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EFFECT OF DIFFERENTIATED ORGANIC TREATMENT ON INSECTICIDE ACTIVITY OF ENTHOMOPATHOGENIC FUNGI AND NEMATODES

M. Jaworska[1], *D. Ropek*[1], *K. Mazur*[2], *B. Filipek-Mazur*[2]

[1]Department of Agricultural Environment Protection; [2]Department of Agricultural Chemistry
Agricultural University of Cracow
Al. Mickiewicza 21, 31-120 Kraków, Poland

A b s t r a c t. The present work aimed at investigating diversified organic treatment including sewage sludge on the insecticide activity of enthomopathogenic fungi and nematodes. Crude sludge from biological tannery sewage treatment plant and vermicomposts based on the sludge with added straw and fruit tree leaves were used for the experiment. Organic treatment significantly affected pathogenic properties of enthomopathogenic nematodes naturally present in the soil of the investigated plots. The highest death rate among trap insects was detected in the soil from untreated plots or fertilised by farmyard manure and vermicomposts. Nematodes from examined plots reproduced in varying numbers depending on the treatment applied on individual plots. The lowest number of invasive larvae was obtained for nematodes from plots fertilised by straw vermicompost, (crude)sewage sludge and hair. The applied organic treatment influenced also insecticide activity of the applied nematodes towards *G. mellonella* larvae. The highest death rate of *G. mellonella* larvae was observed in the soils treated with organic fertilisers and farmyard manure. Clear decrease in *B. bassiana* pathogenic properties was detected in soil fertilised with sewage sludge and hair. Chemical sludge and fertilisation with hair caused also a clear decrease in *S. carpocapsae* pathogenic properties towards *G. mellonella* larvae. The obtained results reveal unfavourable effects of crude sewage sludge on the pathogenic properties of enthomopathogenic fungi and insects. Vermicomposts obtained from this sludge revealed a positive influence on the microorganisms. This fact may indirectly improve health conditions of plants.

K e y w o r d s: enthomopathogenic fungi and nematodes, insecticide activity, organic fertilization, soils.

INTRODUCTION

Interest in sewage sludge application as a treatment for arable lands and meadows has increased lately. Application of this sort of fertilization may bring benefits of utilising sewage wastes produced in large quantities. While using sewage sludge, one should consider not only the effect of this kind of fertilisation on the

amount and quality of yield but also biological activity of soil. Sewage sludge discharge into soil may have unfavourable impact on beneficial microorganisms.

Enthomopathogenic fungi and nematodes play an important role in natural ecosystems and agrocenoses. Many harmful insect populations are controlled by enthomopathogenic microorganisms naturally present in soils. Most insects are related to soil environment. Part or a whole life-cycle of many pests undergo in the soil. There are many enthomopathogenic fungi and nematodes in arable and meadow soils [2]. Miętkiewski *et al.* [5,6] mention four enthomopathogenic fungi species commonly isolated from the soil: *Beauveria bassiana, Metarhizium anisopliae, Paecilomyces farinosus* and *P. fumosoroseus*. A commonly used method for isolating enthomopathogenic microorganisms is a technique proposed by Zimmerman [7], in which larvae of *G. mellonella* are used as trap insects.

Activity of enthomopathogenic fungi and nematodes is affected by many abiotic factors, like temperature, soil moisture or soil reaction. Mineral and organic treatment also influence activity and infectivity of enthomopathogenic microorganisms [1,3].

The present work aimed at investigating diversified organic treatment including sewage sludge on the insecticide activity of enthomopathogenic fungi and nematodes.

MATERIALS AND METHODS

The present experiment was carried out in Sędziszów on brown, medium cohesive soil, containing 55% fine fraction and pH_{KCl} 5.29. The content of organic carbon was 2.17% and organic matter 3.91%.

Crude sludge from a biological tannery sewage treatment plant and vermicomposts based on the sludge with added straw and fruit tree leaves were used for the experiment. Crude sludge from a chemical sewage treatment plant and hair wastes from tannery were also utilised. The dose of organic fertilizers was set according to nitrogen content in them and the dose of 100 kg N/ha. Doses of phosphorus and potassium were supplemented with mineral fertilizers up to the highest content in organic fertilizers. Corn was cultivated on the field and the forecrop were fodder beets. Soil samples for laboratory analyses were taken in September.

Soil from the plots was placed into sterile Petri dishes (diameter 10 cm). Five *Galleria mellonella* larvae were put on soil surface. The mortality of *G. mellonella* larvae was evaluated daily. Dead larvae were transferred to separate dishes to determine death cause. Larvae bearing nematode infestation symptoms were placed on watch glasses in Petri dishes and the reproduction rate of nematodes coming from the plots with different fertilization was determined. Larvae with grown

mycelium were placed in Petri dishes laid with wet filter paper. Afterwards mycelium was inoculated on glucose-potato agar in order to carry out systematic determination.

