Deciduous	Nuts	Coniferous	Ornamental	Pieces
	SUM	66,17 %	2,19 %	8,04 %	23,60 %	100,00 %
AREA	57180	53,19 %		2,13 %	44,68 %	100,00 %
	59962	65,98 %	2,30 %	8,38 %	23,34 %	100,00 %
	63924	93,94 %			6,06 %	100,00 %

		Dendrological density [pieces / 100 m²]					Surface returned to a specimen [m²/specimen]
		Deciduous	Nuts	Coniferous	Ornamental	Pieces	
	SUM	1,35	0,04	0,16	0,48	2,04	49,06
AREA	57180	1,26		0,05	1,06	2,37	42,21
	59962	1,56	0,05	0,20	0,55	2,36	42,29
	63924	0,24			0,02	0,26	389,27

Figure 10. Domain tree balance after place work

The following figure shows a general balance and an analysis by areas and by the whole domain that took into account the number of inventoried and identified specimens as well as the areas on which they are found.
The part of the calculation that highlights the inventoried and identified tree material
Using this centralized data, we made an analysis that highlights the categorization of the evaluated vegetation and we calculated the category density globally and by areas.

		VALUE ESTIMATION [Lei]							
		16,38	1 403 786	9 465 560	8 607	741	529	9 877	9 455 683	7
			VALOAREA ESTIMATĂ A ARBORILOR [Lei]							
		% evaluation	The value of the nursery	Value of trees	Devaluation from trunk injuries	Devaluation from crown injuries	Devaluation from root injury	TOTAL damage	The value of the identified trees	Increasing the value of the nursery
AREA	57180	87,23	6 969	29 985	140	121	87	348	29 637	4
	59962	12,85	218 847	1 473 036	1 270	0	0	1 270	1 471 766	7
	63924	87,88	4 136	47 517	0	0	0	0	47 517	11

Figure 11. Tree material value

We centralized all this information in a table that gives the inventory value of the evaluated ornamental trees located on the analyzed area of the Marghiloman Domain.
Figure 11 shows the estimated values for all tree material in the park. This was done with two parameters:
the percentage resulting from the ratio between the identified specimens and the evaluated specimens;
the value of the specimens evaluated for the three areas (cadastral numbers).

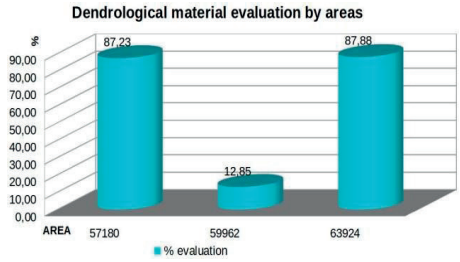

Figure 12: The stage of the field analysis at the date of making this material

From Figure 12 it can be seen that the estimated value of the tree material would be 9,456,238 lei at an evaluated specimen percentage of 16.38%. From the table you can read both the percentage of specimens assessed for each area and the damage due to injury and damage to the trunk, crown and roots.
Also, from this table you can see that most of the damage is in AREA 59962.

The calculations took into account the B.E.V.A. evaluation method and scales according to this method were used to calculate the indices.

CONCLUSIONS

The Marghiloman Domain Park in Buzău is a challenge regarding the reorganization in the idea of restoring the atmosphere specific to the interwar period of maximum flowering.
In a way, this park had special characteristics, somewhat unique, in that it combined an ornamental vegetation with many acclimatized species to that specific to the area, to which was added the presence of animals, a sector where vegetables were grown and let's not forget the existence of a heated greenhouse.
Also noteworthy is the organization of the park on well-defined principles that regulated both the organization of the vegetation and that of the animal sectors, the organization of routes and that of the buildings.
It is remarkable for the positioning and the logic of their placements and included:
living spaces; relaxation sectors; agro-zootehnical sectors; technical buildings.
By restoring the characteristic historical vegetation, to which the principles of permaculture can be associated, the biodiversity of both the vegetation and that of insects and animals attracted will be greatly increased. All these actions must comply with the regulations regarding the historical character as well as those regarding protected areas. Only through a documented, scientific approach with the application of the principles that govern biodiversity will the results be as desired. Establishing the value of ornamental trees framed in a certain context can play an important role in establishing the value of the domain, in establishing premiums in the case of insurance services and a role before legal bodies in case of litigation. Normally, in the case of administrations, it should provide a basis for discussions in establishing construction, road, municipal regulations, etc.
Tree evaluation is an entrepreneurial opportunity by providing services of:
-Tree inspection;
-Assessing the health and structure of a tree;
-Risk assessment for tree-related properties and people;
-Evaluation of the monetary value of trees and wood lots in the urban perimeter and landscaped areas;
-Assessment of the monetary value of damage to trees;
-Inventory and prescriptions for trees
-Supervision of arboricultural works (planting, cutting, cutting of trees along alleyways and roads, etc.)
This assessment comes in support of:
-Inspection of urban area trees that grow in parks, green spaces and on the street;
-Approval of applications for authorization to cut down trees;
-Legal disputes involving trees;
-Expropriations;
-Damage insurance files for affected trees;
-Judicial expertise;
-Purchase/sale of spaces with tree;
-Providing trees to urban spaces;
-Development of municipal policies on urban trees and forests;
-Elaboration of urban arboriculture plans.

All this should play an important role in assessing biodiversity, in maintaining the balance and health of an environment conducive to life.
Biodiversity would create an environment that brings joy and health benefits and a compensation for anthropogenic aggression

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EVALUATION OF SENSORY PROPERTIES AND CONSUMER PERCEPTION OF MIXTURE TEAS, OBTAINED FROM ORGANIC FREEZE DRIED FRUITS AND VEGETABLE

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Abstract

The health effects of the active components in organic fruits and vegetables are well known. Our goal was to obtain a mixture of organic powders, from fruits and vegetables, to be enjoyed as tea for both personal satisfaction and health. The ecological powders were realized at the Research Centre for Studies of Food Quality and Agricultural Products, USAMV Bucharest. This study was conducted to identify the sensory characteristics of two kind of mixture teas containing organic ingredients and analyse sensorial attributes for the products based on consumer acceptance. The attributes were analysed by classifying them as appearance, colour and texture for dehydrated fruit and vegetable blended teas, infusion observation and tasting tea infusions from fruit and vegetable powder ecological by on appearance, flavoured, aroma, texture or mouthfeel, and any aftertaste. Principal component analysis results showed that the mixture teas analysed are generally tasty, with a discreet sweet taste, sour and aromatic sweetness due to the dehydrated apple and was well appreciated by the consumer.

Key words*: antioxidant activity, consumer perception, dried ecological powder, mixture of teas, total polyphenols.*

INTRODUCTION

The society is constantly changing and, at the same time, its preferences. Consumption preferences are varied and profoundly changed compared to the past; consumers are no longer classified only in terms of income, demographic variables and lifestyle (Popa et al., 2017; Farruggia et al., 2016). The modern consumer is more attentive to his and his family's health, to sustainable and sustainable consumption, is thirsty again and innovative (Dragomir et al., 2017). Also, consumer attitudes and beliefs are factors that influence the acceptance or rejection of new foods (Stan et al., 2017).

The concern for health also translates into a higher and varied consumption of teas, consumed for therapeutic or relaxation purposes. In the tea beverage industry, it is known that the characters of tea change from season to season, from process to process from time to time. In search of new solutions, manufacturers mix different types of teas from different fields and come up with a mixture of products that conform to the color, taste, aroma and other acceptable perceptions of the consumer. Thus was born - the mixing of tea - a new tool for tea marketing (Gogoi, 2014; Kim et al., 2019).

Blending is a much highly elevated art form; though not exactly scientific, it's a highly skilled art practiced by a few who truly know their craft. Simply mixing of more than a single kind or grade of tea cannot fulfil the demand and perception of the consumers (Gogoi, 2014). The mixing of tea make from fruit gives us the opportunity to enjoy a complete, sensory and mental experience. We can thus observe the characteristics of each type of fruit and plant mixture, quality, fragrance, texture, color, etc. To taste the fruit infusion, a taster needs a lot of attention and, especially, some exercise.

New flavored powdered beverages now contain a variety of healthful ingredients from fruit and fiber to protein, and these mixes are now the

right products at the right time in a growing era of label-friendly, functional and sustainable products. Choosing the right ingredients for this mixture is essential and the ticket to success will be a combination of functional and label-friendly ingredients (Asioli et al., 2017; Dragomir, 2019).

What's more, powdered mixes now provide a wide range of nutritional benefits with a high degree of functionality. They are also cost-effective, easy to store and more sustainable when packaging doesn't rely on single-use plastic bottles. And in these times of pandemic, consumers will also appreciate the convenience and safety of buying in bulk and mixing their own beverages as opposed to popping into the store for an RTD (ready to drink) beverage.

MATERIALS AND METHODS

Materials

The ingredients of organic farming offer the advantage of providing the consumer with clean, healthy and tasty products. For this reason, one way to use the ingredients of organic farming is to develop tea formulas from fruits and herbs.

The goal is to obtain a mixture of organic processed (slices, powders) fruits and vegetables, to be enjoyed as tea for both personal satisfaction and health.

In the study we used 3 freeze dried products, respectively:

- Freeze-dried organic apple (pulp and peel pieces and slices) - obtained from apples, from the organic Gala variety;
- Freeze-dried organic raspberry powder; Freeze-dried organic basil powder.

• ***Freeze-dried organic apple*** - Organic ingredient used in study is *freeze-dried organic apple* (pieces and powder), obtained by organic *Gala* variety, which was dehydrated by the lyophilisation process. The powder was obtained from peel, pulp, and mixture of both and their characterization is comprised most the antioxidant ability and free radical scavenging capacity, with correlation with content of polyphenolics and ascorbic acid, according to (Li et al., 2014; Badulescu et al., 2019). Drying using low temperatures represent a simple and easy way for minimally processing of organic fruits, moreover this procedure is accepted in organic agriculture (Stan et al., 2020). Apple powder it is an important source of polyphenols with high antioxidant capacity. Its presence in the recipe, balances the taste and aroma of the finished product, and the pieces of lyophilized apple give a pleasant texture and aroma to the product (Bădulescu et al., 2019).

Freeze-dried organic raspberry powder - Raspberries (*Rubus idaeus* L.) are one of the most economically important soft fruit due to their taste, appearance and composition. Organic raspberries represent a good source of anthocyanins, vitamins and mineral elements. The postharvest storage of organic fresh raspberries is relatively short and the injuries, rapid spoilage, nutritional and moisture loss lead to dramatically reduction of their commercial value (Stan et al., 2019; Roopesh et al., 2012).

Freeze-dried organic basil powder - Basil (*Ocimum basilicum* L.) belongs to aromatic plants due to their volatile compounds presented especially in leaves and flowering tops. These basil parts are used since antiquity for food preservation, flavouring, and as medicine, because of high antioxidant, antibacterial and antifungal activity of volatile oils, being good sources of natural antimicrobial and antioxidant agents, with possible application in food industry, cosmetics or medicine (Avetisyan et al., 2017).

By lyophilised basil retains the characteristics intense colour and flavour. Lyophilised basil powder is aromatic, slightly sweet, with spicy notes in taste. Because, it has a great capacity to rehydrate in the presence of water; its original character, such as the taste, colour and aroma specific to the basil, will be present in the new preparation. Added the powder from the lyophilized basil aromatizes to the product, balances the taste and increases the presservation of final product (William et al., 2019; Di Cairano et al., 2018).

Freeze-dried organic fruit and vegetable powders are obtained within the SusOrgPlus project at the Research Center for Studies of Food Quality and Agricultural Products of University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Methods

The scope of research is to obtain a mixture of organic processed (slices, powders) fruits and

vegetables, to be enjoyed as tea for both personal satisfaction and health.
Will be followed: - sensory characteristics of two mixtures of teas containing organic ingredients, - sensorial attributes for the products based on consumer acceptance, and - nutritional characteristics of two blended teas.

Development of mixing teas
The blended fruit and vegetable powders, have been chosen according to the behavior of the new ingredients that are the object of the present study, respecting all the requirements provided by the legislation.
Sensory analysis of the products obtained during the study. Sample presentation: each sample was provided to the panelists in white plastic cups of about 75 ml. About 50 ml of each infusion were served and were approximately 60-70°C at the time of tasting. The panelists received mineral water to cleanse their palates between samplings of tea.

Consumer acceptance analysis
The sensory analysis was performed with the help of 50 tasters, in the *Laboratory for quality control of agri-food products*, within the University of Agronomic Sciences and Veterinary Medicine of Bucharest and within the Workshop *Consumer Acceptance Analysis*, held at the USAMV Bucharest Conference, University Research - Support for organic agriculture on October 30, 2019 at the INDAGRA 2019 International Fair.
Determination of the total content of polyphenolic compounds by the Folin-Ciocâlteu method. For the preparation of the extract was used the recommendation for preparation: Pour 200 ml of hot water at 100°C over a 2 grams of blended tea. Allow to infuse for at least 5-10 minutes (average 6 min), covering the cup. The extract was cooled to room temperature in order to be able to quantitatively determine the total polyphenol content using the Folin-Ciocâlteu method following a protocol adapted by Georgé et al., 2005.
Determination of antioxidant activity using the DPPH method. The antioxidant activity of the samples is determined based on the DPPH test, using the stable free radical 2,2-diphenyl-1-picrilhydrazyl - DPPH, according to a method Bujor et al., (2016).
To determine the antioxidant activity, use a volume between 200 µL of infusion and add 2 mL of DPPH solution (0.2 M) in methanol. Shake magnetically in the dark for 30 minutes. After incubation, the absorbance at 515 nm is measured.
Antioxidant activity is expressed as a percentage (%) of inhibition of DPPH radicals relative to the reference solution using the equation:

$$\%I = \frac{A_0 - A_c}{A_0}$$

where:
A_0 - absorbance of the reference sample at t = 0 minutes ;
A_c - absorbance of samples (with polyphenolic extract) after 30 minutes of rest (t = 30 minutes).

Statistical analysis
All the data represent the average of three replicates with independent sample preparation. Standard deviation was calculated using incorporated function of Microsoft Excel.

Nutrient Content
Nutrient content it was calculated using a program nutritional development tool, *Softmedia programme* (http://softfedima.ro/).
Softmedia programme makes it easy to prepare a nutrition facts panel, nutrition data sheet, ingredient statement for any food product. Formulas can be adjusted for moisture and/or fat content. Information can be printed, saved as a PDF document.

RESULTS AND DISCUSSIONS

1. Development of blended
Freeze dried organic fruit and vegetable powders are obtained within the SusOrgPlus project at the Research Center for Studies of Food Quality and Agricultural Products of University of Agronomic Sciences and Veterinary Medicine of Bucharest.
For blended the following powders were used:
- Freeze-dried organic apple (pulp pieces and peel) is obtained from apples, from the organic *Gala* variety;
- Freeze-dried organic basil powder;
- Freeze-dried organic raspberry powder.

By mixtures different types of dehydrated fruits, two products were obtained:

Table 1. Codes used for samples analysis

Sample	Main ingredients
T1	95% piece of freeze dried apple pulp and peel, 5% raspberry powder (figure 1)
T2	95% piece of freeze dried apple pulp and peel, 4% raspberry powder, 1% basil powder. (figure 2)

Figure 1. T1 sample- Organic fruit tea with pieces of freeze-dried apple and freeze-dried raspberry powder (Original photo)

Figure 2. T2 sample - Organic fruit tea with pieces of freeze-dried apple, freeze-dried raspberry powder and basil powder (Original photo)

The method consists in extracting of soluble substances in dried ingredients, containing in a porcelain or earthenware kettle, by means of freshly boiling water, pouring of the liquor into a white porcelain, examination of the organoleptic properties of the infused (adapted according to ISO 3103: 2019).

The dry mixture of fruits with particles of different sizes has a pleasant and homogeneous appearance. From the apples from the *Gala* variety, pieces of pulp and pieces of peel were used. Apple peel freeze-dried is extremely valuable from a nutritional point of view and comes in solving environmental problems.

Table 2. Characteristics of preparation (mixture fruits tea) products used in this study

Preparation conditions			
Sample	**Water (mL/tea bag)**	Temperature (°C)	Time (min)
T1	200	100±2°C	5-10
T2	200	100±2°C	5-10

The mixture powder fruits are recommended to store in airtight containers and kept at room temperature, without high humidity fluctuations.

2. Sensory analysis of the organic mixture fruit teas obtained during the study

Consumer preference is influenced by intrinsic quality attributes discovered before (colour, taste, flavour, and texture) (Sulistyawati et al., 2020) as well as on tea itself, its taste and smell. The sensory analysis performed along the way included: dry mixture teas analysis, and sensory analysis of mixture teas infusion.

Dry mixture teas analyzis

The shape of the pieces of size must be uniform, also in the case of powders. The color must be uniform and correspond to the type of ingredient; the texture of the ingredients must be in the case of pieces of crumbled dehydrated apple, easily broken.

The freeze-dried raspberry powder adheres to the surface of the apple pieces and creates a unitary whole. In sample T2, the lyophilized basil particles stand out quite well due to the color. Table 3 shows the results of the sensory assessment of the two types of tea made.

Table 3. Sensory attributes for the mixture tea organic fruits

Attributes	**T1**	**T2**
Overall appearance	Particle shape: raspberry powders are fine, easily adhere to the surface pieces of apple pulp and peel	Particle shape: raspberry powders are fine, easily adhere to the surface pieces of apple pulp and peel. The basil particles are visible and beautifully distributed in the mixture
Color	The color is specific to the freeze dried fruits present in the mixture (yellow to pink)	The color is specific to the freeze dried fruits present in the mixture (yellow with pink and green dots)
Texture	The pieces of apple stand out with a gummy texture and slightly sticky to the touch. Apple peels appears as hard particles. Raspberry powders have a nice appearance that adheres nicely to the surface of other products in the mixture.	The pieces of dehydrated apple stand out with a gummy texture and slightly sticky to the touch. Dehydrated apple peels appears as hard particles Raspberry powders have a nice appearance that adheres nicely to the surface of other products in the mixture. The basil powder has a very small size that adheres to the surrounding surfaces.

Sensory analysis of mixture teas infusion

For preparation of the infusion it is recommended to use white porcelain cups to taste the infusion color. We must follow exactly the preparation instructions of each type of tea: the amount of fruit, the water temperature and, especially, the infusion time.

When straining the tea, it is recommended not to squeeze fruits, because substances can be released which change taste.

Recommendation for preparation: Pour 200 ml of hot water at 100°C over a 2 grams of fruits tea. Allow to infuse for at least 5-10 minutes (average 6 min), covering the cup. Tea tasters and specialists in the field call fruit liqueur "*liqueurs*".

The observation of the infusion consists in appreciating the general appearance, the color of the liqueur and its clarity. Tasting the tea infusion, we must start by smelling it, and then taste it, because if we tasted it first, the initial perception could create a wrong impression. Thus, the information received by the brain through smell and taste forms the general impression about the profile of the tea we tasted.

Infusion must to be served and tasted at approximately 60-70°C at the time of tasting. The panellists received mineral water to cleanse their palates between samplings of tea.

Table 4 shows the results of the sensory assessment of the two types of tea made.

Table 4. Visual attributes for the infusion of mixture teas

Attributes	T1	T2
Appearance of infused fruits	The obtained tea has a slightly pink color; the fruits were rehydrated, the apple pieces and peel returned to their initial form. The raspberry particles were dispersed throughout the liquid mass. At the bottom of the cup a sediment is formed consisting of rehydrated raspberry and apple powders. At the top, gather the rehydrated pieces of apple.	The obtained tea has a slightly pink color, the pieces of apple and peel return to their original volume. The raspberry and basil particles were rehydrated but are dispersed throughout the liquid mass. The basil particles have rehydrated but tend to remain in suspension. At the bottom of the cup a sediment is formed consisting of rehydrated raspberry and apple powders. At the top, gather the rehydrated pieces of apple.
Clarity	The infusion shows hydrated fruit particles in suspension. The liquid is relatively clear. After separating the fruit, the liqueur becomes clear.	The infusion shows hydrated fruit particles in suspension. The liquid is relatively clear. After separating the fruit, the liqueur becomes clearer.
Color	The color is yellowish white with a pale pink tinge. It has a bright color, and the rehydrated fruits from tea have regained their native, beautiful shape.	The color is yellowish- white, slightly greenish with a shade of pale pink. It has a bright color, and the rehydrated fruits from tea have regained their native, beautiful shape. The basil particles are found at the top, to a large extent.

The smell or aroma of tea can be determined by two methods by which we can feel the smell of the tea infusion:

1) deep inhalations: keep the cup or bowl of tea as close as possible to the nose and inhale deeply;

2) quick inhalations: we quickly and superficially inhale the smell of the prepared tea.

The taste and aroma of fruit liqueur is determined by sipping a small amount of liquid, inhaling air and exhaling through the nose. By absorbing it in this way, the oxygen combines with the infusion, highlighting its distinctive notes. He then exhales through his nose, keeping his mouth closed, to discover the retro-olfactory characteristics. While tasting the tea, pay attention to the: sweet, tasty or slightly bitter. Table 5 shows the results of sensory assessment of the two types of tea made.

Table 5. Sensory attributes for tasting the infusion of mixture fruit teas

Attributes	T1	T2
Flavor and smell	Delicate apple, sour and fragrant. Predominates the smell of apples.	Delicate apple, sour and fragrant. Predominates the smell of basil.
Taste	The general impression is tasty, discreetly sweet, slightly sour and mild due to the dehydrated apple powder. The taste and aroma are balanced.	The general impression is tasty, sweet and sour, with a hint of aroma specific to basil.

3. Consumer acceptance analysis

A sensory evaluation for consumer acceptance testing was performed consumer groups of 50 tasters, using a 5-point Hedonic scale to determine the level of acceptance of mixture teas by organic freeze-dried fruits (Woods et al., 2016; Spence et al., 2021).

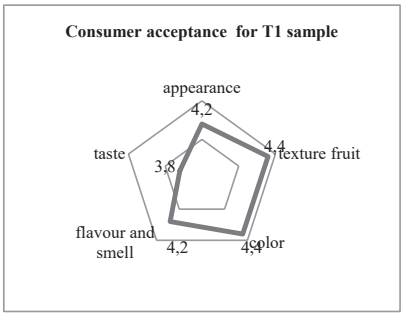

Figure 3. Consumer acceptability scores on a 5-point Hedonic scale for T1 sample

At tasting the T1 sample (Figure 3), consumers appreciated this assortment of organic tea, where they found the flavor of each fruit and a harmony between tastes and smells. The pieces of freeze dried apple rehydrated very well giving the liqueurs a special look, so that the two attributes received a weighted average grade of 4.4. Regarding the taste of T1 samples, the appreciation was very low because the apple taste is very discreet.

