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TESTING OF CERTAIN PHYSIOLOGIC PROPERTIES OF MYCORRHIZAL FUNGUS *AMANITA MUSCARIA* (LINN. EX FRIES)

Vesna GOLUBOVIĆ-ĆURGUZ[1], Vera RAIČEVIĆ[2], Ljubinko JOVANOVIĆ[3]

Abstract: *This paper presents laboratory results of the research on certain physiologic properties of Amanita muscaria (Linn.ex Fries). The MEA, PDA and modified MEA media were used for testing the influences of different media on the growth of mycelia. In order to test the influences of fungicides and heavy metals in medium on the fungus growth we have set the experiments where nutrient media was enriched by fungicides Befungin and Captan FL, or solutions of heavy metals Pb, Cu, Cd, and Zn in three different concentrations (3ppm, 33ppm, and 100ppm).*

Key words: Amanita muscaria, mycelia growth, nutrient media, heavy metals.

ISPITIVANJE NEKIH FIZIOLOŠKIH OSOBINA MIKORIZNE GLJIVE *AMANITA MUSCARIA* (LINN. EX FRIES)

Izvod: *U radu su prezentovani rezultati laboratorijskih ispitivanja nekih fizioloških osobina gljive Amanita muscaria (Linn. Ex Fries)***.** *Za ispitivanje uticaja različitih podloga na porast micelije korišćene su MEA, PDA i modifikovana MEA*

[1] Vesna Golubović-Ćurguz, Ph.D., Institute of forestry, Belgrade
[2] Vera Raičević, Ph.D., Faculty of Agriculture, Belgrade
[3] Ljubinko Jovanović, Ph.D., Institute for multidisciplinary research, Belgrad

podloga. Da bi se ispitalo kakav uticaj ima prisustvo fungicida i teških metala u podlozi na rast ove gljive postavljeni su ogledi u kojima je hranljivoj podlozi dodati fungicidi Benfungin i Kaptan FL ili rastvori teških metala Pb, Cu, Cd i Zn u tri različite koncentracije (3 ppm, 33 ppm i 100 ppm).

Ključne reči: Amanita muscaria, porast micelije,hranljive podloge, teški metali.

1. INTRODUCTION

Amanita muscaria (Linn.ex Fries) is a very poisonous fungus of *Amanita* genus, with a wide diffusion area. It can develop in a variety of habitats; it is found in oak, beech and even in coniferous stands. Although, certain fungi types of this genus can form endomycorrhizae, most frequently it forms ectomycorrhizae on the root of various species. Ectomycorhizae is mostly present on forest trees in the areas with moderate climate. It is connected to a number of coniferous species including all genera from *Pinaceace* family, including slightly less deciduous, tropical trees, woody bushes and some grass species (Bruns et al. 2002). The fungi forming ectomycorrhizae belong to the species from *Agaricales* order, (families *Boletaceae, Tricholomataceae, Amanitaceae. Cortinariaceae, Paxilaceae, Gomphidiaceae*), *Gasteromycetes* class and from the subclass of *Deuteromycotina, Ascomycotina* and *Mastigomycotinae* (Agrios, 1997).

Fungus *A. muscaria* biologic properties through laboratory research of certain physiologic properties. These results may be applied in the production process of mycorrhizaed seedlings for afforestation of long deforested terrains, degraded The mycorrhizae presence on the tree seedling root is often the only precondition for seedling survival and growth under unfavorable conditions of the environment (Rudawska et al. 2001). The fungus (mycobiont) contributes to improvement of plant supply with soil nutrients, root mass enhancement (Dahm, 2005), root system protection from pathogens (Marx, 1973). It also helps in absorbing and trans-locating water in plants, drought protection, temperature extremes and the reduction of heavy metal influence on plants (Godbold et al 1998, Rudawska et al 2001).