It was also examined how organic treatment affects enthomopathogenic activity of nematodes and fungi applied into soil (strains reared in laboratory condition). Fungus *Beauveria bassiana*, which is a Polish strain from *Leptinotarsa decemlineata* and nematode *Steinernema carpocapsae* were tested. Nematodes were reared on *G. mellonella* larvae. Liveliness of the tested nematodes and concentration of invasive larvae in water suspension was checked prior to their use in the experiment. The experiment was carried out in Petri dishes. 300 invasive larvae of *S. carpocapsae* were applied into the soil. Five *G. mellonella* larvae were put onto the soil. Petri dishes were stored at 25 °C in the dark. Mortality of *G. mellonella* larvae was checked daily. Dead larvae were put into separate dishes on watch glasses to determinate the reproduction rate.

Fungus *B. bassiana* was reared on glucose-potato agar in 25 °C in the dark. After three weeks conidia were harvested. The conidia concentration in water suspension was determined using hemocytometr according to the technique described by Lipa and Śliżyński [4]. The soil was mixed with the water suspension of 1 million spores. Pathogenicity of *B. bassiana* in soil treated with different organic fertilizers was evaluated on the grounds of the mortality of *G. mellonella* larvae.

The results were statistically studied by analysis of variance, and means were separated according to the Duncan's multiple-range test.

RESULTS AND DISCUSSION

Mortality of trap insects was in most cases caused by enthomopathogenic nematodes and only in small degree by enthomopathogenic fungi (Table 1). Therefore it can be assumed that the mortality of *G. mellonella* larvae shows the influence of different organic fertilization on the pathogenic properties of nematodes naturally present in the soil of investigated plots.

Organic treatment significantly affected pathogenic properties of enthomopathogenic nematodes naturally present in the soil of the investigated plots (Table 1). The highest death rate among trap insects was detected in the soil from untreated plots or fertilised by farmyard manure and vermicomposts. Fertilisation with hair caused a decrease in the pathogenic properties of enthomopathogenic nematodes, which was visible as lower death rate among trap caterpillars. There are not many publications concerning the influence of mineral and organic fertilization on enthomopathogenic nematodes. Long lasting mineral fertilization causes

Table 1. Influence of organic fertilization on the occurrence and pathogenic properties of fungi and nematodes naturally present in the soil of the investigated plots (% of dead caterpillars of *G. mellonella)*

Fertilization	Mortality of trap insects - *G. mellonella* (%)	Occurrence of entomopathogenic microorganisms in the soil of investigated plots	
		Fungi	Nematodes
Control untreated	85c*	+	+
NPK - mineral	60a	-	+
Farmyard manure	80bc	-	+
Vermikompost + straw	85c	-	+
Vermikompost + leaves	75abc	+	+
Raw sludge from biological sewage treatment plant	70abc	-	+
Raw sludge from chemical sewage treatment plant	65ab	-	+
Hair sludge + NPK	60a	-	+

*Means followed by the same letters within a column are not significantly different (P=0.05) according to the Duncan's multiple-range test.

lower pathogenic properties of nematodes [3]. Also Bednarek [1] established that long lasting contact with mineral fertilizers decreased infectivity of nematodes against *G. mellonella* larvae.

Enthomopathogenic fungi were found only on control plots without fertilization and on plots treated with vermicompost based on leaves (Table 1). Fungus *Paecilomyces fumosoroseus* was found on the plots treated with vermicompost based on the leaves and *Beauveria bassiana* on the control plots. Jaworska *et al.* [3] stated that mineral fertilization did not affect the occurrence of enthomopathogenic fungi. Enthomopathogenic nematodes were present in soil of all plots irrespective of used fertiliser.

Nematodes from the examined plots reproduced in varying numbers depending on the treatment applied on the individual plots (Table 2). The lowest number of invasive larvae was obtained for nematodes from plots fertilised by straw vermicompost, (crude) sewage sludge and hair. Vermicomposts based on sewage sludge and straw or leaves affected less disadvantageously the nematode reproduction. Nematodes from the untreated plots (control) reproduced in the largest numbers. Both mineral fertilization (NPK) and farmyard manure caused enthomopathogenic nematodes reproduce in a smaller number in comparison to the control. It betoken of unfavourable influence of both organic and mineral fertilization on the reproduction of enthomopathogenic nematodes naturally present in the soil of investigated plots.

T a b l e 2. Influence of organic fertilization on the reproduction of enthomopathogenic nematodes naturally present in the soil of the investigated plots

Fertilization	Nematodes/ *G. mellonella* caterpillars
Control untreated	50 243 d*
NPK - mineral	43 295 cd.
Farmyard manure	35 490 bcd
Vermikompost + straw	17 900 abc
Vermikompost + leaves	29 081 abc
Raw sludge from biological sewage treatment plant	642 a
Raw sludge from chemical sewage treatment plant	19 40 abc
Hair sludge + NPK	15 561 ab

*Means followed by the same letters within a column are not significantly different (P=0.05) according to the Duncan's multiple-range test.

