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SUSTAINABLE FORESTRY **ODRŽIVO ŠUMARSTVO**
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Original scientific paper

THE INHIBITORY EFFECT OF FUNGICIDES BENFUNGIN WP AND KAPTAN FLON ON THE GROWTH OF MYCELIUM OF MYCORRHIZAL FUNGI

Vesna GOLUBOVIĆ-ĆURGUZ[1], *Mara TABAKOVIĆ-TOŠIĆ*[1], *Dragan KIKOVIĆ*[2], *Ljubinko JOVANOVIĆ*[3]

Abstract: *The paper presents the results of the laboratory studies of the effects of two types of fungicides (Benfungin WP and Kaptan FL) on the growth rate of the mycelium of the following species of mycorrhizal fungi: Suillus luteus (L. ex Fr.) S. F. Gray, Suillus bovinus (L.ex Fr) Roussels, Suillus granulatus (L. ex Fr.)O. Kuntze, Paxillus involutus (Batsch ex fr.), Hebeloma spp., Amanita muscaria (L. ex Fr.) Hook. The observed fungicides were used in two concentrations - Benfungin WP in 0.04 and 0.06% concentrations and Kaptan FL in 0.2 and 0.3% concentrations.Benfungin had no effect on the growth of mycelium of Hebeloma spp., whereas in the direct contact in both concentrations it affected all other fungi, by stopping the growth of the mycelium.*

Kaptan FL had the similar effect in both concetrations, by stopping the growth of mycelium of Hebeloma spp., A. muscaria, P. involutus and S. luteus. It had no effect on the growth of mycelium of S. bovinus and S. garnulatus.

Key words: forest seedlings, mycorrhizal fungi, growth inhibition, fungicides

1. INTRODUCTION

The rhizosphere, the zone surrounding the root, is inhabited by numerous microorganisms which form saprophytic, pathogenic and symbiotic associations.

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The symbiosis between non-pathogenic fungi and roots of host plants is called the mycorrhizal association. In this association both components benefit from cohabitation. The fungus (mycobiont) contributes to the increase of root weight (Dahm, 2005), which enables the plant to adopt the nutrients that are located at the greater depth than the root. Along with the absorption of the nutrients, ectomycorrhiza can protect the root system from the invasion of pathogens and saprophytic microorganisms (Zak, 1964; Froidevaux, 1975; Marx et al., 2002), which makes it easier for the seedlings of coniferous tress, whose root was attacked by pathogenic fungi, to survive (Chakravarty i Sidhu, 1987; Haug i sar., 1988; Marx, 2002). In addition, it has a role in the absorption and water translocation in the plants, protection from drought, temperature extremes, and the alleviation of the influences of the heavy metals on plants (Godbold et al., 1998; Rudawska et al., 2001). For the reforestation of the terrains which have been deforested for a long time, degraded shallow soil without humus horizons, the mycorrhizal seedlings which overcome the problems regarding the surviving of seedlings used for reforestation by the presence of the fungi as the symbiont on the root, should be applied. The production of mycorrhizal seedlings should be initiated in the nurseries of forest seedling material in Serbia, which has been already present in many countries in Europe and America.

The usual application of pesticides and additional nutrition in the nursery production can have the adverse effects on the formation and development of mycorrhizal fungi, by affecting their germination, sporulation and root colonization (Hetrick and Wilson, 1991; Perrin et al., 1996). Numerous researches of these effects worldwide were aimed at finding the suitable ways of using pesticides, by monitoring the growth and development of mycorrhizal association on the root of plants upon the application of different fungicides or herbicides (Chakravarty and Sidhu, 1987; Castellano, Molina, 1993; O´Neill and Mitchell, 2000). In Serbia the sufficient attention has not been paid to the effect of the application of pesticides in the production of forest seedling material on the development of mycorrhizal fungi. Veselinović et al. (1976) analyzed the effect of fumigation with methyl bromide on the mycorrhizal inoculum. It is one of the frequent treatments in the nurseries which is applied in order to eliminate the weeds, nematodes and pathogenic fungi in the soil, but they concluded that it frequenly led to the destruction of the inoculum of mycorrhizal fungi. The similar effects on the beneficial microflora were also reported in the French nurseries (Tacon et al., 1986). Fraedrich and Dwinwill (2003) reported that after the application of soil fumigation with methyl bromide, the pathogenic species *Fusarium* sp., and *Pythium* spp. were no longer presented in the treated sites, but that, at the same time, their antagonist, *Trichoderma* spp. was destroyed.