Overall, following the centralization of the grades given during the sensory analysis, a weighted average grade of 4.2 was obtained.

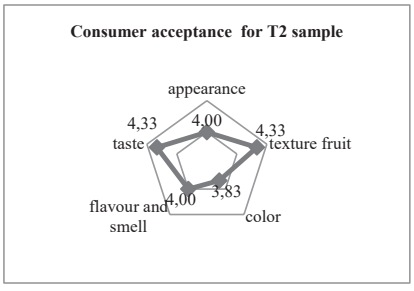

Figure 4. Consumer acceptability scores on a 5-point Hedonic scale for T2 sample

At the consumer's acceptance (Figure 4.), the T2 sample was extremely appreciated. The tasting found the flavor of each fruit but also a harmony between tastes and smells. The pieces of dehydrated apple rehydrated very well, giving the liqueurs a special look. The freeze-dried basil powder used as an ingredient slightly changed the appearance and color of the liqueur, and at the tasting it had a fine basil taste appreciated by a lower category of consumers. Overall, following the centralization of the grades given during the sensory analysis, a weighted average grade of 4.15 was obtained.

Total content of polyphenolic and antioxidant activity

The infusion obtained from the T1 sample shows a higher total polyphenol content, with 2.03 mg/mL of infusion more than T2 sample.

Table 6. Total content of polyphenolic and antioxidant activity

Tea infusion	Total polyphenol content (mg/mL of infusion)	Antioxidant activity (%)
T1	14.73 ± 1.28	48.93 ± 4.48
T2	12.71 ± 0.20	39.95 ± 0.04

Both T1 and T2 showed high antioxidant activity, with T1 having the higher value of 48.93 % inhibition

Even though the addition of 1% basil powder should increase the polyphenol and antioxidant capacity, it cannot compensate the high polyphenol and antioxidant activity of raspberry powder.

This result are in compliance with sensory analysis, for which T1 also received the highest general score 4.2.

Nutrient Content

The nutritional content and energy value is shown in the Table 7. Energy value is 1245,6 kcal per 100 g of product for both samples.

Table 7. Nutritional content for both mixture tea

Nutritional value for 100 g product		
	T1	**T2**
Energy	1245.6 KJ	1245.6 KJ
	294.4 kcal	294.4 kcal
Total fat	0.4	0.4
Saturated fat	0	0
Carbohydrates	66.6	66.6
Sugar	54	54
Fiber	10.2	10.2
Protein	1	1
Salt	0.1	0.1

Can be mentioned, as a product with allergenic potential, due to the raspberry powder. It is recommended to consume 1-2 cups a day.

CONCLUSIONS

The consumption of products from organic agriculture has increased a lot, on the current climate of the sanitary crisis and the preoccupation of the consumers for a healthy diet. This also translates into a series of by-products resulting from processing. To reduce waste, it is necessary to make new assortments of value-added products.

Obtaining mixture fruit teas from preserved organic ingredients, through various dehydration processes, is a sustainable and economically feasible option.

Blended fruit infusion is easily accepted by the modern consumer, especially because they are mindful about health. The same it is an option for a healthy diet.

The infusion obtained from the T1 sample shows a higher total polyphenol content, with 2.03 mg/mL of infusion more than T2 sample. Both T1 and T2 showed high antioxidant activity.

Addition of 1% basil powder should increase the polyphenol and antioxidant capacity, it cannot compensate the high polyphenol and antioxidant activity of raspberry powder.

Sensorial analysis show that T1 sample was more easily accepted by consumers than T2 sample, which felt the taste and aroma of basil.

The predominant taste of apple in infusion, which is sweet and slightly aromatic, can be enriched by high additions of raspberry, basil powder or another fruit and/or plant powders

Developing strategy for developing a new product, must be take account the characteristics of the products preferred by consumers and their eating habits.

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PHYSICOCHEMICAL CHARACTERISATION OF VINE WASTE USED FOR PRODUCING BIOCHAR

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Abstract

Plant wastes are often burned, leading to air pollution and significant loss of potential soil nutrients. In order to mitigate these drawbacks, the waste can remain or be added to the soil, but this may increase crop diseases and also greenhouse gas (GHG) emissions (e.g., CO_2, CH_4, N_2O). Pyrolysis of vine waste is a promising and relevant technique, and the obtained biochar can be further used as a soil amender, can enhance soil C sequestration and water holding capacity, reduce GHG emissions and nutrient leaching, increase soil fertility, resulting in agronomic, environmental, and economic benefits. The aim of this study was to characterise vine waste from a physicochemical point of view in order to be used as raw material for producing biochar, which will be applied as soil amender. Plant waste material (grapevine prunings and marc) was received from Pietroasa-Istrita Research Station for Viticulture. The materials were characterised in terms of dry matter, loss on ignition, surface morphology, total carbon and nitrogen, bulk density, water holding capacity, pH, electrical conductivity, and mineral content. The obtained results indicate that grapevine prunings and marc are suitable materials for obtaining biochar.

Key words*: biochar; grape marc; grapevine prunings; physicochemical characterisation.*

INTRODUCTION

Conversion of organic materials to biochar via pyrolysis provides an alternative to manage various wastes. Crop- and forestry-waste, vegetal and animal waste resulting from industrial processing, urban-yard waste, animal manure, organic fraction of household solid waste municipal sewage sludge have been widely used to obtain biochar (Kung et al., 2015; Purakayastha et al., 2019).

Waste valorisation by pyrolysis can have significant agronomic, environmental, and economic benefits. In addition to biochar, pyrolytic bio-oil and gases are produced by pyrolysis. They can be further upgraded to obtain fuels and different chemicals, enhancing the process efficiency (Ceatra et al., 2016). Pyrolysis can be slow, fast or flash, depending on the heating rate and residence time. Slow pyrolysis, also called conventional carbonization, produces biochar by heating biomass at a low heating rate (0.1-1°C/s) and relatively long residence time (up to several days) (Qian et al., 2015). Pyrolysis process is usually performed in the presence of an inert (nitrogen, argon) or oxidizing (steam, carbon dioxide) carrier gas (Dobre et al., 2010; 2012; Pârvulescu et al., 2016).

Biochar is a highly porous carbon-based material that has significant aromaticity and anti-decomposition capabilities (Wang et al., 2017). Biochar has a high potential as soil amender, to improve soil properties and its capacity for increasing nutrient retention (*i.e.*, decreasing nutrient leaching and gaseous nutrient emission), while also allowing nutrient release (Yang et al., 2017). Biochar can

improve the physical and chemical characteristics of soil and promote crop yield (Schulz et al., 2013). Change in water holding capacity of soil by adding biochar is one of the key factors that can explain the crop growth (Karhua et al., 2011).

According to the International Organisation of Vine and Wine (https://www.oiv.int/), in Romania, in 2016, there were up to 191 356 ha of vineyards. Up to 20% of the harvested wine grape becomes waste during wine production. Grape marc can be used for compost and substrate in ornamental plants (Madjar et al., 2014a) and vegetables (Carmona et al., 2012), as well as for obtaining biofuel (Xu et al., 2009) and biochar (Ibn Ferjani et al., 2019). The use of vine pruning materials in pyrolysis processes solves several environmental problems, including managing large volumes of waste generated annually and reducing CO_2 emissions during uncontrolled waste burning (Nunes et al., 2021). The aim of this study was to characterise the wine waste from a physicochemical point of view, in order to be used as raw material for producing biochar, which will be further applied as soil amender.

MATERIALS AND METHODS

Biomass waste materials

Both grape marc and grapevine prunings came from Pietroasa-Istrita Research Station for Viticulture and Pomiculture, part of the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Grape marc came from Cabernet Sauvignon wine production. The wine was separated from the marc after 14 days of fermentation by draining. After separation, the marc was pressed to about 1.8 bar, using a pneumatic press, discharged and stored in plastic bags until it was shipped to the laboratory.

Grapevine prunings came from a viticulture area of 2.29 ha, cultivated with the Cabernet Sauvignon variety. The grapevine prunings were cut at the beginning of March 2021 and ropes with a diameter between 9-14 mm were selected from the fruit rings left after the cutting process was carried out.

In order to be further processed, both samples were analysed in terms of dry matter. After dry matter determination, grape marc was directly placed in an oven at 105°C until constant mass was achieved and then stored in a desiccator for further characterization. Grape prunings were cut at a length of 1-2 cm and further processed similar to grape marc.

Proximate and ultimate analysis

The determination of the dry matter (*DM*) was performed in a Memmert UN110 using the following steps: first step - 1 h at 70 °C, second step - at 105 °C until constant mass was achieved. The results were expressed as mass percentage.

Ash content (*AC*) was determined by ignition of 1 g of sample at 650°C for 6 h in an oven (Nabertherm, B150) until all carbon was removed. The final calculation was based on the percentage of ash from the original compound.

The volatile matter (*VM*) analysis method was based on ASTM D5142. 1 g of sample was weighed in a specific crucible with cover and placed in the oven (Nabertherm, B150). The furnace was heated (50°C/min) to a temperature of 950 ± 20°C and it was held for 7 min at this maximum temperature. *VM* was calculated with Eq. (1), where m_1 (g) is the mass of sample after drying in moisture test and m_2 (g) is mass of sample after heating in volatile matter test (Aller et al., 2017).

$$VM(\%) = \frac{m_1 - m_2}{m_1} \times 100 \quad (1)$$

Fixed carbon content (*FC*) was calculated based on the average obtained from three determinations of ash content and volatile matter. Fixed carbon content is the difference between 100 and the sum of the percentages of moisture, ash, and volatile matter. Since prior to analysis both samples were dried to constant mass, moisture was not taken into consideration.

For ultimate analysis of samples, an amount of 1-3 mg was used to determine the C, N, H, and S content. The analysis was performed using the CHNS elemental analyser (EuroVector EA3100 Elemental Analyzer), with cystine as standard reference material. Oxygen (O) was calculated by difference from the obtained results.

Mineral content

The mineral content was determined using Inductively Coupled Plasma - Mass Spectrometer (NexION 300S, PerkinElmer) for Co and Mo, and Inductively Coupled Plasma – Optical Emission Spectrometer (Optima 8300, PerkinElmer) for Ca, K, P, Mg, Fe, Al, Mn, Zn, B, and Cu. Briefly, 0.5 g of sample was mineralised with 5 mL of HNO_3 65 % and 0.5 mL of H_2O_2 30% using a Anton Paar PROSOLV microwave oven. After digestion, the samples were diluted to a final volume of 25 mL with ultrapure water and quantified based on an external calibration curve.

Physicochemical characterization

The dry bulk densities (*BD*) of the material were determined on the previously prepared samples, using cylinder method. The bulk densities were calculated using Eq. (2), where m_2 (g) is the mass of oven-dried sample within the cylinder, m_1 (g) the mass of the empty cylinder, and V (cm^3) the volume of the cylindrical core.

$$BD = \frac{m_2 - m_1}{V} \quad (2)$$

Electrical conductivity (*EC*) and pH of grape marc and grapevine prunings were determined by blending 0.5 g of milled sample with 20 mL of distilled water for 1 h using a magnetic homogenizer (IKA C-Mag HS7). *EC* and pH of the suspension were recorded using a Mettler Toledo SevenExcellence Multiparameter.

A Carl Zeiss EVO LS 15 scanning electron microscope, at accelerating voltages of 5 kV, 2001 Pa, and different magnifications was used in order to observe the morphology of the samples.

Measurement of water holding capacity (*WHC*) of plant material was performed as follows: around 20 g of each sample was introduced in a container with glass wire mesh at the bottom and the container was placed in a glass beaker with water for 24 h. The samples were then fixed in a larger recipient to let excessive water drain for 6 h. Wet sample was then weighed and oven-dried at 105 °C until no more weight (Bikbulatova et al., 2018). *WHC* was calculated using Eq. (3), where m_1 is the mass of glass container, m_2 the total mass of wet material and glass container, and m_3 the mass of oven-dried material sample and glass container.

$$WHC(\%) = \frac{m_2 - m_3}{m_2 - m_1} \times 100 \quad (3)$$

All experimental determinations were conducted in triplicate and the results were expressed as the mean values ± SD.

RESULTS AND DISCUSSIONS

Proximate and ultimate analysis

Both grape marc and grapevine prunings were conditioned prior characterization. Grape marc came with a dry mass content of 38.74 ± 4.94% compared to 82.80 ± 1.38% of grapevine prunings, therefore both were dried in an oven to a constant mass.

Proximate analysis offers primary information about biochar when it is used as a solid fuel, but can also offer information about the transformation of waste material into biochar. Grape marc had a higher content of ash compared to grapevine prunings, up to 5.2 times higher. Ash content can be correlated with a higher mineral content of the raw material (Figure 1). Volatile matter is the organic fraction of moisture-free biochar that can migrate into the soil and become a source of food for soil microbes (Zhu et al., 2017). Grapevine prunings had a higher volatile matter content (83.03 ± 3.34%) compared to grape marc (75.92 ± 3.15%), but part of this volatile matter can be lost in pyrolysis process resulting in a biochar with higher fixed carbon content. Grapevine prunings also had a higher fixed carbon content (14.98 ± 3.45%) compared to grape marc (13.81 ± 3.13%), as shown in Table 1. According to Sun et al. (2017), the content of volatile and fixed matter is higher in plant biomass, such as woody pruning wastes, compared to other types of materials (agricultural waste, aquatic waste, nutshells and fruit peel, livestock manure, and residual sludge).

Biochar prepared from crop residues and woody materials also has a higher carbon content than biochar prepared from other sources, *e.g.*, manure (Tomczyk et al., 2020). Spokas (2010) stated that biomass composition in terms of O/C molar ratio is between 0.6 and 1 depending on the main component type (*e.g.*, cellulose, hemicellulose, lignin, starch).

Table 1. Proximate and ultimate results of waste vegetal material

	Sample	
	Grape marc	**Grapevine prunings**
***DM* (%)**	38.74 ± 4.94	82.80 ± 1.38
***AC* (%)**	10.27 ± 0.04	1.99 ± 0.12
***VM* (%)**	75.92 ±3.15	83.03 ± 3.34
***FC* (%)**	13.81 ± 3.13	14.98 ± 3.45
C (%)	50.10 ± 0.57	48.39 ± 0.61
H (%)	6.30 ± 0.27	6.65 ± 0.28
N (%)	2.20 ± 0.27	0.49 ± 0.02
O (%)	41.40 ± 1.79	44.47 ± 0.85

In our case, the O/C molar ratio of grapevine prunings was slightly higher (0.69) than that of grape marc (0.62), but the final O/C molar ratio of biochar will largely depend on the pyrolysis conditions. According to Budai et al. (2013), biomass with high values of H/C and O/C ratios exhibits low resistance to degradation, hence these values should be taken in consideration when the material will be subjected to pyrolysis, to obtain a graphite-like biochar.

Mineral content

As expected, grape marc had a high content of K, N, and P compared to lignocellulosic material (grapevine prunings). Grape marc contains almost 11 times more K than grapevine prunings, as shown in Figure 1. P content was 0.34% in grape marc compared to 0.12% in grapevine prunings and also N content was higher in grape marc. Both N and P are volatile and can be lost during pyrolysis, depending on the operating temperature.

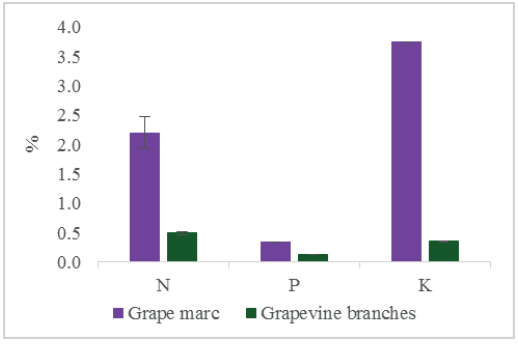

Figure 1. NPK results of waste vegetal material

Both samples were characterized in terms of content (mg/kg) of Ca, Mg, Fe, Al, Mn, Zn, B, Cu, Co, and Mo. Higher levels of mineral content were in grape marc samples (Table 2), except for Mg (1246.03 ± 29.76 mg/kg in grapevine prunings compared to 799.37 ± 3.39 mg/kg in grape marc). Grape marc had a total content of Ca of 5790.25 ± 2.85 mg/kg, higher than that of grapevine prunings (4799.20 ± 37.69 mg/kg). The large amount of calcium in the raw materials will be found in large proportion in the final product (biochar), which makes it suitable to be used as an amender for acid soils.

Grape marc also contains up to 12 times more Fe and up to 18 times more Al compared to grapevine prunings. Mineral content of the biomass feedstock can be correlated with the mineral content of biochar, taking into account that there are little losses of minerals during pyrolysis. Similar content of Mn was observed in both samples, *i.e.*, 26.87 ± 0.80 mg/kg for grape marc and 25.63 ± 0.02 mg/kg for grapevine prunings. Slightly differences were also observed in Zn content, *i.e.*, 13.79 ± 0.86 mg/kg for grape marc and 10.26 ± 0.00 mg/kg for grapevine prunings. Grape marc had higher content in B (45.58 ± 1.28 mg/kg), Cu (19.75± 0.37 mg/kg), Co (0.064 ± 0.001 mg/kg), and Mo (0.064 ± 0.001 mg/kg), compared to the content of B (4.74 ± 0.40 mg/kg), Cu (3.96 ± 0.01 mg/kg), Co (0.027 ± 0.000 mg/kg), and Mo (0.128 ± 0.001 mg/kg) in grapevine prunings.

Physicochemical characterization

Electrical conductivity (*EC*) of grape marc was more than 4 times higher than that of grapevine prunings. *EC* of grape marc was of 2.09 ± 0.05 dS/m compared to 0.50 ± 0.01 dS/m for grapevine prunings, with more plant-available nutrients (Madjar et al., 2014b). Higher levels of *EC* of grape marc are due to its higher values of nutritive element content.

Table 2. Mineral content in biomass waste material

	Sample	
	Grape marc	**Grapevine prunings**
Ca (mg/kg)	5790.25 ± 2.85	4799.20 ± 37.69
Mg (mg/kg)	799.37 ± 3.39	1246.03 ± 29.76
Fe (mg/kg)	148.89 ±4.14	12.72 ± 0.02
Al (mg/kg)	168.75 ± 2.59	9.38 ± 0.02
Mn (mg/kg)	26.87 ± 0.80	25.63 ± 0.02
Zn (mg/kg)	13.79 ± 0.86	10.26 ± 0.00
B (mg/kg)	45.58 ± 1.28	4.74 ± 0.40
Cu (mg/kg)	19.75± 0.37	3.96 ± 0.01
Co (mg/kg)	0.064 ± 0.001	0.027 ± 0.000
Mo (mg/kg)	0.213 ± 0.004	0.128 ± 0.001

Bulk density (*BD*) of raw material depends on the chemical composition, but also on the size and distribution of the particles. Grape marc had a higher density (0.36 ± 0.01 g/cm^3) than grapevine prunings (0.33 ± 0.01 g/cm^3), mainly due to sample preparation. Moreover, grape marc had a higher acidity (pH = 3.85 ± 0.01) compared to grapevine prunings (pH = 5.41 ± 0.53). During pyrolysis, the pH of the biochar increases with operating temperature (Yang et al., 2017), so the initial pH value of the grape marc and grapevine prunings is not highly significant in characterization of biochar.

SEM images shown in Figure 2 indicate a higher porosity of grapevine prunings, with pore diameters ranging from 40.46 µm to 120.7 µm at the center of the prunings and from 40.66 µm to 69.86 µm at the edge. For grape marc, it was difficult to determine surface porosity due to its complex mix (waste seeds, skin, and stalks). Grape marc has a higher water holding capacity (*WHC*), *i.e.*, 71.60 ± 3.43%, compared to 55.41 ± 1.43% for grapevine prunings.

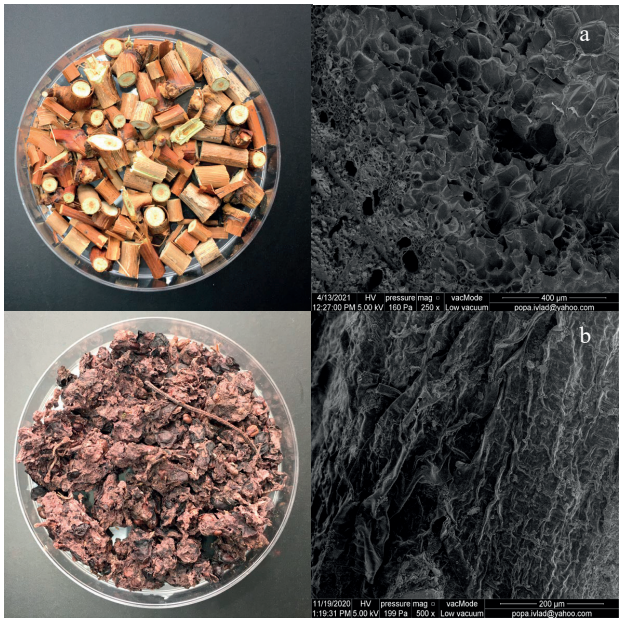

Figure 2. SEM analysis of vegetable waste material: (a) grapevine prunings and (b) grape marc

CONCLUSIONS

The properties of the biochar produced by pyrolysis can be significantly influenced by feedstock type, reactor design, pyrolysis temperature, and heating rate.

Characterisation of pyrolysis feedstock is essential to obtain a biochar applied for specific purposes. Several analytical techniques were used to characterise two types of biomass waste, *i.e.*, grape marc and grapevine prunings.

Proximate and ultimate analyses, SEM analysis, measurements of mineral content, pH, *EC*, *BD*, and *WHC* were performed. Both vegetal materials had high content of N, P, K, Ca, Mg and also micronutrients. A high content of C and nutrients as well as values of *WHC* over 50% suggest that grape marc and grapevine prunings are suitable for producing biochar which could be applied as soil amender.

Further analysis should be conducted to determine the available plant nutrients and their variation during pyrolysis.

