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EXPERIMENTAL RESEARCH ON BIOINSECTICIDE ACTIVITY OBTAINED BY USING AN OLEIC EXTRACT FROM DWARF SILVER FIR ON SOME VEGETABLE CROPS

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Abstract

The article presents a series of experimental studies on the use of an oleic extract, produced by steam separation utilizing a prototype distillation equipment. The separation process is performed with the use of wet saturated steam under pressure, using as raw material twigs and buds of dwarf silver fir. Since the volatile extracts of aromatic plants do not bring a direct nutritional contribution to the seeds subjected to germination, the germination increase and the root growth of the plants can be obtained only from the exercise of the anti-microbial, anti-oxidant and insect-repellent effect. The use of essential oils in the protection of seeds and rhizosphere is an ecological method because it does not negatively affect the number and frequency of species of useful microorganisms. The nutritional relationship that is established between the seeds treated with volatile oils and the germination bed can be influenced by the changing proportions of microbial populations with beneficial effects on productivity.

Key words: *bioinsecticide activity, oleic extract, dwarf silver fir, vegetable crops.*

INTRODUCTION

The chemical fertilizer industry is known for its high energy consumption (especially methane gas) and for its potentially high environmental risk (Manea, 2016; Mircea, 2020; Nenciu, 2022). The decrease of global fuel resources and several environmental challenges are raising the pressure on manufacturing costs. This highlights the potential presented by some organic alternatives, such as biofertilizers, that can be produced from various plant extracts (Muscalu, 2018; Tudora, 2021; Nenciu & Vladut, 2021). Biofertilizers present the great advantage of being non-polluting for terrestrial flora and fauna. In addition to their basic soil fertilizing effect, they also show a significant role as an inhibitor of pathogenic fungi and bacteria that can affect agricultural crops. Organic farming is a growing industry in Romania, that has seen an upward trend in recent years, for both vegetable and animal production sectors (Butu, 2022; Nenciu, 2022). In the organic farming industry, product marketing is highly important. Only MADR-registered merchants can sell organic products through different market channels, including sales at the farm gate, retail stores, specialized stores, the online exchange market for organic products (Bougherra, 2015; Cardei, 2021), and seasonal marketplaces. One of the essential conditions for the development of organic farming is promoting the concept, in order to make consumers aware of the benefits of these products. The higher price for organic products is justified by the quality, which is guaranteed by inspection and a certification system (Tisserand, 2014).

The use of fertilizers/pesticides in organic farming must leave no residues of chemicals, according to European regulations. Therefore, identifying new substances and compounds with fertilizing / insecticide action represents a continuous challenge for the field of agricultural scientific research in the 21st century (Udayashankar, 2022).

The insecticide industry produces compounds that may have harmful effects on insect populations, generating odors, vapors, gases, smoke, heat, oils, soap, etc. Natural chemicals, on the other hand, can partially or completely replace these substances. For example, the strong smell of garlic, tobacco, rhubarb and other similar plants is repulsive to some insects. Hot peppers, alcohol, salt and other substances can burn or destroy several pests. The oils suffocate certain insects, while the soap or detergents added to the solution have the role of making the substances stick better to the leaves and stems.

An important direction in today's agriculture is the use of biofertilizers made from plant extracts, containing bioactive substances. These essential oils are produced from medicinal, aromatic, farmed, or perennial plants. The advantage of using bioactive substances to the detriment of synthetic chemicals include the reduced contamination of the aquatic and terrestrial environments, the suppression of insect populations resistant to classical control measures, and the lack of unfavorable human and animal impact.

The use of essential oils to preserve seeds and the rhizosphere is an environmentally friendly strategy since it does not reduce the amount or frequency of beneficial microorganisms. The nutritional interaction that develops between seeds treated with volatile oils and the germination bed can be altered by changes in microbial population levels. In some cases, the complex action of volatile oils stimulates the development of microbial populations capable of solubilizing nitrogen, phosphorus, and potassium from insoluble chemical combinations, thereby increasing the reserve of nutrients available to plants without the use of conventional fertilizers. The goal of the research for the action of fir essential oil was to find a balance between preventing pathogen growth and causing phytotoxic effects. The main components of silver fir essential oil are Limonen 70%, α-Pinen 10%, Mircen 2.8% (Anna Wajs, 2010) and its action is a repellent for nematodes (*Meloidogyne incognita*).

