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Experimental results concerning the biofertilization of mulberry cuttings with Endorize sol product – 2002

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Abstract

The sustainable agriculture promoted at the present time in our country includes the utilization of some agricultural techniques in order to increase the usefully productive capacity of all agrosystems, by developing ecopedological and biological factors, in the conditions of keeping the natural balance.

The sustainable agriculture is based on economical-ecological principles of avoiding any form of pollution, in the conditions of obtaining benefits.

The sericulture is an agricultural activity which, due to its biotechnological characteristics, can respond to the sustainable agriculture standards.

The aim of these researches performed in the frame of the Project "The utilization of vesicular-arbuscular mycorrhizas in mulberry culture" – Contract no. 6/2001 – Program AGRAL, is to establish the utilization method of the Endorize SOL biofertilizer, the doses and its influence on the biotechnological parameters of the mulberry plants – seedlings and mulberry cuttings.

Keywords: mulberry, cutting, Endorize

Introduction

The product Endorize SOL, utilized as VAM (vesicular-arbuscular mycorrhizas) biofertilizer in the Project "The utilization of vesicular-arbuscular mycorrhizas in mulberry culture" – Grant no. 6/2001 – Program AGRAL – is homologated in France under the license INRA no. 981002, being produced and commercialized by the company Biorize –France, www.biorize.com.

This product, commercialized in the E.U. countries (Germany, Italy, Ireland, Scotland) presents the following characteristics:

Presentation card

Product name:	Endorize SOL
Presentation form:	Solid, granulated
Content:	Useful VAM flora, including active and selected lines of Glomus mosseae, G. fasciculatum, G. sp. and Sclerocystis sp.
Destination:	- Individual inoculation in planting holes for seedlings and mulberry cuttings from nursery

- Inoculation of substratum from root system zone
- Inoculation of disinfected soil from nursery

Doses: - Nursery seedlings: 25-50 ml/plant
- Planting trees: 200-300 ml/tree

Expected effects: - Improving of rooting
- Accelerated rhythm of growing - Phytoprotection (increasing of plant immunity), increasing of useful soil flora

Preservation conditions: - Medium temperature 10-15^{o}C.

Guarantied: - Maximum 24 months.

The characterization of vesicular-arbuscular mycorrhizas

The mycorrhizae is a symbiotic association between the plants' roots and fungus, with a major role in many plants' essential functions, the most important being the nutrition with mineral elements from soil and the resistance at environmental conditions.

As the relation soil-plant is important for the agricultural production, also the mycorrhizas represent a relation soil-fungus-plant important in developing of a new strategy in sustainable agriculture, the mycorrhizas determining the decreasing of the quantities of chemical fertilizers and pesticides and implicitly the reducing of their negative impact on the environment [1].

The symbiotic fungus have extra cellular mycelium, causing the extension with few cm long of the colonized plants' roots, these fungus producing organic substance (exudates) which can be used as substratum for other germs from soil. This mycelium improves soil aeration, water circulation and soil stability.

These two component organisms of mycorrhizas are mutually dependent at a nutritional level. There is also an exchange of substances, like vitamins and amino acids, between these two organisms. In the roots' tissues there are produced vitamins like thiamine and biotin and some amino acids, while the fungus determines an increasing of plant's supply with K, Ca, Cu, Fe, Mg and Mn, but especially P, in soils with deficit of these substances.

Important in the establishing of the work methodology have been the data form the specific literature concerning the biologic cycle of VAM mycorrhizas for Glomus fasciculatum species, which presents the following dynamic of colonization – 10 days for mycorrhizas initiation - 50 days for the intense growing of inter cellular mycelium and intra cellular vesicles – at the end of vegetation period of the host plant it appears the physiologic decline of the mycorrhyzal fungus, decreasing the number of intra cellular vesicles, but intensifying the spores formation on the extra radicular mycelium. The maximum percentage of colonization appears at 45-50 days after inoculation, this being the moment of taking soil samples to determine the colonization ratio with mycorrhizas.

Material and method

The evaluation of vesicular-arbuscular mycorrhizas (VAM) by coloration

The plants with VAM contain arbuscular and vesicular mycelium, outside and inside of active roots' cells. In the arbusculs take place the transfer of mineral nutrients and the intracellular mycelium vesicles are responsible for the carbohydrates transfer. Therefore, in order to evaluate the roots' colonization with VAM, the coloring methods must be able to distinguish the different structures formed by fungus.

