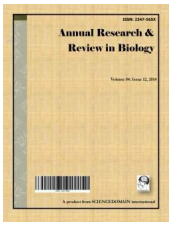

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Influence of Different Auxins on Rooting of Rosemary, Sage and Elderberry

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Authors' contributions

This work was carried out in collaboration between all authors. Author LKG designed the study, wrote the protocol and wrote the first draft of the manuscript. Author FT performed the statistical analysis and managed the analyses of the study. Author LM managed the analyses of the study. Author JT managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of research is to examine the influence of auxins IAA, IBA, NAA and commercial rooting product on rooting of cuttings from rosemary (*Rosmarinus officinialis* L.), sage (*Salvia officinialis* L.) and elderberry (*Sambucus nigra* L).
Study Design: Completely randomized design.
Place and Duration of Study: The experiment was conducted in a plastic tunnel with three beds in Stip, Republic of Macedonia, between October 2012 and January 2013.
Methodology: Fifty cuttings from each experimental species (*Rosmarinus officinialis* L., *Salvia officinialis* and *Sambucus nigra* L.), with height of 15 cm, were treated with freshly made solutions of different auxin and planted for rooting in the rooting beds. After 100 days of planting we

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measure: height of cuttings, number of rooted cuttings, number of roots, root length and rooting percentage.
Results: The conducted research shows that growth regulators applied during the experiment, including the commercial rooting product, positively influenced rooting process of vegetative cuttings of rosemary and sage. Application of auxins as IAA, IBA and NAA in concentration of only 5 ppm have stimulating effect on rooting of the rosemary (IBA – 92%), sage (IBA – 54%; NAA – 50%) and elderberry cuttings (K1 and IAA – 14%).
Conclusion: This study is first report for application of auxins on rosemary, sage and elderberry cuttings collected from local populations in Republic of Macedonia. Application of auxins in concentrations of 5 ppm positively affected rosemary, sage and elderberry. The results presented in this paper show that the vegetative propagation of medicinal and aromatic species might be stimulated by auxins utilization even in very low concentrations. It should be noted that further research should be extended to more medicinal and aromatic plants with significant economic importance as well as with application of several higher concentration of auxins.

Keywords: Cuttings; rooting; vegetative propagation; auxins.

ABBREVIATIONS

IAA : Indole-3-acetic acid,
IBA : Indole-3-butric acid,
NAA : α-naphthaleneacetic acid

1. INTRODUCTION

The three species under study, rosemary (*Rosmarinus officinialis* L.), sage (*Salvia officinialis* L.) and elderberry (*Sambucus nigra* L.) are wild flora species and/or introduced and cultivated in Republic of Macedonia. Rosemary (*Rosmarinus officinialis* L.) and sage (*Salvia officinialis* L.) are members of family Laminaceae, both naturally growing in Mediterranean region. They are evergreen perineal woody shrubs, highly aromatic; thus, they are cultivated since ancient times as spice and as source of etheric oils and antioxidants for pharmaceutical and medicinal purposes. Elderberry (*Sambucus nigra* L.) is a member of family Caprifoliaceae, native to most of Europe and North America. It is deciduous shrub or small tree growing tall from 3 m to maximum 10 m in a variety of conditions. Different parts of the plant as stembark, leaves, flowers, fruits and root are used in in beverage industry and traditional medicine. The wild sage has limited distribution on few locations in the western part of the country, while rosemary is an introduced plant species, grown as ornamental or cultivated for different pharmaceutical and culinary purposes. Elderberry is found usually as solitary small tree in suburban and rural areas [1-2].

Republic of Macedonia, although a small country, is characterized by rich diverse of medicinal and aromatic species which has contributed for development of traditional medicine into modern pharmaceutical industry as important economic sector in the country. Medicinal and aromatic plants and their parts are mostly used medicinally, for cosmetics, as herbal teas, as spices, as insecticides and fungicides etc. Up to 95% of the total plant material used in pharmaceutical industry is from wild origin and 80% of it is collected and/or cultivated in Macedonia. On one hand, the need of pharmaceutical industry for raw material of medicinal and aromatic plants stimulates uncontrolled collection of wild species and makes pressure to the native plant biodiversity, but on the other hand raises a need for organized cultivation of such plants [3]. Yet, there is no institution in the country, or company, which supplies the market with seed and/or seedlings of medicinal and aromatic plants. This shortage of medicinal and aromatic starting material makes their organized production difficult from the very beginning. Vegetative propagation is a tool to improve this situation and it is very much utilized for rooting of plants in plant nursery businesses and acquisition of planting material (plantlets) formany economically important medicinal and aromatic plants [4-8]. The vegetative propagation is utilized especially when seed germination of specific species is less than 50%, which applies to the most of the aromatic and medicinal species, which additionally makes the process of cultivation of these plants complicated [4,9].