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IDENTIFICATION AND QUANTIFICATION OF HERBICIDE CONTAMINANTS FROM INPUTS USED FOR ORGANIC AGRICULTURE

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Abstract

Organic agriculture is increasing in Romania, especially due to a better understanding of the effect of conventional agriculture on biodiversity. Being a relatively new implemented concept, there is a gap in the regulations regarding contaminants in organic inputs. Therefore, there are no available standard methods used to quantify contaminants such as pesticides from inputs (fertilisers) used for organic farming. This study aims to develop and to validate a quantification method to analyse the triazine contaminants (Hexazinone, Simazine, Simetryne, Atrazine, Ametryn, Propazine, Terbuthylazine, Prometryn) from organic foliar fertilizer using UPLC-PDA. Commercialy available types of fertilizer were used as complex matrix (Codamix). The quantification method was evaluated in terms of linearity, limit of detection and limit of quantification, BIAS, recovery, precision of repeatability and precision of reproducibility. Based on the acquired data, the standard uncertainty of the method was also evaluated. The developed and validated method can be successfully optimised for other type of organic fertilisers.

Key words*: triazine; organic inputs; UPLC-PDA; method validation.*

INTRODUCTION

In Romania they are over 3000 certified producers in organic agriculture in 2021 (MADR, 2021), which in order to maintain their certification they need to use special fertilizers according to regulation EC 2008/889. Fertilizer obtained from biomass waste, using different types of processing (Aro Fatehi, 2017), may contain pesticide residues. In order to be used in organic agriculture all fertilizers should be tested to make sure that no contamination is present.

Triazines were one of the most frequently used group of herbicides, until they were suspected to be endocrine disrupters, moreover to cause different types of cancers and birth defects (Baranowska et al., 2012). The most extensively applied triazine prior to 2003 were Atrazine (2-chloro-6-ethylamino-4-isopropylamino-1,3,5-triazine) and Simazine (2-chloro-4,6-bis-ethylamino-1,3,5-triazine), members of s-triazine groups. Although atrazine has been banned since 2003 in EU, the parent compound and its metabolites are still detected in waters (Sass & Colangelo, 2006), soil and plants.

At EU level several triazines are still allowed in food products in different maximum residue amounts, for example: Atrazine - 0.05 mg/kg (EC 2016/440), Simazine - 0.02 mg/kg (EC 2011/310), Terbuthylazine - 0.01 mg/kg (EC 2021/618), depending on commodity type. Simetryne, Hexazinone, Ametryn, Prometryn, and Propazine are not accepted in food products, according with EU MRL database vers.2.2 (EU, 2021).

According to Romanian regulation regarding the triazine contaminants in environment, the normal values of total triazines is < 0.1 mg/kg of dry matter in soil (Order 756/1997). In case of alert steps should be less than 1 mg/kg of dry matter of in case of sensible soils and be less than 2 mg/kg of dry matter in case of less

sensible soils. Specifications regarding pesticides in the fertilizers used for organic agriculture are missing from organic regulation, mainly because no one will expect to find pesticides in this area.

Currently, herbicides are mainly determined by gas (GC) (Dou et al., 2020) and liquid (LC) chromatographic methods coupled with different detectors such as, UV (Khana et al., 2018), PDA (Baranowska et al., 2012), tandem MS/MS (Patrudu et al., 2020), but also by special methods such as surface-enhanced Raman spectroscopy coupled with an advanced chemometric model (Chen et al., 2015). High pressure liquid chromatography (HPLC) coupled with UV-Vis detection is still widely used technique for the analysis of non-volatile and high molecular weight pesticides which depend on its solubility in the mobile phase (Leong et al., 2020). Furthermore, HPLC is known to be more rapid and efficient than the GC-MS. Shah et al. (2011) determined triazine herbicides (Atrazine, Metribuzin, Ametryn, and Terbutryn), in soil samples with HPLC-UV detection. In another study, the HPLC coupled with MS analysis was used for the evaluation of the sorption and persistence of Atrazine, Propazine, Simazine, Terbuthylazine, Prometon, Prometryn, Simetryne, Terbutryn, Metamitron and Metribuzin in agro-industrial and composted organic wastes (sheep manure, spent coffee grounds, composted pine bark and coir) (Fenoll et al., 2014).

The aim of this study was to develop and to validate an analytical method based on UPLC-PDA for the identification and the quantification of eight most common triazine contaminants in organic fertilisers. This method can be successfully optimised for other type of organic fertilisers based on biomass waste material.

MATERIALS AND METHODS

Chemicals and reagents

The standard solution was prepared using reference materials of Hexazinone, Simazine, Simetryne, Atrazine, Ametryn, Propazine, Terbuthylazine, and Prometryn with a purity of more than 98%, purchased from Dr. Ehrenstorfer. Acetone, ethyl acetate, acetonitrile (ACN), sodium sulphate and sodium chloride were purchased from Merck KGaA. All of the other chemicals used were of analytical grade.

Samples

The development and validation of the analytical method was performed using lignosulfonate fertilizer CODAMIX as test matrix which was purchased from agrochemical market. CODAMIX is a formulation of trace elements chelated with citric acid, lignosulfonic acids, which is completely soluble in water and specially created to complement NPK fertilizers for both hydroponic and fertilized crops. The tested matrices were not contaminated with any of the herbicides investigated in this study and therefore they are suitable to be used as blank samples. The samples were stored at room temperature during the analysis.

Extraction and concentration

The extraction and concentration method were developed based on ISO 11264:2005. In our case, 5 ± 0.05 g of sample was weighed in a 100 mL Erlenmeyer flask, and the necessary water was added in order to keep a ratio between 10: 20: 15 of water: acetone: ethyl acetate. A volume of 20 mL of acetone was added and the samples were homogenised for six hours on a mechanical stirrer (IKA KS 260). After 5 h, 3 g of sodium chloride was added to the samples and 15 mL of ethyl acetate. The samples were stirred again for 5 minutes, and then 14 mL of the extract was transferred through a filter paper containing anhydrous natrium sulphate in order to remove the water excess into a 50 mL pear shaped rotavapor flask. Extract was concentrated to 1 mL volume on a Heidolph Laborota 4000 rotavapor, then 2 mL of acetonitrile was added in the rotavapor flask, and concentrated under 1 mL. The procedure was repeated 2 times. The final extract was adjusted with acetonitrile to 1 mL and additional 1 mL of ultrapure water was added. The final volume was mixed, filtered through a 0.45 μm filter, collected in a 1.8 mL vial, injected and subjected to UPLC-PDA analysis (Figure 1).

Lignosulfonate fertilizer samples were spiked with herbicides standard at a concentration of 2.5 mg/kg. The spiked samples were subjected to extraction according to the method described

above in order to assess the 3 important R: repeatability, reproducibility and recovery.

UPLC-PDA instrument and chromatographic conditions

A Waters Acquity I chromatographic system was used for herbicide analysis, equipped with a binary pump, autosampler, PDA detector and a Zorbax Eclipse Plus C18 4.6 x 100 mm, 5 µm column. The following chromatographic conditions were used: the samples were maintained at 10°C and the injection volume was 10 µL. Column was kept at 30°C during the analysis and the PDA detector was set to register the spectrum from 210 to 320 nm, and also at wavelengths of 245 nm for Hexazinone herbicide and 220 nm for the other 7 herbicides. The flow rate of the mobile phase was 1 mL/min and the gradient is presented in the Table 1.

Method validation

For the method validation several parameters were taken into account: linearity, limit of detection, limit of quantification, accuracy, precision of repeatability, precision of reproducibility and recovery (EURACHEM, 2014).

Table 1. Gradient condition

Time (min)	A% (H_2O)	B% (ACN)	Curve
Initial	85	15	Initial
5	85	15	6
25	40	60	6
30	85	15	6
33	85	15	6

Linearity

For the calibration curve, five concentrations in the range 0.1-10 µg/mL of herbicide standard solution in methanol were used. First, a stock solution of individual herbicide Hexazinone, Simazine, Simetryne, Atrazine, Ametryn, Propazine, Terbuthylazine, and Prometryn in 2-propanol or acetone with a concentration of 1 mg/mL was prepared. The equipment response and the calibration curve validity were checked with a different herbicide solution at five levels of concentration (0.1, 0.5, 1, 5 and 10 µg/mL for Simazine, Simetryne, Atrazine, Ametryn, Propazine, Terbuthylazine, Prometryn and 0.2, 1, 2, 10, 20 µg/mL for Hexazinone). For linearity evaluation, two coefficients were calculated, correlation coefficient (r) and coefficient of variation, in order to obtain the best result.

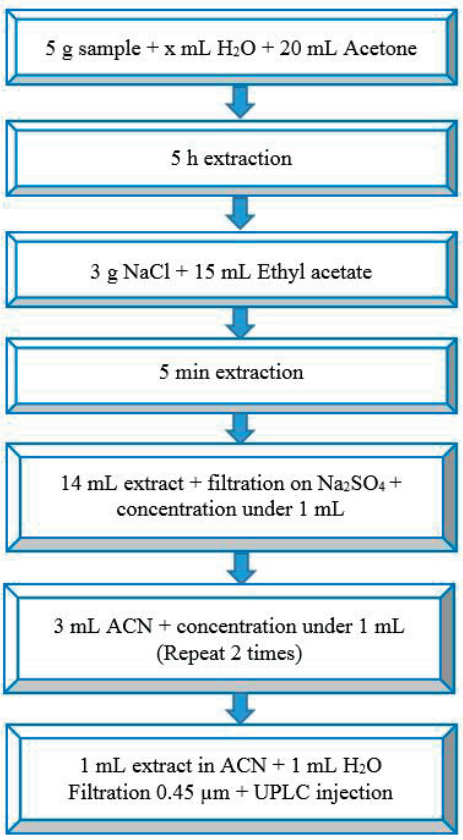

Figure 1. Schematic representation of the extraction and concentration of fertilizer samples

Precision and recovery

The precision of the method was evaluated based on the relative standard deviation (RSD) of the intra-day precision (RSDr %) and the inter-day precision (RSDR %), both for standard solutions and spiked samples. The RSDR values are calculated using Horwitz equation, a generalised precision equation which has been found to be independent of analyte and matrix but dependent on concentration.

One concentration of herbicide standard mix solution was prepared, namely 2.5 µg/mL for Simazine, Simetryne, Atrazine, Ametryn, Propazine, Terbuthylazine, Prometryn and 5 µg/mL for Hexazinone). The accuracy and precision of repeatability was determined based on the analysis of 10 freshly prepared standards on the same day by one analyst, and the precision of reproducibility was determined by analysing the mix solutions in different days using 2 operators. Also, recovery and precision tests were conducted by analysing spiked blank samples at the concentration of 2.5 mg/kg, since there are no imposed MRL, and the only specification in European regulation regarding the use of mixed inputs is "*the use of the EU fertilising product as specified in the use instructions must not lead to the exceedance of those limit values in food or feed*" (EC 2019/1009). For the precision of repeatability six samples have been analysed and for the precision of reproducibility the samples were analysed in eleven different days with three replicates.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The detection limit is the lowest amount of herbicides in a sample which can be detected, under well-specified conditions, but not necessarily quantified as an exact value, and the quantification limit represents the lowest amount of herbicides in a sample which can be quantitatively determined with suitable precision and accuracy. The LOD value is the point at which a true signal is detectable from the noise of the detector, the ratio has to be larger than a three to one signal to noise, and for the LOQ the determined value of signal to noise ratio is larger than 10. For this analytical method, samples were spiked at low concentrations until a detectable signal was obtained, and the results were repeatable.

Measurement Uncertainty

Quantification of measurement uncertainty was determined taking into consideration the following sources: standard uncertainty from control solution, uncertainty derived from linearity (coefficient of variation), uncertainty derived from precision of repeatability of control solution, uncertainty derived from BIAS and uncertainty derived from precision of reproducibility of spiked samples.

RESULTS AND DISCUSSIONS

Optimization of extraction and analysis method

Water, acetone, 2-propanol and ethyl acetate are considered to be chemicals that present the least negative environmental impact. Due to their low environmental impact, they are also popularly denoted as a 'Green Solvent' (Welton, 2015; Byrne et al., 2016). Water has a great importance in extraction of pesticides, low water content can lead to low recoveries in triazine herbicide extraction.

As guided by ISO 11264:2005 different solvents were tested for triazine extraction from lignosulfonate fertilizer, each with different effect on the matrix. Dichloromethane has a polymerization effect on the lignosulfonate fertilizer, making it impossible to separate the liquid phase from the solid phase in order to proceed with pesticide extraction. Petroleum ether showed a good recovery, but is preferred to be avoided due to its high volatility. Ethyl acetate is recognized for its lower recovery (about 5-10% lower) compared to other solvents (petroleum ether, hexane, dichloromethane, and so on) but it was selected due to his smaller impact on environment. Taking into consideration good results for the intra-day precision (RSDr %) and the inter-day precision (RSDR %), the recovery can be introduced in calculation of the final amount of pesticides in the fertilizer matrix.

Another important parameter in the triazine extraction from fertilizer matrix is the mixing time between sample, water and acetone. Lowering the contact time between samples and extraction solvent under 5 h could lead to lower recoveries (ISO 11264:2005), hence when developing a new method, careful consideration should be applied to adjusting the extraction time to obtain an acceptable recovery (in general higher of 70%).

Good separation between the triazine herbicide was obtained as showed by Figure 2, with the following elution order: Hexazinone (13.107 min), Simazine (13.531 min), Simetryne (16.323 min), Atrazine (16.899 min), Ametryn (19.381 min), Propazine (19.954 min),

Terbuthylazine (20.817 min), and Prometryn (22.222 min). Even though Hexazinone was quantified at 245 mn, the herbicide showed lower intensity in absorbance compared to the other herbicides, therefore the concentration of the analytical standard and spiked sample had to be doubled.

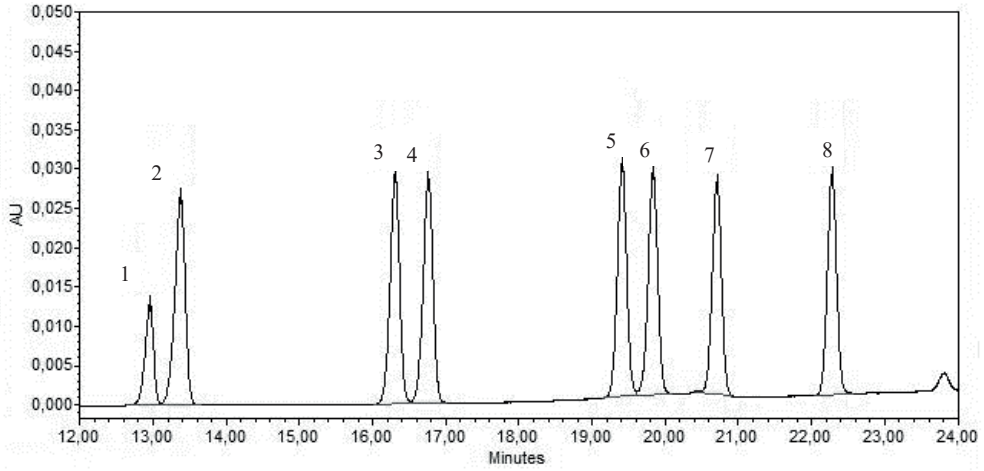

Figure 2. Analytical standard chromatographic profile at 220 nm at a concentration of 2.5 µg/mL (1-Hexazinone, 2-Simazine, 3-Simetryne, 4-Atrazine, 5-Ametryn, 6-Propazine, 7-Terbuthylazine, and 8-Prometryn)

Linearity

Under the described optimum conditions, the plotting of the calibration curves (Figure 3) was achieved using five concentration levels of the reference material of herbicide solutions, with two replications each. An excellent linearity over the relevant working range of 0.1-10 µg/mL for Simazine, Simetryne, Atrazine, Ametryn, Propazine, Terbuthylazine, Prometryn and 0.2-20 µg/mL for Hexazinone, as shown by correlation coefficient r, which was higher than 0.9999 (Table 2) was obtained. The values of the parameters that characterize the calibration curve for triazine herbicides are also listed in the Table 2. Another coefficient taken into consideration for the linearity of an analyte, is the coefficient of variation, calculated based on ANOVA, which shows a good linearity for all herbicides, with values from 0.441% for Atrazine to 1.866% for Simetryne (Table 2).

Accuracy and BIAS

The lowest accuracy was observed for Simazine, 89.47% mainly due to a poor dissolution of the powdered standard in the organic solvent. Even though the other 7 triazine herbicides were dissolved in 2-propanol, Simazine shows a low solubility to this solvent, hence acetone was used instead. Particular care should take place when preparing the intermediary solution, due to Simazine tendency to precipitate when the stock solution is stored at 4 ± 2°C.

Table 2. Linearity criteria of triazine herbicide

Herbicide	RT (min)	Coefficient of correlation (r)	Coefficient of variation (%)
Hexazinone	13.107	1.0000	0.571
Simazine	13.531	1.0000	0.528
Simetryne	16.323	0.9999	1.866
Atrazine	16.899	1.0000	0.441
Ametryn	19.381	1.0000	1.101
Propazine	19.954	1.0000	1.307
Terbuthylazine	20.817	1.0000	1.405
Prometryn	22.222	1.0000	0.571

All other triazines had a good accuracy, in the interval of 100 -110% (Table 3), with respect to EURACHEM Guide, which recommends that the accuracy should be between 70-110%. For the selected triazines BIAS or trueness showed good values, between 0.90% for Hexazinone

and 5.44% for Prometryn, with the exception of Simazine, as explained above (Table 3).

Table 3. Accuracy and BIAS of triazine herbicide obtained at a concentration of 2.5 µg/mL

Herbicide	Mean concentration (µg/mL)	*Accuracy (%)*	*BIAS (%)*
Hexazinone	5.04	100.90	0.90
Simazine*	2.04	89.47	-10.53
Simetryne	2.53	101.08	1.08
Atrazine	2.55	102.03	2.03
Ametryn	2.63	105.10	5.10
Propazine	2.57	102.76	2.76
Terbuthylazine	2.57	102.64	2.64
Prometryn	2.64	105.44	5.44

**Special consideration should be paid to Simazine due to difficulty of dissolution in solvent*

Precision and recovery

The intra-day and inter-day precision of triazine mix in samples was evaluated using standard addition in the blank samples. The results obtained for both standard solutions and spiked samples are showed in Table 4.

Table 4. Intra-day and inter-day precision

Herbicide	Conc. (µg/mL)	*Standard solution*		*Spiked samples*	
		RSDr (%)	*RSDR (%)*	*RSDr (%)*	*RSDR (%)*
Hexazinone	5	0.89	1.39	10.2	16.3
Simazine	2.5	0.91	1.45	7.4	11.9
Simetryne	2.5	0.94	1.50	11.8	18.9
Atrazine	2.5	0.99	1.59	9.5	15.3
Ametryn	2.5	1.41	2.25	6.8	10.8
Propazine	2.5	2.23	3.56	8.3	13.3
Terbuthylazine	2.5	1.15	1.85	8.1	13.0
Prometryn	2.5	0.96	1.53	6.7	10.7

The obtained RSDr for standard solutions ranged from 0.89 % for Hexazinone to 2.23% for Propazine, which indicated that the equipment method is highly repeatable. Moreover, the RSDR ranged from 1.39% for Hexazinone to 3.56% for Propazine, emphasizing a good precision of selected method, and also showing a low influence on the overall uncertainty. When we analyse the results of RSDr % of the spiked matrix, it can be observed that the highest value (11.8%) was obtained for Simetryne, followed by Hexazinone with 10.2%. The lowest RSDr % were obtained for Prometryn (6.7%), closely followed by Ametryn with 6.8%.

The RSDr % values obtained after spiking lignosulfonate fertilizer the triazine herbicide were less than 20% which indicated a good precision of repeatability, within the acceptable limits imposed by the referential in the pesticide field (SANTE, 2017). The same trend is observed also for RSDR % values, the obtained results, even though higher, are still in compliance with validation criteria imposed by SANTE (20%), therefore the overall method doesn't require optimisation or improvement of the extraction method. The recovery values for all the triazine herbicide in spiked lignosulfonate fertilizer were 70%. Simazine coelutes with a compound found in the lignosulfonate fertilizer, the analyte being selected based on the full spectra of the peak at the known retention time (Figure 4). The matrix effect can be removed using SPE (solid phase extraction) but lower recoveries should be expected due to retention of analyte on the sorbent (such as silica, florisil). The best recovery was obtained for Prometryn with 100.82%, followed by Simetryne (80.35%) and Terbuthylazine (80.34%). The rest of the triazine herbicide had a recovery lower that 80% as follows: Hexazinone - 78.07%, Simazine - 79.24%, Atrazine 71.83%, Ametryn - 71.89%, and Propazine - 75.58%. Even though the recoveries were in the accepted interval for pesticide residues analysis, we recommend that when real unknown samples are received, spike with the concern analyte should be performed, in order to obtain a result closer to the true value.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The values of LOD and LOQ parameters were found by reporting the processed integrated peak from the UPLC chromatograms to the noise. Thus, for the LOD value the ratio has been 3: 1 signal to noise ratio, and the LOQ value was determined to be larger than 10 values.

The LOD was established as 1 mg/kg for Hexazinone and 0.5 mg/kg for all other triazine herbicides, while the LOQ value was 2 mg/kg for Hexazinone and 1 mg/kg for all other triazine herbicides. Spiked samples were extracted and analysed in triplicates in order to obtain the minimum reliable results. The values of LOD and LOQ can be improved by increasing the injection volume or by decreasing the concentration volume, in case the new regulations will require smaller concentrations to be determined in organic fertilizers.

