THE INFLUENCE OF THE USE OF PESTICIDES ON PRINCIPAL GROUPS OF MICROORGANISMS PRESENT IN THE EUTRIC CAMBISOL

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Abstract

To produce a healthy and vigorous afforestation material, besides the technical works are needed measures to prevent and control the pathogenic agents presents in forestry crops. Pest control and pesticide management are integral parts of the best management practices during the growing period of nursery crops. Pesticides used in nursery settings should be selected not only to control the pest, but also to limit its ability to negatively impact the environment.

The objective of this investigation was to determine the effects of the fungicide Microthiol on principal groups of microorganisms determined in eutric cambisol collected from a nursery. The fungicide was applied at recommended dosages. The effects of this pesticide on total viable bacteria were investigated in laboratory experiments. Enumeration of total number of microorganisms, Actinomycetes, yeast-mold, nitrogen fixing bacteria and nitrifying bacteria was accomplished by serial dilution and plate count technique or most probable number method. Based on the obtained results, was calculated the bacterial indicator of the soil quality (BISQ).

The results were analyzed with the ″Student″ test used to determine the significance or non-significance of differences between the values. The statistical interpretation of the results suggest that the fungicide Microthiol had no influence on the physical and chemical properties of the eutric cambisol and also there were no significant differences between the untreated and treated soil variant.

The microorganisms are capable to grow in the presence of several commercial pesticides. Catabolism and detoxification metabolism occur when a soil microorganism uses the pesticide as a carbon and energy source.

Key words: fungicide, nursery, microorganisms, soil, properties.

INTRODUCTION

Some of the most serious pests affecting the health of the holm oak seedlings, often occurring together, include: *Rosallinia quercina, Alternaria solani, Fusarium sp., Botrytis sp., Phytophtora sp.*

The presence of the pathogens in forestry crops occurs regularly and preventive treatments of the seeds are needed in nurseries. The strong attacks of phyto-pathogens cause the so-called mildews and the most important of them is *Microsphaera abbreviata* (Holonec et al., 2007).

The effects of mildews are manifested by the decrease of growing and biomass accumulations, with direct repercussions on the further quality of the attacked exemplars. As the age of the attacked exemplars is younger, as the intensity and degrees of attack are stronger. In this phase, the only measure of quick intervention, with direct effects is the chemo therapy (Holonec et al., 2007). *Microsphaera abbreviata* develops itself on the aerial organs (leaves and sprouts) of different species of oaks, that they cover on a more or less great surface with a mildly tissue, whitish, with an aspect similar to the manna of grape vine.

The fungi attack since the first leaves of seeding plants or spouts up to leaves and sprouts of mature trees. Infections are influenced by the conditions of the environment, state of vegetation and the consistency of the undergrowths.

The spreading of the fungi is very intense in years with gentle springs, with high temperatures, moderate rainfall; they even spread during May and June, when the optimum conditions are met for the development of leaves and sprouts. (Holonec et al., 2007).

Dryness may impair the attack due to the decrease of the fragility of leaves and oaks. It has to be remarked that a dryness period followed by rainfalls may stimulate the attacks and increase their intensity. The worst effects of the attack are registered in the damaged undergrowths, in seed planting cultures and especially in young natural regenerations that are present in the form of a concentrated mass of leaves on open surfaces (Georgescu et al., 1957).

It is important to use only biodegradable substances, with no remanence on soil level. There are a lot of substances that may be used in fighting against mildew such as the fungicides: *Systhane, Karathane, Champion, Trifmine and Alert.* The concentrations and doses for applying are differently established for each substance depending on the technical instruction for the use of products (Marcu Olimpia, Dieter S., 1995).

MATERIALS AND METHODS

The research was conducted in autumn 2009 on eutric cambisol. To investigate the influence of the fungicide *Microthiol* on the eutric cambisol properties, the soil was collected from a forest nursery located in Sudrigiu village.

The fungicide *Microthiol* (0.3-0.4%) was applied on the treated soil variant to combat the powdery mildew of holm oak seedlings produced by *Microsphaera abbreviata.* The control soil samples were collected from a surface where the fungicide was not applied.

Microthiol (wettabla sulphur) is a unique formulation of sulfur used for controlling powdery mildew. *Microthiol* is produced by a patented micronizing process that ensures uniform hyper-dispersible granules.This results in superior dry flowable sulfur that can be measured more accurately and with virtually no dust. *Microthiol* goes into suspension quickly and completely, providing more consistent coverage and greater surface contact compared to traditional sulfur products.

The soil was collected from 0-20 and 20-40 profile. Plant material and soil macrofauna were removed and the soil samples were sieved (<2mm) and mixed in the laboratory.

