

THE EFFECT OF NATURAL BIOSTIMULATORS AND SLOW-
-DISINTEGRATING FERTILIZERS ON THE QUALITY OF ROSEMARY
SEEDLINGS (*Rosmarinus officinalis* L.)

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Abstract: In the present work the effect of natural biostimulators and different doses of slow disintegrating fertilizer on the quality of rosemary seedlings was studied. Rosemary seedlings were produced in containers, according to the »speedling system«. During the production of seedlings natural biostimulators *Megafol* and *Viva* and microbiological fertilizer *Slavol* were added. The applied biostimulators made a significant effect on the quality of rosemary seedlings. Different doses of the slow disintegrating fertilizer *Scotts (Osmocote Extact)* were applied (0, 1, 2, 3 and 4 g/l), which also produced a significant influence.

Key words: rosemary, seedling, natural biostimulators, slow disintegrating fertilizer.

I n t r o d u c t i o n

Rosemary (*Rosmarinus officinalis* L.) is a perennial evergreen bush, 1 to 2 meters high. It is mostly grown in the Mediterranean countries as a decorative, aromatic and seasoning plant (Westervelt, 2003). Rosemary leaf (*Rosmarini folium*) and etherial oil (*Rosmarini aetheroleum*) are applied in cosmetic and perfume industry (Kovačević, 2000). In folk medicine rosemary leaf is used as an ingredient of tea mixtures for baths (*pro balneo*) and improvement of blood circulation.

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In our country the predominant way of rosemary production is its propagation by cuttings. With increasing interest in this plant species there is a need for intensifying its production. Recently, in modern plant production, apart from the already known hormones, microbiological fertilizers, different biostimulators and bioregulators affecting plant development, have been used (Poincelot, 1993; Maksimović, et al., 2001; Nelson, 2003).

Biostimulators affect better seed germination (Yildirim et al., 2002, Jelačić et al., 2006a), and represent stimulators of plant biological activity, having simultaneous effects on the plant and on the soil microflora.

The use of slow-disintegrating fertilizers in the production of seedlings in containers (*speedling system*) and pots (*pot system*) has so far found its application in the production of pot decorative plants (Belger et al., 1989). One of the most important advantages of the use of these fertilizers is almost complete satisfaction of the plants' requirements for mineral nutrients in a certain period of time by a single use of fertilizers, which is achieved by mixing fertilizers with the substrate prior to planting (Hanić, 2000).

The aim of this work was to observe the effect of different natural biostimulators and slow disintegrating fertilizers on the quality of rosemary seedlings.

Material and Methods

The researches were done during of 2006 in the glass house of the Faculty of Agriculture, Belgrade. Rosemary seedlings were produced in polystyrene containers, with the capacity of cells (76 cm³). A commercial substrate composed of: white peat (40%) and mixed peat (60%) with the content of salt of around 0.9 g/l and pH value of 5.3 to 5.8 was used as a substrate for seed sowing and production of seedlings. Rosemary seed sowing in the containers was conducted on February 22, 2006. One plant was left in each cell after germination. When conducting the experiment, natural biostimulators and different doses of slow-disintegrating fertilizer were added to plants.

The experiment was divided into two parts; the following treatments (biostimulators) were applied in the first part of the experiment: Control group (where no biostimulator was applied), biostimulators *Megafol*, *Viva* and microbiological fertilizer *Slavol*.

Biostimulator *Megafol* was applied through the leaf (foliary) in the dose of 1.4 ml/l of water, while *Viva* was applied to the soil, in the dose of 3ml/l of water. Biostimulators were added to the plants from the 2 pairs of permanent leaves phase, in 12 days intervals.

Natural bio-organic fertilizer *Slavol* consisting of nitrogen-fixing bacteria (*Azotobacter chroococum* and *A. Vinelandi*) and phosphomineralizers (*Bacillus megaterium* and *B. subtilis*) was also applied. *Slavol* was applied to the soil in the dose of 5 ml/l of water in intervals of 7 days.

In the second part of the experiment different doses of slow disintegrating fertilizers (treatments) were used in the following variants: control (without fertilizer), 1 g/l, 2 g/l, 3 g/l and 4 g/l of substrate. *Scotts (Osmocote Exact)* slow disintegrating fertilizer, with the formula 15:9:9:MgO+Me was applied. Fertilizer was added to the plants in the 2 pairs of leaves stage.