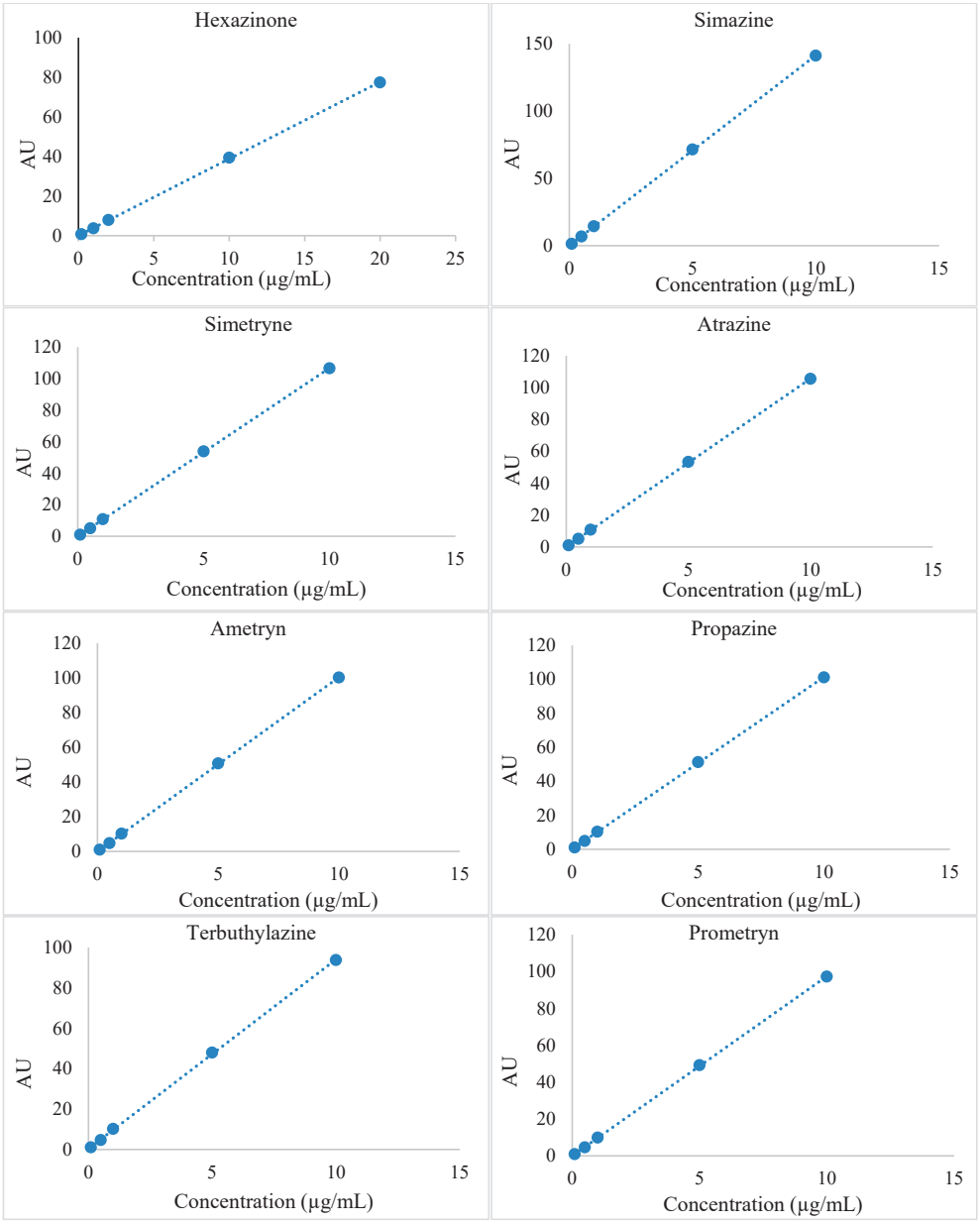

Figure 3. Calibration curves of 8 mixed triazine herbicides

Measurement Uncertainty

The main important factors which influenced standard method uncertainty with more than 30% are BIAS and sample RSDR. From the obtained results it can be observed that higher standard method uncertainty was obtained for Simetryne 19.4%. Similar values of standard uncertainty were obtained for Hexazinone (16.8%), Simazine (16.3%), and Atrazine (15.9%).

The lowest uncertainty was obtained for Prometryn (12.6%), followed by Ametryn (12.7%), Terbuthylazine (13.9%) and Propazine (14.5). For unknown samples the standard uncertainty (U_{sd}) will be reported as expanded uncertainty (U_{ex}) using a coverage factor of 2 (SANTE, 2017):

$$U_{ex} = 2 \times U_{sd} \quad (1)$$

Based on this estimation, the highest applied expanded uncertainty will be for Simetryne, with a value of 40 %.

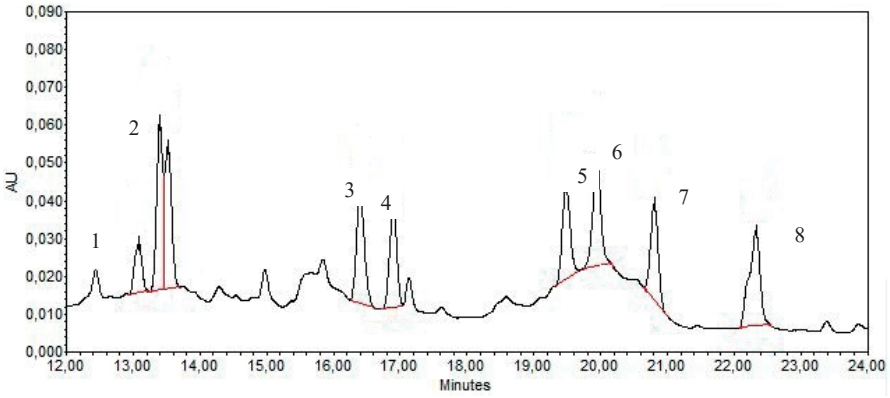

Figure 4. Spiked lignosulfonate fertilizer with triazine herbicide (1-Hexazinone, 2-Simazine, 3-Simetryne, 4-Atrazine, 5-Ametryn, 6-Propazine, 7-Terbuthylazine, and 8-Prometryn)

CONCLUSIONS

In this study an analytical method based on UPLC-PDA for the identification and the quantification of eight most common triazine contaminants (Hexazinone, Simazine, Simetryne, Atrazine, Ametryn, Propazine, Terbuthylazine, and Prometryn) in organic lignosulfonate fertilizer was developed. The method showed good results in terms of linearity, accuracy, precision of repeatability, precision of reproducibility, and recovery. The method can be successfully optimised for other type of organic fertilisers based on biomass waste material. Further investigation should be conducted in order to decrease the limit of detection and quantification, and to improve the sensibility of the overall method.

ACKNOWLEDGEMENTS

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SOME ASPECTS REGARDING THE MORPHO-ANATOMY AND ANTIOXIDANT POTENTIAL OF THE MEDICINAL PLANT *EUCOMMIA ULMOIDES* OLIV.

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Abstract

Eucommia ulmoides Oliv. - Gutta-percha, Hardy rubber tree, known as Du Zhong in China, is commonly used in Chinese herbalism, being considered to be one of the 50 fundamental herbs. E. ulmoides *is native to China and has been widely cultivated in central and south-eastern regions of China and other countries worldwide. The studied individuals have been cultivated in the Bucharest, at the University of Agronomic Sciences and Veterinary Medicine's Botanical Garden which maintained this species for over 40 years. Different studies have been carried out with regard to leaf morphology, as well as the anatomy of the stem, leaf and petiole. Morphological analyses show that leaves vary in length between 12 and 15 cm, in width between 5.2 and 8 cm, and the petiole length measures 1.5-2 cm. The leaves of Eucommia ulmoides Oliv. feature an epidermis (upper and lower) covered by cuticle. Their mesophyll is bifacial. Ethanolic extracts from the fresh leaves and fruits were prepared in order to determine the total phenolic, flavonoid and associated antioxidant activity. The results showed that leaves had higher total polyphenol and flavonid content than fruits and fruits had higher antioxidant activity than leaves. The findings of this study suggest that Eucommia ulmoides can be also used as a dietary source of phenolic compounds with antioxidant potential.*

Key words: *Eucommia ulmoides, leaf anatomy, stem anatomy, polyphenol, trichomes.*

INTRODUCTION

Eucommia ulmoides (*E.u*.) (Gutta - Percha, The Hardy Rubber Tree) is a small dioecious tree, the only species of genus *Eucommia* (Family *Eucommiaceae*), being native to China. It has been widely cultivated in central and south-eastern regions of China and other countries worldwide (Li & Du, 2001). Gutta-percha, known as Du Zhong in China, is commonly used in Chinese herbalism, where it is considered one of the 50 fundamental herbs (Duke & Ayensu**,** 1985). *Eucommia* is a primitive angiosperm and fossils reportedly show that a few species were distributed worldwide up to the late around the world about 6 million years ago. Fossils of other *Eucommia* species have been found in 10 -to 35-million-year-old brown coal deposits in central Europe and widely in North America (Call & Dilcher, 1997). Some fossil specimens assigned to the living *Eucommia ulmoides* were found from the Miocene and Pliocene in Germany, Poland and Romania (Guo Shuang - Xing, 2000). The pharmacological properties and efficacy of *E. ulmoides* have been well documented in ancient Chinese medicinal books such as Shennong's *Classic of Materia Medica* and *Compendium of Materia Medica* (Bamba et al. 2002, 2010). Some 112 compounds have been isolated from *E. ulmoides*, including lignans, iridoids, phenolics, steroids and other compounds. Delicious tea formula made from *E. ulmoides* leaves was reported to reduce fattiness and enhance energy metabolism (Hussain et al., 2016). The leaf of *E. ulmoides* has been found to be a rich source of aminoacids, vitamins, minerals and flavonoids, such as quercetin, rutin and geniposidic acid (Chen et al., 2004; Takamura

et al., 2007). The leaves of *E. ulmoides* have been reported to enhance bones' strength and body muscles, thus leading to longevity and promoting fertility in humans. Flavonoids are important compounds which are common in nature and are considered as secondary metabolites. Analyses of *E. ulmoides* isolated a total of 7 flavonoids (Cheng et al., 2000).

The effect of *Eucommia ulmoides* substances is antibacterial (against *Acinetobacter baumannii* and *Staphylococcus aureus*), antifungal (against *Aspergillus fumigatus*), antiviral and anti-inflammatory (Nakano, 1997; Lv et al., 2008; Kim et al., 2009; Tsai et al., 2010; Zang et al., 2013; Peng et al., 2014; Kwon, 2016). Two antifungal peptides from the bark of *E. ulmoides* inhibited 8 pathogenic fungi from cotton, wheat, potato, tomato, and tobacco, including *Phytophthora infestans*, *Ascochyta lycopersici*, *Verticillium dahlia*, *Gibberella zeae*, *Alternaria nicotianae*, *Fusarium moniliforme*, *Fusarium oxysporum* and *Colletotrichum gossypii* (Huang et al., 2002). In the Chinese traditional medicine, *Eucommia* is considered a major herbal tonic for cardiac patients (Luo et al., 2010; Greenway et al., 2011). Antioxidant compounds from the *Eucommia* plant reduced the level of free radicals (Cai et al., 2004) and improved the disease condition caused by oxidative stress (Akinmoladun et al., 2010). The *Eucommia* cortex extract can be used in the control of osteoporosis. Therefore, the mentioned extract can be established as a therapeutic agent under conditions of osteoporosis (Ha et al., 2003). Antioxidant properties of the *Eucommia* leaf extract were also reported to contribute positively to the promotion of bone growth by improving cell integrity during oxidative stress (Lin et al., 2011). Previous studies have shown that *E. ulmoides* has also properties that help the human body fight against obesity and the antimetabolic syndrome (Hirata et al., 2011; Kobayashi et al., 2012; Dai et al., 2013). Stem bark extract of *E. ulmoides* showed higher protection activity against memory dysfunctions (Kuon et al., 2010; 2013). It has also been shown that the activities of sex hormones in the body are optimized with the application of *E. ulmoides* (Ong & Tan, 2007). *Eucommia* also increases the level of other antioxidant enzymes in the blood to neutralize free radicals (Park et al., 2006). Aucubin, Geniposidic contained in *E. ulmoides* may be therapeutic candidates for non-alcoholic fatty liver disease (Lee et al., 2014). β-carotene may also be relevant to the anti-cancer effects of *Eucommia*. The leaf and bark of *E. ulmoides* have been widely used in traditional Chinese medicine for the treatment of hypertension (Deyama et al., 2001; Tagawa et al., 2005). The leaves are also used as the basic ingredient of Tochu tea (Du zhong tea) and the plant is cultivated in Japan. Numerous studies have been conducted to reveal the medicinal properties of the species (Duke & Ayensu, 1985; Yeung Him-Che, 1985; Li Dong et al., 1986; Hong et al., 1987; Chevallier, 1996; Yen & Hsieh, 1998; Stuart, 1998; Zang et al., 2007; Choi et al., 2008; Zhou et al. 2009; Zang et al., 2009; Horii et al., 2010; Jin et al., 2010; Luo et al., 2010; Jiang et al., 2011; Kwon et al., 2010, 2013; Li et al., 2016; Kim et al., 2012; Fujikawa et al., 2012; Zang et al., 2012; Zang et al., 2014; Guo et al., 2015; Li et al., 2016; Hussain et al., 2016; Do et al., 2018). *E. ulmoides* has excellent resistance to insect and disease problems. Its general morphology, systematics, anatomy, pollen, chemical elements, growth, development and habitat are studied for *E. ulmoides* (Tippo, 1940; Metcalfe and Chalk, 1957; Metcalfe, 1967; Erdtman G., 1969; Zhang Hong-Da et al., 1979; Cronquist, 1981, 1988; Li et al., 1981; Zavada & Dilcher, 1986; Nakazawa et al., 2013; Zhang Yu-Iong et a1., 1988; Zhang Zhi-Yu et al., 1990; Zhang Kang-Jan, 1990; Cheng Jun-Qing et al., 1992; Watson and Dallwitz, 1992; Yan, 1999; Yu Pang et al., 2008). In Romania, to date no studies have been conducted regarding the morphology, anatomy and biochemistry of this species.

MATERIALS AND METHODS

The material of *Eucommia ulmoides* Oliv. (female plants) originated from the field of the Botanical Garden of the University of Agronomical Sciences and Veterinary Medicine in Bucharest, Romania. The species exhibits good adaptation here, having been cultivated for more than four decades. It showed immunity to various pests. The material used in this study was sectioned by hand using razor blades to obtain

semipermanent and permanent slides for microscopic studies. Fresh leaves, stems and petioles were collected for anatomical study in the year 2019. Thereafter, the sections were cleansed with chloral hydrate for 24 hours, then washed and stained with carmine alaunate and green iodine (Georgescu et al., 2015). Analyses and observations of the obtained cross-sections were performed at the Center for the Study of Food and Agricultural Products Quality at USAMV - Bucharest. Photos were taken and measurements were made using the Leica DM1000 LED, the Leica DFC295 Video Camera and the Leica S8 APO Stereo Microscope, Novex Holland, Optika Microscope, as well as a Sony photocamera. Photos were taken using a light microscope with different magnifications. The physicochemical analysis were carried out in the laboratories of the Research Center for Studies of Food and Agricultural Products Quality, University of Agronomic Sciences and Veterinary Medicine of Bucharest. The extracts were prepared using 50% (v/v) ethanol, after the pharmacopoeia method. The total phenolic content was determined using Dobrin et al. (2018) method, employing the Folin-Ciocalteu reagent and expressed as mg gallic acid equivalents per grames of fresh material (mg GAE/g FW). Calibration curve of the gallic acid had the folowing concentrations: 0, 5, 10, 20, 30, 40, 50, 60, 70 and 80 µg/ml. The $AlCl_3$ modified assay after Dobrin et al. (2018) was used for quantifying the total flavonoid content of the ethanolic plant extracts. The standard catechine solutions for the calibration curve were 0, 0.01, 0.05, 0.10, 0.20, 0.30, and 0.40 mg/ml. The total flavonoid content was expressed as mg catechin equivalents/g fresh weight (Xu et al., 2010). The radical scavenging activity (RSA) assay was made after Dobrin et al., 2018. The results were expressed in DPPH inhibition percentage. All the solvents used were of the analytical grade. All the absorbances were measured using a Specord 210 Plus UV/VIS spectrophotometer. All the samples were analyzed in triplicates.

RESULTS AND DISCUSSIONS

Stem anatomy

The stem has a circular shape (Figures 1 and 2).

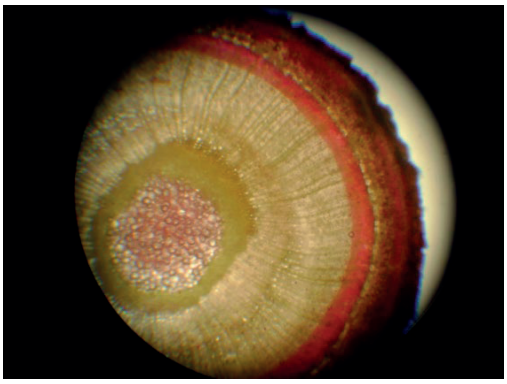

Figure 1. Stem anatomy - cross section

Cross-sections performed in young stem branches reveal an outer epidermis consisting of a polygonal cells layer, covered by a smooth cuticular layer.

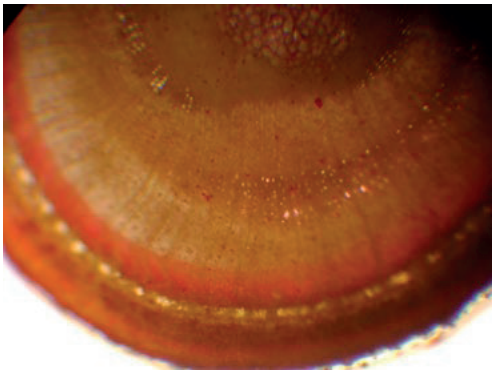

Figure 2. Stem anatomy: cross section details

Under the epidermis there is the primary cortex, made up of 8-12 layers of cells (see Figure 3).

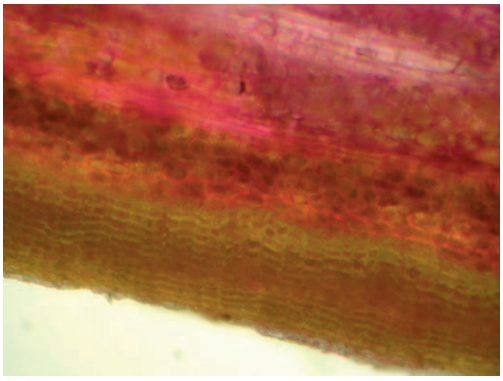

Figure 3. Stem cross-section: details of the outer region

More specifically, the primary cortex is made up of collenchyma tissue, phellogen consisting of 1 or 2 oblong cells, phelloderm, perycicle with loose ring of fibers and parenchymatous cells, xylem and phloem, as continuous cylinders traversed by narrow rays, and heterogeneous pith cells in the middle (see Figure 4).

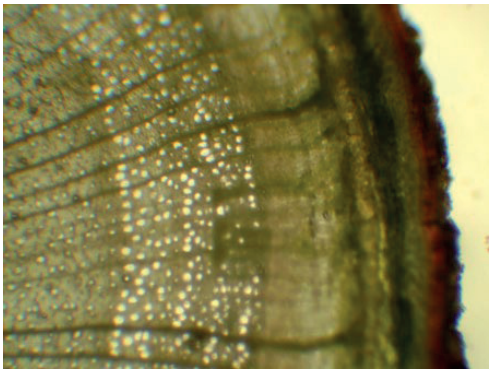

Figure 4. Stem cross-section: phloem, xylem and rays

Between the phelloderm and phloem there are several layers of cells and fibre. The phloem rays are made up of 1 to 3 cell columns. There are articulated laticifers present in the phloem and cortex, as well as scattered latex-cells in some of the other tissues. The wood is of a diffuse-porous type with distinct growth rings as shown in the cross section in Figure 5.

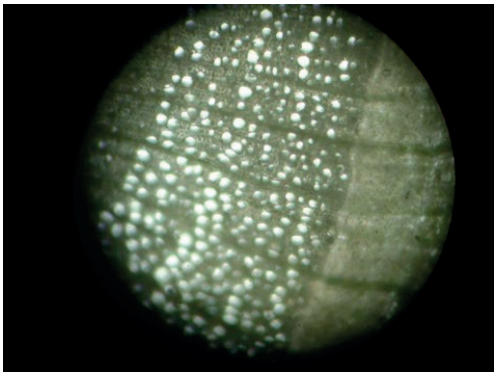

Figure 5. Diffuse-porous wood

There are some solitary vessels with a circular to angular contour. The xylem rays are uni and biseriate. Laticiferous cells are present in the primary cortex, phloem and pith (Metcalfe & Chalk, 1957). The xylem has small vessels (the average tangential diameter measures 9.43-23.28 mm), nearly all are solitary, in shape of half ring, porous type, with spiral thickening, simple perforation plate (Figure 6), ovate and elliptic, lateral wall with bordered pits and spiral thickenings, intervascular pitting uncommon owing to the solitariness of the vessels; small, usually opposite, scalariform perforation plate (Figure 7).

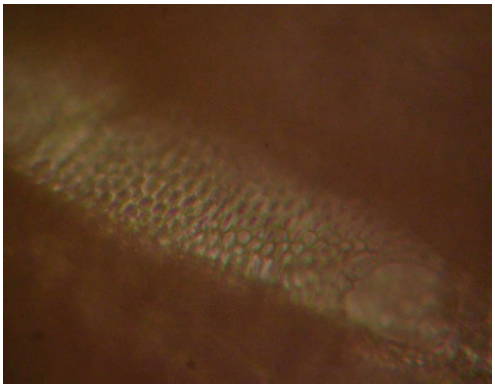

Figure 6. Cross section: simple perforation plate

The rays are almost homocellular or slightly heterocellular.

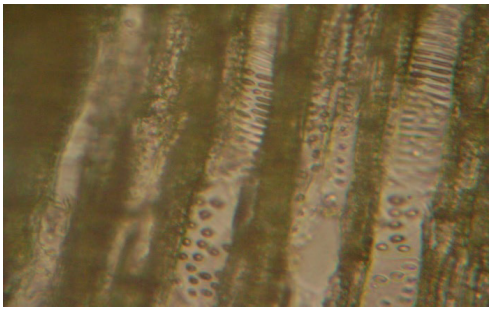

Figure 7. Cross section: simple and scalariform perforation plate

Leaf morphology

Leaves are simple, alternate (Figure 8), deciduous, serrate, astipulate; the blade has an elliptical, ovate or oblong shape, varying in length between 12 and 15 cm and in width between 5.2 and 8 cm; the length of the petiole measures 1.5-2 cm; hairs are simple, unicellular; the edge is simple-serrate and pinnate venation.

Figure 8. Leaf

If a leaf is torn across, strands of latex exuded from leaf veins solidify into rubber and hold the two parts of the leaf together (Figure 9).

Figure 9. Leaf with rubber

Leaf anatomy

The leaves of *E. ulmoides* Oliv. feature an epidermis (upper and lower) covered by cuticle. Their mesophyll is bifacial; it consists of a pallisade tissue (63.32 µm), just beneath the upper epidermis, whereas there are more chloroplasts and a spongy tissue (87.10 µm) toward the lower epidermis. The dorsi-ventral blade is 174.27-130 µm thick; the upper and lower epidermis is somewhat irregular in shape. The upper epidermal layer consists of compact cells (11.70 µm with cuticle), while the lower epidermis consists of one layer of cells (7.28 µm), with stomata and no glandular trichomes,unicellular The palisade tissue has two cell layers. The cells of the secondary layer are shorter, irregular, columnar. The spongy tissue consists of 5-6 cell layers, with intercellular spaces (Figures 10 and 11).