Fungicides can cause the increase of the degree of granulation and degradation of cytoplasms of symbiotic fungus on the mycorrhizal root (Manninen et al., 1998). Upon the application of fungicides, due to the “fungicidal stress”, the presence of several mycorrhizal fungi at the same time makes them easier to survive, than their individual presence (Schreiner and Gabor, 1997). The results of the researches by Testa et al. (2006) suggest that the adverse effect of the different fungicides on the spread of mycorrhizal fungi should be used when it is needed to

limit the development of some undesirable mycorrhizal association under the controlled conditions.

The usual measures of protection which are applied in Serbia in the nurseries for the production of forest seedling materials refer to the treatments of substrates and seedlings prior to sowing and four additional application after the sprouting in order to prevent the occurrence of rot and lodging of seedlings. The constant use of these preparations is the hazard for the ecosystem since it has the adverse effect on the beneficial microflora in the soil. This paper presents the research results of the effect of the most frequently used fungicides Benfungin WP and Kaptan FL on the most significant species of mycorrhizal fungi present on the forest seedling material: *Suillus luteus* (L. ex Fr.) S. F. Gray, *Suillus bovinus* (L.ex Fr) Roussels , *Suillus granulatus* (L. ex Fr.)O. Kuntze, *Paxillus involutus* (Batsch ex fr.), *Hebeloma spp., Amanita muscaria* (L. ex Fr.) Hook.

2. MATERIAL AND METHODS

It was studied in the laboratory conditions whether the application of fungicide Benfungin WP (Galenika, Belgrade) (0.04 and 0.06 % concentrations) and Kaptan FL (Zorka, Šabac) (0.2 and 0.3 % concentrations) affects the growth of mycorrhizal fungi.

The following mycorrhial fungi were used in the experiments: *Suillus luteus, S. bovinus, S. granulatus, Paxillus involutus, Hebeloma spp., Amanita muscaria.* The isolates of fungi were obtained from Professor Doctor M. Rudawska from the Institute of Dendrology of the Polish Academy of Sciences.

The isolates of fungi, which were used in the experiment, were developed on the nutrient medium (2 %) in petri dishes. After pouring the standard MEA medium containing 20 grams of mulch (Sigma-Aldrich, USA) and 20 grams of agar (Torlak, Belgrade, Serbia) in petri dishes, the fragments of the observed fungi sized 8 x 8mm were sown. Prior to the inoculation of medium, the isolates were 21 years old. The fragments were placed in the center of petri dishes, and around them square-shaped filter paper, which after the sterilization was soaked in the certain concentration of the same type of fungicide (modified method by Uščuplić, M., Lazarev, V.,1983), was placed. The growth of the observed mycelia and reaction around the filter paper was monitored. The incubation was perfomed at $23^0C \pm 1^0C$ temperature. During the growth on MEA medium the growth rate for all species was monitored, and the inhibition of the growth of fungi was determined based on the growth rate of the control isolate on the pure MEA medium.

The experiment was conducted in five repetitions, and the obtained results were statistically processed. The mean values and mean errors were determined, and the statistical significance of the differences in the variances by using the method of variance analysis (ANOVA-Duncan method).

3. RESULTS

The experiment was controlled every seven days over a two-month period, how long the experiment lasted. These types of the observed fungicides contain the active ingredients which belong to the different chemical groups - Benomil belongs

to the group of benzimidazoles, and Kaptan is from the group of phthalimides). During the experiment the different reactions depending on the type of fungicides and their concentrations were reported.

The influence of fungicide Benfungin on the growth of colony of mycorrhizal fungi *IN VITRO*

Fungicide Benfungin affected the observed species of fungi in different ways. At the beginning of the experiment the normal growth and development of mycelium of all observed species of fungi were reported. At the end of the experiment Benfung in the lower concentration (0.04%) affected all observed species of fungi by stopping the growth of mycelium, i.e. the mycelia grew up to the filter paper and their growth was stopped in the direct contact with it. It did not have any influence only on the mycelium of the species *Hebeloma* spp, the development of which was undisturbed, i.e. it completely overgrew the filter paper. The growth rate of mycelium was the same in the species *S. luteus* and *S. bovinus*, with the growth on the control medium, whereas in all other observed species the slight deceleration of growth rate was reported. When the concentration of it was higher (0.06%), the reaction all species of fungi was the same as in the case of the lower concentration of fungicides (Table 1), i.e. the different concentrations of preparations did not have any influence on the obtained results.

