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VEGETATIVE PROPAGATION SYSTEM FOR *HELICHRYSUM ITALICUM* PLANTS WITH ORNAMENTAL AND MEDICINAL PROPERTIES

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Abstract

Market demand for Helichrysum italicum (Asteraceae) is growing, especially for its valuable essential oils; however, seed quality and crop growth are variable. Among the various methods of vegetative propagation, propagation by stem cuttings is one of the most viable techniques that allow multiplication of genotypes of interest to achieve crop uniformity. Therefore, the aim of this work was to develop a sustainable vegetative multiplication system under controlled growing conditions. For this purpose, H. italicum seedlings were grown on different experimental substrate variants. Growth and vegetative development parameters of the seedlings were monitored, such as: average number of seedlings, average height of seedlings, length and number of roots. The results showed that the best substrates for root growth and rapid seedling production of H. italicum were: perlite and rooting with Radistim 2 (V5-a2b2) followed by perlite with peat and Radistim 2 (V8-a3b2) and perlite with peat and rooting with Atonik solution (V9-a3b3). The results obtained may contribute to the expansion of H. italicum cultivation in Romania for use as a medicinal plant or in the food industry.

Key words*: cuttings, culture conditions, Helichrysum italicum, vegetative propagation system.*

INTRODUCTION

Medicinal and aromatic plants represent a very important pharmaceutical and food raw material, being used since ancient times. However, for their use, scientific knowledge is necessary, the way the species spreads, the identification of plant products, the chemical composition, pharmacological indications, the feeding of medicinal plants, their active principles (Marcelino et al., 2023).

The Food and Agriculture Organization of the United Nations (FAO, IFAD, UNICEF, WFP and WHO, 2021) warns developing countries that 800 million people are malnourished through food and nutritional insecurity (Vassilakou, 2021).

Thus, natural bioactive compounds derived from plants are important resources, which can be sources of both medicines and functional foods (Ghendov-Moşanu, 2018).

The *Asteraceae* family consists of 1,900 genera, 32,000 species spread all over the world (https://en.wikipedia.org/wiki/Asteraceae), growing in various climates, and is also considered the largest family of flowering plants. Important species from this family are used as food, spices or for medicinal purposes, having diuretic, choleretic, anti-inflammatory properties.

The genus *Helichrysum* Miller (family *Asteraceae*) consists of a taxonomic group of plants used in Europe in traditional medicine for their bile-regulating and diuretic effects (Ferraz et al., 2022; Litvinenko et al., 1992). The genus name is derived from the Greek words: "helios" which means sun and "chrysos" which translates to gold, after the color of the yellow-golden inflorescence.

Tutin et al. (1976) mention that the genus *Helichrysum* is a member of the *Asteraceae* family which includes more than 400 members distributed worldwide, with 25 species native to the Mediterranean basin.

Traditional medicine around the world, has been using for about 200 years for medicinal, food and ornamental purposes (Viegas, et al., 2014), some of the species of the *Helichrysum* genus, the best known are those widespread in Europe, Central Asia and China: H arenarium (L. Moench), *H. plicatum, H. italicum* (Roth),

H. bracteatum, H. gymnocephalum, H. splendidum. Helichrysum spp., also offers a variety of phenolic extracts and pure compounds, among which are also some species of *Helichrysum* belonging to the Turkish flora: *H. arenarium* (L.) Moench subsp. *aucheri* (Boiss), *H. armenium, H. artvinense* (David and Kupicha), *H. chionophilum* (Boiss and Bal), *H. compactum, H. goulandriorum, H. graveolens, H. heywoodianum, H. noeanum, H. orientale* (L.) DC, *H. pallasii* (Sprengel) Ledeb (Albayrak et al., 2010).

Helichrysum italicum (Roth.) G. Don is an aromatic halophyte plant widespread in Europe in countries such as Italy, Spain, Portugal and Bosnia and Herzegovina, being traditionally used in human nutrition as a spice (Viegas, et al., 2014). The plants of this species especially show antioxidant, antibacterial, antifungal properties. This species in the past was used as a culinary spice due to its curry flavor, having a strong, pungent and spicy smell, and is currently processed to prepare *Helichrysum italicum* essential oil (EO) and to obtain extracts with fragrance compositions and preparations from the cosmetics industry, aromatherapy (Ferraz et al., 2022; Andreani et al., 2019). In the studies developed by Andreani et al. (2019) it is concluded about the species and subspecies of *H italicum* essential oils (EO) that they contain numerous terpenic and nonterpenic constituents, which give a characteristic, aromatic smell, from which the popular name of curry plant.