Therefore, mycorrhizaed plants should be formed in the nursery during production of sowing material. The production of mycorrhizaed forest seedlings is much more expanded across American and European countries (Castellano and Molina, 1993). There are small records on the use of mycorrhizae in forest sowing material production and afforestation in Serbia. Veselinovic and associates (1976) have among the first published the results of artificial inoculation with mycorrhizaed fungi in *Pinus nigra* Arn production. Later, Peno and Veselinovic (1984) have with their papers contributed to the significance of mycorrhiazeing the root system of *P. nigra* and *P. sylvestris* in afforestating the Ibar gorge.

The aim of this paper is better familiarization with mycorrhizal shallow soils without humus horizon where has the forest micro flora completely disappeared.

1. MATERIAL AND METHOD

We have tested the influence of different nutrient media to the mycelia growth of this fungus, than fungal fermenting activity, firstly, production of oxidase and reductase. In the experimental group we have controlled the present amounts of herbicide and heavy metals in nutrient medium and how much it can reduce this mycorrhizal fungus mycelia growth. All the isolates used in the experiments were developed on the MEA (2%) nutrient media in Petri dishes.

2.1 The mycelia of mycorrhizal fungus growth on different types of nutrient media

The mycelia of mycorrhizal fungus growth was marked on three nutrient media: MEA (malt-extract agar), PDA (potato-dextrose agar), and modified agar medium (Rudawska et al., 2000 cit. Tomaszewski and Wojciechowska, 1974) (55 mM glucose; 14 mM maltose; 6.25 mM NH_4NO_3; 3.7 mM KH_2PO_4; 2 mM $MgSO_4$; 0.15 μM thiamine chloride; 0,02 μM biotin; agar $9gL^{-1}$). All media prepared were spread over in Petri dishes, and after sowing with mycelia fragments placed in thermostat, in no light conditions on 23 $\pm$ 1°C. The mycelia growth was observed for two months, and the measurements of the two crossed diameters were conducted on every other day during the period. The experiment was repeated five times.

2.2 The fungal fermenting activity

The fungal fermenting activity was analyzed by testing the influences of isolated fungi on the media oxidation degree. The Bavenndamm's method, later on elaborated by Davidson et al (1938), was used for testing oxidase. The MEA medium, to which was added 0.5% of gallic or tannic acid was used as a medium. This experiment was repeated five times. The diffusion zone size, color and tone were used as a criterion for grading the oxidase secretion. The oxidase degree, according to Davidson et al, was expressed in the following way:

- negative, lack of brown agar coloration under or around inoculum;

\+ light to dark brown diffusion zone, created under the inoculum in colony center visible from the bottom side of the Petri dish; when the colony is not formed the zone under the inoculum receives brown coloration;

++ light to dark brown diffusion zone, formed beneath the largest part of the colony, but not reaching its borders;

+++ light to dark brown diffusion zone spread on a short distance from the colony edge visible from the upper side;

++++ dark brown diffusion zone, opaque, considerably spreading across the line designating the inoculum zone;

+++++ highly intensive diffusion zone, dark brown, opaque, forming a wide wreath around the colony; usually this strong reaction have those species not grown on the medium with gallic acid added.

According to the colony growth rate on malt-agar medium with 0.5% gallic or tannic acid added, we have determined on the basis of key by Davidson et al the relation to fungi group in the following way:

Negative or non-reacting fungi

Group 1 - mycelia growth in gallic or tannic environment is nearly equal;

Group 2 - growth in gallic environment good, colony diameter longer than the one in tannic acid environment;

Group 3 - good growth in gallic acid environment, there is no growth or is found only in traces in tannic acid environment;

Positively reacting fungi

Group 4 - not growing or growing only in traces on both media;

Group 5 - not growing or growing only in traces in gallic acid environment, mycelia diameter up to 25mm in tannic acid environment;

Group 6 - not growing or growing only in traces in gallic acid environment, growth 25-50mm (after 7 days) in tannic;

Group 7 - Mycelia have similar diameter on both acid environment;

Group 8 - clear growth in gallic acid environment, good growth in tannic acid environment;

Group 9 - good growth in gallic acid environment, not growing or growing only in traces in tannic acid environment; most frequently these fungi are vaguely reacting and for definite results it is necessary to wait 14 days;

Fungi having negative or positive reaction depending on the environment

Group 10 - negative reaction on gallic acid environment, positive in tannic acid environment, with good growth in both.