Table 1. *The growth of mycelium of mycorrhizal fungi on MEA medium and media with the added fragments soaked by Benfungin WP in the different concentrations*

Medium	*S. luteus*	*S. bovinus*	*S. granulatus*	*P. involutus*	*Hebeloma* spp.	*A. muscaria*
control (MEA)	2.9 ± 0.02^{a}	3.1 ± 0.03^{a}	5.6 ± 0.03^{a}	5.64 ± 0.02^{a}	3.88 ± 0.04^{ab}	4.37 ± 0.02^{a}
Fragments with Benfungin WP in 0.04 concentration	2.9 ± 0.03^{a}	3.1 ± 0.05^{a}	4.9 ± 0.12^{b}	5.55 ± 0.03^{ab}	3.97 ± 0.05^{ab}	4.0 ± 0.14^{b}
Fragments with Benfungin WP in 0.06 concentration	2.9 ± 0.02^{a}	3.1 ± 0.05^{a}	4.9 ± 0.12^{b}	5.55 ± 0.03^{ab}	3.9 ± 0.12^{ab}	4.0 ± 0.01^{b}

The mean values marked with different letters in the same row are significantly different (Tukey HSD test of multiple ranks $P<0.05$)

The influence of fungicide Kaptan FL on the growth of colonies of mycorrhizal fungi *IN VITRO*

The presence of fungicide Kaptan FL had the different influence on the growth of mycelia of mycorrhizal fungi (Table 2).

Table 2. *The growth of mycelia of mycorrhizal fungi on MEA medium and media with the added fragments soaked by Kaptan FL in different concentrations*

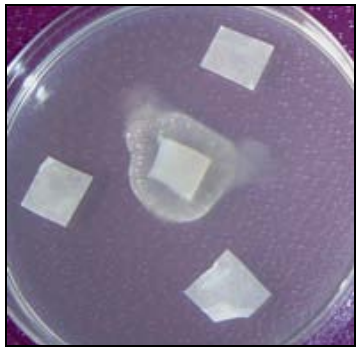
Medium	*S. luteus*	*S. bovinus*	*S. granulatus*	*P. involutus*	*Hebeloma* spp.	*A. muscaria*
Control medium (MEA)	2.9 ± 0.02^{a}	3.1 ± 0.03^{ab}	5.6 ± 0.03^{ab}	5.64 ± 0.02^{a}	3.88 ± 0.04^{a}	4.37 ± 0.02^{a}
Fragments with Kaptan FL in 0.2 concentration	2.9 ± 0.03^{a}	3.3 ± 0.05^{a}	5.9 ± 0.12^{a}	5.55 ± 0.03^{ab}	3.71 ± 0.05^{ab}	4.0 ± 0.14^{b}
Fragments with Kaptan FL in 0.3 concentration	2.9 ± 0.02^{a}	3.3 ± 0.05^{a}	5.9 ± 0.12^{a}	5.55 ± 0.03^{ab}	2.8 ± 0.12^{b}	3.3 ± 0.01^{c}

The mean values marked with different letters in the same row are significantly different (Tukey HSD test of multiple ranks $P<0.05$)

Kaptan FL in the lower concentration affected *Hebeloma* spp., *A. muscaria, P. involutus* by decelerating the growth of mycelium at the beginning of the experiment. It had no influence on the growth of *S. luteus*, and in species *S. bovinus* and *S. garnulatus* the slight stimulating effect on the growth rate was reported. When the mycelia developed up to the filter paper, there were two reactions of fungi. The mycelia of fungi *S. bovinus* and *S. Granulatus* continued to grow after the contact and completely covered the filter paper, i.e. the presence of fungicides did not have any influence on these fungi. The growth of mycelia of other fungi stopped in the contact with the filter paper.

The higher concentration of Kaptan had the similar effect on the growth of mycelia of all fungi except for *Hebeloma* spp.and *A. muscari,* in which the inhibition of growth was more intensive and the clearly differentiated inhibition zone between the mycelium and filter paper was formed. In the comparison with Benfungin, Kaptan FL had more intensive unfavourable effect on the observed mycorrhizal fungi.

Picture 1. *The growth of mycelium Hebeloma spp.and filter paper soaked by solution of fungicide Benfungin (0.06%)*



Picture 2. *The growth of mycelium Amanita muscaria and filter paper soaked by solution of fungicide Kaptan FL (0.30%)*

4. DISCUSSION

Undoubtedly, certain types of fungicides will be always applied in the process of production of forest seedling material. However, during the selection of fungicides for treatments the knowledge on the effect of these preparations on mycorrhizal fungi are necessary, i.e. the selected preparations must enable the undisturbed development of mycorrhizal fungi on the root of the seedlings. The results obtained in these researches point out to the different reactions of mycorrhizal fungi to the presence of fungicide Benfungin WP (0.04 and 0.06% concentrations) and to Kaptan FL (0.2 and 0.3% concentrations). Benfungin had no influence only on the growth of mycelium *Hebeloma* spp., whereas in the direct contact in both concetrations it affected all other fungi by stopping the growth of

mycelium. The presence of different concetrations of Benfungin WP did not have any influence on the growth of the observed mycorrhizal fungi.