The species *Helichrysum italicum* (Roth) G. Don, popularly called immortelle or curry plant, has from a food and pharmaceutical point of view numerous therapeutic and seasoning properties in food flavoring, as well as a potential antioxidant that prevents the accumulation of free radicals (Węglarz et al., 2022; Andreani et al., 2019).

Natural antioxidants prevent deterioration of products: food, beverages, pharmaceutical products. In the cosmetic industry, products containing α-pinene prevent the aging process of the skin. The flowers of the species *Helichrysum italicum* (Roth) G. Don, present antibacterial constituents, bitter substances for the treatment of gout, rheumatism of hepato-biliary diseases, gastrointestinal disorders, hepatitis, combating intestinal worms, antifungal. Neryl acetate present in *H. italicum* EO from Italy (Tuscany) has orange blossom, sweet and fruity rose scent.

Authors such as Andreani et al. (2019), as well as Leonardi et al. (2013), mention that the Ar - curcumin present gives a "ginger" aroma, and the italidiones identified are anti-inflammatory agents, which provide protection to the skin against pollution and UV radiation. Also, *Helichrysum italicum* essential oils (EO) Corsican contains oxygenated compounds in greater quantity appreciated in the perfume industry and aromatherapy, (neryl acetate-40%), β-diketones ("italidiones") (5-10%) (Andreani et al., 2019).

According to what was stated in different studies, *Helichrysum italicum* essential oil (EO) has many biological and pharmacological properties (Viegas et al., 2014; Maksimović et al., 2017), collagen regeneration and is used in anti-aging creams (Andreani et al., 2019).

After 2005, the interest in curry plant (*Helichrysum italicum*) increases and at the same time the market price €2000/kg for the essential oil, and the annual production values are estimated at 1.34 tons (France AgriMer, 2017).

Taking into account the previously presented arguments, the growing interest in *Helichrysum italicum* species is also reported in S-E European countries, especially for its valuable essential oils. Reports on the establishment of new crops with *Helichrysum italicum* plants have been mentioned recently by researchers from Croatia (Tomičić et al., 2022) and the Republic of Moldova (Nartea et al., 2023; Cojocaru-Toma, & Nartea, 2022). Thus, the objective of this work was to develop an effective propagation method under controlled culture conditions in a greenhouse with a controlled atmosphere, for curry plant (*Helichrysum italicum*).

MATERIALS AND METHODS

At present, the information with reference to propagation by seeds and by vegetative propagation of *Helichrysum italicum* plant, mentioned in Romania, is only at the level of popularization of offers by commercial companies. To expand the culture, with plants

that are marketable, a sustainable production system is needed to ensure a constant supply of good quality biological material.

Among the different methods of vegetative propagation, in this research the propagation by stem cuttings was studied, as it is considered one of the most viable techniques that allow the multiplication of the genotypes of interest and to obtain a uniformity of the culture (Melnic et al., 2022).

The experiments were carried out in a compartment of the block greenhouse for research, an automated unit and with self-control functions of the air conditioning provided by the Research Center for the Study of the Quality of Food Products - QLAB of the USAMV of Bucharest (Romania), during the period October-December 2023. The compartments of this greenhouse are modern constructions with a height of 6.2 m, recently equipped with all the facilities for monitoring and maintaining the climatic factors through the funding obtained for the ClimaGreen project. (Project Cod: RO-NO-2019-0420, https://www.climagreen.eu/), held in the period 2021-2023. ClimaGreen is a multidisciplinary research project between the Research Center for the study of the quality of agro-food products from the University of Agronomic Sciences and Veterinary Medicine (USAMV) of Bucharest, the research institute SINTEF Energy Research in Trondheim (Norway) and the SME Gether AS in Oslo (Norway).

The source of mother plants necessary for the vegetative propagation experiments with Immortelle (*Helichrysum*) from the *H. italicum* species, originally came from seedlings sold by the "Lavandă de Romania" company, from Timiș county (region located in the west of Romania; https:// www.butasidelavanda.ro). Planted seedlings were transplanted and maintained for fortification under field conditions between July and October 2023. The biological material, consisting of cuttings, was taken from the level of the mother plants, both from the top of the main shoots and the side branches, as well as from the length underlying it with standard dimensions of 4- 5 cm long.