2.3 The influence of heavy metals on mycorrhizal fungi mycelia growth

This influence was marked by sowing fungi on standard MEA medium to which, after being sterilized in autoclave, were added solutions of heavy metals Pb, Cu, Cd, and Zn in three concentrations (3ppm, 33ppm, and 100ppm)(Dunabeitia et al, 2004). These solutions were prepared from the salts of these metals in the following compounds $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $CdSO_4 \cdot 8H_2O$ and $Pb(COOH)_2$. The measured pH value for all solutions was 5.5 except for copper solutions pH 4.5. We have observed the mycelia growth and the values obtained were compared with the growth values on standard MEA medium.

2.4 The influence of fungicides on mycelia growth

Fungicides are applied in nursery production to eliminate pathogenic organisms. Whether their use can have negative influence on the development of mycorrhizal fungi and how great that influence is was examined in laboratory conditions using fungicides Benfungin (Galenika, Belgrade), and Captan FL (Zorka, Sabac) in two concentrations, mostly applied in nurseries.

MEA medium was sown with fragments of the tested fungus, and around them was placed filter paper – three pieces cut in square shapes. Each piece of filter paper was soaked in certain concentration of the same type of fungicide. The growth of the tested mycelia was observed, as well as the reaction around the filter paper. That is a modified method taken from Karadzic's PhD paper (1982).

3. RESEARCH RESULTS AND DISCUSSION

3.1 Effect of different media on mycelial growth

The growth of the mycorrhizal fungi mycelia was observed on three nutrient media. Two standard nutrient media MEA (malt-extract agar), PDA (potato-dextrose agar), and modified agar medium (mod.MEA).

Table 1. *Effect of different media on mycelial growth (mm /1 week)*

Week of growth	Type of media		
	MEA	PDA	Mod.MEA
1	-	-	-
2	-	6.37	-
3	7.88	7.77	18.00
4	7.35	6.53	14.58
5	9.04	7.09	10.00
6	2.29	5.21	7.50
7	6.08	1.70	2.50
8	10.91	2.06	
9	8.79	0.57	
10	4.56	0.42	
11	4.39	0	
12	2.89	0	

The tested mycorrhizal fungus differently reacted to nutrient media used (table 1). The growth in MEA and mod.MEA medium was noted three weeks after sowing, while the fungus began to grow after a week on the PDA medium. The fungus grew up to the edges of the Petri dish in MEA medium, while the PDA medium did not suit *A. muscaria*, so owing to extremely slow growth it had not managed to fill the Petri dish.

Table 2. *Daily and weekly growth of mycelia A. muscaria on different media*

Type of media	growth of mycelia	(mm)
MEA	Daily	0.98
	weekly	5.35
PDA	Daily	0.02
	weekly	2.69
Mod. MEA	Daily	1.79
	weekly	8.76

A. muscaria did not have a steady growth rate on all media (table 2), the daily growth difference measured ranged from 0.02mm to 1.79mm, on the weekly basis it ranged from 2.69 to 8.76mm. The fungus has filled the Petri dishes in the shortest period on modified MEA medium, so the fastest daily growing rate was recorded (1.79mm), as well as the fastest weekly rate (8.76mm).

3.2 Fungal fermenting activity and medium oxidation degree

Thanks to the synthesis ability of different extra cellular enzymes mycorrhizal fungi can use various energy sources from humus components and plant tissue and in this way provide themselves with nitrogen and phosphorous (Cairney and Burke, 1998).

The research degree of gallic and tannic acid oxidation degree in these media 7 and 14 days after incubation are presented in table 3. After 7th day isolates show slow growth in gallic acid oxidizing it during the process. Tannic acid is faintly oxidized by this fungus and shows faint mycelia growth. After 14 days this fungus shows faint mycelia growth in both gallic and tannic acid. The oxidation intensity of gallic acid is slightly pronounced. The most intensive medium coloration is beneath inoculum, gradually reducing toward the rim of diffusion zone.