The application of phytohormones, especially auxins as growth regulators, in nursery rooted production is used to increase the number of seedlings and plants, to shorten the rooting time, to increase the root number per plant and uniformity of the root system [3]. IBA and NAA

are the most commercially utilized growth regulators, mostly because of their steadiness during water irrigation of the plant cuttings [10-12].

The aromatic and medicinal species are characterized by short seeds longevity, as well low germination capacity, and it is a result of deficiency of breeding programs and seed processing [13] although the application of some biostimulators can promote their seed germination [14]. Some plant species are rooted difficultly without application of growth regulators, thus the application of optimal concentration of IBA and/or NAA is mandatory [15]. The auxins are supporting translocation of nutrition elements from the upper parts of the plant cutting to the basal parts, where the activity of enzymes is increased. It is increasing the hydrolysis of carbohydrates which leads to development of enough energy in the cells responsible for rhizogenesis [16-17].

The aim of this study was to determine the rooting effect of auxins IAA, IBA, NAA and commercial rooting product on cuttings from rosemary (*Rosmarinus officinialis* L.), sage (*Salvia officinialis* L.) and elderberry (*Sambucus nigra* L.). It is a first study for application of auxins on cuttings from medicinal and aromatic collected from local population in Republic of Macedonia.

2. MATERIALS AND METHODS

The experiment was conducted in a plastic tunnel with three rooting beds in Stip, Republic of Macedonia (41.7375° N, 22.1936° E; altitude: 288 m ASL) from October 2012 to January 2013. The ground of the plastic tunnel was cleaned from weeds and top-soil layer, disinfected by solarization and the beds filled with mixture of peat and perlite with ratio 1:1.

The plant material used in this research was collected from different sites using field seizers. The rosemary (*Rosmarinus officinialis* L.) cuttings were collected from a shrub, while the elderberry (*Sambucus nigra* L.) cuttings were collected from an individual tree, both in near vicinity of experimental site. Sage (*Salvia officinialis* L.) cuttings were collected from a commercial field which is cultivated with sage plants originated from natural populations.

The collected material of each species was cut to 15 cm long cuttings. The leaves of rosemary and sage cuttings were cleaned from base up to 5 cm in height, while the elderberry cuttings were leafless. Once the plant cuttings of each species were prepared as described above, the lower 3-5 cm of the cuttings, were immersed in a solution of different auxin for 5 seconds. For each species under this research, there were 5 treatments applied on 50 plant cuttings, in total 250 plant cuttings of plant species:

- K - control, the cuttings were not treated with any solution
- K1 - commercial rooting product (powdery growth regulator IBA, applied as 0,003% solution)
- IAA - 5 ppm IAA
- IBA - 5 ppm IBA
- NAA - 5 ppm NAA

Collection of plant material, preparation, treatment with growth regulators and plantation in beds was performed in the same day. There was no sterilization of cuttings applied.

After the treatment with auxins, the cuttings were planted in beds, with 10 cm distance in rows and between rows. The cuttings were irrigated manually depending on the weather conditions and wetness of rooting beds. There were no diseases symptoms and insects registered during the experiment.

The effect of the treatments was observed after 100 days from the experimental setting. After 100 days, the cuttings were taken out of the rooting beds. Roots of each plant were cleaned and washed with distilled water and dried with paper. At this stage, the height of cuttings and root length were measured, as well as the number of rooted cuttings and number of roots was recorded.

2.1 Walter Diagram – Presentation of Climate Conditions at the Experimental Site

The experiment was conducted under protected conditions where the environmental conditions were not regulated; consequently the weather conditions influence the experiment material. Climate parameters as average monthly temperatures and monthly rainfall sum in the experimental period are presented in Walter climate-diagram (Fig. 1.).