Rooting pots with dimensions of 10x10 cm, soil, peat, perlite and rooting hormones (Atonik and Radi-Stim no. 2) were used as materials for this experiment. Radi-Stim no. 2 produced in Romania, is a biopreparation, marketed in powder form, intended for rooting lignified and semi-lignified cuttings, which can be used for rooting vine cuttings, fruit tree rootstocks and ornamental plant cuttings (https://viasigradina.ro/radi-stim-nr-2-pudra-10-gr-radistim.html).

For the choice of this rooting preparation, the manufacturer's recommendation was taken into account, which mentions that due to the complex composition, the cuttings are protected during the period of callusation and the emission of roots against the attacks of various pathogens, thus reducing the mortality caused by molds. Atonik biostimulator is a systemic product formulated as an aqueous solution with the following chemical composition: sodium orthonitrophenolate (0.2%), sodium paranitrophenolate (0.3%) and sodium 5-nitroguaiacolate (0.1%). The detachment of the cuttings was made with scissors through a straight cut, after which they were immersed for a length of 1 cm at the base through the rhizogenic stimulant variants.

In the experimental scheme applied to *Helichrysum italicum*, two experimental factors were studied: Factor A - the rooting substrate represented by 3 gradations: a1 - peat substrate 100% (the control); - variant control (Vm); a2 - 100% perlite substrate; a3 - perlite mixture substrate 50% + peat 50%; and the experimental Factor B, represented by 2 rooting stimulators 3 gradations: b1 - no rooting treatment (Vm - Control); b2 - rooting treatment with powder-Radi-Stim 2; and b3 - rooting treatment with Atonik solution. In order to estimate the effect of the B-experimental Factor represented by the stimulating products on the rooting process and after rooting, the cuttings were studied on the 3 types of substrate used for rooting, represented by the A-experimental Factor, in greenhouse cultivation conditions. Growth and vegetative development parameters were monitored at the level of the cuttings, such as: the average number of shoots, the average height of the shoots, the length and the number of roots.

Throughout the 80 days of monitoring rooting and shoot growth development at the level of *Helichrysum italicum* cuttings, controlled conditions in terms of environmental factors

(temperature, humidity, light) were ensured in the greenhouse compartment.

Statistical analysis. The bifactorial experiments with siminoc cuttings were placed according to the method of subdivided plots, in 5 repetitions within each experiment, the influence of the substrate on the rooting of the cuttings and the growth dynamics of the stems, leaves and shoots at the plant level were tested.

As a result of the combination of the two experimental factors Factor A x Factor B, 9 experimental variants resulted in 5 repetitions. The values of the presented determinations represent the averages of the 5 repetitions related to each experimental variant, and the calculation and interpretation of the experimental results was carried out by the variance analysis method for P 5%, P 1%, P 0.1%, in accordance with the field placement method, respectively in subdivided plots.

RESULTS AND DISCUSSIONS

The biometric determinations carried out on *Helichrysum italicum* plants highlighted the fact that treatments with biostimulators had a direct influence on the number of differentiated roots on the plants, all experimental variants surpassing the control variant V1-a1b1 (Control) in which it was used as a culture substrate peat and no rooting stimulation treatments were performed.

Table 1. Influence of experimental factors on the number of differentiated roots on *Helichrysum italicum* plants

Experimental Variant	Shoots number/plant (No.)	Difference (No.)	Significance degrees
V1-a1b1 (Control)	**2.0**	Mt.	Mt.
V2-a1b2	4.0	2	*
V3-a1b3	3.0	1	-
V4-a2b1	8.0	5	***
V5-a2b2	11.5	9.2	***
V6-a2b3	9.5	7.3	***
V7-a3b1	6.5	4.2	***
V8-a3b2	10.9	8.9	***
V9-a3b3	9.0	7	***
$DL_{5\%} = 1.883$; $DL_{1\%} = 2.745$; $DL_{0,1\%} = 4.181$			

Legend for Statistical significance: (-) - insignificant, (*) - significantly positive, (**) - distinctly significant positive, (***) - very significant positive

Thus, based on the results summarized in Table 1, it is observed that the number of roots formed on the plants varied between 2 roots/plant in the case of the control variant (a1b1) and 11.5 roots/plant in the case of the variant in which perlite was used as a culture substrate in proportion to 100%, against the background of the administration of treatment with Radistim-2 (powder) as a rooting biostimulator (V5-a2b2).