Table 3. *Oxidation degree of gallic and tannic acid*

Medium		Reaction	Size of diffusion zone (mm)	Size of Mycelia (mm)	Davidson ՚s group
Gallic acid	7thday	+++	27,67	<20	4
	14thday	+++	36,67	<20	4
Tannic acid	7thday	+	<20	<20	4
	14thday	++	<20	<20	4

Fungi causing intensive oxidation of gallic and tannic acid are grouped among fungi which are intensively secreting ferments from oxidase group, proving their ability to oxidize lignin and decompose wood (Karadzic, 1986). The wood decomposing fungi (*Hypholoma fasciculare, Phanerochaete velutina*), endomycorrhizal fungi (*Hysterangium setchellii, Lactarius affinis, Lactarius controversus*) and ericoidal mycorrhizae *(Hymenoscyphus ericae*) decompose hydrolyzed polyphenol – protein complex which is built from gallic acid (Bending and Read, 1997; Waterman and Mole, 1994.).

3.3 The influence of heavy metals on fungal growth

Amanita muscaria is tolerant to the presence of tested metals, mycelia has grown on media with metals added in all concentrations. The average daily fungal growth on control medium (MEA) was 0.98mm/day, and on media with presence of heavy metals it varied depending on the metal type and its concentration, so the growth rate doubled in the highest concentrations in the presence of Zn, Pb, and Cd. Cooper had the strongest inhibition influence in 100ppm concentration (table 4).

Table 4 *Effect of heavy metals added to media on the mycelial growth of A. muscaria (mm/1 day)*

Concentrations (ppm)		Mycelial growth of *Amanita muscaria*
Zn	3	1.90
	33	2.27
	100	1.90
Pb	3	2.27
	33	2.27
	100	2.27
Cu	3	1.90
	33	1.65
	100	0.98
Cd	3	1.90
	33	1.65
	100	1.65
MEA	0	0.98

Similar results have been obtained by Jacob et al (2001) in their research on exposing *P. involutus* mycelia to Cd influence in 0.05ppm concentration in 12 hour time frame. They have proved its tolerance to Cd presence, stating that this type of cell reaction was conditioned by enzyme presence.

Contrary to these results, Dunabeitia et al (2004) have been testing the influence of Pb, Cu, and Cd in three concentrations (3, 33, and 100ppm) in media on growth of different types of mycorrhizal fungi. The highest influence on growth inhibition was exhibited by Cd, slightly less by Cu and Pb least.

Positive experiences with pure mycorrhizal fungi cultures in elevated heavy metal content conditions in laboratory were confirmed by planting micorrhizaed seedlings on the terrain so the use of these fungi as bio-remedies is recommended (Khan et al, 2000) in polluted soils or as pollution bio-indicators (Leyval et al, 1997). Muzenberger et al (2004) have established that elevated metabolic activity of mycorrhizae conditions good adapting abilities of mycorrhizaed root systems to the extreme soils on recultivated places. Practical application of certain ectomycorrhizal fungi conditioned better seedling development in conditions of elevated content of heavy metal. Fungi developed in soil containing the elevated cooper content adapt in time to these conditions. Comparing laboratory analysis of these fungi against the fungi from non-polluted soil it was concluded that they keep their properties and show tolerance to Cu presence even when they are out of those conditions (Arnebrant et al, 1987).

3.4 Fungicide influence on the *In vitro* colony growth

The experiment control was performed on weekly bases during two months time while the experiment was monitored. Active substances of these fungicides belong to the different chemical groups (Benomyl belongs to benzimidazole group, and Captan is from ftalamide group) and during experiment different types of reactions were recorded depending on the fungicide type and their concentration.

Mycelia has been growing without interference in the presence of Befungin in both concentration, during the entire test period, the same results were recorded in the first (after two weeks) and second control (in the end of the testing period).