T a b. 1. - Chemical composition of *Megafol* (A.O.A.C. *Sampling procedure* 1990)

Total Aminoacids	Nitrogen (N)		Potassium oxide (K ₂ O)	Organic Carbon
	Total	Organic		
28.0 %	4.5%	4.5 %	2.9 %	15.0 %

T a b. 2. - Chemical composition of biostimulator *Viva* (A.O.A.C. *Sampling procedure* 1990)

Organic Matter OM	Protein, Peptides, Aminoacids	Polisaccharides	Humic acids	Potassium oxide (K ₂ O)	Vitamin complex
12.0 %	12.8 %	2.0%	2.9 %	3.0 %	0.18 %

T a b. 3. - Physical characteristics of *Megafol* and *Viva* biostimulators (A.O.A.C. *S. Procedure* 1990)

	<i>Megafol</i>	<i>Viva</i>
Apparance	Liquid	Liquid
Colour	Brown	Brown
Conductivity (g/cm ³) 20°C	1.26	1.21
pH (1% aquatic solution)	7.6	8.6
Solubility 1 % (mS/cm) 18°C	0.380	0.195
Freezing point	-5°C	-1°C

During the period of seedling production standard care measures were used: watering, shading and ventilation. Seedling production lasted until June 25, 2006. 31 plants from each variant were taken by random sampling.

The following parameters of seedling quality were analyzed: height (cm), number of branches, fresh and dry plant mass (g), root length (cm), fresh and dry root mass (g).

The results of the experiments were presented through key descriptive statistics indicators (variation interval, arithmetical mean and its standard error and variation coefficient). The equality of variances of analyzed treatments was tested by Levene's test and in accordance with the obtained result statistical significance of differences between average values for each tested parameter was tested according to variance analysis method (ANOVA), Kruskal-Wallis' and mediane test.

Results and Discussion

The Effect of Natural Biostimulators on the Quality of Rosemary Seedlings

Research results (Tab. 4) show that with the use of *Viva* biostimulators and microbiological fertilizer *Slavol* highest average values of height of the rosemary

plant were obtained (21.284 and 21.153 cm). The applied biostimulator *Megafol* did not exhibit any significant differences compared to the control variant. Variation coefficient for all tested treatments is relatively uniform (7.93-9.67%). Positive effect of *Viva* biostimulator was also determined in the research conducted with some other medicinal and aromatic species (Beatović et al., 2006).

With the use of *Viva* and *Megafol* highest production of branches per plant was achieved (8.469). For this analyzed parameter of the seedling quality a high variation coefficient was recorded (27.81-38.44%). The highest value of plant mass (both fresh and dry) was obtained through the use of *Viva* (1.694 g and 0.414 g) and microbiological fertilizer *Slavol* (1.669 g and 0.396 g). The lowest variation coefficient for the tested parameters of fresh and dry plant mass was obtained with the use of *Slavol*.

Lower average values of the length of root compared to the control variant were used where natural biostimulators and microbiological fertilizers were applied (Tab. 4).

Microbiological fertilizer *Slavol* showed the greatest effect on the root mass (fresh 0.872 g and dry 0.072 g). Also, the lowest values of variation coefficients were obtained for the tested parameters.

According to the results of Levene's test, variances between the observed groups of plants for the following characteristics were homogenous: the height of plants, fresh plant mass and root length (Tab. 5). Bearing this in mind, the significance of average values of all groups for these characteristics was tested with parametric variance analysis model, and for the other tested characteristics with non-parametric Kruskal-Wallis' test and median test.

The analyzed biostimulators induce statistically highly significant differences between average values of parameters: plant height, number of branches and fresh plant mass. However, according to the median test, the difference in the average number of branches is statistically significant, but not highly significant as with other tests.

The use of biostimulators does not change statistically the average length of root and fresh root mass. Only based on the median test we can conclude that the dry plant mass and the dry root mass do not depend on the applied biostimulator (Tab. 5), while according to the results of ANOVA and Kruskal-Wallis' test this effect has statistical significance.

The calculated F values (Tab. 5) for the characteristics of the length and mass of fresh root were less than 1, which means that the data in the samples were less homogenous than between the samples, so the parametric ANOVA model cannot be used. That is why the length of root parameter should be tested with non-parametric tests, notwithstanding the homogeneity of sample variances.