It is observed, in an overall analysis of the values in Table 1, that the highest number of roots was determined in the experimental variants in which stimulation treatments with Radistim-2 were carried out, against the background of growing the plants in 100% perlite as a substrate of growth. Compared to the control variant not treated with growth biostimulators, the differences recorded in the other experimental variants were, from a statistical point of view, insignificant (-) for the V3-a1b3 variant, dsignificantly positive (*) for the a1b2 variant and very significantly positive (***) in the rest of the experimental variants, treatments with biostimulators stimulating the formation of plant roots, regardless of the culture substrate tested in the research (Table 1). Figure 1 shows *Helichrysum italicum* cuttings from variant V5-a2b2 (a2 - 100% perlite substrate; b2 - rooting treatment with powder-Radi-Stim 2) evaluated for root development 50 days after planting the cuttings *Helichrysum italicum*.

The experimental results summarized in Table 2 reveal the fact that there were no significant differences between the experimental variants in terms of the growth dynamics in the length of the roots, the values of this parameter being

between the limits of 2 cm and 3.4 cm, compared to the experimental control the differences recorded in the rest of the variants experimental having insignificant statistical assurance (-).

Figure 1. Immortelle *Helichrysum italicum* cuttings evaluated for root development, after 50 days from the date of planting the cuttings (photo - 7 December 2023)

Table 2. Influence of experimental factors on the length of differentiated roots on *Helichrysum italicum* plants

Experimental Variant	Roots length (cm)	Difference (cm)	Significance degrees
V1-a1b1 (Control)	**2.0**	Mt.	Mt.
V2-a1b2	2.7	0.7	-
V3-a1b3	2.5	0.5	-
V4-a2b1	2.7	0.7	-
V5-a2b2	3.4	1.4	-
V6-a2b3	2.7	0.7	-
V7-a3b1	2.5	0.5	-
V8-a3b2	3.2	1.2	-
V9-a3b3	2.6	0.6	-
$DL_{5\%} = 1.507$; $DL_{1\%} = 2.290$; $DL_{0,1\%} = 3.765$			

Legend for Statistical significance: (-) - insignificant.

Analyzing the growth and development dynamics of the stems, it is found that their length varied from 4.3 cm in the case of the control variant V1-a1b1 (Control) to 7 cm, the maximum length of the stem determined in the case of the V5-a2b2 variant, variant in which perlite was used as a substrate for plant growth, and Radistim treatments were performed as a plant rooting biostimulator (Table 3).

It can be seen in the results presented in Table 3, that the perlite used as a culture substrate potentiated the effect of the biostimulators tested during the research, resulting in the harmonious growth and development of the vegetative organs of the *Helichrysum italicum* plants, the height of the plants exceeding 6 cm.

The differences recorded following the determination of this biometric parameter compared to the experimental control (V1-a1b1) varied between the limits of 0.27 cm and 2.7 cm, with insignificant statistical assurance (-) in the case of the experimental variants in which peat was used (alone or mixed with perlite) as a culture substrate and significantly positive (*) in the experimental variants in which the culture substrate was perlite (alone or mixed with peat).

Table 3. Influence of experimental factors on the length of differentiated stems on *Helichrysum italicum* plants

Experimental Variant	Stems length (cm)	Difference (cm)	Significance degrees
V1-a1b1 (Control)	**4.30**	Mt.	Mt.
V2-a1b2	6.10	1.80	*
V3-a1b3	4.57	0.27	-
V4-a2b1	5.30	1.00	-
V5-a2b2	7.00	2.70	*
V6-a2b3	5.00	0.70	-
V7-a3b1	6.20	1,90	*
V8-a3b2	6.37	2.07	*
V9-a3b3	5.80	1.50	-
$DL_{5\%} = 1.689$; $DL_{1\%} = 2.822$; $DL_{0,1\%} = 5.349$			

Legend for Statistical significance: (-) - insignificant, (*) - significantly positive.

The degree of branching of the stem in the plants belonging to the species *Helichrysum italicum* did not show significant differences between the 9 experimental variants (Table 4), the plants differentiating between 1.2 ramifications/plant in the case of the control variant V1-a1b1 (Control) and 2.4 ramifications/plant in the variant V8-a3b2, variant in which the administration of the biostimulator Atonik, against the background of the use of perlite as a substrate for plant growth and vegetation, stimulated the vegetative development of the aerial vegetative organs of the plants, thus forming the largest number of branches per plant.

Table 4. Influence of experimental factors on the number of differentiated branches on *Helichrysum italicum* plants

Experimental Variant	Branches number/plant (No.)	Difference (No.)	Significance degrees
V1-a1b1 (Control)	**1.2**	Mt.	Mt.
V2-a1b2	2.0	0.8	-
V3-a1b3	1.4	0.2	-
V4-a2b1	2.0	0.8	-
V5-a2b2	2.0	0.8	-
V6-a2b3	1.7	0.5	-
V7-a3b1	1.5	0.3	-
V8-a3b2	2.4	1.2	*
V9-a3b3	2.0	0.8	-
$DL_{5\%} = 0.999$; $DL_{1\%} = 1.402$; $DL_{0,1\%} = 1.980$			

Legend for Statistical significance: (-) - insignificant, (*) - significantly positive.

