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RESEARCH ON THE USE OF BIOFERTILIZERS IN MULBERRY CULTURE AND SILKWORM REARING

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Abstract

One of the objectives of the sericultural research is the reducing of research field was the reducing of chemical fertilizers quantity by applying some ecological agricultural practices, among which it can be mentioned, next to using extra-radicular fertilizing, also the biofertilizers of vesicular-arbuscular mycorrhize (VAM) type, in the form of commercial products obtained by biotechnologies of high biological performance. The biofertilization of VAM type aims to reduce or eliminate the chemical fertilization, the mulberry being a plant with a high consumption of mineral elements. Also, this type of mycorrhize stimulates the plants growing and development, having a role in soil remediation and nutrition improvement from soil-plant system.

Key words: *mycorrhize, mulberry, silkworm.*

INTRODUCTION

The sustainable agriculture implies the using of agricultural practices which determine the increasing of useful productive capacity for all the agro-ecosystems by making capital out of the ecopedological and biological factors, in the conditions of maintaining the balance of respective agro-ecosystems. The sustainable agriculture is based on economic-ecological principles of avoidance of any form of environment pollution and of making profits (Bethlenfalvay & Schüepp, 1994; Bhale et al., 2018).
The sericiculture constitutes an agricultural activity which by its biotechnological characteristics can meet the requirements imposed by the practice of sustainable agriculture, the silkworms being extremely sensitive to a multitude of pollution factors.
The studies aimed the reducing of the quantities of chemical substances used in the technologies of mulberry exploitation, in particular for fertilization, by the approach of some alternative agricultural practices, among which lately it is mentioned the utilization of biofertilizers of vesicular-arbuscular mycorrhize (VAM) type, in the form of commercial products obtained by biotechnologies of high biological performance (Chikkaswamy, 2015).
As main economic effect, the emphasis of vesicular-arbuscular mycorrhize (VAM) presence in mulberry varieties created the premises of establishing the optimum doses of Endorize SOL and NPK chemical fertilizers, in accordance with the soil fertilization, reducing in this way the doses of chemical fetilizers recommended in the classic technologies of mulberry plantaions, and thus the technological costs (Babu et al., 2013; Baqual, 2013; Moorthi et al., 2016). Also, this type of mycorrhize stimulates the plants growing and development, having a role in soil bioremediation and nutrition improvement from soil-plant system (Pavankumar et al., 2020).
The mycorrhizal associations established those biofertilizers using, increase the absorption of mineral elements from soil and improve the poor absorption of some nutrients (Kumaresan et al., 2010).
The use of vesicular-arbuscular mycorrhize (VAM) determines the reducing of chemical fertilizers, in particular of those with phosphorus, stimulating in the same time the absorption of secondary mineral elements (Mg, Ca, S) and of microelements (B, Cu, Zn, Fe). The presence of vesicular-arbuscular mycorrhize is conditioned by the soil type, the host plant

species, the environment conditions and the agricultural practices used (Chakraborty et al., 2015; Greiss et al., 2003; Petkov et al., 2006).
The mycorrhize is a symbiotic association between the plants'roots and fungous, having a major role in many fundamental functions of plants, of which the most important being the nutrition with mineral elements from soil and the resistance to the environment conditions stress (Begum et al., 2019; Ghulam Hassan Dar & Pankaj Dunge, 2020). As the relationship between soil and plant is important for the agricultural production, thus the mycorrhize represents a soil-fungous-plant relationship of great interest for the development of new strategies in sustainable agriculture, the mycorrhize enabling the reducing of the chemical fertilizers quantity, also of pesticides and thus minimizing their negative impact on the environment (Abbasi et al., 2015).
The researches targeted the method of using Endorize SOL biofertilizer, the doses and its influence on technological parameters in mulberry plants (saplings and adult plants) utilized in silkworm feeding and on biotechnological parameters in silkworms.