The average monthly temperature at the experiment start in October 2012 is 15.3℃, decreasing to 1.5℃ in December, and slightly increasing in January 2013 (2.2℃). Obviously,

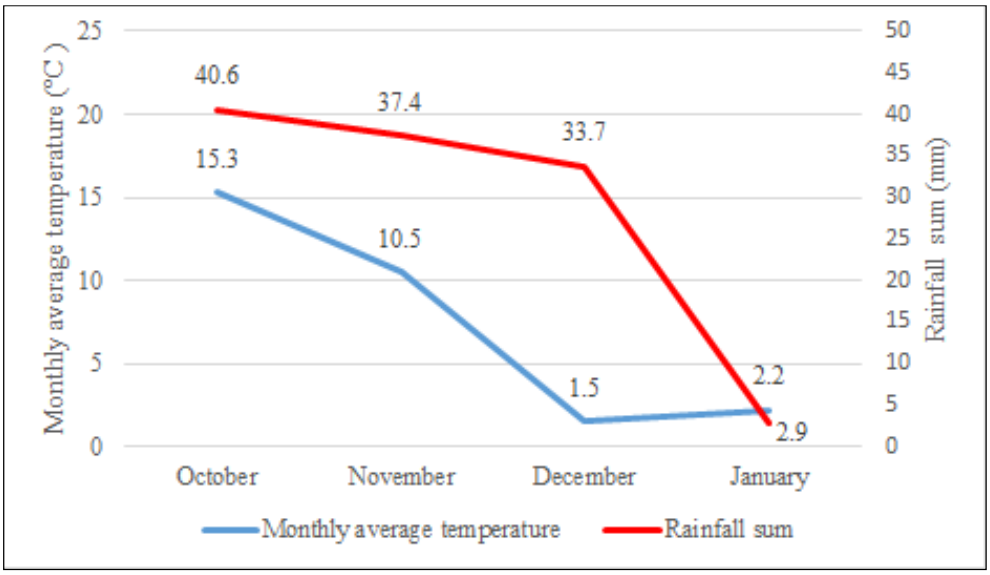

Fig. 1. Walter climate diagram for the period October 2011 - January 2012 at the experimental site

there were no temperatures below zero and such winter temperatures in temperate climate certainly affected the vegetative propagation in positive manner. The rainfall sum was the highest in October (40.6 mm) and the lowest in January (2.2 mm).

2.2 Statistical Data Processing

All data on rooting of rosemary, sage and elderberry were subject to analysis of variance (ANOVA) and mean values were evaluated at the $p<0.05$ level of significance using Duncan's Multiple Range Test (IBM SPSS Statistics 19 Brief Guide, 2010).

3. RESULTS AND DISCUSSION

3.1 Rosemary (*Rosmarinus officinialis* L.)

The results of the experiment conducted to study the influence of different auxins on the rooting of rosemary cuttings are presented in Table 1. IBA treatment showed the best simulative effect on the rosemary cuttings because the height of cuttings (25.17 cm), number of rooted cuttings (46) and the rooting percentage (92%) were significantly the highest as compared to the other treatments, including the control and the commercial rooting product. The number of roots has not shown any significant difference among the treatments, while the cuttings treated with IBA and NAA have significantly shorter roots as compared to the other treatments and the control roots.

3.2 Sage (*Salvia officinialis* L.)

The experimental results for sage are presented in Table 2. The stimulation effect of applied auxins was lower for sage cuttings as compared to rosemary. The best rooting effect was reached with IBA (54%) and NAA (50%) treatments, although the longest roots were observed in the cuttings treated with commercial rooting product (5.60 cm) and control cuttings (5.25 cm). The highest mean height of cuttings was observed in the treatment with the commercial product (21.63 cm). Auxin treatments did not affect significantly the number of roots of sage cuttings.

3.3 Elderberry (*Sambucus nigra* L.)

The results of the examination of auxins influence on rooting of the elderberry cuttings during the vegetative propagation are shown in Table 3. The elderberry cuttings did not show any growth in height because they were leafless, consequently no photosynthesis during the propagation period. The shortest roots were registered for cuttings treated with NAA. The applied auxins have shown the lowest stimulation effect on rooting process in elderberry cuttings compared to rosemary and sage. The rooting percentage (14%) is the highest for the commercial product treatment and IAA treatment and the lowest for NAA treatment (6%).

Table 1. Rooting of rosemary cuttings (*Rosmarinus officinialis* L.) during vegetative propagation stimulated by auxins

	Cuttings at the experiment start		Cuttings after 100 days				
	Number	Height (cm)	Height (cm)	Number of rooted cuttings	Number of roots	Root length (cm)	Rooting (%)
K	50	15	18.95d	43b	21.00a	5.57a	86b
K1	50	15	21.52c	42b	23.00a	6.10a	84b
IAA	50	15	23.44b	38c	22.00a	5.10a	76c
IBA	50	15	25.17a	46a	15.33a	3.10b	92a
NAA	50	15	23.00b	39c	25.33a	2.80b	78c

Means within each column having different letters are significantly different according to Duncan's test at P=.05

Table 2. Rooting of sage (*Salvia officinialis* L.) during vegetative propagation stimulated by auxins