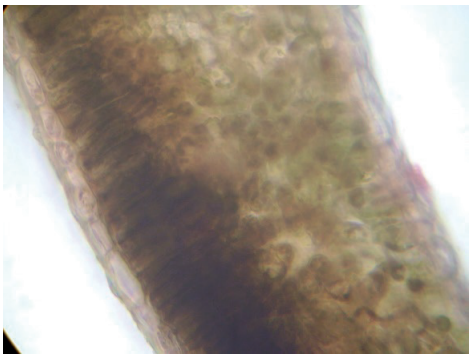

Figure 10. Leaf anatomy: lamina

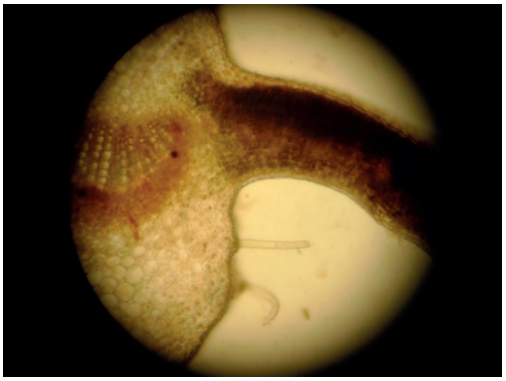

Figure 11. Leaf anatomy: lamina with midrib

The vascular bundle in the middle vein has an arc shape, with several layers of parenchyma cells on the exterior enclosing them. There are scattered rubber cells, 2-4 layers of collenchyma cells under the middle vein/ midrib epidermis (Figure 12).

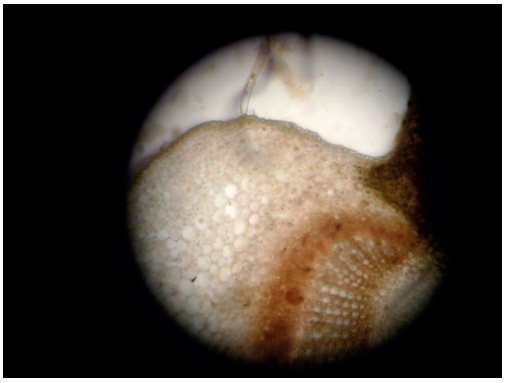

Figure 12. Midrib

The leaf epidermis was scanned by through electron microscopy (SEM) (Figures 13, 14, 15, and 16).

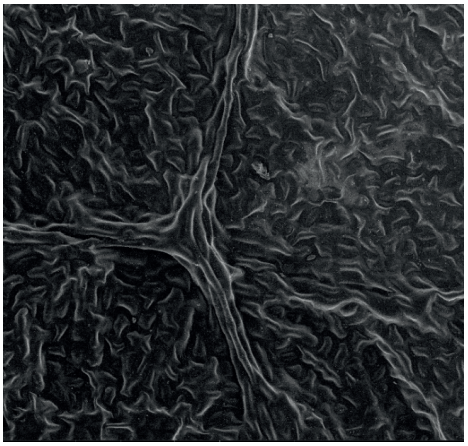

Figure 13. Upper epidermis (SEM)

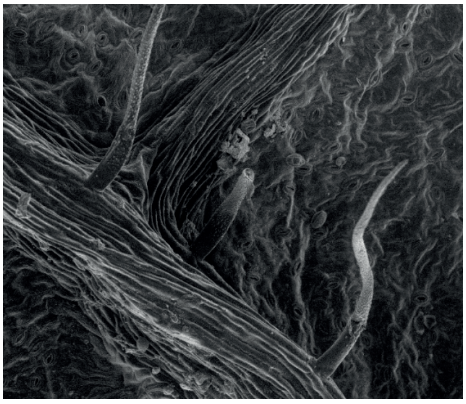

Figure 14. Lower epidermis: stomata, no glandular trichomes

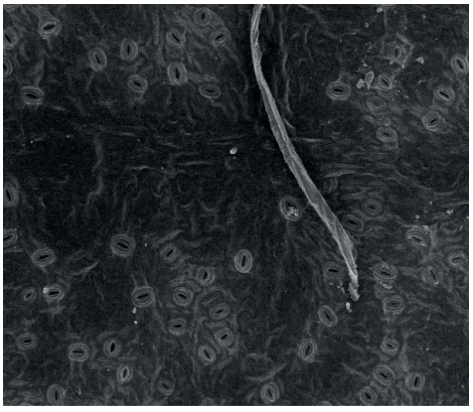

Figure 15. Lower epidermis: stomata, no glandular trichomes

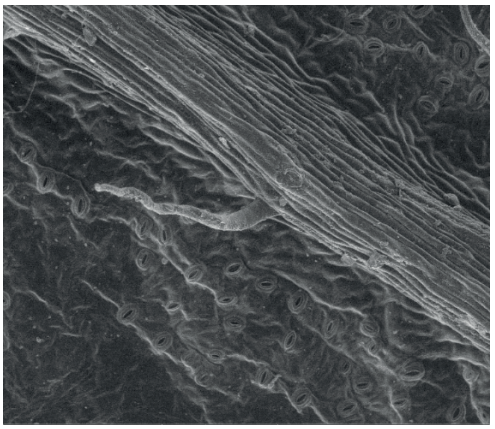

Figure 16. Lower epidermis: stomata, no glandular trichomes

Petiole anatomy

The petiole is thick, crescent-shaped, with 1-layer cuticled epidermis; the sclerenchyma, consisting of 4-6 layers, is present on the lower surface of epidermis, cortex parenchyma inside, the cells are large, 6-7 layer cells thickness. The vascular bundle of midrib has an arc shape (Figure 17).

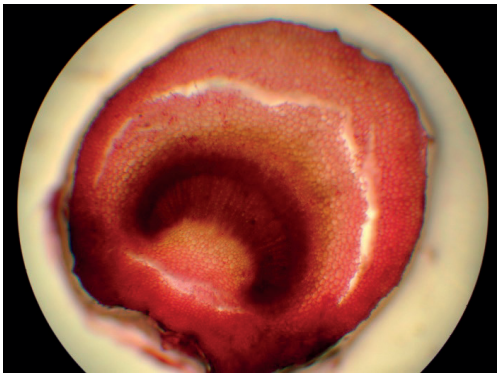

Figure 17. Petiole anatomy

Phenolic, flavonoids and antioxidant activity

It was found that the total phenolic content was higher in leaves (1398.65 mg GAE/g FW) than in fruit (803.22 mg GAE/g FW).

Total flavonoid content was found also in a very hight content in leaves (10,24 mg CE/g FW) than in fruit (5.33 mg CE/g FW).

The scavenging efect on DPPH radical of *E. ulmoides* leaves extract (61.40 %) was lower than in fruits (69.79%). This results are in accordance with the studies of Xu et al., 2018,

Wang et al., 2012, and Xu et al., 2018, who found DPPH scavenging activity of 65.9% in leaves extracts and Wang et al., 2012, who found that DPPH scavenging activity of *E. ulmoides* varied between 56.32% and 90.37%.

CONCLUSIONS

The morphological and anatomical studies that we carried out on stem and leaf cross-sections, as well as on leaf surface sections, demonstrate for the first time in Romania the anatomy of organs of *E. ulmoides* Oliv. specimens growing in our country.

The microscopic observations performed on leaves, petioles and stems of *Eucommia ulmoides* are very significant and useful, representing relevant information to the specialists of systematic botany and to the taxonomists.

These preliminary results suggest that *E. ulmoides* can be an ideal candidat for capitalizing its potential in pharmaceutical, food and biomedical industries.

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EFFECT OF GAMMA IRRADIATION ON BIOACTIVE COMPOUNDS OF MEDICINAL PLANTS

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Abstract

Gamma irradiation has various applications in the agricultural, medical and industrial fields. It is widely used as a method of microbial decontamination and for mutation breeding in plants. Particular emphasis is placed on the ability of gamma rays to stimulate cellular metabolism to synthesize medicinally valuable secondary metabolites. The aim of this paper is to review the published results on the effect of gamma irradiation on bioactive compounds of medicinal plants. Comparison of different studies showed that the effects observed after radiation exposure depended on several factors, such as species, radiation dose, sample type, sample state (solid or liquid, fresh or dry), extraction solvent and extraction method. Current findings are good indicators of the potential application of gamma irradiation to produce high quality raw materials to meet the requirements of the food and pharmaceutical industries.

Key words*: radiation dose, phenolic compounds, volatile compounds, biological activity.*

INTRODUCTION

In human society, gamma irradiation has various applications in agriculture, medicine and other biotechnological processes. In agriculture, it is used for microbial decontamination, to reduce post-harvest losses by suppressing sprouting and to extend the shelf life of food (Emovon, 1996). Gamma irradiation is also used to improve the physiological and biochemical processes in plants, an example being the stimulation of the synthesis of medicinally valuable secondary metabolites (Aly, 2010).

Gamma rays are ionizing radiation that contains high energy photons with a higher penetration capacity than alpha and beta rays, causing the ionization of matter and plants through indirect interaction (Kovács & Keresztes, 2002; Vandenhove et al., 2010). Gamma radiation interacts with atoms or molecules in the cell, especially with water, producing free radicals that can change important components of plant cells, affecting differently the morphology, physiology, anatomy and biochemistry of plants depending on the radiation dose (Kovács & Keresztes, 2002; Ashraf et al., 2003). High doses of gamma radiation disrupts hormonal balance, protein synthesis, enzymatic activity, gas exchange in leaves and water exchange in plants (Esfandiari et al., 2008; Kiong et al., 2008). These effects induce changes in cell structure and plant metabolism; for example alteration of photosynthesis, dilatation of thylakoid membranes, accumulation of phenolic compounds and modulation of the antioxidant system (Kim et al., 2004; Wi et al., 2006; Ashraf, 2009).

According to the World Health Organization, irradiation doses up to 10 kGy are considered non-toxic to food products (WHO, 1999). However, irradiation can produce qualitative and quantitative changes in plant phytochemicals.

The aim of this paper is to review the published results on the effect of gamma irradiation on bioactive compounds of medicinal plants.

MATERIALS AND METHODS

A systematic and comprehensive research of the literature was conducted using a set of representative keywords, such as “gamma irradiation”, “medicinal plants”, “bioactive compounds” and “biological activity”. From

the accessed scientific databases, 67 studies were used in this review study.

RESULTS AND DISCUSSIONS

The results of studies on the effect of gamma irradiation on bioactive compounds of medicinal plants are contradictory.

Regarding the effects produced by gamma radiation on the phenolic content, different results were reported. Irradiation at 10, 20 and 30 kGy did not produce significant changes on phenolic compounds and flavonoids in some Brazilian medicinal herbs (Koseki et al., 2002). There were also no changes in the phenolic content of thyme and peppermint irradiated at 2, 5 and 10 kGy (Pereira et al., 2016). Instead, irradiation at 10 kGy increased the concentration of phenolic compounds in ginkgo extract (Pereira et al., 2015). Pereira et al. (2018) also reported an increase in lithospermic acid A in lemon balm and 5-O-caffeoylquinic acid in bastard balm irradiated at 1 and 10 kGy. Irradiation at 15 kGy increased the phenolic content in *Brassica nigra* L. (0.1%), *Cassia senna* L. (1.3%), *Cymbopogon schoenanthus* L. (24.9%), *Lepidium sativum* L. (25.6%), *Cassia senna* L. (70%) and decreased the phenolic content in *Trigonella foenumgraecum* L. (4.1%), *Hibiscus sabdariffa* L. (5.1%), *Acacia nilotica* L. (14%) and *Cymbopogon citrates* L. (33%) (Polovka & Suhaj, 2010).

There are reported studies in which gamma irradiation had a different effect on different phenolic compounds. Variyar et al. (1998) found that irradiation at 10 kGy led to an increase in the content of gallic acid and syringic acid in clove, while the content in synapic acid, ferulic acid and p-coumaric acid decreased by about half and that of caffeic acid and gentisic acid remained unchanged. In the case of nutmeg, with the exception of p-coumaric acid and protocatechuic acid, which remained unchanged, irradiation produced changes in the content of other phenolic acids compared to the control samples.

Campos et al. (2005) reported a 5.2% decrease in tannic acid in *Maytenus aquifolium* Martius irradiated at 100 Gy.

The effect of gamma irradiation on phenolic compounds also depended on the radiation dose applied. Kiong et al. (2008) reported that rosamarinic acid content was lowest (5.27 mg/g fw) in *Orthosiphon stamineus* plantlets irradiated at 10 Gy and highest (8.40 mg/g fw) in plants irradiated at 30 Gy.

The different effects of gamma irradiation on phenolic compounds can be attributed to their different extraction capacity. Also, the increased phenolic content may be associated with the degradation of tannins due to irradiation, plants with large amounts of hydrolysable tannins being more sensitive to irradiation compared to those containing condensed tannins (Variyar et al., 1998; Khattak et al., 2008).

Regarding the effect of gamma radiation on antioxidant activity, there are studies that have shown a stimulatory effect of irradiation (Variyar et al., 2004; Štajner et al., 2007; Suhaj & Horvathova, 2007, Khattak et al., 2009; Mohamed, 2009; Khattak & Simpson, 2010; Mali et al., 2011; Taheri et al., 2014; Ashouri Sheikhi et al., 2016) or no effect of irradiation observed in other studies (Byun et al., 1999; Chatterjee et al., 1999; Horvathova et al., 2007; Thongphasuk et al., 2008; Brandstetter et al., 2009; Kim et al., 2009; Akshatha et al., 2013), while other studies have indicated a decrease in antioxidant levels (Lampart-Szczapa et al., 2003; Gumus et al., 2011; Yalcin et al., 2011).

Research results on the effect of gamma irradiation on volatile compounds are also contradictory. There are studies that have shown an increase in the content of volatile compounds after gamma irradiation (Gyawali et al., 2006), insignificant effects of irradiation (Chatterjee et al., 2000; Zareena et al., 2001; Koseki et al., 2002; Haddad & Quetin-Leclercq, 2007; Gyawali et al., 2008; Ryu et al., 2008; Sádecká & Polovka, 2008; Shim et al., 2009), while one study has shown a decrease in the content of volatile compounds (Salum et al., 2009).

Some research has shown that irradiation had different effects on different essential oil compounds. Variyar et al. (1998) reported that the content of myristicin, 1-terpinene-4-ol and á-terpeniol increased, while that of elemicin, α-pinene and sabinene decreased in the irradiated nutmeg compared to the control sample. Among the constituents of Korean angelica essential oil, oxygenated terpenes, such as verbenone, α-eudesmol and β-eudesmol

increased after gamma irradiation (Seo et al., 2007). Irradiation led to an increase of trans fatty acids and a decrease of unsaturated fatty acids in *Nigella sativa* L. (Arici et al., 2007). Gamma irradiation also significantly decreased the content of unsaturated fatty acids in *Mucuna pruriens* L. Although linoleic acid was not detected in the non-irradiated sample, it was detected in the irradiated samples (Bhat et al., 2008).

The effect of gamma irradiation on volatile compounds also depended on the radiation dose applied. Ilyas & Naz (2014) reported that maximum amount of essential oil and curcuminoids in the rhizomes of turmeric was recorded at 50-60 Gy, while higher doses of gamma irradiation (100 Gy) reduced the amount of oil and curcuminoids.

Studies on the effect of gamma radiation on other chemical compounds have also been reported.

For example, irradiation at 10 kGy led to a significant decrease in the total ascorbate content in oregano, sage, nutmeg, cinnamon and black pepper, and a significant decrease of carotenoids in sage, rosemary, oregano, cinnamon, parsley and bird pepper (Calucci et al., 2003).

Chung et al. (2006) reported a 400% increase in the total shikonin content in *Lithospermum erythrorhizon* irradiated at 16 Gy, by 240% in plants irradiated at 2 Gy and by 180% in plants irradiated at 32 Gy.

Gamma irradiation at 110 Gy doubled the value of the alkaloid in *Atropa belladonna* L. (Abdel-Hady et al., 2008).

The gamma radiation dose of 15 Gy slightly increased the stevioside content, while other doses (5, 10 and 20 Gy) showed a negative effect on the stevioside content in the callus of *Stevia rebaudiana* Bert. (Khalil et al., 2015).

No changes were reported in the sennoside content in senna irradiated at 10-25 kGy, in the curcumin content in turmeric irradiated at 10-50 kGy and in the paeoniflorin content in *Paeonia* Radix irradiated at 10 kGy (Van Doorne et al., 1988; Chosdu et al., 1995; Yu et al., 2004). Also, after irradiation up to 17.8 kGy, the content of flavonol glycosides and caffeine in *Ginkgo biloba* L. and *Paullinia cupana* H.B.K., respectively, was not modified (Soriani et al., 2005).

Byun et al. (2004) reported that irradiation had no effect on xanthine oxidase and the nitrite scavenging capacity in *Lonicera japonica* Thunb.; however, tyrosinase inhibition was increased.

Studies on black pepper, cinnamon, paprika, caraway, pimento, thyme, coriander and fenugreek irradiated at 10 kGy showed no qualitative or quantitative chemical changes compared to the control sample (Josimović & Čudina, 1987; Gupta et al., 2009). Instead, phytic acid was not detected in velvet bean seeds irradiated at 15 kGy (Bhat et al., 2007).

Regarding the effect of gamma irradiation on biological activities, the studies did not show any influence of irradiation on the anti-inflammatory and antimicrobial activity (Mamatha et al., 2010; Abd EI-Aziz & Abd EI-Kalek, 2011).

The main reported results on the effect of gamma irradiation on bioactive compounds of medicinal plants are presented in Table 1.

Table 1. Effect of gamma irradiation on bioactive compounds of medicinal plants

Medicinal plants	Radiation dose	Results	Authors
Capsicum annuum var. *angulosum* Mill. (paprika) *Carum carvi* L. (caraway) *Cinnamomum verum* J. Presl (cinnamon) *Coriandrum sativum* L. (coriander) *Pimenta dioica* L. Merr. (pimento) *Piper nigrum* L. (black pepper) *Thymus vulgaris* L. (thyme)	10 kGy	No significant qualitative or quantitative chemical changes.	Josimović & Čudina (1987)
Senna alexandrina Mill. (senna)	10-25 kGy	No changes in the content of sennoside.	Van Doorne et al. (1988)
Curcuma longa L. (turmeric)	10-50 kGy	No significant changes in curcumin content.	Chosdu et al. (1995)
Syzygium aromaticum L. (clove)	10 kGy	Increases in gallic acid and syringic acid content; decreases in the content of synapic acid, ferulic acid and p-coumaric acid; no effect on the content of caffeic acid and gentisic acid.	Variyar et al. (1998)
Myristica fragrans Houtt. (nutmeg)	10 kGy	Increases in the content of myristicin, 1-terpinene-4-ol and á-terpeniol; decreases in the	Variyar et al. (1998)

Medicinal plants	Radiation dose	Results	Authors
		content of sabinene, α-pinene and elimicin; no effect on the content of p-coumaric acid and protocatechuic acid.	
21 Korean medicinal herbs	10 kGy	No significant effect on antioxidants, nitrite scavenging and electron donation capacity.	Byun et al. (1999)
Curcuma longa L. (turmeric)	10 kGy	No effect on antioxidant activity.	Chatterjee et al. (1999)
Curcuma longa L. (turmeric)	10 kGy	No significant changes in aromatic compounds.	Chatterjee et al. (2000)
Crocus sativus L. (saffron)	2.5-5 kGy	No significant changes in the constituents of essential oil.	Zareena et al. (2001)
Cynara scolymus L. (artichoke) *Nasturtium officinale* R. Br. (watercress) *Ocimum basilicum* L. (sweet basil) *Rosmarinus officinalis* L. (rosemary)	10, 20 and 30 kGy	No significant changes in essential oils, phenolic compounds, flavonoids and tannins.	Koseki et al. (2002)
Capsicum frutescens L. (bird pepper) *Cinnamomum verum* J. Presl (cinnamon) *Myristica fragrans* Houtt. (nutmeg) *Ocimum basilicum* L. (basil) *Origanum vulgare* L. (oregano) *Petroselinum sativum* Hoffm. (parsley) *Piper nigrum* L. (black pepper) *Rosmarinus officinalis* L. (rosemary) *Salvia officinalis* L. (sage)	10 kGy	Significant decrease in total ascorbate in oregano, sage, nutmeg, cinnamon and black pepper; significant decrease in carotenoids in sage, rosemary, oregano, cinnamon, parsley and bird pepper.	Calucci et al. (2003)
Lupinus angustifolius L. (lupin) *Lupinus albus* L. (white lupin) *Lupinus luteus* L. (yellow lupin)	1-10 kGy	Increasing irradiation doses reduced the antioxidant effects.	Lampart-Szczapa et al. (2003)
Lonicera japonica Thunb. (japanese honeysuckle)	10-30 kGy	No influence on xanthine oxidase and the nitrite scavenging capacity. Tyrosinase inhibition increased with radiation dose.	Byun et al. (2004)
Paeonia albiflora var. *trichocarpa* Bunge (*Paeoniae* Radix)	10 kGy	No significant changes in the amount of paeoniflorin.	Yu et al. (2004)
Glycine max L. Merr. (soybean)	0.5-5 kGy	Increases in DPPH free radical-scavenging activity; decreases the content in glycosidic conjugates; increases the content in aglycons.	Variyar et al. (2004)
Ginkgo biloba L. (ginkgo) *Paullinia cupana* H.B.K. (guarana)	5.5-17.8 kGy	No significant difference in the flavonol glycosides content of ginkgo and the caffeine content of guarana.	Soriani et al. (2005)
Maytenus aquifolium Martius ("espinheira-santa")	10, 20, 40, 60, 80 and 100 kGy	Decreases the tannic acid content by 5.2 % at 100 Gy.	Campos et al. (2005)
Lithospermum erythrorhizon (purple gromwell)	2, 16 and 32 Gy	Increases in total shikonin content.	Chung et al. (2006)
Allium fistulosum L. (welsh onion)	1, 3, 5, 10 and 20 kGy	Increasing the total content of volatile compounds by 31.60% at 10 kGy and by 24.85% at 20 kGy.	Gyawali et al. (2006)
Angelica gigas Nakai (Korean angelica)	1, 3, 5, 10 and 20 kGy	Increases in oxygenated terpenes.	Seo et al. (2007)
Mucuna pruriens L. (velvet bean)	2.5-30 kGy	Increases in total phenolic content; no significant difference in tannin content up to 7.5 kGy; significant increase in tannin content at higher doses.	Bhat et al. (2007)
Nigella sativa L. (black cumin)	2.5-10 kGy	Decreases unsaturated fatty acids content; increases the content of trans fatty acids.	Arici et al. (2007)
Eucalyptus radiate A. Cunn. ex DC. (narrow-leaved peppermint) *Lavandula angustifolia* Mill. (lavender) *Thymus vulgaris* L. *thymoliferum* (thyme)	25 kGy	No significant qualitative or quantitative changes in the compounds of essential oils.	Haddad & Quetin-Leclercq (2007)
Origanum vulgare L. (oregano)	30 kGy	Insignificant effect on DPPH radical-scavenging capacity.	Horvathova et al. (2007)
Rosmarinus officinalis L. (rosemary)	30 kGy	Increases in total phenolic content in water extracts; no effect on the total phenolic content of methanol and ethanol extracts.	Pérez et al. (2007)
Glycine max L. Merr. (genotype Ana)(soybean)	1-10 kGy	Increases in total phenolic content and antioxidant activity.	Štajner et al. (2007)
Syzygium aromaticum L. Merr. & L.M. Perry (clove) *Zingiber officinale* Roscoe (ginger)	5-30 kGy	The increase in the oxidative substances content with the radiation dose.	Suhaj & Horvathova (2007)
Nigella sativa L. Kalungi (black cumin)	2, 4, 8, 10, 12 and 16 kGy	Increases in total phenolic content and enhances free radical-scavenging activity.	Khattak et al. (2008)
Glycyrrhiza uralensis Fisch. (licorice)	1, 3, 5, 10 and 20 kGy	No major qualitative and quantitative diferences in volatile compounds.	Gyawali et al. (2008)
Atropa belladonna L. (belladonna)	50, 80, 110 and 150 Gy	Doubling the alkaloid value at 110 Gy.	Abdel-Hady et al. (2008)
Orthosiphon stamineus (java)	0, 10, 20, 30,40, 50, 60 and 70 Gy	Rosamarinic acid content was lowest in plants irradiated at 10 Gy and highest in plants irradiated at 30 Gy.	Kiong et al. (2008)
Andrographis paniculata (Burm.f.) Nees (King	10 kGy	No significant difference in the DPPH radical-	Thongphasuk et al.