Volatile oils are the product of the extraction process of volatile aromatic compounds that are found naturally in different parts of plants. A typical volatile oil is a mixture of chemical compounds such as esters, oxides, aldehydes, phenols, alcohols, terpenes, etc., which provide the oil its special characteristics. These complex mixtures make the essential oil an effecttive agent with effective bioinsecticide action.

There are several methods of extracting volatile aromatic compounds from plants: cold pressing, steam distillation, solvent extraction, fat extraction, filtration, etc. Pure volatile oils are only those obtained by steam distillation or cold pressing, because only these methods maintain the therapeutic properties of the oils unaltered (Kopaczyk, 2022).

Most industrially produced volatile oils are obtained by extraction with water vapor. All components of a volatile oil have higher boiling points than water but have high volatility at temperatures below the boiling point and can be easily entrained by water vapor at a lower temperature than the boiling point. Separation is made easier by their insolubility in water and their distinct densities.

The migration of volatile oil from plant cells to the surface layers precedes its production. The length of this process is determined by the type of the raw material being used. Diffusion is easier on green plants than in dried plants, and in flowers rather than in roots, seeds, or wood.

Vapor entrainment is the most widely used process for obtaining volatile oils, where steam is generated using an ancillary installation. This ensures a uniform temperature and the possibility of an efficient control of the steam flow. Furthermore, the degradation of the volatile oil is avoided due to the direct contact of the plant with the superheated walls of the extractor. These are large-scale systems that can handle many tons of raw material.

The vegetal material is positioned at the top, being supported by a perforated metal basket. Steam production can be performed either with direct steam technology (less often used), or indirect steam. Direct steam is introduced to the bottom of the container through a distributor (perforated spiral). When heated with indirect steam, the thermal agent circulates in a jacket and ensures the vaporization of a quantity of water that is introduced in the driving vessel, together with the raw material. The plant material comes into contact only with the generated water vapors, which ensures a gradual and controlled heating. The separation is performed with a modified Florentine distiller vessel, in order to extend the residence time and ensure a better separation of the volatile oil.

In order to reduce the losses caused by the partial dissolution of the volatile oil in the floral water, in most cases the product obtained in the Florentine distiller vessel enters a recirculation cycle.

Silver fir or white fir (*Abies alba* Mill.), is a fir native to Europe, whose area is limited by the Pyrenees Mountains, north of Normandy, east of

the Alps and Carpathian Mountains, to southern Italy and northern Serbia. It is a massive, evergreen conifer, growing up to 40–50 m tall (rarely 60 m), with trunk diameter of up to 1.5 m.
The goal of the present research was to test several recipes, composed of active natural compounds, that could be successfully applied to improve the development of tomatoes plants. The present paper is oriented towards Silver fir extract, however, our extensive research consists in combining several volatile oils for stimulating the plants growth, or to be used as repellent for some pests.

MATERIALS AND METHODS

A Wet Saturated Steam Distillation Equipment (E.D.A.S.U.P.) was designed for the current experiment, in order to optimize the characteristics of the oil for the field of bio-insecticides. The wet saturated steam distillation equipment has the following main elements: Electric Steam generator (1), Pressure regulator (2), Distillation vessel (3), Distillation vessel holder (4), Connecting hoses between vessels and steam generator (5), Serpentine (6) Cooling vessel (7), Flexible hose emptying cooling vessel (8), Cooling vessel holder (9), Flexible hose for draining hot water (10), Volatile oil collection vessel (11), Hot water drain (12), Exhaust volatile oil (13) (Figure 1).

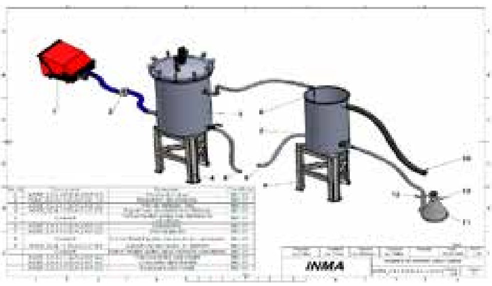


Figure 1. Wet saturated steam distillation equipment functioning under pressure **E.D.A.S.U.P**

Electric steam generator PULSE 7K, has supply voltage of 400 V, absorbed power 7000 W, degree of protection IPX5, supply flow of 3 l / min, max. supply pressure 6 bar.
Silver fir twigs were used to experiment the wet saturated steam distillation equipment, functioning under pressure E.D.A.S.U.P. (Figure 2).