The applied organic treatment influenced also insecticide activity of the applied nematodes towards *G. mellonella* larvae (Table 3). The highest death rate of *G. mellonella* larvae was observed in the soils treated with mineral fertilisers and farmyard manure. Clear decrease in *B. bassiana* pathogenic properties was detected in soil fertilised with sewage sludge and hair. *S. carpocapsae* nematodes revealed the highest pathogenic properties in the untreated soil and in soil from plots fertilised with straw vermicompost. Chemical sludge and fertilization with hair caused a clear decrease in this nematode pathogenic properties towards *G. mellonella* larvae. Sewage sludge utilisation by preparing vermicompost decreased the unfavourable effect of this waste on *B. bassiana* and *S. carpocapsae* pathogenic properties.

T a b l e 3. Influence of organic fertilization on the pathogenic properties of fungi and nematodes applied into the soil (% of dead caterpillars of *G. mellonella*)

Fertilization	Mortality of trap insects - *G. mellonella* (%)	
	B. bassiana	*S. carpocapsae*
Control untreated	86.6c*	100b
NPK - mineral	100c	93.3ab
Farmyard manure	93.3c	93.3ab
Vermikompost + straw	80bc	100b
Vermikompost + leaves	60ab	93.3ab
Raw sludge from biological sewage treatment plant	46.6a	80a
Raw sludge from chemical sewage treatment plant	80bc	73.3a
Hair sludge + NPK	60ab	73.3a

*Means followed by the same letters within a column are not significantly different (P=0.05) according to the Duncan's multiple-range test.

T a b l e 4. Influence of organic fertilization on the reproduction of *Steinernema carpocapsae* applied into the soil

Fertilization	Nematodes/ *G. mellonella* caterpillars
Control untreated	98450 d*
NPK - mineral	82120 cd.
Farmyard manure	90744 bcd
Vermikompost + straw	80131 abc
Wermikompost + leaves	78081 bc
Raw sludge from biolog. sewage treatment plant	60292 a
Raw sludge from chemical sewage treatment plant	68434 ab
Hair sludge + NPK	69391 ab

*Means followed by the same letters within a column are not significantly different (P=0.05) according to the Duncan's multiple-range test.

Influence of organic fertilization on the reproduction of *S. carpocapsae* applied into soil was also examined (Table 4). Fertilization with sewage sludge, chemical sludge and hair caused lower reproduction of nematodes. Nematodes reared in laboratory were less sensitive to the sewage treatment than nematodes naturally present in the soil. It can be connected with shorter contact of applied nematodes (strain reared in laboratory) with the tested soil.

CONCLUSIONS

The obtained results revealed unfavourable effects of crude sewage sludge on the pathogenic properties of enthomopathogenic fungi and insects. Sewage sludge disadvantageously affected both microorganisms naturally present in the soil and the strains reread in laboratory. Vermicomposts obtained from those sludge revealed positive influence on the microorganisms and due to this fact one may indirectly expect better plant healthiness. It indicates the necessity of utilising these sewage sludge prior to their use as fertilizers by preparing composts or vermicomposts.

REFERENCES

1. **Bednarek A.:** Ecological factors affecting the biological activity of entomogenous nematodes in soil habitats. Wyd, SGGW-AR, Warszawa, 1990.
2. **Jaworska M., Dudek B.:** Występowanie owadobójczych nicieni w glebach wybranych warzyw. Zesz. Nauk. AR, Kraków, Ogrodnictwo, 20, 267, 131-140, 1992.
3. **Jaworska M., Ropek D., Gorczyca A., Mazur K.:** Occurrence of enthomopathogenic microorganisms in the soil of mountain meadow of long-term mineral fertilization. Proc. Symp. "Effectiveness and practical application of biological control in plant protection". Skierniewice, 92-98, 1997.

4. **Lipa J.J., Śliżyński K.:** Wskazówki metodyczne i terminologia do wyznaczania średniej dawki śmiertelnej (LD_{50}) w patologii owadów i toksykologii. Prace Nauk. Inst. Ochrony Roślin, XV, 1, 59-82, 1973.
5. **Miętkiewski R., Żurek M., Tkaczuk C., Balazy S.:** Występowanie entomopatogenicznych grzybów w glebie ornej, leśnej oraz ściółce. Roczn. Nauk Roln., E, 21, 1/2, 61-68, 1992.
6. **Miętkiewski R., Tkaczuk C., Zasada L.:** Występowanie grzybów entomopatogennych w glebie ornej i łąkowej. Acta Mycologica, 27(2), 197-203, 1992.
7. **Zimmermann G.:** Galleria bait method for detection of enthomopathogenic fungi in soil. Zeitsh. Angew. Entomol., 2, 213-215, 1986.