The diversity of fungus structure and their quantity determine the choice of the evaluation method for the colonization degree, without taking into account the physiological activity of fungus inside the roots. The different types of investigation can also use specific method of alive coloring.

The following table presents the characteristics of some coloring methods and their use in accordance with the proposed aim.

Table 1 The choice of colorant and their capacity
for different types of investigation

Availability for	Alive species			Non-vital species		Dual species
	FDA	ALP	AF	Tryphan blue	CBE	NBT/AF
Survivors	No	No	Yes	Yes	Yes	Yes
Experiences on nutrients adsorption	Yes	Yes	(No	No	No)	Yes
Colonization rate	No	Yes	Yes	No	Yes	Yes
Biodiversity (structure comparison)	**No**	**No**	**Yes**	**Yes**	**Yes**	**Yes**
Extern mycelium	Yes	Yes	Yes	No	No	No
Using of confocusing microscope	Yes	No	Yes	No	No	Yes

where: FDA = fluorescent diacetate; ALP = alkaline phosphatose; AF = fucsinic acid
CBE = chlorazol black E; NBT/AF = nitroblue tetrazolinum/acid fucsinic
NBT – can be used for alive species

The utilization of different colorants is synthesized in the following table (table no. 2) which presents information concerning the colorants utilized in VAM investigation.

Table 2 Information concerning the colorants utilized in VAM investigation

Colorant	Alive species			Non-vital species		Dual species
characteristics	FDA	ALP	AF	**Tryphan blue**	CBE	NBT/AF
Coloring	UV Dark yellow	Violet	Pink	**Blue**	Black	Purple/ pink
Toxicity	SUSPICIOUS OF INDUCING CANCER					
Utilization for sections or segments	only for sections	both		**segments**	both	both
Method of coloring	The methods are not complicated if the roots are nor strongly pigmented					
The type of UV microscope	**Field strongly lighted**					Contrast

Methods of coloring for unyielding tissues

All the samples for investigation which are resulted from roots must be clarified with KOH, taking into account the material used, the time and the equipment available. During clarifying, KOH must replace the cytoplasm and any other colored material from the root. The unyielding tissues for coloring can have a high content of phenol and phenolic compounds, either they are very thin or woody. This type of material requires strong clarifying and autoclaving and also an additional covering with alkaline peroxide in water. It is recommended a pre-coloring before clarification to help the process of fungus fixing.

The quantitative estimation can be realized by Giovannetti and Mosse method [2] or by recording, on root sample, the number of vesicles and contact points on radicular segments of 1 cm length.

Experimental results concerning the obtaining of mulberry cuttings inoculated with VAM

The experimental stage of the applicative research for the obtaining of mulberry cuttings inoculated with VAM has taken place into the greenhouse, utilizing the following variants:

V1 – Ukraine 107 variety, with mycorrhizass;
V2 - Ukraine 107 variety, fertilized NPK;
V3 – Kokuso 21 variety, with mycorrhizass;
V4 – Kokuso 21 variety, fertilized NPK;
V5 – Ichinose variety, with mycorrhizass;
V6 – Ichinose variety, fertilized NPK;
V7 – China 32 variety, with mycorrhizass;
V8 – China 32 variety, fertilized NPK;
V9 – Olteni variety, with mycorrhizass;
V10 – Olteni variety, fertilized NPK;
V11 – Ken Mochi variety, with mycorrhizass;
V12 – Ken Mochi variety, fertilized NPK.