Some physical and chemical properties of the soil samples were determined as follows: soil moisture using gravimetrically method by oven-drying fresh soil at 105^0C, pH in 1:2:5 soil water suspension by pH-meter, organic material by using Walkley-Black method, nitrate (NO_3-N) determination by colorimetric method, ammonium with Nessler reagent and mobile P, mobile K, using extraction with Egner–Riehn–Domingo method.

Total number of microorganisms, *Actinomycetes*, yeast-mold, nitrogen fixing bacteria and nitrifying bacteria were determined using the dilution method. The soil samples (10 g) were suspended in 90 ml distilled water. Dilutions (of 10^{-6}) were prepared from the soil samples using distilled water and these were dispersed with a top drive macerator for 5 min. The soil samples taken from suitable dilution were planted in or on the solid or liquid feeding medium as required.

Plate count method was used to estimate the total number of microorganisms on a solid nutrient medium containing meat extract (Atlas, 2004), total number of *Actinomycetes* on agar with glucose and asparagines and total number of yeast-mold on Sabouraud Agar. Also, total number of *Azotobacter* was revealed on Ashby's glucose agar. After incubation the counts obtained were multiplied by the dilution factor to obtain the number of colony forming unit per gram of soil.

To estimate the number of nitrifying bacteria the most probable number method (MPN) was used. Nitrate bacteria and nitrite bacteria were cultured in a liquid culture medium containing Winogradsky's salt solution (Drăgan-Bularda medium, 2000). After incubation the most probable number of nitrifying bacteria was calculated according to the statistical table of Alexander (1965).

The bacterial indicator of the soil quality (BISQ) after Muntean (1995-1996) was calculated based on the number of total viable bacteria, total number of *Actinomycetes,* yeast-mold, *Azotobacter* and total number of nitrifying bacteria.

The results were analyzed with the ″Student″ statistics method to determine the significance or non-significance of the differences between the values.

RESULTS AND DISCUSSION

The moisture values of the eutric cambisol ranged between 4,62% and 8,69% with no significant differences ($p>0,10$) between the two soil variant such as, untreated eutric cambisol and treated eutric cambisol

(Table1). The pH values determined in the two variants of the eutric cambisol indicate a strong acid reaction. In the treated eutric cambisol, where the fungicide *Microthiol* was applied, the pH values were much higher (insignificant) compared with the values of untreated eutric cambisol. The content in $N\text{-}NO_3$ and $N\text{-}NH_4$ was much higher in the untreated eutric cambsiol and both variants of soil presented a low supply with mobile P and mobile K (table 2). The statistical interpretation of the results suggest that the fungicide *Microthiol* had no influence on the physical and chemical properties of the eutric cambisol and there were no significant differences between the untreated and treated soil variant ($p>0.10$) (table 2).

Table 1

The mean values of the eutric cambisol moisture (% of weight)

Vegetation period	Soil variant	Depth (cm)	Average values
Autumn 2009	Untreated eutric cambisol	0-20	4,62
		20-40	7,35
	Treated eutric cambisol	0-20	8,42
		20-40	8,97

Table 2

The mean values of the chemical parameters of eutric cambisol

Vegetation period	Soil variant	Depth (cm)	pH	N-NO_3 ppm	N-NH_4 ppm	mobile P ppm	mobile K ppm	Organic matter %
Autumn 2009	Untreated eutric cambisol	0-20	4,4	5,95	2,7	14	70	3,93
		0-40	3,7	5,90	5,9	18	120	4,17
	Treated eutric cambisol	0-20	2,3	5,70	3,3	11	120	3,60
		20-40	3,6	5,90	2,3	9	60	2,22

Table 3

The influence use of the fungicide *Microthiol* on principal groups of microorganisms (cells $\cdot g^{-1}$ dry matter soil) present in eutric cambisol

Vegetation period	Soil variant	Depth (cm)	Total microflora	*Actino-mycetes*	Yeast-mold	*Azoto-bacter*	Nitrifying bacteria
Autumn 2009	Untreated eutric cambisol	0-20	6,88	5,39	4,65	2,52	2,37
		20-40	6,65	4,47	3,81	-	-
	Treated eutric cambisol	0-20	6,82	5,33	4,66	1,17	2,02
		20-40	6,38	4,17	3,54	-	-