The lower concentration of Captan FL influenced *A. muscaria* conditioning mycelia growth reduction. The presence of higher concentration of Captan FL acted in the same way as the lower, only the influence was stronger and formed inhibition zone. Castellano and Molina (1993) and Lazarev (1998) have through research reached the conclusion that certain fungicide types might have a negative influence on mycorrhizal fungi i.e. reducing mycorrhizae development, they have reached the same results with captan based preparations.

The usual pesticide application in nursery production might affect the formation and development of mycorrhizal fungi, influencing mycorrhizal fungi germination, sporangia as well as root colonization (Hetrick and Wilson, 1991). Negative effects of pesticide application are manifested through mycorrhizal fungi destruction pointing the research in the direction of controlled application of chemical preparations and biological fighting measures.

The application effects of fungicides Benomyl and Captan to the development of mycorrhizal fungi which colonize *Picea sitchensis* and *Fraxinus excelsior* were tested by O'Neill and Mitchell (2000). Benomyl application has 2-3 times influenced the ectomycorrhizal fungi species reduction number on the root, and on the reduced root colonization. Soil fumigation applied in nurseries in order to eliminate pathogenic fungi *Rizoctonia solani, Pythium sp., Fusarium oxysporum* etc. has positive influence on spruce and coast Douglas – fir seedling mycorrhization. These seedlings could be successfully inoculated with mycorrhizal fungus only in those conditions because saprophytic organisms and naturally developed ectomycorrhizae show antagonist properties in introduction of this specie (Tacon et al. 1986). Soil fumigation frequently results in plant growth underdevelopment, as well as in mycorrhizal fungi inoculum destruction. However, if the soil is rich in organic matter the mycorrhizae regeneration on the root of the sown black pine is established by the end of the year after sowing (Veselinovic et al. 1976; Lazarev, 2005). The mycorrhizal seedlings *Pinus elliotti* have, on the soil fumigated with methyl bromide, had higher survival rate, the root was branched better, and the ratio of nitrogen and phosphorous in pine needles and root was more favorable than in non – mycorrhizaed seedlings (Shoulders, 1972).

4. CONCLUSIONS

The results of these researches have led to the following conclusions:

-Mycorrhizal fungus *Amanita muscaria* has been growing in different rates on nutrient media. The fastest daily and weekly growth rates were recorded on modified MEA medium.

-This fungus has shown oxidation of gallic and tannic acid. The gallic acid oxidation intensity was slightly more pronounced.

-Befungin fungicide did not affect the growth of mycelia in both concentrations. Captan FL has equally affected the growth in both concentrations conditioning slower *A. muscaria* mycelia growth rate.

-*Amanita muscaria* is tolerant to the presence of metals tested, thus mycelia grew in media with addition of metals in every concentration.

LITERATURE

Agrios, G. N. (1997): Plant pathology, Academic Press, San Diego, USA

Arnebrant, K., Baath, E., Nordgren, A. (1987): Copper tolerance of microfungi isolated from polluted and unpolluted forest soil, Mycplogia 79(6): 890-895

Bending, D.G., Read, D.J.(1997): Lignin and soluble phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. Mycological Research 101, 1348–1354.

Bruns, T. D., Bidartondo, M. I., Taylor, D. L. (2002): Host specificity in ectomycorrhizal communities: What do the exceptions tell us? Integ. And Comp. Biol. 42:352-359

Castellano, M. A., Molina, R. (1993): Mycorrhizae. In: Landis, TD., Tinus, RW, McDonald SE, Barnett JP(eds) The container tree nursery manual. Vol 5t Agriculture handbook 674. USDA Forest Service, Washington D.C. pp.101-167

Cairney, J.W.G., Burke, R.M. (1998): Extracellular enzyme activities of the ericoid mycorrhizal endophyte *Hymenoscyphus ericae* (Read) Korf & Kernan: their likely roles in decomposition of dead plant tissue in soil, *Plant and Soil* 205: 181–192