Medicinal plants	Radiation dose	Results	Authors
of Bitters) *Curcuma longa* L. (turmeric)		scavenging ability. No significant changes in the curcuminoids content of turmeric and in the total lactone content of King of Bitters	(2008)
Houttuynia cordata Thunb. (chameleon)	10 kGy	No significant differences in the following volatile oil compounds: decanoic acid, dodecanoic acid, 2-undecanone, octadecanol, caryophyllene oxide, phytol, menthol and hexahydrofarnesyl acetone.	Ryu et al. (2008)
Origanum vulgare L. (oregano)	10 kGy	No changes in the content of volatile compounds.	Sádecká & Polovka (2008)
Mucuna pruriens L. (velvet bean)	2.5-30 kGy	Significant decreases in unsaturated fatty acids content. Although linoleic acid was not detected in the non-irradiated sample, it was detected in the irradiated samples.	Bhat et al. (2008)
Eryngium foetidum L. (culantro)	10, 20 and 40 Gy	Increases in the total phenolic content, flavonoids, tannin and saponin; enhances reactive scavenging capacity.	Mohamed (2009)
Trigonella foenum-graecum L. (fenugreek)	10 kGy	No changes in the content of phytochemicals.	Gupta et al. (2009)
Origanum vulgare L. (oregano) *Salvia officinalis* L. (sage) *Thymus vulgaris* L. (thyme)	10 kGy	No significant effect on antioxidant capacity.	Brandstetter et al. (2009)
Nelumbo nucifera Gaerth (lotus)	1-6 kGy	Increases in phenolic content with increasing radiation dose; enchances DPPH scavenging activity.	Khattak et al. (2009)
Cuminum cyminum L. (cumin)	1-10 kGy	No significant differences in the content of antioxidant compounds.	Kim et al. (2009)
Paenia albiflora var. *trichocarpa* Bunge (*Paeoniae* Radix)	1-10 kGy	No differences in the content of volatile compounds.	Shim et al. (2009)
Cinnamomum verum J.Presl (cinnamon)	10-25 kGy	Decreases in the content of volatile compounds.	Salum et al. (2009)
Andrographis paniculata (Burm.f.) Nees (King of Bitters)	5-10 kGy	No influence on anti-inflammatory activity.	Mamatha et al. (2010)
Glycyrrhiza glabra L. (licorice)	5-25 kGy	No significant differences in phenolic content at 5-15 kGy; increases in phenolic content at 20-25 kGy; significant increases DPPH scavenging activity in all irradiated samples.	Khattak & Simpson (2010)
Brassica nigra L. Koch (black mustard) *Cassia senna* L. (senna) *Cymbopogon schoenanthus* L. (lemon grass) *Lepidium sativum* L. (garden cress)	5-30 KGy	Slight increase in phenolic content at 15 kGy.	Polovka & Suhaj (2010)
Acacia nilotica L. (gum arabic tree) *Cymbopogon citrates* L. (lemon grass) *Hibiscus sabdariffa* L. (roselle) *Trigonella foenum-graecum* L. (fenugreek)	5-30 KGy	Decreases in phenolic content at 15 kGy.	Polovka & Suhaj (2010)
Satureja hortensis L. (summer savory) *Thymbra spicata* L. (Mediterranean thyme) *Thymus vulgaris* L. (thyme)	5.1 kGy	Decreases in total phenolic content and DPPH radical scavenging activity.	Gumus et al. (2011)
Punica granatum L. (pomegranate)	5-25 kGy	Significant increases in total phenolic content and antioxidant activity at 10 kGy.	Mali et al. (2011)
Salvia sclarea L. (clary sage)	2.5-7 kGy	Negative effect on antioxidant activity.	Yalcin et al. (2011)
Cucurbita moschata Duchesne (pumpkin)	10 kGy	No effect on antimicrobial activity.	Abd EI-Aziz & Abd EI-Kalek (2011)
Terminalia arjuna Roxb. (arjuna)	25, 50, 100, 150 and 200 Gy	No significant increases in DPPH scavenging activity levels; increases in proline and phenolic content.	Akshatha et al. (2013)
Curcuma longa L. (turmeric)	10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy	Maximum yield of essential oil and curcuminoids at 50-60 Gy; decreases the amount of oil and curcuminoids at higher doses (100 Gy).	Ilyas & Naz (2014)
Curcuma alismatifolia var. *Sweet pink* (Siam tulip)	10, 15 and 20 Gy	Increases the content of phenolic compounds, flavonoids, fatty acids and antioxidant activity as the radiation dose increases.	Taheri et al. (2014)
Stevia rebaudiana Bert. (stevia)	5, 10, 15 and 20 Gy	Slight increase in stevioside content at 15 Gy; negative effect of the other doses on stevioside content; increases in total phenolic content and total flavonoid content at 15 Gy; enhances antioxidant activity at 20 Gy.	Khalil et al. (2015)
Ginkgo biloba L. (ginkgo, maidenhair tree)	1 and 10 kGy	Increases the concentration of phenolic compounds at 10 kGy.	Pereira et al. (2015)
Ferula gummosa Bioss. (galbanum)	10, 15, 20 and 25 Gy	Increases in phenolic content and radical scavenging activity.	Ashouri Sheikhi et al. (2016)
Mentha x piperita L. (peppermint) *Thymus vulgaris* L. (thyme)	2, 5 and 10 kGy	No change in phenolic content and bioactive properties.	Pereira et al. (2016)
Melissa officinalis L. (lemon balm) *Melittis melissophyllum* L. (bastard balm)	1 and 10 kGy	Increases in lithospermic acid A in lemon balm and 5-O-caffeoylquinic acid in bastard balm.	Pereira et al. (2018)

Given the contradictory results of the studies reviewed, the effects of gamma irradiation on bioactive compounds of medicinal plants are quite difficult to conclude. These effects depend on several factors, such as species, radiation dose, sample type, sample state (solid or liquid, fresh or dry), extraction solvent and extraction method (Pérez et al., 2007; Khattak et al., 2008; Alothman et al., 2009; Polovka & Suhaj, 2010). For this reason, it is not possible to identify common trends to all compounds and/or plant species.

CONCLUSIONS

The effect of gamma irradiation on the bioactive compounds of medicinal plants depends on several factors, but the main ones are the species and the dose of radiation applied.

Current findings are good indicators of the potential application of gamma irradiation to produce high quality raw materials to meet the requirements of the food and pharmaceutical industries.

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ENERGY CONSUMPTION AND GREENHOUSE GAS EMISSIONS IN MECHANIZATION APPLICATIONS FOR TOMATO PRODUCTION

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Abstract

This study was aimed to determine the energy use and equivalent carbon dioxide emissions (CO_2-equivalent) in per unit production area (ha) considering the petroleum products (PU) directly used for the production of table tomatoes in open field conditions in Adana province. Energy consumption values related to diesel fuel and engine oil (PU) usage were calculated based on the quantities used for the unit area (ha) of PUs and lower energy values (MJ/L), which are an important indicator of the energy content of these products (PU). As the carbon dioxide emission (kg CO_2) related tothe use of PU, kg CO_2 values are taken into consideration depending on the energy content (MJ) of the PU. A total of 249.6 L/ha diesel fuel is consumed when using tools and machinery in tomato production. As a result of diesel fuel consumption, a total of 685.34 kg CO_2/ha CO_2 emission occurs in tomato production. As a result of engine oil consumption in open field tomato production, using tools and machinery, a total of 0.1153 kg CO_2/ha CO_2 emission occurs.

Key words*: tomato, fuel consumption, CO_2 emission.*

INTRODUCTION

The carbon dioxide (CO_2) comes first among the greenhouse gases and this effect is global. Greenhouse gases are released through both natural processes and human activities. The most important natural greenhouse gas in the atmosphere is water vapor. However, humanitarian activities increase the atmospheric concentrations of these gases, causing large amounts of greenhouse gases to be released. This situation warms the climate by increasing the greenhouse effect.

According to the data of Turkey Statistical Institute (TUIK, 2019), total greenhouse gas emissions in Turkey was determined as 496.1 million tonnes of carbon dioxide equivalent in 2016. In this period, the largest share of CO_2 emissions in total emissions was energy-related emissions with 72.8%, followed by industrial processes and product use with 12.6%, agricultural activities with 11.4% and waste with 3.3%, respectively. Total greenhouse gas emissions in 2016 as CO_2 equivalent increased by 135.4% compared to 1990. While the carbon dioxide equivalent emission per capita was calculated as 3.8 tons/person in 1990, this value was determined 6.3 tons/person in 2016. In 2016, 33.1% of total CO_2 emissions were from electricity and heat production, 86.1% was from energy, 13.6% was from industrial processes and product use, 0.3% was from agricultural activities and waste. 55.5% of methane emissions originated from agricultural activities, 25.8% from waste, 18.6% from energy and 0.03% from industrial processes and product use. Agricultural activities constituted the biggest proportion in diazo monoxide (NO_2) emissions with 77.6%. This was followed by energy with 12.1%, waste with 6.5%, and industrial processes and product use with 3.8%.

Tomato is a cultivated plant all over the world and its production and consumption continues to increase. Thanks to its unique nutritional value, that is, it contains lycopene, beta carotene and flavonoids, it is considered as a protective plant. In particular, it has gained a huge popularity in recent years due to the antioxidative activities and anti-cancer functions of lycopene (Raiola et al., 2014). Therefore, its

production and consumption is constantly increasing. Tomatoes contain many phytochemicals, but the most well-known is lycopene. Lycopene; although it is found in carrot, watermelon, rosehip, chestnut, pink grapefruit, papaya and pink guava, even some of them are more than tomatoes, tomato is considered as the "source of lycopene" due to huge amount of its consumption within the year. Lycopene provides the conversion of ripe tomato fruit to red colour and is important (Figueiredo-Gonzalez et al., 2016). Today, there are many studies investigating the effects of lycopene, which has a strong antioxidant feature, on health. Antioxidants ensure that free radicals are neutralized, preventing cell components such as DNA, protein and lipids from being damaged by free radicals.

In this study, determining energy use and carbon dioxide emissions (CO_2) were aimed taking into consideration the consumption of petroleum products (PU; Diesel fuel and lubrication oil) used directly for unit production area (ha) in table tomato production in mechanization applications in open field conditions in Adana province.

MATERIALS AND METHODS

Determining the Number of Company to be Surveyed

The primary data of the study consisted of data collected by making face-to-face surveys with the producers of table tomatoes in Adana. A survey was conducted with a total of 125 tomato producers in 15 districts of Adana province, and the companies to be surveyed were determined by using stratified random sampling method.

The questionnaires applied consist of 2019 production year data. The sampling size was calculated by the *Neyman* method, the formula of which is given below.

$$n = (\sum N_h S_h)^2 / (N^2 D^2 + \sum N^h S^2{}_h) \quad \text{.....(1)}$$

Where:

n = sample volume,
d = projected deviation,
N = total number of producers,
z = standard normal distribution value and
N_h = number of producers in the stratified,
S_h = stratified variance.
$D = d/z$

In determining the number of samples, it was studied with 5% deviation from the average and 95% confidence level. As a result of the calculations, the number of sample companies that should be worked was found as 123.5. In the research, the number of questionnaires applied to growers producing tomatoes in the open field is 125.

Tomato Production in Open Field in Adana Province

Soil Preparation: The impermeable layer is broken in early autumn by using subsoiler in order to break the impermeable layer called plow base 50 cm below the soil base. In the fall, 3-4 tons of well-burned barn manure is applied to the decare and a deep release is made. Before planting the seedlings, the field is plowed 20-30 cm deep and a disc harrow is passed.

Growing Seedlings: Generally, peat-perlite-vermiculite or peat-perlite-soil or mixtures such as burnt animal manure-sand-soil are used in seedling.

Seed Sowing: After soaking the seeds for 3-4 hours, 1-3 cm. planted in depth. Lightly watering is done after sowing seeds. When the soil temperature is 12-15ºC, the seeds germinate in 5-13 days.

Fertilization: In the spring, before preparing the furrow, the bottom fertilizer is given. All of the phosphorous fertilizer, one third of the others, is given as base fertilizer. The remaining fertilizers are given when the fruits begin to appear on the plants. When the fruits take the hazelnut size, magnesium nitrate (400-600 g/100 L water,) is applied at 2-3 times with an interval of 10-15 days. Top fertilizers are divided and divided into soil, after each irrigation, when the soil comes to the appropriate conditions are mixed. Generally, Ammonium sulfate, NPK compound fertilizers, Magnesium nitrate, Calcium nitrate, Zinc phosphate, Magnesium phosphate and plant growth regulators are frequently used during the production process.

Seedling Planting: Planting seedlings is done when the risk of spring frost is completely passed and the soil and air temperature reaches 12-15ºC. When seedlings are about 15-20 cm height, they are usually ready for planting. Planting is usually done about 7-8 weeks after sowing seeds. Seedlings are planted in Adana region from the end of February to the beginning of March. Following the planting,

enough water is given. Together with first watering, necessary sprayings are made against root and shoot, in which close to the root, diseases. In tall tomato varieties, it is 60-80 cm between rows in pole varieties, 50-60 cm above rows, 140 cm between rows in floor varieties, 40-50 cm above rows.

Hoeing: The first hoeing is done 2 weeks after the seedlings are planted. The second hoeing is achieved 2-3 weeks after the first hoeing. In this period, when the plants reach a length of 30-35 cm, the tall tomato variety is planted together with the second hoeing in the tall varieties. Filling the root area is also done during these hoes. After the second hoe, weeds are removed, the creamy layer is broken, the soil is aerated and the moisture in the soil is preserved.

Irrigation: An average of 7-10 times watering is done in the region.

Pruning: In open field tomato cultivation, in order to obtain quality products pruning is achieved. Breaking the top shoot of the plant over two leaves of the last bunch is desired to be taken. The first unwanted shoot removal process starts and repeats every 10-15 days.

Disease and Pest Control: Early leaf blight, mildew, leaf mold, bacterial speckle, bacterial cancer and wilt, tomato mosaic virus and tomato yellow leaf curl virus are the main diseases, nematodes, wireworm, aphids, whitefly, thrips, leaf gallery flies, red spiders, mitesand other sucking insects are the main pests.

Harvest: Tomato fruits that are at harvest maturity are harvested by hand.

Diesel Fuel Amount Used in Open Field Tomato Production.

Diesel fuel quantities used in mechanization applications in tomato production in Adana province are given in Table 1.

Table 1. Amount of Diesel Fuel Used in Tomato Production in Open Field

Fuel	Applications	Amount used (kg/ha)
Diesel	Soil cultivation Toprakişleme	40.8905
	Fertilization (tractor) Gübreleme (traktör)	17.5245
	Pest control (tractor) Ziraimücadele (traktör)	35.049
	Irrigation+Fertilization+Spraying Sulama+Gübreleme+İlaçlama	114.8272
	Total	208.2912

Analytical Approach

The number and features of production processes in open field tomato production affect the energy efficiency of the production. Information on the processes applied in open tomato production was obtained through a survey conducted with the producers. Depending on the information obtained from the producers of open field tomatoes, the main production method for open tomato production in Adana province was determined. Diesel fuel and engine oil quantities, which are petroleum products (PU), which are directly used for tomato production, mechanization applications, for unit production area (ha), have been determined.

Energy related to the use of diesel fuel and engine oil (PU), depending on the quantities used in open-field tomato production per unit area (ha) of petroleum products (PP) and their lower heat values (MJ/L), which is an important indicator of the energy content of these products (PU) consumption values were calculated. The amount of energy consumed for the packaging, transportation, and distribution of these products was not taken into account in determining the energy values related to PP. As the carbon dioxide emission (kg CO_2) related to the use of PP, kg CO_2 values are taken into consideration depending on the energy content (MJ) of the PP.

Consumption of Petroleum Products

In the mechanization processes for tomato production in the open field, fuel consumption is consumed by tractors and irrigation pump engines in the use of tools and machinery.

- Diesel fuel consumption,
- Lubrication oil consumption and
- PP (Diesel fuel + lubricant oil) is considered as consumption.

The Diesel fuel and lubrication oil values per unit production area (ha) used by the tractor engine used during the tomato production processes in the open area were evaluated as total PP consumption.

$m_{PP} = m_D + m_L$ …….(2)

Where;

m_{PP} = Total petroleum products consumption (L/ha),

m_D = Diesel fuel consumption (L/ha) and

m_L = Engine oil consumption (L/ha).

Hourly oil consumption of the tractor engine, which is used for mechanization processes in tomato production in the open field, has been determined depending on the rated power of the tractor. In order to estimate hourly engine oil consumption in diesel fuel tractor engines, the following linear equation, which is dependent on Engine Rated Power (P_e) and specified in ASABE Standard D497.7 Section 3.4 (2011), is used as a reference model.

$$m_L=0.00059\times P_e+0.02169 \quad (3)$$

Cancante et al. (2017) using the MINITAB 17.0™ data processing software, the coefficients specified in the linear regression (LRA) and variance analysis (ANOVA) and equation (3.3) are as follows:

$$m_L=0.000239\times P_e+0.00989 \quad (4)$$

Where;

m_L = Hourly oil consumption of the tractor engine (L/h) and
P_e = The tractor's rated power (kW).

The *Pearson correlation coefficient* for the variables in Equation (4) is r = 0.90 ($p < 0.05$). In the developed model, the standard errors of the constant term and the linear coefficient are $1.50\ 10^{-3}$ L/h and $9.0\ 10^{-6}$ kW, respectively.

Consumption of Petroleum Products Energy

Total petroleum products energy consumption in the process of tomato production in the open field, consumed by the tractor and irrigation pump engines in the use of tools and machinery;

- Energy consumption related to diesel fuel consumption,
- Energy consumption related to lubrication oil consumption and
- It has been taken into consideration as the total energy consumption of PP (Diesel fuel + Lubrication oil) consumption.

The PP energy consumption (EC_{PP}, MJ/ha) related to diesel fuel and engine oil consumption consumed per unit production area (ha) by tractor and irrigation pump engines used during the tomato production processes in the open area is determined as follows.

$$EC_{PU}= EC_D+EC_L \quad (5)$$

Where:

ET_{PP} = Total energy consumption for PP (MJ/ha),
EC_D = Energy consumption related to Diesel fuel (L/ha) and
EC_L = Energy consumption related to lubrication oil (L/ha).

Diesel fuel energy consumption (EC_D, MJ/ha) for diesel fuel consumption per unit production area (ha) has been determined by tractor and irrigation pump engines used during open field tomato production processes as follows.

$$EC_D=m_D\times LHV_D \quad (6)$$

Where:

EC_D = Energy consumption related to Diesel fuel (MJ/ha),
m_D = Diesel fuel consumption (L/ha) and
LHV_D = The lower heating value of Diesel (MJ/L).

The lower heating value of diesel fuel consumed during agricultural production with agricultural tools and machinery was taken into account as LHV_D = 37.1 MJ/L (Table 2) (IPCC, 1996).

The lubrication oil energy (EC_L, MJ/ha) related to lubrication oil consumption per unit production area (ha) has been determined by tractor and irrigation pump engines used during the mechanization processes in open tomato production as follows.

$$EC_L=m_L\times LHV_L \quad (7)$$

Where:

EC_L = Energy consumption related to lubrication oil (MJ/ha),
M_L = Lubrication oil consumption (L/ha) and
LHV_L = The lower heating value of lubrication oil (MJ/L).

The lower heating value of the engine oil consumed during agricultural production with agricultural tools and machinery was taken into account as LHV_L = 38.2 MJ/L (Table 2) (IPCC, 1996).

CO_2 Emission Regarding Petroleum Consumption

Carbon dioxide (CO_2) emission during the mechanization processes in tomato production, consumed during the use of tools and machinery;

- CO_2 emission related to diesel fuel consumption,

- CO_2 emissions related to engine oil consumption and
- It has been taken into account as the total CO_2 emission of PP (Diesel fuel + Lubrication oil) consumption.