Figure 2. Silver fir raw material in process and oil production

Since the volatile extracts of aromatic plants do not bring a direct nutritional intake to the seeds subjected to germination, the increase of germination and root growth of plants can be obtained only from the exercise of the anti-microbial, anti-oxidant and insect-repellent effect. Seeds prepared for sowing undergo a series of physical-mechanical processes that damage the morphological structures of the surface in order to achieve the required quality standards. Although these seeds possess high levels of purity and germination under controlled conditions, they may be susceptible to disease in the field due to phytopathogen colonization of injured tissue.
The protocol for preparing the mixture based on plant extracts has been prepared as follows: 0.2 g of agar is dissolved in 95 ml of distilled water at a temperature of 95-100ºC, then is cooled to room temperature, 5 ml of plant extract is added and stirred on a magnetic plate until homogenizes, while keeping away from the light.

Preparation of batches for testing the effect of plant extracts at different concentrations.
A batch of 1200 seeds is prepared (with its own unaltered microbiological load). The seeds are being mixed and divided into 24 batches of 50 seeds each (for small seeds e.g., peppers, tomatoes) or 48 batches of 25 seeds each (large seeds e.g., cucumbers). Petri vessel (90 mm x 16.2 mm, with ventilation) are being labeled from V1R1 to V6R4, or V6R8.
The seeds are displayed in the Petri vessel, then in the lid are inserted five disks of industrial paper (three layers) of cellulose.

Performing the treatment:
Four batches of 50 or 25 seeds are weighted each to determine the average mass, then is calculated the equivalent amount of mixture of 500 ppm, 1000 ppm, 2000 ppm, 3000 ppm and

5000 ppm for the average seed mass. Next is added the test dose of the vegetal extract mixture to one side of the plate without touching the seeds. Then is added the amount of water needed for homogenization with the plant extract, which is mixed well with the seeds. The cellulose discs are being placed on top of the seeds, and the cellulose discs are soaked with 10 mL of water. After that enters into an incubation process at the optimum temperature, for three days.

Performing measurements and observations after the treatment:

- The seeds are being weighted;
- Germinated seeds are counted;
- The length of the root / hypocotyl is measured;
- Embryos are weighed;
- Observations are made on visible microbial activity;
- CIM (minimum inhibitory concentration) is determined.

RESULTS AND DISCUSSIONS

The results of experiments performed using wet saturated steam distillation equipment functioning under pressure (E.D.A.S.U.P), using as raw material silver fir are briefly presented in Table 1.

Figure 4 describes the treatment on tomato seeds with silver fir essential oil (*A. alba* Mill.). The test showed that at concentrations between 1000 and 3000 ppm, tomato seeds did not produce negative effects on root growth in the first four days compared to the untreated control sample. The presence of a substantial standard deviation in both control and sample measures indicated that the batch of seeds lacked morphological uniformity and structural integrity. In comparison to the other situations, the concentration of 3000 ppm was observed to enhance the formation of absorbent filaments. This result might be a form of drought adaptation for conifers, and it could be used in groundwater studies.

Table 1. Experimental setup and results obtained using Wet Saturated Steam Distillation Equipment Functioning Under Pressure

Parameters	Sample Silver fir
Batch mass [kg]	6
Air	27
Temperature [°C]	
- cooling water	26.7
- steam pipe at the outlet of the generator	128.1
- steam pipe at the entrance to the distillation vessel	95.2
- steam in the distillation vessel	80.2
Air humidity [% RH]	61.6
Test duration [min]	50
Volume of resulting products	
- oil [ml]	27.1
- floral water [l]	4.6

In the germination phase, a Gaussian response curve was found, with the peak at 200 ppm, depending on the concentration of the extract used. When seedlings were treated with silver fir essential oil at a dose of 2000 ppm (depicted in Figure 5), germination increased by 10% over the control sample, with the least standard variation across the observed tests. This indicates that the seed's morphological structure is influenced and vulnerable to microbial invasion, as depicted in Figure 3.