Dahm, H. (2005): Role of Mycorrhizae in Forestry. In Rai, M.K.(eds) Handbook of microbial biofertilizers. pp. 241-270

Dunabeitia, M. K., Hormilla, S., Garcia-Plazaola, J. I., Txaterina, K., Arteche, U., Becerril, J. M. (2004:: Differential responses of three fungal species to environmental factors and their role in the mycorrhization of *Pinus radiata* D.Don. Mycorrhiza. 14:11-18

Godbold, D. L., Fritz, E., Huttermann, A. (1998): Aluminium toxicity and forest decline. Ecology Vol.85 pp. 3888-3892

Hetrick, B.A.D., Wilson, G. W. T. (1991): Effects of Mycorrhizal Fungus Species and Metalaxyl Application on Microbial Suppression of Mycorrhizal Symbiosis Mycologia, Vol. 83, (1):97-102

Jacob, C., Courbot, M., Brun, A., Steinman, H. M., Jacquot, J-P., Botton, B., Chalot, M. (2001): Molecular cloning. characterization and regulation by cadmium of a superoxide dismutase from the ectomycorrhizal fungus *Paxillus involutus.* Eur. J. Biochem. 268: 3223-3232

Karadžić, D. (1986): Study of Biecology of Fungus Dothistroma pini Hulbary – agent of Austrian pine needle shedding. Doctoral thesis, Faculty of Forestry. The University of Belgrade, p. 328 *(Original: Proučavanje bioekologije gljive Dothistroma pini Hulbary-prouzrokovača osipanja četina crnog bora. Doktorska disertacija Šumarski fakultet. Univerzitet u Beogradu pp.328)*

Khan, A. G., Kuek, C., Chaudhry, T. M., Khoo, C. S., Hayes,W. J.(2000): Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere. 41:197-207

Leyval, C., Turnau, K., Haselwander, K. (1997): Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7: 139-153

Lazarev, V. (1998): Mycorrhiza and the Impact of some Pesticides on the Occurrence of It. Plant Doctor 26, pp. 261-266 *(Original: Mikoriza i uticaj nekih pesticida na njenu pojavu. Biljni lekar 26. pp.261-266)*

Lazarev, V. (2005): Forest Phytopathology. University textbook. Art Print Banja Luka. pp. 1-595 (Original: Šumska fitopatologija. Univerzitetski udžbenik. Art Print Banja Luka. 1-595)

Münzenberger, B., Golldack, J., Ullrich, A., Schmincke, B., Hüttl, R. F. (2004) :Abundance, diversity, and vitality of mycorrhizae of Scots pine (*Pinus sylvestris* L.) in lignite recultivation sites. Mycorrhiza 14:193-202

O·Neill, J. J. M., Mitchell, D. T. (2000): Effects of benomyl and captan on growth and mycorrhizal colonization of Sitka-spruce (*Picea sithensis*) and ash (*Fraxinus excelsior*) in Irish nursery soil. For. Path. 30:165-174

Peno, M., Veselinović, N. (1984): The Importance of Mycorrhization of Root System Pinus nigra and P. sylvestris in Reforestation of Barren Land of Ibarska Gorge. The Collection of Papers of the Institute of Forestry and Wood Industry Belgrade. Vol. XXII-XXIII. pp. 57-69 *(Original: Značaj mikorizacije korenovog sistema Pinus nigra i P. sylvestris u pošumljavanju goleti ibarske klisure. Zbornik radova Instituta za šumarstvo i drvnu industriju Beograd. Vol. XXII-XXIII. 57-69)*

Rudawska, M., Kieliszewska-Rokicka, B., Leski, T. (2000): Effect of aluminium on *Pinus sylvestris* seedlings mycorrhizal with aluminium-tolerant and aluminium-sensitive strains of *Suillus luteus*. Dendrobiology. vol. 45. 89-96

Rudawska, M., Leski, T., Gornowicz, R. (2001): Mycorrhizal status of *Pinus sylvestris* L. nursery stock in Poland as influenced by nitrogen fertilization. Dendrobiology. vol.46: 49-58