CO_2 emissions from all motor vehicles burning fossil fuels can be calculated taking into account the amount of fuel consumed and the distance travelled. In the method of calculating CO_2 emissions taking into account the amount of fuel consumed, the value of fuel consumption is multiplied by the CO_2 emission factor for each type of fuel. This emission factor is developed depending on the heat value of the fuel and the carbon fraction oxidized in the fuel and the carbon content. This approach is defined as the fuel-based CO_2 emission calculation method as it uses average fuel consumption data. The fuel consumption-based approach can be applied taking into account vehicle effectiveness data and fuel economy factors that enable the calculation of fuel consumption. In calculating emissions using the distance-based method, distance-based emission factors are taken into account. The fuel-based CO_2 emission calculation method is the preferred approach, since data on the fuel consumed is generally more reliable. However, since the uncertainty level in CO_2 estimates can be quite high, the distance-based method should be used as a last solution (IPCC, 1996).
Taking into consideration the lubrication oil consumption value of the tractor engine, CO_2 emissions related to oil consumption can also be calculated. The values given in Table 2 are used for the heat values of Diesel fuel and lubrication oil and CO_2 emission factors depending on the type of fuel.

Table 2. Heating Values and CO_2 Emission Factors (IPCC, 1996)

Fuel	Lower heating value (MJ/L)	CO_2 emission factor (kg CO_2/MJ)
Diesel fuel	37.1	0.07401
Lubrication oil	38.2	0.07328

In the calculations made to determine the CO_2 emissions released regarding the use of PP as a result of open field tomato production, the fuel-based CO_2 emission calculation method proposed in the Intergovernmental Climate Change Panel has been taken into consideration (IPCC, 1996). The proposed approach to calculate CO_2 emissions based on fuel consumption is summarized in equations (10) and (11).
The total CO_2 emission per unit production area (ha) of PP consumption (TCO_2E_{PP}, kgCO_2/ha) has been determined by the tools and machines used during open tomato production.

$$TCO_2E_{PP}=CO_2E_D+CO_2E_L \quad (8)$$

Where:
TCO_2E_{PP} = Total CO_2 emission related to PP consumption (kg CO_2/ha),
CO_2E_D = CO_2 emissions related to Diesel fuel consumption (kg CO_2/ha) and
CO_2E_L = CO_2 emission related to lubrication oil consumption (kg CO_2/ha).

CO_2 emission (CO_2E_D, kg CO_2/ha) for Diesel fuel consumption per unit production area (ha) was determined by agricultural tools and machinery used during open tomato production processes as follows.

$$CO_2E_D=m_D\times LHV_D\times EF_D \quad (9)$$

Where:
CO_2E_D = CO_2 emissions related to Diesel fuel consumption (kg CO_2/ha),
m_D = Diesel consumption (L/ha),
LHV_D = Lower heating value of Diesel (37.1 MJ/L) and
EF_D = CO_2 emission factor for Diesel fuel (0.07401 kg CO_2/MJ).

The CO_2 emission factor of Diesel fuel consumed during agricultural production with agricultural tools and machinery is taken into account as EF_D = 0.07401 kgCO_2/MJ (Table 2) (IPCC, 1996).
CO_2 emissions (CO_2E_L, kgCO_2/ha) for lubricant oil consumption per unit production area (ha) was determined by agricultural tools and machinery used during open tomato production processes.

$$CO_2E_L=m_L\times LHV_L\times EF_L \quad (10)$$

Where:
CO_2E_L = CO_2 emission related to lubrication oil consumption (kg CO_2/ha),
m_L = Lubrication oil consumption (L/ha),

LHV_L = Lower heating value of lubrication oil (37.1 MJ/L) and
EF_L = CO_2 emission factor for lubrication oil (0.07401 kg CO_2/MJ).

The CO_2 emission factor of the lubrication oil consumed during the production processes in the field with agricultural tools and machinery is taken into account as EF_L = 0.07328 kg CO_2/MJ (Table 2) (IPCC, 1996).

RESULTS AND DISCUSSIONS

Diesel Fuel Consumption

Diesel fuel consumption values in the use of tools and machinery during the mechanization processes in open-field tomato production in Adana province are given in Figure 1. Diesel fuel consumption values given in Figure 1 indicate the average values of diesel fuel consumption values determined from the districts of Adana province. It is seen that diesel fuel consumption values are in parallel with the change in the usage time of the tools and machines used in the open tomato production process and the loading rates of the tractor engine. The highest diesel fuel consumption is in pump irrigation applications, which includes irrigation + fertilization + spraying with 137.6 L per unit area (ha). Soil cultivation applications take the second place in diesel fuel consumption with 49 L/ha. Diesel fuel consumption in open tomato production is 42 L / ha in tractor and plant protection products (PPP) applications and 21 L/ha in PPP applications. A total of 249.6 L/ha diesel fuel is consumed when using tools and machinery in tomato production.

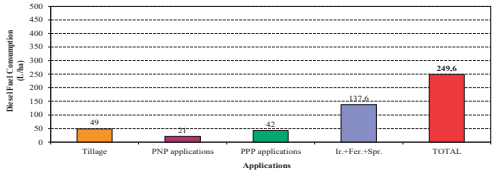

Figure 1. Change of Diesel Fuel Consumption in Open Field Tomato Production (Own findings)

Diesel Fuel Energy Consumption

Diesel fuel energy consumption values in the use of tools and machinery during open field tomato production processes in Adana province are given in Figure 2. The highest diesel fuel energy consumption is determined in pump irrigation applications, which includes irrigation + fertilization + spraying operations with 5104.96 MJ per unit area (ha). Soil cultivation applications take the second place in diesel fuel energy consumption with 1817.9 MJ/ha.

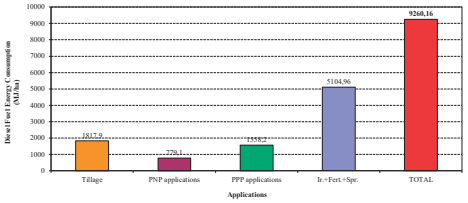

Figure 2. The Change of Diesel Fuel Energy Consumption in Open Tomato Production (Own findings)

Diesel fuel energy consumption in open field tomato production is 1558.2 MJ/ha in tractor and BMU applications and 779.1 MJ/ha in BBU applications. A total of 9260.16 MJ/ha diesel fuel energy is consumed when using tools and machinery in tomato production.

CO_2 Emission Regarding Diesel Fuel Consumption

CO_2 emission values resulting from Diesel fuel consumption in the use of tools and machinery during the mechanization processes in open field tomato production in Adana province are given in Figure 3. As a result of the highest diesel fuel consumption, the maximum CO_2 emission per unit area (ha) is realized in pump irrigation applications, which includes irrigation + fertilization + spraying processes with a value of 377.82 kgCO_2. Soil cultivation practices take the second place in CO_2 emission with the value of 134.54 kg CO_2/ha.

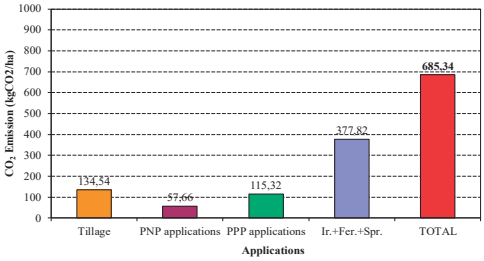

Figure 3. CO_2 Emission Regarding Diesel Fuel Consumption in Open Field Tomato Production (Own findings)

As a result of diesel fuel consumption in open tomato production, 115.32 kg CO_2/ha CO_2 emission occurs in tractor and PPP applications and 57.66 kg CO_2/ha CO_2 in plant nutrition products (PBU) applications. As a result of diesel fuel consumption, a total of 685.34 kg CO_2/ha CO_2 emission occurs in open field tomato production.

Lubrication Oil Consumption

The lubrication oil consumption values of the engines that run the tractor and irrigation pumps in the process of tomato production in the open field are given in Figure 4. The lubrication oil consumption values given in Figure 4 indicate the average values of lubrication oil consumption values determined from the districts of Adana province. It is observed that the lubrication oil consumption values are in parallel with the usage time of the tools and machines used in the open tomato production process and the loading rates of the tractor engine, as in the Diesel fuel consumption values.

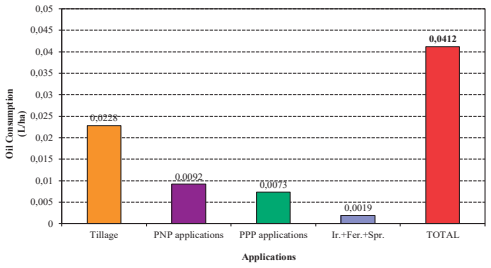

Figure 4. Change of Average Oil Consumption in Open Field Tomato Production (Own findings)

The highest oil consumption is realized in soil cultivation applications with 0.0228 L per unit area (ha). PBP applications take the second place in oil consumption with a value of 0.0092 L/ha. In tomato production in open air, lubrication oil consumption in BBP applications is 0.0073 L/ha. A total of 0.0412 L/ha of engine oil is consumed when using tools and machinery in tomato production.

Energy Consumption Related to Lubrication Oil Consumption

Lubrication oil energy consumption values in the use of tools and machinery during open tomato production processes in Adana province are given in Figure 5. The highest lubrication oil energy consumption is determined in soil cultivation applications with 0.87 MJ per unit area (ha). PBP applications take the second place in lubrication oil energy consumption with 0.35 MJ/ha.

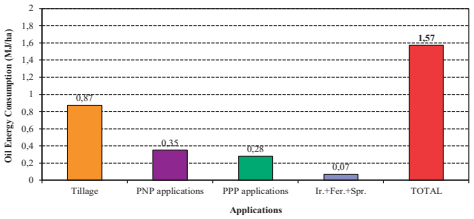

Figure 5. The Change of Lubrication Oil Energy Consumption in Open Field Tomato Production (Own findings)

Lubrication oil energy consumption in open tomato production is 0.28 MJ/ha in tractor and PPP applications. A total of 1.57 MJ/ha engine oil energy is consumed when using tools and machinery in tomato production.

CO_2 Emission Regarding Engine Oil Consumption

CO_2 emission values that occur as a result of engine oil consumption in the process of tomato production in open field in Adana province are given in Figure 6. As a result of maximum engine oil consumption, the highest CO_2 emission per unit area (ha) is realized in tillage applications with a value of 0.0638 kg CO_2. The second place in CO_2 emission is PBP applications with the value of 0.0258 kg CO_2/ha.

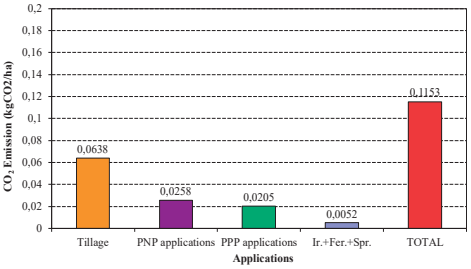

Figure 6. CO_2 Emission related to Engine Oil Consumption in Open Field Tomato Production (Own findings)

As a result of engine oil consumption in open field tomato production, 0.0205 kgCO_2/ha CO_2

emission occurs in tractor and BKU applications. As a result of engine oil consumption in open tomato production, using tools and machinery, a total of 0.1153 kg CO_2/ha CO_2 emission occurs.

CONCLUSIONS

In open field tomato production; 249.6 L/ha diesel fuel and 0.0412 L/ha lubrication oil are consumed in the use of tools and machinery.

In open field tomato production; a total of 9260.16 MJ/ha diesel fuel energy and 1.57 MJ/ha lubrication oil energy are consumed in the use of tools and machinery. In open field tomato production; As a result of diesel fuel consumption, a total of 685.34 kg CO_2/ha CO_2 and 0.1153 kg CO_2/ha CO_2 emission occur as a result of engine oil consumption. To produce 1 kg of fresh tomatoes in open field tomato production, a total of 2.625 g of Diesel fuel and engine oil are consumed in mechanization applications. As a result of Diesel fuel and engine oil consumption to produce 1 kg of fresh tomatoes, 115.77 kJ energy consumption 8.568 kg CO_2 emission occurs. Technologies with high energy efficiency should be used for the mechanization infrastructure of the companies. Tools/machines with a capacity suitable for the power supply should be used. Necessary power optimization for the companies should be provided. For example, operations that require less power should not be performed with larger powerful tractors. Agricultural tools/machines should be operated at full load and efficiently. With the agricultural tools/machinery used in open field tomato production processes, fuel consumption during the work should be carefully monitored. Measures to reduce fuel consumption should be taken by evaluating the power requirements of the machines used.

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LEAF MICROMORPHOLOGY OF JUTE (*CORCHORUS OLITORIUS* L.) IN CONDITIONS FROM CLUJ COUNTY

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Abstract

With a long history of cultivation in warm climates, Corchorus olitorius is a versatile plant with many utilizations. This species has been used for fibres, as leafy vegetable, for medicinal purposes, has phytoremediation potential and could present interest for a variety of emerging applications from agriculture to biotechnology. The aim of this research was to provide a micro-morphological characterization for leaves of jute plants grown for the first time in conditions from Cluj county, Romania. Microscopic analysis revealed that jute leaves displayed paracytic stomatal complexes, average leaf stomata density of 518.31 per mm^2, and characteristic capitate glandular trichomes less than 50 µm in size. Leaf insertion exercised a significant influence on stomata density, with an increased stomata density in leaves from secondary branches compared to leaves from first order stems, fact that is consistent with Zalenski's law. Plants developed a bushy habitus in the field and produced flowers and fruits towards the end of the summer. It is proposed that C. olitorius to be introduced in cultivation in local conditions for culinary and medicinal purposes.

Key words*: stomata density, trichome, leaf, abaxial, food, utilization.*

INTRODUCTION

Jute belongs to genus *Corchorus* from family *Malvaceae*, subfamily *Grewioideae* (Benor, 2018). *Corchorus olitorious* is one of the most cultivated species from this genus and is originally from Africa (Benor, 2018; Mukul, 2020). *C. olitorious* has a variety of utilizations and potential applications. There are two cultivar groups within *C. olitorious*: Olitorius and Textilis. First group has small habitus and is used as vegetable, while second group grows up to 4 m in height and is farmed for fibres (https://pfaf.org). Currently, jute is cultivated throughout the tropical and subtropical regions of the world: Asia, Africa, Central and South America (Mukul, 2020), where is recognized for the culinary and medicinal purposes and it is commonly consumed as leafy vegetable (Traoré et al., 2019; Chigurupati et al., 2020; Samuel et al., 2020). Also, jute occupies second place after cotton as most important fibrous plant crop in the world (Mukul, 2020). Jute has been used traditionally for ropes and hessian fabrics. Due to the remarkable durability of jute bast fibres, these have started to be used in automobile industry at lining the interior panels and trucks as well as for clothing the seat backs (Chandekar et al., 2020). Recent advancements indicate that jute polymer composites have the potential to further extend the utilization of these fibres due to improved properties with promising opportunities particularly for constructions industry among others (Chandekar et al., 2020). The plant was shown to be host for many useful endophytic microorganisms that can find applications in agriculture or biotechnologies (Ahmed et al., 2016; Das et al., 2017; Haidar et al., 2018). The wide possibilities for utilization explain the expansion of jute crops.

Most important physiological process that sustains the life of plants is photosynthesis. This process relies on gas exchange that is mediated by small openings on the leaf surface called stomata (Xiong and Flexas, 2020). The pattering of leaf surface with stomata is a complex process controlled by genetic mechanisms (Baillie and Fleming, 2019), and strongly influenced by environmental factors (Veselý et al., 2020). Studies on stomata parameters have been considered relevant for characterization of genotypes (Miljkovi et al., 2013), were proposed as selection criteria in plant breeding programs for the identification of drought resistant genotypes (Li et al., 2017),

have been used to study adaptability of plants across altitudinal gradients (Holland and Richardson, 2009), acclimatization (Rodrigues et al., 2020), or were put in relationship with various treatments, such as inoculation with beneficial microorganisms (Chitarra et al., 2016). As key structures that are in relationship both with assimilation and transpiration rate (Xiong and Flexas, 2020), stomata parameters are important for the characterization of plants and their adaptability to the environmental conditions. The study of the stomata parameters such as density could reveal important information about species proposed to be introduced into cultivation in non-native geographical regions.

The aim of this research was to screen the stomata density of *Corchorus olitorius* plants grown in conditions from Cluj County Romania. The research had two objectives: 1) analysis of leaf micro-morphology, 2) identification of the histological gradient for leaf stomata density in jute plants.

MATERIALS AND METHODS

Corchorus olitorius L. plants from this study were obtained from seeds belonging to two accessions: XX-0-GZU-07100734, XX-0-CLA-4158, provided through International Plant Exchange Network. Seeds were sown on 13th March 2019 in pots with eutrophic peat, in greenhouse. Seeds sprouted on 25th March 2019 and seedlings were replanted in larger pots on 5th April 2019. Because in the month of May were heavy rains, the obtained jute plants were transplanted in field conditions from Agro-Botanical Garden of USAMV Cluj-Napoca on 2nd June 2019. Distance between rows was 50 cm and between plants per row 40 cm. Plants were watered until became established. Weed control was performed mechanically and manually. Soil in the Agro-Botanical Garden has clay-loam type, neutral pH, low humus level, good nitrogen, phosphorus, and potassium content (Crișan et al., 2018).

Samples for microscopic analysis were collected on 3rd September 2019. Leaves chosen had roughly similar size. Imprint samples collection and analysis was conducted following similar procedure as Crișan et al. (2018). Observation was performed on abaxial surface imprints under Bresser Biolux NV microscope with camera. In total were analysed under microscope 48 leaf imprints (6 plants × 8 leaves, where 4 leaves from first order stems and 4 leaves from second order branches of each plant). Half of the analysed samples belonged to first accession and the other half belonged to the second accession. Stomata density was determined on 240 fields of view, corresponding to 5 fields of view analysed per leaf sample. Field of view at 640× magnification had Ø 0.235 mm, measured with stage micrometre. Density of stomata determined corresponded to $\pi r^2 = 0.04337$ mm^2 and then calculated for 1 mm^2. Stomata were counted only for the areas between leaf veins and marginal stomata were considered in the field of view only if whole ostiole was visible. Images were taken using Photomizer 2 Bresser edition software and further processed with ArcSoft Photo Impressions 5 software. Statistical analysis was conducted with Origin software by OriginLab.

RESULTS AND DISCUSSIONS

Climatic data from the experimental year are presented in Figure 1.

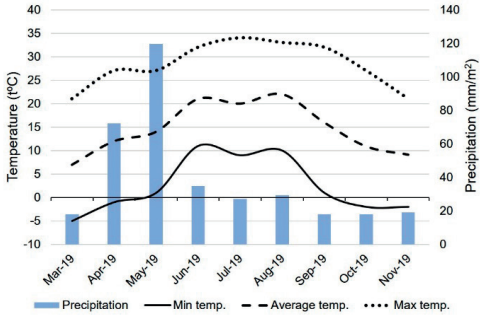

Figure 1. Climatic data for interval March-November 2019 in Cluj-Napoca, Romania (https://en.tutiempo.net, https://www.wunderground.com)

During 2019 in Cluj-Napoca, the last spring month with frost was April while highest precipitation levels were registered in May, before planting the jute plants in the field. Maximum temperatures exceeded 30°C for the months of June-September, and the highest temperature (of 34°C) occurred in July. In

October, the temperatures dropped below 0°C, marking the end of vegetative season. The accessions developed in Agro-Botanical Garden for a duration of 4 months (June-September), were healthy and displayed a bushy-habit. The flowering and fruit development occurred towards the end of summer and beginning of September.

Microscopic analysis of leaf imprint samples revealed the leaf surface micromorphology. It was determined that *Corchorus olitorius* accessions presented paracytic stomata complexes, because stoma was surrounded by two subsidiary cells that were parallel to the longitudinal axes of the pore and guard cell pairs (Figures 2e, 2f). Leaf surface presented characteristic capitate hairs having <50 µm in length. These trichomes presented a basal cell, a short unicellular stalk and enlarged tip, aspect that suggests to the secretory function as glandular trichomes (Figures 2c, 2d). Epidermal cells were preponderantly isodiametric (Figures 2e, 2f). Analysis of variance revealed that leaf insertion had a highly significant influence on leaf stomata density (p<0.001), explaining 79% of variance registered by this parameter. Average stomata density on first order stems was 463.26 per mm^2 and increased to an average of 573.36 per mm^2 on leaves of second order branches (Tables 1 and 2).

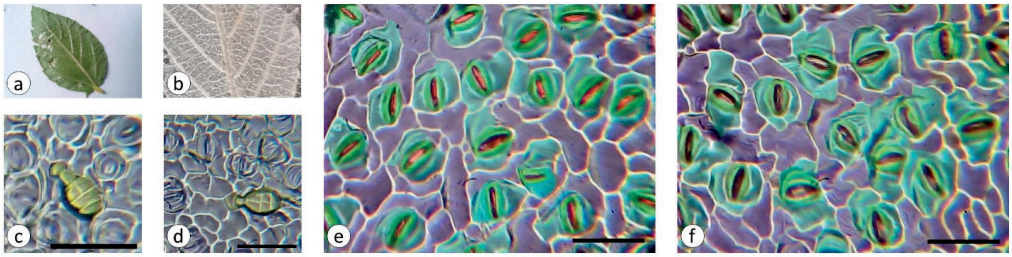

Figure 2. *Corchorus olitorius* samples: a) leaf with applied polish; b) abaxial leaf imprint; c), d) capitate glandular trichomes highlight in yellow; e), f) microscopic observation of stomata, where: red - stoma, green - guard cells, light blue - subsidiary cells, purple - epidermal cells, magnified 640×, bar = 50 µm (Original)

Table 1. Influence of leaf insertion and individual plant on abaxial stomata density of *Corchorus olitorius*

Factors	*F* value	*p* value	Significance	η^2
Leaf insertion (L)	47.968	<0.001	***	0.79
Plant (P)	1.005	0.368	n.s.	0.03
Interaction (L × P)	5.486	0.005	**	0.18

Note: Two-way ANOVA, p<0.05 (*), p<0.01 (**), p<0.001 (***); η^2 - partitioning of variance

Table 2. Stomata density variation according to leaf insertion in *Corchorus olitorius*

Leaf insertion	Mean per mm^2	±SD	±SE	Variance (s^2)
1st order stems	463.26	93.24	8.51	8693.91
2nd order branches	573.36	150.91	13.78	22775.22
Overall	518.31	136.79	8.83	18711.87

Note: standard deviation (SD) and standard error of mean (SE)

This gradient corresponds to an increase of stomata density according to leaf insertion, higher stomata density being associated with leaves from higher order branches. However, one can notice that higher stomata density variability among fields of view analysed were present on samples from second order branches compared to first order stems (Table 2). There was no significant difference among individual plants regarding stomata density (p = 0.368) (Table 1), thus the overall average stomata density of 518.31 per mm^2 determined in this study can be considered representative for these accessions in local conditions. Frequency histogram revealed that on the first order stems most leaf samples had a stomata density

between 400-500 per mm^2 while for second order branches most samples had a stomata density between 500-600 per mm^2. It can also be observed that leaves from higher order branches had lower densities of stomata (<500/mm^2) as well as samples with densities exceeding 600 per mm^2 (Figure 3).