MATERIALS AND METHODS

The method of vesicular-arbuscular mycorrhize using in mulberry cultivation proposed for homologated constituted the primary documentation for the development of testing methodology of biological fertilizers for mulberry cultivation. The product Endorize SOL, utilized as biofertilizer in the project, contains the beneficial flora of vesicular-arbuscular mycorrhize from Glomus genus - *Glomus mosseae*, *Glomus fasciculatum*, *Glomus occulatum*, *Glomus heterogama*, *Glomus microcarpum* și *Sclerocystis* sp.
The experimental device in field was realized in an uniform plot, the vegetal biological material being represented by cuttings and mature mulberry plants fertilized with Endorize SOL product during the period of starting in vegetation (April) in order to establish the mycorrhizal associations in 40-50 days, corresponding to the phenophase of intensive growth of shoots, when the consumption of nutrients from soil is maximum.
The experimental variants were the following:
V_0 - control - mature mulberry plants - unfertilized;
V_1 - mature mulberry plants - NPK fertilized, dose 240: 120: 120 kg a.s./ha;
V_2 - mature mulberry plants - fertilized with Endorize SOL, dose 25 mg/plant.
The determination of the production of mulberry leaves/bush for each experimental variant was made by harvesting the whole mulberry leaves from 3 specimens/variant and the experssion of production by the quantity of leaves per bush. Another agro-productive parameter was the weight of 25 leaves with/without petiole, as average sample for each variant.
The silkworm rearings were made in two series, spring - summer, during June, 1st - July, 5th, and respectively summer - autumn, during August, 15th - September, 15th. For the first rearing series the biological material was represented by silkworms from the simple hybrid Select, and for the second series, silkworms from Băneasa 75 (B75) race and from simple hybrid Select, were used.
Each variant of rearing had 3 repetitions obtained by the distribution of larvae resulted from 1 g silkworm eggs. The feeding was made with mulberry leaves harvested according to the experimental variants and given *ad libitum* to silkworms until cocoons have been formed.
The methodology to determine the influence of Endorize SOL product on the rearing parameters consisted in the individual weighing of 25 raw silk cocoons for each variant of rearing and the average value experssion for raw silk cocoon weight and shell cocoon weight (raw silk cocoon weight after extracting the chrysalis).
For the IIIrd and Vth ages were collected samples for veterinary health testing of silkworms from the experimental variants fertilized with Endorize SOL product.

RESULTS AND DISCUSSIONS

Results on the use of vesicular-arbuscular mycorrhize in producing mulberry cuttings
The mulberry cuttings inoculated with vesicular-arbuscular mycorrhize constitute mulberry planting material which respects the technical characteristics of quality and certification of mulberry rooted cuttings concerning the somatic parameters - root, stem, crown - and the biological authenticity of 100%

of the initial material. The mulberry planting material represented by mulberry cuttings inoculated with vesicular-arbuscular mycorrhize allows the application of some doses of chemical fertilizers reduced at ½ of the recommended doses, decreasing the risks of soil and groundwater pollution. These inoculated cuttings are intended for establishment of mulberry plantations for sericultural exploitation, respectively silkworm rearings and for establishment of plantations of grafted branches, as source of initial biological material. After using Endorize SOL product for mulberry cuttings' inoculation, it was obtained an increase of rooting percentage in Ucraina 107, China 32 and Olteni varieties, in proportion of 64.5-76%, compared to the control of each variety, fertilized according to the traditional technology. Also stands out the improvement of cuttings' quality parameters regarding the number of issued roots, the length of issued roots, the length of cuttings stems and the thickness at bundle, compared to the control obtained in the conditions of current technology application. For the elaboration of the reference for the method of using Endorize Sol product, there were compared the parameters provided in the technical regulations regarding the mulberry planting material for cuttings and rooted marcots, with the results obtained experimentally (Table 1).

The ecopedological data regarding the influence of Endorize SOL product and the NPK chemical fertilization on the development of the radicular system of cuttings are presented in Table 2.

Table 1. Biotechnological data in mulberry cuttings with VAM type mycorrhize

Mulberry variety	Standard stem length (cm)	Standard roots no./ length (cm)	Experiment results dtem length (cm)	Experiment results roots no./ length (cm)
Kokuso 21 mycorrhize	50	5 /10	131.33	5.55/13.00
Kokuso 21 NPK fertilized	50	5 /10	172.00	5.00/16.67
Ucraina 107 mycorrhize	50	5 /10	175.33	6.67 /18.50
Ucraina 107 NPK fertilized	50	5 /10	160.00	2.33/13.50
Ichinose mycorrhize	50	5 /10	158.67	7.67/15.00
Ichinose NPK fertilized	50	5 /10	186.67	4.33 / 8.00
China 32 mycorrhize	50	5 /10	148.67	4.40/18.00
China 32 NPK fertilized	50	5/10	142.67	5.00/14.50
Olteni mycorrhize	50	5 /10	170.00	4.33/16.00
Olteni NPK fertilized	50	5 /10	171.67	5.67/20.00
Ken Mochi - Control	50	5 /10	160.33	3.00/21.33