T a b. 4. - Key descriptive statistics indicators for the tested parameters of rosemary seedling quality with the use of biostimulators and critical values of LSD test

Parameters	Biostimulators	Interval of variation I_v	Arithmetical mean $\bar{X} \mp S_{\bar{x}}$	Median M_e	Coefficient of variation C_v (%)
Plant height (cm)	Test	15.1 - 20.9	18.472 \mp 0.277	18.5	8.49
	<i>Megafol</i>	16.0 - 21.8	18.881 \mp 0.265	18.6	7.93
	<i>Viva</i>	17.5 - 25.7	21.284 \mp 0.364	21.5	9.67
	<i>Slavol</i>	18.0 - 23.5	21.153 \mp 0.308	21.2	8.23
		LSD 0.05 0.01	0.883 1.189		
Number of branches	Test	4 - 12	6.436 \mp 0.438	6	38.44
	<i>Megafol</i>	6 - 14	8.469 \mp 0.462	7	30.88
	<i>Viva</i>	6 - 13	8.469 \mp 0.416	8	27.81
	<i>Slavol</i>	5 - 14	8.188 \mp 0.5613	9	2836
		LSD 0.05 0.01	0.720 0.970		
Fresh plant mass (g)	Test	0.775 - 2.020	1.289 \mp 0.060	1.302	26.35
	<i>Megafol</i>	0.589 - 2.059	1.405 \mp 0.072	1.455	29.11
	<i>Viva</i>	0.973 - 2.569	1.694 \mp 0.078	1.638	26.17
	<i>Slavol</i>	1.179 - 2.164	1.569 \mp 0.051	1.533	18.31
		LSD 0.05 0.01	0.191 0.258		
Root length (cm)	Test	11.0 - 19.6	14.084 \mp 0.422	13.35	16.93
	<i>Megafol</i>	10.5 - 18.0	13.209 \mp 0.344	12.65	14.72
	<i>Viva</i>	9.5 - 18.1	13.853 \mp 0.506	14.75	20.67
	<i>Slavol</i>	9.0 - 18.0	13.656 \mp 0.444	13.50	18.40
		LSD 0.05 0.01	1.250 1.683		
Fresh root mass (g)	Test	0.336 - 1.167	0.798 \mp 0.034	0.802	24.26
	<i>Megafol</i>	0.464 - 1.420	0.837 \mp 0.044	0.833	29.51
	<i>Viva</i>	0.478 - 1.320	0.838 \mp 0.040	0.760	26.83
	<i>Slavol</i>	0.459 - 1.145	0.872 \mp 0.032	0.881	20.55
		LSD 0.05 0.01	0.109 0.146		
Dry plant mass (g)	Test	0.226 - 0.528	0.347 \mp 0.016	0.324	26.87
	<i>Megafol</i>	0.254 - 0.543	0.380 \mp 0.017	0.328	25.65
	<i>Viva</i>	0.158 - 0.601	0.414 \mp 0.021	0.416	28.41
	<i>Slavol</i>	0.274 - 0.557	0.396 \mp 0.012	0.397	17.62
		LSD 0.05 0.01	0.049 0.066		
Dry root mass (g)	Test	0.045 - 0.091	0.062 \mp 0.002	0.060	19.86
	<i>Megafol</i>	0.034 - 0.102	0.065 \mp 0.003	0.068	29.90
	<i>Viva</i>	0.047 - 0.094	0.070 \mp 0.002	0.071	19.17
	<i>Slavol</i>	0.054 - 0.090	0.072 \mp 0.002	0.071	13.45
		LSD 0.05 0.01	0.007 0.010		

Tab. 5. - The results of Levene's variance homogeneity test, parametric ANOVA test and non-parametric tests for the use of biostimulators

Parameters	Levene's test		ANOVA		Kruskal-Wallis ANOVA		Median test	
	F	p	F	p	H	p	χ^2	p
Plant height (cm)	0.931	0.428	23.369	0.000	-	-	-	-
Number of lbranch	3.573	0.016	4.007	0.009	12.948	0.005	8.633	0.035
Fresh plant mass (g)	2.139	0.099	7.264	0.000	-	-	-	-
Root lenght (cm)	2.049	0.110	0.655	0.582	1.842	0.606	1.250	0.741
Fresh root mass (g)	3.806	0.012	0.736	0.532	2.887	0.409	4.345	0.227
Dry plant mass (g)	3.606	0.014	2.788	0.043	8.117	0.044	6.845	0.077
Dry root mass (g)	8.485	0.000	3.199	0.026	9.198	0.027	4.250	0.236

p<0,05 (*) the difference is significant

p<0,0 (1**) the difference is highly significant

The Effect of Different Dosages of Slow Disintegrating Fertilizer on the Quality of Rosemary Seedlings

Highest average values (22.209 cm) for the height of plant (Tab. 6) were obtained with the dose of 3 g/l of slow disintegrating fertilizer. Variation coefficient for the analyzed parameter ranges from 5% to 10%. The dose of 4 g/l had the greatest effect on the production of branches per plant (11.75). The highest variation coefficient for the number of branches was recorded in the control variant (38.44%).