	Cuttings at the experiment start		Cuttings after 100 days				
	Number	Height (cm)	Height (cm)	Number of rooted cuttings	Number of roots	Root length (cm)	Rooting (%)
K	50	15	19.40b	20c	8.66a	5.25a	40c
K1	50	15	21.63a	19c	10.33a	5.60a	38c
IAA	50	15	19.41b	24b	12.33a	4.10b	48b
IBA	50	15	20.29b	27a	10.66a	3.10c	54a
NAA	50	15	19.48b	25a	9.00a	2.30c	50a

Means within each column having different letters are significantly different according to Duncan's test at P=.05

Table 3. Rooting of elderberry (*Sambucus nigra* L.) during vegetative propagation stimulated by auxins

	Cuttings at the experiment start		Cuttings after 100 days				
	Number	Height (cm)	Height (cm)	Number of rooted cuttings	Number of roots	Root length (cm)	Rooting (%)
K	50	15	15a	5ab	8.00a	3.88a	10.0ab
K1	50	15	15a	7a	8.66a	3.65a	14.0a
IAA	50	15	15a	7a	6.00a	3.66a	14.0a
IBA	50	15	15a	5ab	7.33a	3.68a	10.0ab
NAA	50	15	15a	3b	8.33a	2.3b	6.0b

Means within each column having different letters are significantly different according to Duncan's test at P=.05

Among the most important functions of plant hormones is controlling and coordinating cell division, growth and differentiation [18]. Auxins are well known plant hormones which are tightly connected to root development which is proven by genetic, molecular and cellular experiments [19-22]. The results of our research show that auxins IAA, IBA and NAA in concentration of only 5 ppm have stimulating effect on rooting of the rosemary, sage and elderberry cuttings.

The results of this study are in agreement with the results presented in scientific publication by other researchers. Application of rooting powder containing 0.5% IBA positively affected on sage and rosemary cutting growth and development, thus plants under auxin treatment had significantly higher values for plant height, root length, fresh weight of plant and dry weight of plant compared to control plants [11]. Application of IBA on rosemary cuttings in series of 0, 20, 40, 60, 80 and 100 ppm, showed stimulating impulse on number of roots formed with concentrations higher than 40 ppm [23]. Other authors [24-26] presented application of auxins in concentration of 100, 200, 300, up to 1000 ppm, and 1000, 3000 and 5000 ppm [27-32] for stimulation of rooting of species that is difficulty to root, where as a rule of thumb, the most medicinal and aromatic species are listed. Application of NAA, IBA and NAA in concentrations of 1000 ppm and 5000 ppm on *Melissa officinalis* L. stem cuttings

did not have apparent effect on rooting rate but they influenced the morphological characteristics of newly generated plants [30].

The effects of auxin hormones on rooting and plant development were researched in studies of other species. The effect of IAA, IBA and NAA hormones on rooting and morphological characteristics were researched in many species such as *Lilium artvinense* [27], *Prunus spp.* and *Pseudotsuga menziesii* [33], *Lilium martagon* [28], *Oryza sativa* [34], *Pisum sativum* [35], *Robinia pseudoacacia* [36], *Ficus benjamina* [29] and *Melissa officinalis* [30-31]. These studies show that, generally, auxins affect the rooting process which is in compliance with our results.

Still, a successful rooting of plant cuttings is depending not only on external application of auxins, but also on numerous external factors as soaking time in growth regulators, propagation time, growing media, propagation system [6,23,37-38].

Finally, the presented results give an important applicative point that weather conditions in late autumn to early winter in Republic of Macedonia are still favorable for vegetative propagation of medicinal and aromatic species under protected conditions without heating. Hence, this study is a solid basis for experimentation and application of growth regulators for vegetative propagation of many plant species during different weather seasons.

4. CONCLUSION

Application of auxins in concentrations of 5 ppm positively affected rosemary, sage and elderberry. IBA treatment showed the best rooting effect on rosemary and sage cuttings, 92% and 54% respectively. Elderberry cuttings gave the highest 14% of root formation when treated with the commercial product and IAA. Number of roots was not affected by the auxins treatments, while NAA treatment resulted in shortening of roots for the tree species under study. The results presented in this paper show that the vegetative propagation of medicinal and aromatic species might be stimulated by auxins utilization even in very low concentrations. It should be noted that further research shall be extended to more medicinal and aromatic plants with significant economic importance as well as an application of higher concentration of auxins. A reason more for extension of this research is the fact that soil and climate characteristics in Republic of Macedonia give many opportunities for production of high quality raw materials of medicinal and aromatic plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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