Kaptan FL had the similar effect in both concentrations by stopping the growth of mycelium of *Hebeloma* spp., *A. muscaria, P. involutus* and *S. luteus*. It had the most adverse effect on the fungi *Hebeloma* spp., and *A. Muscaria*, in which the inhibition zone was formed. It had no influence on the growth of mycelium of *S. bovinus* and *S. garnulatus*.

These results point out to the fact that the application of fungicide Benfungin in the process of the production of forest seedling material had less adverse effects on the development of mycorrhizal fungi in comparison with the application of Kaptan FL. It is necessary to study the results obtained in the laboratory conditions in the field conditions.

The same results by multiple application based on kaptan were obtained by Castellano and Molina (1993) and Lazarev (1998). These scientists by different studies determined that some types of fungicides based on kaptan can have the adverse effect on mycorrhizal fungi, i.e. decrease the development of mycorrhiza. Somewhat different results were obtained by O´Neill and Mitchell (2000) during the researches of the effects of application of fungicides Kaptan and Benomyl on the development of mycorrhizal fungi which colonize *Picea sitchensis* and *Fraxinus excelsior*. The application of kaptan had the stimulating effect on the root length and ectomycorrhizal colonization of spruce and ash seedlings, whereas the application of benomyl 2-3 times caused the decrease of the number of ectomycorrhizal species of fungi on the root, as well as the reduction of the colonization of root.

The possible adverse effects of the application of pesticides, reflected in the destruction of mycorrhizal fungi, direct the further researches aimed at the controlled application of chemical preparations and the integral measures of fight. The same mycorrhizal fungi can have the important place in the biological measures of fights since they can provide the protection by secretion of antibiotic mucus from fungi or by stimulating the root cells of host plants which secrete antimicrobial metabolites by which some pathogens are killed (Dehne, 1982) or inhibited (Graham i Menge, 1982). The positive results were also obtained in the mycorrhization of seedlings by the selected species of mycorrhizal fungi upon the application of treatment of elimination of pathogenic fungi *Rizoctonia solani, Pythium sp., Fusarium oxysporum*, etc. In these conditions the seedlings are successfully inoculated by the selected mycorrhizal fungi as the saprophytic organisms are destroyed and the ectomycorrhizae which are antagonistic in the introduction of the selected species are naturally developed (Tacon et al., 1986). Regardless of the fact whether the mycorrhization is perfomed by the selected symbionts or it enables the development of the present fungi as the symbiont on the root, their presence helps to overcome the problem regarding the reforestation reflected in the inability of the transplanted plants to use the micro and macroelements from the soil (Lazarev, 2005). In numerous unfavourable conditions the presence of mycorrhiza on the root of tree seedlings is the only precondition for the surviving and growth of these seedlings (Rudawska et al, 2001).

5. CONCLUSION

The possible adverse effects of the application of pesticides, which are reflected in the inhibition of the growth of mycorrhizal fungi, direct the further researches aimed at the selection of the controlled application of chemical preparations and the increasingly significant application of biological measures of fight by knowing the specific requirements of all ligneous species individually.The pesticides should be applied only in the sensitive phases of the development of plants when they needed it most in the doses which significantly reduce the inoculums of mycorrhizal fungi.

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Summary

In the nurseries for the production of forest seedling material the different types of fungicides are applied as a part of the regular measures of tending and protection from the potential diseases. The most frequently used fungicides are Benfungin WP and Kaptan FL. The application of these fungicides along with the positive effect on the elimination of pathogens can have the adverse effect on the beneficial microflora in the soil. In order to study the influence of these fungicides on the most significant species of mycorrhizal fungi, the experiment was set in the laboratory conditions in which the most frequently isolated species of mycorrhizal fungi on the forest seedling material were used: *Suillus luteus* (L. ex Fr.) S. F. Gray, *Suillus bovinus* (L.ex Fr) Roussels, *Suillus granulatus* (L. ex Fr.)O. Kuntze, *Paxillus involutus* (Batsch ex fr.), *Hebeloma spp.*, *Amanita muscaria* (L. ex Fr.) Hook. The applied concentrations of fungicides were determined by the recommendation of the producer for the use of Benfungin (0.04 and 0.06 % concentrations) and Kaptan FL (0.2 and 0.3% concetrations).

Kaptan FL had the similar effect in both concentrations on *Hebeloma* spp., *A. muscaria*, *P. involutus* and *S. luteus*, by stopping the growth of them. It had no influence on *S. bovinus* and *S. garnulatus*.

Benfungin had the similar effect on the growth of mycelia of all observed species of fungi in both higher and lower concentrations. It affected all observed species of fungi by inhibiting the growth of mycelium, except for the mycelium of the species *Hebeloma* spp, the development of which was undisturbed.

Reviewer: **Ph. D. Vera Raičević**