The technology of mulberry varieties multiplication by cutting method contains the following techniques:

- digging at 50 cm deepness, with turning up the double furrows;
- bringing to level the furrows and fertilizing with manure on 15 cm high;
- raising the furrows with sifted fallow and calcium carbonate with ratio 1: 30, at 10 cm high;
- repeated wetting of the furrow with 10 l/sq.m. water;
- superficial breaking up of the planting layer;
- covering with a thin poliethylene stratum when the medium temperature from soil is 20^{o}C.
-

The cuttings for the obtaining of mulberry hardwood cuttings were obtained as follows:

- taking the stratified cuttings from cellar;
- disinfection of the cuttings with Carbetox 1% and Ridomil 0.1%;
- forming of the cuttings in pieces containing 4-5 buds;
- apical paraffination of the cuttings;

- treatment of stimulation for callus forming and rooting by storing the cuttings in solution of IBA 150 ppm/l during 24 h;
- realization of planting holes;
- adding Endorize SOL products 15 g/cutting in the planting holes;
- planting of cuttings in the planting holes followed by the soil sifting around the cuttings (Photo no. 1);

Photo no. 1 – The planting mulberry cuttings

- keeping at high humidity of air, through daily wetting of the plastic stratum;
- elimination of poliethylene stratum;
- daily wetting with 5-10 l/sq.m. water.

The procedure for VAM distinguishing

The choosing of root and soil samples

The choosing of root and soil samples was effectuated taking into account the collecting technique proposed by Timonin (1940):

1- carefully digging around the roots of the analyzed plant;
2- elimination of soil among the roots;
3- placing the roots with the afferent soil in vessel of 100 ml capacity with distilled water – in the laboratory phase.

Distinguishing of VAM in young mulberry roots [3], contains the following steps:

1 – fixing of roots in FAA solution (13 ml formalin + 5 ml glacial acetic acid + 200 ml ethanol 50%), followed by repeated rising of the fixed roots with normal water (30 min.);

2 – clarifying in order to eliminate the pigmentation of the cytoplasm and the vegetal cell nucleus, by keeping the young roots in KOH 10%, on water bath at 90°C for 2 h;

3 – rising of roots in a fresh solution of KOH 10% for 10-20 min. and immersion for 1 h in alkaline solution of H_2O_2 (3 ml NH_40H + 30 ml H_2O_2 10% + 567 ml normal water);

4 – rising of roots in normal water for the removing of H_2O_2 for 10-20';

5 – acidifying of the clarified roots in a solution of 100 ml HCl + 900 ml distilled water for 10 min;

6 – removing of acid solution by repeated rinsing with normal water;

7 – coloring with 0,05% Tryphan blue in lactic acid;

8 – sample observations have been realized at the optic stereomicroscope IOR and their photos have been made at the optic microscope MC-1.

Results and discussions

The biometrical data and the agroproductive and ecopedological parameters determined at the end of vegetation period indicate the following results:

Table no. 3 Mulberry planting material – cuttings inoculated with VAM

No.	Specification	Cuttings length - cm -	Diff. ± abs. val. - cm -	Dif. ± %	Rooting %	Dif. ± abs. val. - % -
1	Ukraine 107 variety with mycorrhizas 15 g/cutting	193.5	+ 20.7	+ 11.98	46.5	+ 16.5
2	Ukraine 107 variety fertilized NPK 10 g/ cutting	172.8	-	-	30	-
3	Kokuso 21 variety with mycorrhizas 15 g/cutting	174.0	- 29.5	- 14.5	28	- 4
4	Kokuso 21 variety fertilized NPK 10g/cutting	203.5	-	-	32	-
5	Ichinose variety with mycorrhizas 15 g/cutting	158.0	- 37.2	- 19.1	8	- 24
6	Ichinose variety fertilized NPK 10 g/cutting	195.2	-	-	32	-
7	China 32 variety with mycorrhizas 15 g/cutting	167.5	+ 63.3	+ 60.74	42	+ 10
8	China 32 variety fertilized NPK 10g/cutting	104.2	-	-	32	-
9	Olteni variety with mycorrhizas 15 g/cutting	220.0	+ 43.8	+ 24.85	40	+ 12
10	Olteni variety fertilized NPK 10 g/cutting	176.2	-	-	28	-
11	Ken Mochi variety– Control	151.3	-	-	20	-

The data of the table show the following aspects:

- the varieties Ukraine 107 and China 32, which did not present VAM in native status, had a favorable answer to biofertilizing with Endorize SOL product, in comparison with the chemical fertilized variants, the length of cuttings at the end of vegetation period being 193.5 cm and 167.5 cm, in comparison with 172.8 cm and 104.2 cm respectively;
- the varieties Kokuso 21 and Ichinose, which present VAM in native status, have presented a developing in the VAM fertilized variant smaller than the chemical fertilized variant, the colonization rate being of 3% and 2% respectively. The maximum developing of the cuttings from the two varieties, of 203.5 cm and 195.2 cm respectively, was recorded in the chemical fertilized variant.