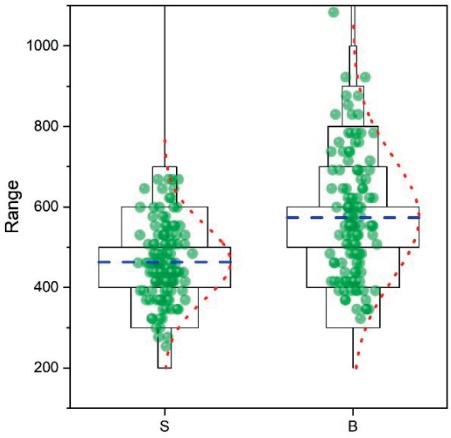

Figure 3. Frequency histogram showing classes for leaf stomata density (number per mm^2) in *Corchorus olitorius*: first order stems (S), second order branches (B), blue dash line - average, red dotted line - normal distribution curve.

Similar with the observation from this study, Ghosh et al. (2004), also described stomata complexes in *Corchorus olitorius* as paracytic. As for stomata density obtained in Cluj conditions, the abaxial surface average is comparable to the abaxial density of 497.70 per mm^2 reported for *Corchorus olitorius* in conditions from India (Maity and Datta, 2011). However, according to data from literature, stomata density can vary widely between *Corchorus* species and hybrids. Abaxial stomatal densities between 300-400 per mm^2 were identified in species: *C. pseudocapsularis, C. trilocularis, C. fascicularis, C. pseudo-olitorius, C. tridens*, between 500-600 per mm^2 in *C. trilocularis* × *C. capsularis, C. capsularis* and high stomatal densities of over 600 and respectively 700 per mm^2 in *C. urticaefolius* and *C. aestuans* (Maity and Datta, 2010; 2011). Compared to these species, *Corchorus olitorius* stomata density situates within a middle range. Because the stomata distribution in *Corchorus olitorius* is amphistomatic and anisostomatic (Ghosh et al., 2004), the measurements of stomatal parameters on abaxial surface are highly relevant from physiological perspective. The rate at which stomata facilitates gas exchange is determined by stomata density as well as size (Baillie and Fleming, 2019), indicating to the importance of this leaf micromorphological parameter. Previous research in various species, revealed that insertion gradients influence stomata densities, with an increase from bottom to middle insertion levels. This histological gradient for stomata parameters is explained through Zalenski's law as a function of distance from the root (Sesták, 1985). In Cluj conditions, *Corchorus olitorius* presented an increase of stomata density from first order stems to secondary branches. Trichomes identified on the leaves of *Corchorus olitorius* from this study resemble those described in other species from family *Malvaceae*. Study on several *Hibiscus* species from Pakistan, has put in evidence in all taxa studied the presence of glandular capitate trichomes with unicellular stalk and multicellular uniseriate or biseriate head, with length up to 50 µm (Shaheen et al., 2009). A few other trichome morphologies described by the cited authors were identified in same *Hibiscus* species but such morphologies were not observed in *Corchorus olitorius* leaves from this study. Leaf micromorphological studies revealed the presence of capitate glandular trichomes in *Malva sylvestris* (Romitelli and Martins, 2013), as well as in *Theobroma* spp. (Garcia et al., 2014), that are also from family *Malvaceae*. Glandular trichomes occur repeatedly across various plant families and species, and their morphology is related to the type of metabolites they secrete. Capitate trichomes usually are associated with non-volatile compounds, that are released directly at the surface by the cells from the tip. This is a major difference compared to volatile producing-trichomes, that present a storage space that might be subcuticular or between secretory cells, while the compounds get released only when trichomes are damaged (Schuurink and Tissier, 2020).

Latest insights related to the potential use of *Corchorus olitorius* plants, as well as many emerging applications (Table 3), are justifying

the revived interest for the study and cultivation of this plant. From this perspective future efforts should be directed towards testing and monitoring acclimatization of genotypes as well as defying the most suitable utilizations based on characterization of the locally grown plants.

Table 3. Recent insights related to applications and potential utilization of *Corchorus olitorius*

Importance	Findings	Sources
fibres and fabrics	Jute fibres can be used as sound absorber material efficient above 3500 Hz frequency.	Sengupta et al., 2021
	Jute fibre reinforced composites present opportunities for wider utilizations due to improved properties.	Chandekar et al., 2020
	Plant breeding selection schemes for improved fibre production can be based on anatomical study of stems.	Mukul et al., 2020
	Jute fibre bonded with polyvinyl alcohol can produce a highly functional non-woven fabric.	Sengupta et al., 2020
food	Fruits soup showed good nutritional value and acceptable sensory characteristics.	Samuel et al., 2020
	Leaves showed significant decrease of β-carotene following cooking. Optimizing the cooking method is important.	Traoré et al., 2019
medicinal	Liposomes proved entrapment efficiency >80% for phytol extracted from leaves, enhancing delivery and pharmacological activity.	Mohammad et al., 2021
	Leaves ethanolic extract demonstrated antioxidant and anti-diabetic effect.	Chigurupati et al., 2020
	Hydroalcoholic extract of leaves showed neuromodulator potential.	Wagdy et al., 2019
corrosion inhibitor	Plant stem extract is inhibitor of mild steel corrosion to H_2SO_4 solution, with up to 93% efficiency.	Gobara et al., 2017
biosynthesis of Au and Fe nanoparticles	70% aqueous ethanolic leaves extract reduced gold aureate and ferric chloride solutions with formation of gold and iron oxide nanoparticles in one step.	El-Rafie et al., 2016
phytoremediation	Plants survived concentration of Cd 20 mg/kg in soil; translocation factor was >1 regardless of concentration.	Ndlovu et al., 2020
	Absorbent obtained from jute stick charcoal was demonstrated to have potential for dye removal from water.	Chakraborty et al., 2020
anti Cu-stress treatment	Jute seeds extract demonstrated protective effect against copper stress in tomato seedlings.	İşeri et al., 2018
useful endophytes	Out of the 27 bacteria species isolated from various plant organs, some demonstrated plant growth enhancing capabilities.	Haidar et al., 2018
	Grammothele lineata isolated from jute plant produced taxol that exhibited *in vitro* cytotoxic affect against HeLa cancer line.	Das et al., 2017
	Aspergillus terreus KP900973 isolated from jute stem produced xylanase enzyme, that could be used as dough-raising agent.	Ahmed et al., 2016

It is proposed that in local temperate-continental conditions of Cluj-Napoca, acclimatization and cultivation of *Corchorus olitorius* may present increased interest particularly due to the culinary and medicinal value of these plants.

CONCLUSIONS

This research provides a leaf micro-morphologic characterization of jute plants (*Corchorus olitorius*) grown for the first time in conditions from Cluj county, Romania.
Results indicate that in local conditions, jute plants can develop in field for at least four months, reaching bloom and producing fruits. Plants had an average stomata density of 518.31 per mm^2, comparable with densities reported in conditions from India where jute is well established in cultivation.
It is proposed that this species can be grown in local conditions for culinary and medicinal purposes.

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BIOTECHNOLOGICAL APPROACHES FOR *EX SITU* CONSERVATION OF MEDICINAL SPECIES *LIGULARIA SIBIRICA* (L.) CASS.

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Abstract

Currently, habitat destruction, exploitation, and climate change are driving biodiversity loss. Glacial relict species - remnants from the last glaciation - most of them cold-adapted, are particularly vulnerable to climate change. Ligularia sibirica (L.) Cass. is a typical glacial relict plant species with medicinal uses, protected in situ within Natura 2000 sites. Despite in situ conservation, in Romania the species is declining, therefore complementary conservation measures should be taken. In this respect, we have tested and selected some efficient biotechnological tools such as in vitro culture and cryopreservation, for medium and long-term ex situ conservation of L. sibirica germplasm.

Key words*: conservation biotechnology, Ligularia sibirica.*

INTRODUCTION

According to Millennium Ecosystem Assessment substantially and largely irreversible biodiversity loss has been occurring over the past 50 years. The most recent report of Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES, 2018) estimates that 33 per cent of the assessed vascular plants living exclusively in Europe and Central Asia are threatened. Moreover, the world's largest and most recent plant survey reveals that since 1900, nearly 3 species of seed-bearing plants have disappeared per year, showing that the extinction rate is 500 times faster than would be expected as a result of natural forces (Humphreys et al., 2019).

The main causes of plant endangerment include habitat alteration, spread of invasive species overexploiting, urbanization, unsuitable agriculture, pollution, global warming and loss of genetic diversity (Pitman and Jorgensen, 2002). Particularly vulnerable are species from wetlands mainly because wetland spread has declined by 50 per cent since 1970 (IPBES, 2018). Moreover, if adapted to cold and wet climate conditions, as glacial relicts are, species are under the pressure of both habitat loss and global warming.

Addressing the conservation and sustainable use of biological diversity a broad international strategy was established- Convention on Biological Diversity (CDB) - ratified by Romania since 1994. Under CDB an agreement was developed for integrated plant conservation, the Global Strategy for Plant Conservation, where target 8 established for period 2011-2020 stipulates that at least 75 per cent of threatened plant species in ex situ collections, preferably in the country of origin, and at least 20 per cent available for recovery and restoration programmes (GSPC, 2011). Following the targets of GSPC all over the world strategies were developed for endangered plant conservation in terms of in situ and ex situ conservation.

Ligularia genus (Asteraceae) originates from Central China in mid-Cretaceous and includes 129 species (Liu et al., 1994; Liu, 2004) with a large distribution range from Asia to Europe. Species *Ligularia sibirica* (L.) Cass. has a main continuous distribution from east Asia to Southern Siberia and a fragmented range with disjunct populations in Europe - Estonia, Latvia, Poland, Hungary, Romania, Croatia, Bulgaria, the Slovak Republic, the Czech Republic, Austria and France - (Meusel and Jager, 1992). The populations with European distribution originated in the early postglacial period and thus represent rare remnants of a former continuous distribution (Šmídová et al., 2011) being protected at European level under the Habitats Directive, Annex II of the Council of the European Community (1992). In

Romania, species is protected exclusively in situ under Natura 2000 network but latest assessment suggests this standalone measure is less than optimal (Mânzu et al., 2013).
Conservation measures should be taken for *L. sibirica* not only because of its scientific importance but also for species medicinal value (Manole et al., 2019). Studies on chemical constituents showed that *L. sibirica* is an important source of bioactive secondary metabolites like sesquiterpenes and pyrrolizidine alkaloids with cytotoxic, antibacterial, antitumor and anti-HIV effect (Kapas et al., 2009; Wu et al., 2016; Şuţan et al., 2020).
In order to complement *in situ* conservation of this vulnerable species and in response to the main targets of Global Strategy for Plant Conservation, the aim of our study was to add new conservation strategy to previously developed seed banking protocol (Manole et al., 2019) by the means of available biotechnological tools.

MATERIALS AND METHODS

Field collection
Mature achenes were collected in October 2015 from Natura 2000 site - ROSCI 0055 *Dealul Cetății Lempeș - Mlaștina Hărman*. In order to ensure coverage of maximum variability, achenes were collected from at least 50 individuals, sampled randomly from the entire population. From each individual the achenes were collected in separate sealed envelopes. To prevent fresh material hydration, in each envelope a few grains of silica gel were disposed (about ¼ of volume of collected achenes).
Dormancy breaking
Achenes were stratified for 30 days at 4°C
In vitro culture
For medium-term *ex situ* conservation of *L. sibirica* germplasm an *in vitro* culture protocol was established, following multiple stages as presented in Figure 1 and Table 1.
Ex vitro acclimatization
Rooted plantlets were transferred onto glass tubes (3 cm diameter, 14 cm high) filled with MS liquid media with low sugar content (2mg/l sucrose), without hormones and maintained at 20°C and 70% humidity. After for two weeks plantlets were transferred onto culture pots filled with aseptic substrate mixture (peat: sand: perlite/2: 1: 1) and maintained at 20°C and 70% humidity. After four weeks in solid substrate plantlets were transferred onto culture pots with acidic substrate mixture (soil: peat/ 1: 1).
Seed cryopreservation
For long-term *ex situ* conservation was developed a protocol for seed preservation under extremely low temperature at -196°C. The main stages for both seed cryo-storage and recovery are shown in Table 2.

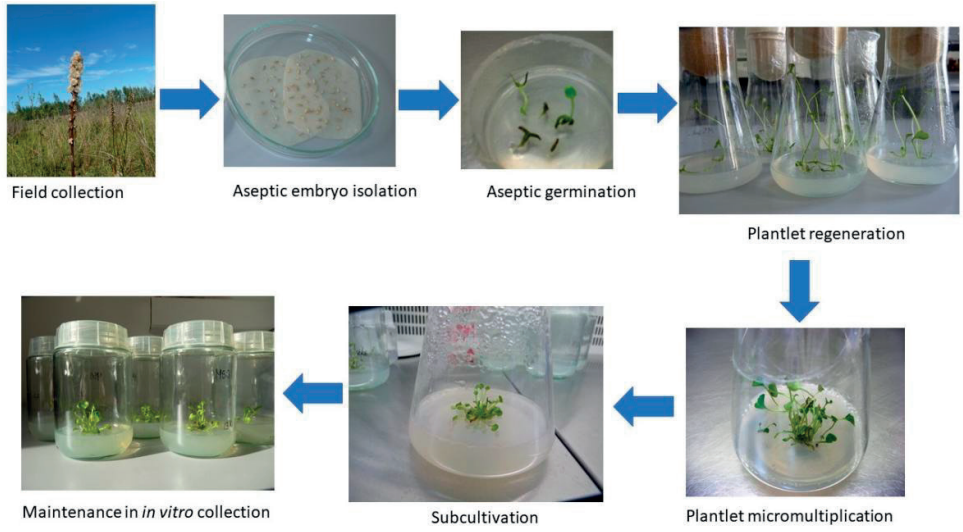


Figure 1. Diagram for medium term *ex situ* conservation protocol

Table 1. Stages for an *in vitro* collection establishment

Stages/sub-stages	A	b	c	d	E
Sterilization	pre-sterilisation pre-imbibition in $KMnO_4$ 0.5% (w/v), 1 hour	imbibition at 20°C for 24 hours in double distilled sterile water	pre-sterilization post-imbibition in ethylic alcohol 70° for 30 sec	sterilization for 2 min in $HgCl_2$ 0.1% (w/v)	3 washes with double distilled sterile water, each for 10 min
Plant regeneration	aseptic embryo isolation	embryo culture on Petri dishes onto MS media without hormone	culture maintenance at 20°C and 16 hours photoperiod	-	-
Micropropagation	single plantlet culture on Petri dishes onto MS[1] media supplemented with 0.1 mg/l IBA[3] and 2.5 mg/l BAP[4]	culture maintenance at 20°C and 16 hours photoperiod	-	-	-
Subcultivation	single shoot culture on Petri dishes onto MS basal media supplemented with 15 g/l manitol and low sugar content (15 g/l sucrose)	culture maintenance at 20°C and 8 hours photoperiod	subcultivation on same condition at 60 days interval	-	-
Rooting	shoot separation	single shoot culture on Petri dishes onto ½ MS[2] basal media supplemented with 0.3 mg/l NAA[5]	culture maintenance at 20°C and 16 hours photoperiod		

[1]MS basal media - Murashige Skoog media formulation (1962), [2]½ MS - Murashige Skoog media with half-strenght salt content; [3]IBA- Indole-3-butic acid; [4]BAP - 6- benzylaminopurine; [5]NAA - 1-naphthylacetic acid

Viability test

Fluorescence-based live assay was used to evaluate the seed viability post-cryotreatment. Seeds recovered after 3 days of storage in liquid nitrogen were immersed in a 0.1% solution (w/v) of fluorescein diacetate (FDA) which stains viable cells. FDA is taken up by cells which convert the non-fluorescent FDA into the green fluorescent metabolite fluorescein. Treated seeds were hand sectioned and analysed by fluorescent microscopy using a Zeiss Axio Imager M2 microscope equipped with fluorescent lamp and multiple fluorescence channels. Taking into account that fluorescein has a maximum absorption at 495 nm and maximum emission at 517 nm we have used a filter with wavelength range between 457 and 538 nm.

Germination test

In order to asses germination potential after cryogenic storage, samples of seeds were placed onto sterile Petri dishes (6 cm diameter) with humid cotton substrate. The samples were placed in the growth chamber set to a temperature of 20°C and an 8 hours photoperiod.

Table 2. Stages for seed cryo-storage

Stages/ sub-stages	A	b	c	d	e
Sample preparation	fresh seed colected from field were placed in an air-tight desiccator jar over calcium sulphate desiccant at 12% relative humidity for 1week	samples of 5 seeds were disposed in cryogenic vials of 2 ml	-	-	-
Cryoprotective treatment	in each cryogenic vial was added 1ml of 0.5M sucrose for 24 hours	solution removal	in each cryogenic vial was added 1ml of 1M sucrose for 24 hours	solution removal	in each cryogenic vial was added 1ml of PVS2[6] for 30 min
Controlled freezing onto *Freeze Control CL-3300* (Cryologic) system	CryoGenesis Programme version 5.0	cooling samples to 0°C cooling rate at 2°C/min	freezing samples to -4°C freezing rate at 1°C/min	2 min pause	freezing samples to -45°C freezing rate at 1°C/min
Freezing in liquid nitrogen and storage	immersion in liquid nitrogen for 1 hour	storage at -196°C in MVE Cryosystem 4000 containers	-	-	-
Seed recovery	vials with samples were thawed by direct immersion in water bath at 37°C	-	-	-	-

[6]PVS2 - Plant Vitrification Solution 2 (Sakai and Engelmann, 2007)

RESULTS AND DISCUSSIONS

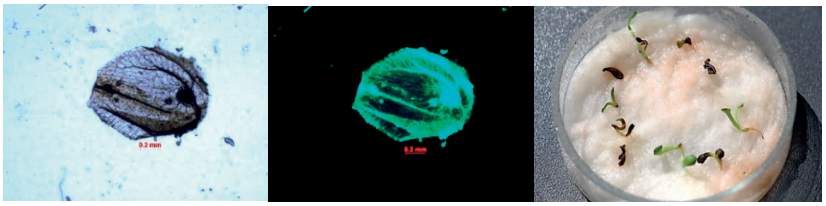
In vitro culture

One of the critical stages when initiating an *in vitro* culture is explant sterilization which has to be efficient to ensure an aseptic culture but also mild in order to ensure explant viability (Sarasan et al., 2006). The proposed sterilization protocol proved to be efficient resulting in 89% embryo survival and also, no contamination (Table 1, *Sterilization* stage). After 30 days of culture the embryos developed plantlets which served as source of plant material for micro multiplication (Table 1, *Plant regeneration* stage). When cultivated on multiplication media plantlets developed multiple shoots, after 4 weeks of culture multiplication efficiency being an average of 5 shoots/single plantlet (Table 1, *Micropropagation* stage). Under slow growth conditions provided by cultivation on maintenance media regenerants showed reduced growth and could be maintained without any alteration by periodical subcultivation at minimum 60 days period (Table 1, *subcultivation* stage). When necessary, shoots could be rooted in about 4 weeks of culture on rooting media (formulated as shown in Table 1), with an efficiency of 97% (Table 1, *Rooting* stage).

Another critical phase when developing an *in vitro* protocol is acclimatization (Hazarika, 2003). Our findings showed that rooted plants could be successfully acclimatised ex *vitro* within 8 weeks with a survival rate of 87%. As a result, following the proposed protocol plant material maintained in *in vitro* collection could be ready for field cultivation in approximately 4 months.

Seed cryopreservation

When treated with FDA, cryostored seed displayed fluorescence meaning that seed tissues are viable (Figure 2 a and b). Seed viability was validated also by germination test which showed that after 4 weeks on humid media 79% of tested seeds were able to germinate (Figure 2 c)



a b c

Figure 2. Viability test - Seed tissues after cryo-treatment a - in direct light, b - in fluorescence, c - germination test

Ex situ collections are valuable sources to increase populations that are declining or to restore the lost populations from rehabilitated habitats (Puchalski et al., 2014). *L. sibirica* populations are declining most probably because of different herbivores that can have a dramatic effect on the fitness of plant populartions (Heinken-Šmídová and Münzbergová, 2012). Consequently, with habitat degradation the moss layerproved to be the most suitable microhabitat for *L. sibirica* seed germination (Heinken-Šmídová and Münzbergová, 2012), is disturbed and appropriate germination and seedling recruitment is hampered. In this context developing *ex situ* collection of good quality material of *L. sibirica* as reserve for future actions for population reinforcement are necessary. Moreover, establishing an *in vitro* collection of a threatened plant species responds to target 8 of *Global Strategy for Plant Conservation* being an active contribution towards the aim "*20 per cent available for recovery and restoration programs*".

Acclimatization is considered a major bottleneck in the micropropagation of many plants that imply complex adaptive processes (Hazarika, 2003; Pospíšilová et al., 2007). When transferred from an atmosphere with high level of humidity and a medium rich in nutrients, including sugars, that allow heterotrophy, to external conditions, plantlets survival becomes critical. Although the proposed *ex situ* conservation protocol comprises the stages shown in Figure 1, we have also provided an efficient method to achieve roots and to acclimatize the micro-multiplicated plantlets in order to make plant material maintained in *in vitro* collections proper for transfer in natural habitats. In addition, the protocol developed within the present study could provide high quality plant material as an important source of bioactive compounds without any collection pressure from natural populations.

In order to back-up *in vitro* collections of *L. sibirica* germplasm the present study provides an efficient protocol for cryogenic storage of this species seeds (Table 2). Cryopreservation provides a safe and cost-effective method for long term-storage of plant tissues (Kaczmarczyk et al., 2011; Panis and Lambardi, 2006). Some estimations showed that until 2010 a few reports were published regarding only 52 wild endangered plant species for which cryoconservation protocols were established (Berjak et al., 2011). Enriching the knowledge in the field is also very important since currently there are still unavailable effective cryoconservation protocols for diverse plant tissues and genotypes (Popova et al., 2015).

CONCLUSIONS

Present paper provides for the first time, two simple and efficient methods, using biotechnological tools, to establish *ex situ* collections for medium and long-term conservation of *L. sibirica* germplasm. Live material preserved in collection may constitute a valuable source of plants for further restoration programs or for valuable bioactive metabolites extraction. These methods also provide insight into the development of protocols for the establishment of *ex situ* collections of other endangered species from *Ligularia* genus.

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