The differences compared to the control variant not treated with biostimulators were insignificant (-) in all experimental variants, with the exception of the V8-a3b2 variant in which there were significantly positive differences (*) of 1.2 cm, compared to the untreated control (Table 4)

The number of leaves formed on *Helichrysum italicum* plants after 80 days of vegetation varied between 18.9 leaves/plant and 26.2 leaves/plant, the lowest number of leaves (18.9 leaves/plant) being determined in the control variant (variant in which the growth substrate was represented by peat 100%) and in variant a3b1 (variant in which the substrate used consisted of a mixture of peat 50% + perlite 50%), in both experimental variants no treatments were administered to stimulate the growth and vegetative development of plants (Table 5).

It is also observed in the results presented in the significant effect of the use of the biostimulators tested in the research, biostimulators which, based on the use of 100% perlite or a 50% mixture of peat and perlite as a substrate for plant growth: 50% led to the optimal growth and development of the plants, with statistically guaranteed differences from distinctly significantly positive (**) in the experimental variant a2b2 (V5), to very

significantly positive (***), a result recorded in the V1 variants, V2,V5 as well as the plants from variants V8 and V9 compared to the untreated control variant (Table 5)

Table 5. Influence of experimental factors on the number of differentiated leaves on *Helichrysum italicum* plants

Experimental Variant	Leaves number/plant (No.)	Difference (No.)	Significance degrees
V1-a1b1 (Control)	**18.9**	Mt.	Mt.
V2-a1b2	21.3	2.4	***
V3-a1b3	20.7	1.8	***
V4-a2b1	19.1	0.2	-
V5-a2b2	26.2	7.3	***
V6-a2b3	19.5	0.6	**
V7-a3b1	18.9	0.0	-
V8-a3b2	22,5	3.6	***
V9-a3b3	20,5	1.6	***
$DL_{5\%} = 0.374$; $DL_{1\%} = 0.561$; $DL_{0,1\%} = 0.904$			

Legend for Statistical significance: (-) - insignificant, (*) - significantly positive, (**) - distinctly significant positive, (***) - very significant positive.

In the case of variants a2b1 and a3b1, insignificant (-) differences were recorded in terms of the number of differentiated leaves on *Helichrysum italicum* plants, compared to the experimental control (Figure 2).

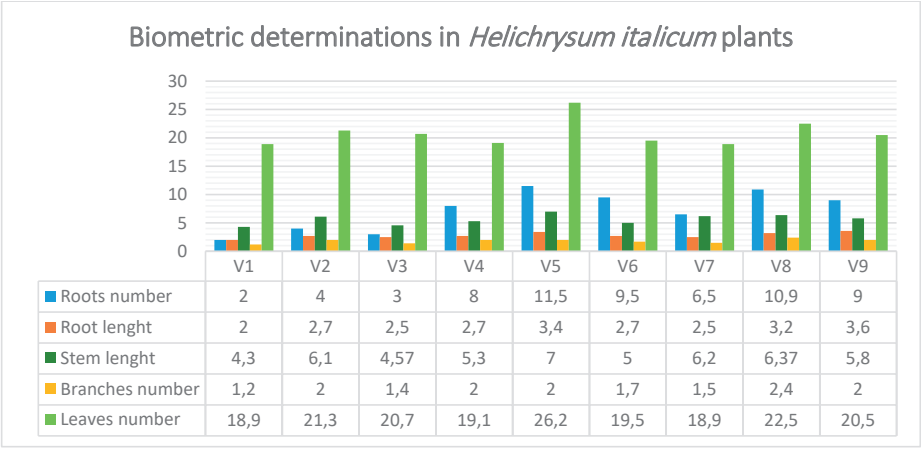

Figure 2. Experimental results recorded for root growth and rapid seedling production of *H. italicum*

CONCLUSIONS

The work brings to attention a special potential of medicinal plants that can be exploited commercially as nutraceutical foods. Through the research carried out, the results of previous research are confirmed, according to which *Helichrysum* sp. species are difficult to multiply by conventional vegetative methods. The arguments for continuing research in order to spread it as a decorative and aromatic plant in Romania are due to the important content of this species in phytochemical compounds.

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