These data are also presented in Graphic no. 1.

The somatometrical data concerning the influence of the Endorize SOL product and of chemical fertilization NPK on the radicular system of cuttings are presented in the following table:

Table 4

No.	Variety / Specification	Length of cutting - cm -	Length of main root - cm -	No. of secondary roots	No. of total active roots (Φ<1 mm) - cm -	Total weight of active roots - g -
1	Ukraine 107 - mycorrhizas	175.33	18.50	6.67	73.33	0.890
2	Ukraine 107 fertilized NPK	160	13.50	2.33	34	1.16
3	Kokuso 21 - mycorrhizas	131.33	13.00	5.55	45	0.74
4	Kokuso 21 - fertilized NPK	172	18.67	5	74.33	3.75
5	Ichinose mycorrhizas	158.67	15	7.67	53	0.55
6	Ichinose - fertilized NPK	186.67	8	4.33	50	0.71
7	China 32 - mycorrhizas	148.33	18	4.4	32	0.88
8	China 32 - fertilized NPK	142.67	14.5	5	47.67	1.30
9	Olteni – mycorrhizas	170	16	4.33	53	1.27
10	Olteni - fertilized NPK	171.67	20	5.67	53.33	1.25
11	Ken Mochi – Control	163.33	21.33	3	71	1.27

It is to be remarked the high length of cuttings under the conditions of chemical and with Endorize SOL fertilization, at all the varieties, in comparison with the standard length of 60 cm. The number of secondary roots comply with the minimum standards in the Ukraine 107, Kokuso 21 and Ichinose varieties. The varieties Olteni, China 32 and Ken Mochi present values under the standard, being necessary the cuttings' replanting for one more year (Photo no. 2).

Photo no. 2 – Mulberry cuttings in vegetation

For the qualitative evaluation of VAM colonization it was used the method intersection-grid described by Giovannetti and Mosse (1980).

There were obtained the following results:

Table 5

No.	Sample	VAM evaluation	Colonization ratio %
1	Sample no. 1 – Ukraine 107 variety- mycorrhizas	present	27
2	Sample no. 2 – Ichinose variety – mycorrhizas	present	3
3	Sample no. 3 – China 32 variety – mycorrhizas	present	2
4	Sample no. 4 – Kokuso 21 variety – mycorrhizas	present	7
5	Sample no. 5 – Olteni variety – mycorrhizas	present	14
6	Sample no. 6 – Ken Mochi variety - Control	absent	-

It is remarked the presence of VAM in all experimental variants, the colonization ratio presenting values between 2 – 27% at China 32 variety and Ukraine 107 variety, respectively (Photo no. 3).

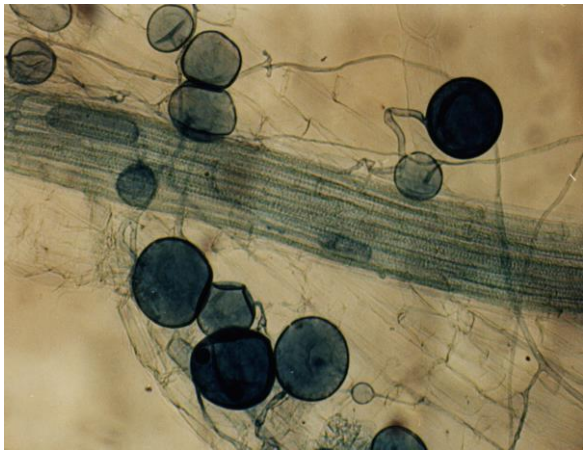

Photo no. 3 – Colonies of VAM

These preliminary data have constituted somatometrical parameters of reference to establish the Standard of mulberry cuttings with mycorrhizas, as a new type of mulberry planting material destined for mulberry plantations utilized in the silkworm rearing (Bombyx mori sp.).

In this type of plantations there will be eliminated the chemical fertilizers with Phosphorous, the planting material inoculated with VAM providing the adsorption of Phosphorous from soil, at optimum level.

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