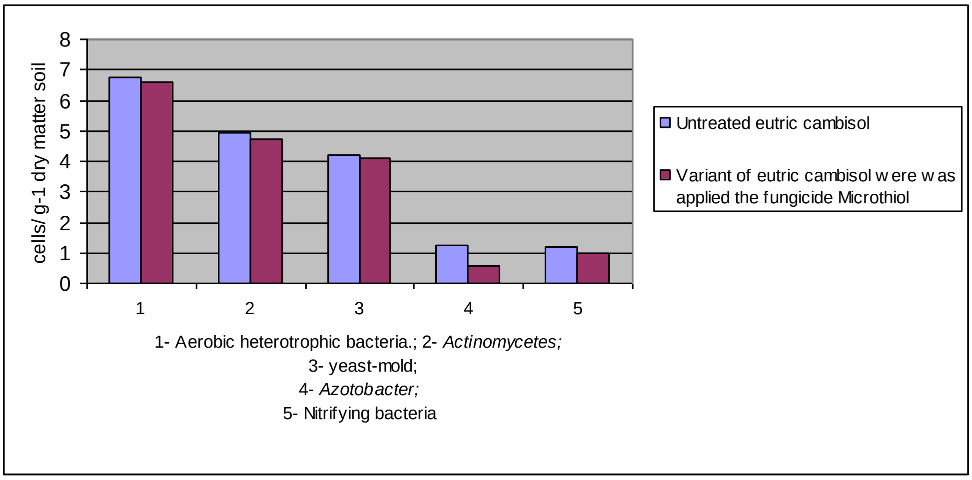

Fig. 1. Principal groups of microorganisms determined in the eutric cambisol from a tree nursery

In the order of their abundance, the aerobic heterotrophic bacteria ($2,4\cdot10^6$-$7,7\cdot10^6$ CFU/g dry matter soil) were followed by the *Actinomycetes* ($15\cdot10^3$-$250\cdot10^3$ CFU/g dry matter soil), yeast-mold ($3,5\cdot10^3$-$45\cdot10^3$ CFU/g dry matter soil). The smallest number was observed for the nitrifying bacteria (10^2 cells/g dry matter soil) and *Azotobacter* (10^1-10^2 CFU/g dry matter soil). As it can be seen (fig. 1) the aerobic heterotrophic bacteria, *Actinomycetes* and yeast-mold are well represented numerically compared with other groups such as *Azotobacter* and nitrifying bacteria which have been inhibited by the strong acid reaction of the eutric cambisol. The development of *Actinomycetes* depends on the presence in soils of plant debris. The yeast-mold uses the pesticides as a carbon and energy source. These microorganisms have an important role in affecting the persistence of pesticides, having the capacity for rapid elimination of highly persistent or toxic chemicals. The treatments with pesticides and chemical fertilizers have inhibitory effect on the development of the *Azotobacter*. *Azotobacter* is widely distributed in soils having a pH value of 6.0 or above.

Azotobacter is able to utilize nitrates, ammonium salts, amino acids and peptones as a source of nitrogen and will only assimilate atmospheric nitrogen when nitrogen in a combined form is absent from the substrate. Factors such as organic matter, water content, oxygen supply, temperature and soil pH can affect the nitrification capacity of the soil. Also the nitrobacteria are affected by the use of chemical fertilizers. The pH value of the soil is correlated with the presence of the nitrobacteria and is an important factor which affects the nitrification rates and losses of N. Many studies have shown significant relationships between soil pH and percentage of nitrification. The nitrobacteria are widely distributed in soils having a pH value of 7.3-8. The pH value of 6-6.5 has inhibitory effects on the development of the nitrobacteria. The effects of soil pH on nitrification, therefore, influenced the amounts of NO_3^- lost by denitrification or leaching during spring rainfall. The observed effects of pH on nitrification rates suggest that economic and environmental benefits of delaying application of fertilizer N may be greater in higher-pH soils than in lower-pH soils.

The main groups of microorganisms studied in the eutric cambisol collected from a nursery have not varied significantly (p>0,10) and the fungicide *Microthiol* used to combat the powdery mildew produced by *Microsphaera abbreviata* did not influence the microbiological properties of the studied soil (table 3).

Based on the number of bacteria belonging to each monitored groups of microorganisms, the bacterial potential of the eutric cambisol was appreciated and the bacterial indicator of soil quality (BISQ) was calculated. In autumn 2009, the control eutric cambisol, in which the fungicide *Microthiol* was not applied, presented a BISQ value (3,674) muh higher than that of the treated eutric cambisol (3,409). The BISQ values indicate a moderate density of the bacterial groups.

CONCLUSIONS

The use of pesticides has become an integral essential part of modern agriculture. Pesticides are often applied several times during one crop season and a part always reaches the soil. The wide use of pesticides has created numerous problems, including the pollution of the environment. The influence of pesticides on soil microorganisms is dependent on physical, chemical and biochemical conditions, in addition to the nature and concentration of the pesticides. Many studies have demonstrated the fact that microorganisms are capable to grow in the presence of several commercial pesticides. Catabolism and detoxification metabolism occur when a soil microorganism uses the pesticide as a carbon and energy source. The main groups of microorganisms studied in the tree nursery eutric cambisol have not varied significantly and the fungicide *Microthiol* used to combat the powdery mildew produced by *Microsphaera abbreviata* did not influence the physical, chemical and biological properties of the studied soil.

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