Shoulders, E. (1972): Mycorrhizal inoculation influences survival, growth and chemical composition of slash pine seedlings. South. Forest Exp. Stn. New Orleans.La.12p. (USDA Forest Serv. Res. Pap. SO-74)

Tacon, F., Bouchard, D., Perrin, R. (1986): Effects of soil fumigatiopn and inoculation with culture of *Hebeloma cylindrosporium* on survival. growth and ectomycorrhizal development of Norway spruce and douglas fir seedlings. Eur. J. For. Path. 16. 257-265

Veselinović, N., Peno, M., Popović, J. (1976): The Influence of Soil Fumigation by Metyl Bromide on the Dynamics of Soil Microflora. Plant Protection. Vol. XXVII (1) Number 135: pp. 99-108 *(Original: Uticaj fumigacije zemljišta metil-bromidom na dinamiku zemljišne mikroflore. Zaštita bilja. Vol. XXVII (1) br.135:99-108)*

Waterman, P.G., Mole, S. (1994): Analysis of Phenolic Plant Matabolites. Blackwell Scientific Publications, London, 238pp

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Vesna GOLUBOVIĆ-ĆURGUZ, Vera RAIČEVIĆ, Ljubinko JOVANOVIĆ

Summary

Mycorrhizal fungus, *Amanita muscaria,* on modified MEA media grows faster that on the MEA and PDA medium. This fungus is tolerant to the presence of heavy metals (Zn, Cu, Pb, Cd) in three concentrations (3ppm, 33ppm, and 100ppm), mycelia grew in media with addition of metals in every concentration. Befungin fungicide did not affect the growth of mycelia in concentrations 0.04 and 0.06. Captan FL has equally affected the growth in both concentrations (0.2 i 0.3) conditioning slower *A. muscaria* mycelia growth rate.

The A. muscaria colony on the media with the addition of gallicic acid and tannin acid shows a positive oxidase reaction and according to the Davidson key this funfus is classified in the four group.

Based on the results obtained during this research the use of this fungus can be recommended in process of forest sowing material mycorrhization. The presence of fungi in a role of root symbiont in these seedlings helps in overcoming afforestation problems manifested in inability of transplanted plants to use micro and macro elements from soil.

ISPITIVANJE NEKIH FIZIOLOŠKIH OSOBINA MIKORIZNE GLJIVE *AMANITA MUSCARIA* (LINN.EX FRIES)

Vesna GOLUBOVIĆ-ĆURGUZ, Vera RAIČEVIĆ, Ljubinko JOVANOVIĆ

Rezime

Mikorizna gljiva, *Amanita muscaria*, na modifikovanoj MEA ima brži porast micelije u odnosu na MEA i PDA podlogu. Ova gljiva je vrlo tolerantna na prisustvo teških metala (Zn, Cu, Pb, Cd) u tri koncentracije (3ppm, 33ppm, 100ppm), tako da je micelija rasla na podlogama sa dodatkom svih metala u svim koncentracijama. Fungicid Benfungin u koncentracijama 0.04 i 0.06 nije uticao na rast micelije. Kaptan FL je podjednako delovao u obe koncentracije (0.2 i 0.3) uslovljavajući sporiji rast micelije *A. muscaria*.

Micelija *A. muscaria* na podlozi sa dodatkom galne ili taninske kiseline pokazuje pozitivnu oksidacionu reakciju i po ključu Davidsona ova gljiva se svrstava u grupu 4.

Na osnovu dobijenih rezultata u toku ovog istraživanja može se preporučiti primena ove gljive u procesu mikorizacije šumskog sadnog materijala. Kod ovih sadnica, prisustvo gljiva kao simbionta na korenu pomaže u prevazilaženju problema pošumljavanja koje se manifestuje u nemogućnosti presađenih biljaka da koriste mikro i makroelemente iz zemljišta.

Reviewer:
Snežana Rajković, Ph.D., Institute of Forestry, Belgrade