The highest value of fresh plant mass (2.22 g) was obtained with the use of the 3 g/l dose. The applied dose of 4 g/l failed to exhibit the expected effect on the fresh plant mass, but it exhibited lower average values compared to the dose of 2 g/l. Based on these results, we can conclude that the dose of 3 g/l was a limiting quantity for the fresh plant mass.

The dose of 3 g/l had also the greatest effect on the fresh root mass (0.931 g). By increasing the dose (g/l) of slow disintegrating fertilizer, lower average values of root mass were obtained compared to the applied doses of 2 and 3 g/l.

The effect of the use of fertilizer was also verified in the researches conducted by Boye et al., 1991; Serrano et al., 2001. Likewise, a positive effect of different doses of slow disintegrating fertilizers was obtained in the research in the production of seedlings and other medicinal plant species: basil, garden balm and sage (Jelačić et al., 2006b).

The applied 3 g/l dose of slow disintegrating fertilizer as the optimal dose for rosemary was verified in the researches conducted with other medicinal and aromatic species (Beatović et al., 2007).

T a b. 6. - Key descriptive statistics indicators for the tested parameters of rosemary seedling quality with the use of slow disintegrating fertilizers and critical values of LSD test

Parameters	Dosage of slow-disintegrating fertilizers	Interval of variation I_v	Arithmetical mean $\bar{X} \pm \text{Standard error}$ $\bar{X} \pm S_x$	Median M_e	Coefficient of variation C_v (%)
Plant height (cm)	0 (test)	15.1 - 20.9	18.472 \pm 0.277	18.50	8.49
	1 g/l	17.0 - 21.1	19.100 \pm 0.182	19.15	5.38
	2 g/l	17.5 - 26.1	21.619 \pm 0.412	21.55	10.78
	3 g/l	18.0 - 27.2	22.209 \pm 0.393	22.50	10.01
	4 g/l	18.5 - 23.5	21.081 \pm 0.272	21.60	7.29
LSD			0.05 0.01	0.921 1.240	
Number of branches	0 (test)	4 - 12	6.436 \pm 0.438	6.0	38.44
	1 g/l	5 - 12	8.219 \pm 0.300	8.0	20.67
	2 g/l	6 - 14	9.125 \pm 0.326	8.5	20.22
	3 g/l	8 - 14	10.688 \pm 0.267	10.0	14.15
	4 g/l	8 - 16	11.750 \pm 0.486	12.5	23.38
LSD			0.05 0.01	1.077 1.450	
Fresh plant mass (g)	0 (test)	0.775 - 2.020	1.289 \pm 0.060	1.302	26.35
	1 g/l	0.849 - 2.301	1.608 \pm 0.061	1.600	21.54
	2 g/l	1.620 - 2.760	2.169 \pm 0.057	2.106	14.98
	3 g/l	1.440 - 3.274	2.220 \pm 0.088	2.111	22.30
	4 g/l	1.145 - 3.018	2.031 \pm 0.094	2.023	26.23
LSD			0.05 0.01	0.213 0.287	
Root length (cm)	0 (test)	11.0 - 19.6	14.084 \pm 0.422	13.35	16.93
	1 g/l	10.2 - 17.4	13.578 \pm 0.364	13.25	15.17
	2 g/l	10.5 - 19.1	14.788 \pm 0.438	14.30	16.76
	3 g/l	9.0 - 19.0	12.819 \pm 0.496	12.55	21.88
	4 g/l	9.0 - 20.0	13.134 \pm 0.452	12.05	19.45
LSD			0.05 0.01	1.257 1.693	
Fresh root mass (g)	0 (test)	0.336 - 1.167	0.798 \pm 0.034	0.802	24.26
	1 g/l	0.698 - 1.078	0.846 \pm 0.016	0.841	10.37
	2 g/l	0.563 - 1.151	0.876 \pm 0.029	0.914	18.71
	3 g/l	0.605 - 1.536	0.931 \pm 0.044	0.892	26.88
	4 g/l	0.602 - 1.224	0.854 \pm 0.028	0.816	18.84
LSD			0.05 0.01	0.091 0.123	
Dry plant mass (g)	0 (test)	0.226 - 0.528	0.347 \pm 0.016	0.324	26.87
	1 g/l	0.326 - 0.518	0.407 \pm 0.008	0.406	11.28
	2 g/l	0.435 - 0.598	0.499 \pm 0.007	0.494	7.44
	3 g/l	0.335 - 0.663	0.525 \pm 0.013	0.518	13.95
	4 g/l	0.226 - 0.733	0.455 \pm 0.018	0.462	22.49
LSD			0.05 0.01	0.038 0.051	
Dry root mass (g)	0 (test)	0.045 - 0.091	0.062 \pm 0.002	0.060	19.86
	1 g/l	0.042 - 0.087	0.069 \pm 0.002	0.070	16.90
	2 g/l	0.044 - 0.131	0.082 \pm 0.004	0.084	28.05
	3 g/l	0.053 - 0.115	0.085 \pm 0.003	0.089	18.89
	4 g/l	0.040 - 0.106	0.066 \pm 0.003	0.062	24.51
LSD			0.05 0.01	0.008 0.011	