Table 2. The influence of biofertilizer Endorize SOL and of chemical fertilization on the development of cuttings radicular system

Variety/cutting's number	Length of cutting (cm)	Length of main root (cm)	Number of secondary roots	Total number of active roots	Total weight of active roots (g)
Kokuso 21 variety with mycorrhize					
- cutting no. 1	116	11	6	36	0.34
- cutting no. 2	98	-	5	43	0.92
- cutting no. 3	180	15	5	56	0.97
Average	**131.33**	**13.00**	**5.55**	**45**	**0.74**
Kokuso 21 variety NPK fertilized					
- cutting no. 1	160	12	7	83	3.08
- cutting no. 2	156	30	2	48	3.35
- cutting no. 3	200	14	6	92	4.82
Average	**172**	**18.67**	**5**	**74.33**	**3.75**
Ucraina 107 with mycorrhize					
- cutting no. 1	220	20	8	88	1.270
- cutting no. 2	200	17	4	46	0.870
- cutting no. 3	106	13	8	86	0.840
Average	**175.33**	**18.50**	**6.67**	**73.33**	**0.890**

Ucraina 107 variety NPK fertilized					
- cutting no. 1	170	11	2	38	1.87
- cutting no. 2	150	-	2	29	0.83
- cutting no. 3	160	16	3	35	0.77
Average	**160**	**13.50**	**2.33**	**34**	**1.16**
Ichinose variety with mycorrhize					
- cutting no. 1	220	24	13	93	1.16
- cutting no. 2	140	6	3	28	0.16
- cutting no. 3	116	7	7	38	0.34
Average	**158.67**	**15**	**7.67**	**53**	**0.55**
Ichinose variety NPK fertilized					
- cutting no. 1	165	-	6	66	0.83
- cutting no. 2	185	10	3	38	0.61
- cutting no. 3	210	6	4	50	0.69
Average	**186.67**	**8**	**4.33**	**50**	**0.71**
China 32 variety with mycorrhize					
- cutting no. 1	232	20	7	60	1.26
- cutting no. 2	117	16	3	24	0.22
- cutting no. 3	98	-	2	12	0.16
Average	**148.33**	**18**	**4.4**	**32**	**0.88**
China 32 variety NPK fertilized					
- cutting no. 1	170	22	10	78	2.57
- cutting no. 2	160	7	2	38	1.15
- cutting no. 3	98	-	3	27	0.19
Average	**142.67**	**14.5**	**5**	**47.67**	**1.30**
Olteni variety with mycorrhize					
- cutting no. 1	190	9	9	102	2.02
- cutting no. 2	160	21	3	32	1.14
- cutting no. 3	160	18	1	25	0.66
Average	**170**	**16**	**4.33**	**53**	**1.27**
Olteni variety NPK fertilized					
- cutting no. 1	200	10	4	48	1.32
- cutting no. 2	160	30	7	80	1.52
- cutting no. 3	155	-	6	32	0.92
Average	**171.67**	**20**	**5.67**	**53.33**	**1.25**
Ken Mochi variety Control					
- cutting no. 1	200	21	1	65	1.45
- cutting no. 2	100	22	-	28	0.19
- cutting no. 3	190	21	5	120	2.18
Average	**163.33**	**21.33**	**3**	**71**	**1.27**

Table 3. The agroproductive parameters specific to mulberry plantations for silkworm rearing

Experimental variant	Leaves production (g/bush)	Difference to the control (g/bush)	Weight of 25 mulberry leaves without petiole (g)	Difference from the control (g)	The unitary production of mulberry leaves (kg/ha)	Difference from the control (kg/ha)
V_0 unfertilized control	1466	-	59.0	-	11728	-
V_1 chemical fertilization	1770	+304	60.0	+1.0	14160	+2432
V_2 Endorize SOL fertilization	1873	+407	60.0	+1.0	14984	+3256

Table 4. The influence of chemical fertilization of NPK type and of biofertilization with Endorize SOL product on the biotechnological parameters of silk cocoons *Bombyx mori* L. sp.