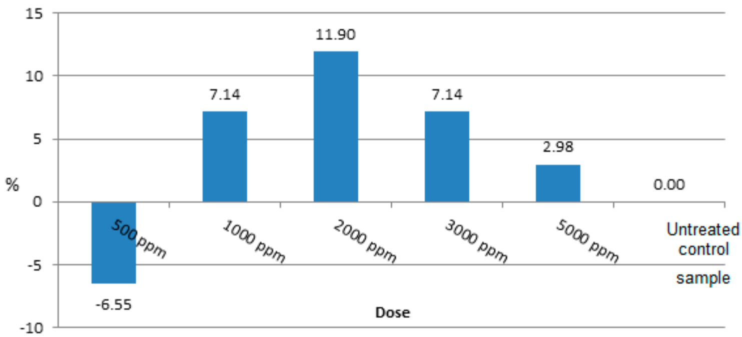
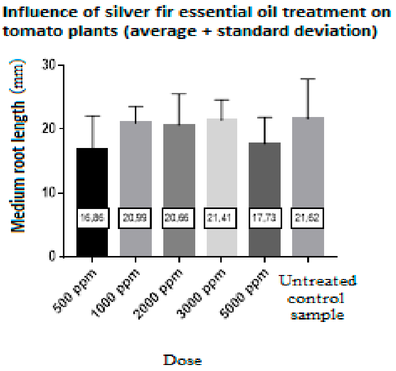
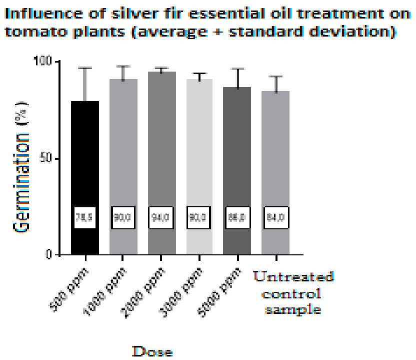


Figure 3. Evaluation of the growth and development of tomato plants in the early vegetative stages influenced by the treatment with silver fir essential oil



Alpha	0.05			
Dunnett's multiple comparison test	Averages difference	Statistical significance	Summary of significance	Adjusted value P
Untreated control sample vs. 500 ppm	4.764	No	ns	0.4614
Untreated control sample vs. 1000 ppm	0.636	No	ns	0.9997
Untreated control sample vs. 2000 ppm	0.9578	No	ns	0.9982
Untreated control sample vs. 3000 ppm	0.2132	No	ns	0.9999
Untreated control sample vs. 5000 ppm	3.894	No	ns	0.6378

Figure 4. Evaluation of silver fir essential oil effect on root development, after treating the tomato seeds. Statistical significance



Alpha	0.05			
Dunnett's multiple comparison test	Averages difference	Statistical significance	Summary of significance	Adjusted value P
Untreated control sample vs. 500 ppm	5.5	No	ns	0.8964
Untreated control sample vs. 1000 ppm	-6	No	ns	0.8617
Untreated control sample vs. 2000 ppm	-10	No	ns	0.4988
Untreated control sample vs. 3000 ppm	-6	No	ns	0.8617
Untreated control sample vs. 5000 ppm	-2	No	ns	0.9984

Figure 5. The influence of silver fir essential oil treatment on tomato seed germination. Statistical significance

CONCLUSIONS

Calculating the combined effect of root growth and germination of tomato seeds, when treated with silver fir essential oil, have been found that a dose of 2000 ppm can increase plant development by 11.9 %, compared to untreated control sample.

Our previous research shown that from the volatile plant extracts (oregano, silver fir, laurel, lavender) tested on tomato crops in the early vegetative stages, oregano extract had a negative influence on growth. Therefore, it is rather recommended for seeds fumigation for preservation (during storage periods), or as an organic herbicide. Silver fir extracts showed positive results and shall be associated with other nutritional extracts to initiate plant protection.

Biochemical content of the volatile extract of fir oil have been determined to possess high quantities of pinene and limonene, both of them having insecticidal, insect-repellent, and anti-microbial properties (Udayashankar, 2022).

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