The results of Levene's test (Tab. 7) show that the variances between the observed groups of plants are homogeneous only for the fresh root mass parameter, so the significance of differences between average values of all groups for this characteristic was tested with parametric variance analysis model and with non-parametric Kruskal-Wallis' test and median test with all other tested parameters. The tested fertilizer doses induce statistically highly significant differences between average values only for the length of root parameter.

T a b. 7. - The results of Levene's variance homogeneity test, parametric ANOVA test and non-parametric tests for the use of slow-disintegrating fertilizers

Parameters	Levene's test		ANOVA		Kruskal-Wallis ANOVA		Median test	
	F	p	F	p	H	p	χ^2	p
Plant height (cm)	5.190	0.001	26.044	0.000	63.499	0.000	48.018	0.000
Number of branches	5.664	0.000	31.112	0.000	69.364	0.000	57.579	0.000
Fresh plant mass (g)	2.711	0.032	29.598	0.000	73.003	0.000	68.750	0.000
Root length (cm)	6.530	0.000	2.354	0.056	4.271	0.371	2.750	0.600
Fresh root mass (g)	0.805	0.523	3.203	0.015	-	-	-	-
Dry plant mass (g)	9.771	0.000	29.007	0.000	69.017	0.000	61.000	0.000
Dry root mass (g)	3.674	0.007	12.307	0.000	37.524	0.000	23.090	0.000

p<0,05 (*) the difference is significant

p<0,0 (1**) the difference is highly significant

Conclusion

The research results lead us to conclude that the use of natural biostimulators *Viva* and *Megafol* and microbiological fertilizer *Slavol* in the production of rosemary seedlings is significant and positive. By applying biostimulators, we can obtain good quality seedlings. The best results were obtained by the use of biostimulator *Viva*.

Also, the application of different doses of slow disintegrating fertilizers had significant influence on the quality of rosemary seedlings. The best results were obtained with the dose of 3 g/l. The results of this research point to a highly significant influence of their application in the production of rosemary seedlings.

The obtained research results have shown that this new approach in the production of rosemary seedlings with the use of natural biostimulators and slow-disintegrating fertilizers is a significant improvement in the production technology used to date.

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UTICAJ PRIRODNIH BIOSTIMULATORA I SPORORAZLAGAJUĆEG
ĐUBRIVA NA KVALITET RASADA RUZMARINA
(*Rosmarinus officinalis* L.)

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R e z i m e

U radu je ispitivan uticaj prirodnih biostimulatora i različitih doza spororazlagajućeg đubriva na kvalitet rasada ruzmarina. Rasad ruzmarina je proizveden u kontejnerima po »*speedling sistemu*«. Tokom proizvodnje rasada dodavani su prirodni biostimulatori *Megafol* i *Viva* i mikrobiološko đubrivo *Slavol*. Upotrebljeni biostimulatori su ostvarili značajan efekat na kvalitet rasada ruzmarina. Korišćene su i različite doze spororazlagajućeg đubriva (0, 1, 2, 3 i 4 g/l) *Scotts (Osmocote Extact)*, koje su također ostvarile značajan uticaj.

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Projekat TR-6900B: "Primena spororazlagajućih đubriva i prirodnih biostimulatora u komercijalnoj proizvodnji rasada cveća, lekovitog, aromatičnog i začinskog bilja". Sredstva za realizaciju projekta obezbedilo Ministarstvo nauke i životne sredine Republike Srbije.