Experimental variant	I^st Series - Select hybrid				II^nd Series - B75 race				II^nd Series - Select hybrid			
	Raw cocoon weight (g)	Difference from the control (g)	Shell cocoon weight (g)	Difference from the control (g)	Raw cocoon weight (g)	Difference from the control (g)	Shell cocoon weight (g)	Difference from the control (g)	Raw cocoon weight (g)	Difference from the control (g)	Shell cocoon weight (g)	Difference from the control (g)
V_0 unfertilized control	1.89	-	0.379	-	0.960	-	0.229	-	1.031	-	0.156	-
V_1 chemical fertilization	2.01	+ 0.12	0.523	+0.144	1.184	+0.224	0.243	+0.016	1.3069	+0.2759	0.371	+0.115
V_2 Endorize SOL fertilization	1.98	+0.09	0.463	+0.084	1.148	+0.188	0.235	+0.006	1.099	+0.068	0.240	+0.084

It stands out the developing in vegetation of the cuttings, the values of inoculated cuttings length exceeding 2-3 times the standard length of cuttings. However, the varieties China 32 and Olteni presented values below the standard for the number of roots. The control used to compare varieties, Ken Mochi, does not meet the standard for the number of roots, such that the cuttings of this variety, along with the 2 others mentioned varieties, will be replanted in the replanted field, to develop at standard parameters.

Results on the use of vesicular-arbuscular mycorrhize mature mulberry plants for silkworm rearing

The results obtained in the production determinations are presented in Table 3.

The experimental data show the positive values of the agroproductive parameters specific to mulberry plantations exploited for silkworm rearing:

- the production of leaves per bush has the maximum value of 1873 g/bush in the variant fertilized with Endorize SOL product;
- the weight of 25 leaves without petiole had values between 59-60 g, specific to Ucraina 107 variety in all variants;
- the production of leaves per hectare registers values significantly positive higher than the control, with 3256 kg/ha in the variant fertilized with Endorize SOL and with 2432 kg/ha in the variant with chemical fertilizers.

Results on the influence of the biofertilizătion with Endorize SOL product on the biotechnological parameters of silk cocoons

The biometric determinations highlighted the following aspects (Table 4):

- normal values regarding the raw cocoon weight for the Ist series of rearing, higher than the IInd series, and among the fertilized variants were observed positive diferences compared to the control, the highest value registering in the chemical fertilized variant, of 2.01 g/raw cocoon, but close in value to the variant fertilized with Endorize SOL product, of 1.98 g/raw cocoon;
- the values regarding the shell cocoon weight and respectively the silk percentage indicate the same maximum values in the chemical fertilized variant, of 0.523 g/cocoon, close in value to the variant fertilized with Endorize SOL product, of 0.463 g/cocoon; the differences from the control in the two fertilized variants, of 0.144 g and respectively 0.084 g for the Select hybrid, are close to the values of the same hybrid in the IInd series, of 0.115 g and respectively 0.084 g;
- in the IInd series of rearing the maximum values are registred for the raw cocoon weight in Select hybrid, compared to B75 race, in both fertilized variants;
- in the IInd series the shell cocoon weight presents higher values in the fertilized variants in Select hybrid, compared to B75 race, and the control presents higher values in B75 race compared to Select hybrid, which indicate a better use of the mulberry leaves from the fertilized variants by the silkworms of Select hybrid;
- comparing the data in both aspects, the cocon weight and the shell cocoon weight, in both rearing series the variant fertilized with Endorize SOL product presents positive values compared to the control, lower than the NPK fertilized variant, but the differences are not significant.

According to the results of veterinary health testing of silkworms in the rearing variant with leaves harvested from the varieties fertilized with Endorize SOL product, the following characteristics of health surveillance became evident:

- uniformly developed larvae, without anatomical-pathological changes;
- negative virological examination for polyhedral crystals in silkworms from B75 race and Select hybrid;
- mycological examination – sterile cultures in both samples;
- negative parasitological examination for *Nosema bombycis* in silkworms from B75 race and Select hybrid.

CONCLUSIONS

The fertilization with Endorize SOL bioproduct does not change the agro-productive parameters in mature mulberry plants and silkworms *Bombyx mori* sp., compared to the NPK chemical fertilization, what influences positively the sericultural economic results by reducing the production costs related to fertilization, having beneficial effects of ecological protection on soil.

The technological parameters of the silk cocoons obtained in the variant fertilized with Endorize SOL product presents positive values compared to the unfertilized control.

For the variant of fertilization with Endorize SOL product, the analysis bulletin concludes negative bacteriological, mycological and parasitological examination for the pathogens specific to silkworms.

The mycorrhize technology has a great potential of application in order to improve the productivity and to reduce the environmental problems associated with the excessive use of pesticides.

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