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THE ROLE OF MAGNESIUM IN THE PROTECTION OF ENTOMOPATHOGENIC NEMATODES FROM SOIL POLLUTION WITH OIL DERIVATIVES*

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Abstract

The aim of the paper was to identify the influence of magnesium on the preservation of pathogenic abilities of entomopathogenic nematodes living in soil contaminated with oil derivatives.

Entomopathogenic nematodes living under natural conditions in soil or applied to soil as biopreparations for plant pest control are sensitive to soil contamination with heavy metals and oil derivatives. These nematodes do not respond directly by higher mortality but by a decrease in their pathogenic abilities, which adversely affects the success in searching and eliminating pests.

A Polish commercial preparation called Owinema SC, containing infective juveniles of *Steinernema feltiae* Filipjev (*Rhabditida: Steinernematidae*), was used to test the effect of oil derivatives on pathogenicity and reproduction of entomopathogenic nematodes.

Soil was polluted with petrol, diesel oil and used engine oil; the control was soil unpolluted with oil derivatives. Magnesium sulphate was also added ($MgSO_4 \cdot H_2O$) in the amount of 160 mg per 1 liter of the suspension. The three oil derivatives in concentrations of: 0.3, 0.6 and 1.0 g or in double doses, i.e. 0.6, 1.2 and 2.0 g were poured over weighted soil batches. The control remained intact. Three replications of each treatment were made. Subsequently, *Tenebrio molitor* L. larvae used as bait inesct were placed in containers with the suspension.

The bait insects were kept in the containers for 7 days, and afterwards they were removed from the soil and taken from the traps in order to check their mortality rate.

Furthermore, to test the reproduction ability of entomopathogenic nematodes, dead larvae were moved to “islands” previously prepared according to the Dutky method.

The results were presented as the number of entomopathogenic nematodes per 1 dead larvae of *Tenebrio molitor* L.

Key words: magnesium, entomopathogenic nematodes, contamination with oil derivatives.

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ROLA MAGNEZU W OCHRONIE OWADOBÓJCZYCH NICIENI W PRZYPADKU ZANIECZYSZCZENIA GLEBY SUBSTANCJAMI ROPOPOCHODNYMI

Abstrakt

Celem pracy było rozeznanie wpływu magnezu na ochronę zdolności patogenicznych owadobójczych nicieni przebywających w glebie skażonej substancjami ropopochodnymi.

Owadobójcze nicienie żyjące w warunkach naturalnych w glebie, a także stosowane doglebowo jako biopreparaty do zwalczania szkodników roślin, są wrażliwe na skażenie gleby metalami ciężkimi oraz substancjami ropopochodnymi. Nicienie te nie reagują wówczas śmiertelnością, ale zmniejszeniem zdolności patogenicznych, co wpływa na ich efektywność w wyszukiwaniu i zabijaniu szkodników roślin.

W doświadczeniu laboratoryjnym wykazano, że siarczan magnezu dodany do gleby skażonej substancjami ropopochodnymi zwiększa zdolności patogeniczne nicieni, ale nie reprodukcje wewnątrz owadów żywicielskich. Owinema S.C. – polski preparat komercyjny zawierający larwy inwazyjne *Steinernema feltiae* (*Rhabditis*, *Steinernematidae*) – używano w testach laboratoryjnych nad oddziaływaniem zanieczyszczenia ropopochodnymi na patogeniczność i reprodukcje owadobójczych nicieni.

Glebę zanieczyszczono benzyną, olejem i olejem silnikowym. Dodano siarczan magnezu ($MgSO_4$ H_2O) w dawce 160 mg l^{-1} zawiesiny. Ropopochodne użyto w dawkach 0,3 g, 0,6 g i 1,0 g lub podwójnej, tj. 0,6 g, 1,2 g i 2,0 g. Gleba nie zanieczyszczona stanowiła próbę kontrolną. W pojemniku z glebą umieszczono owady testowe – larwy mączlika młynarka *Tenebrio molitor* L. Owady po 7 dniach wyjmowano i sprawdzono ich śmiertelność związaną z patogenicznością nicieni i zanieczyszczeniem ropopochodnymi.

Zdolności reprodukcji nicieni badano metodą Dutkego na „wyspach” szkiełek w szalkach. Wyniki przedstawiono jako liczebność larw nicieni z 1 martwej larwy owada *T. monitor*.

Słowa kluczowe: magnez, nicienie owadobójcze, zanieczyszczenie ropopochodnymi.

INTRODUCTION

Under natural conditions, entomopathogenic nematodes of the families *Steinernema* and *Heterorhabditis* live in soil and kill insects by introducing into their bodies *Xenorhabdus* sp. *Photorhabdus* sp. bacteria, which act as pathogens. We have learned to culture these nematodes on a big scale and use them as biopreparations against various plant pests (Bedding 1984, Ehlers 2001). We make use of them for the biological control of harmful insects all over the world (Grewal 2005). Plant protection with these organisms is effective and safe to the environment (Webster 1980). At present, integrated agriculture should combine biological methods, such as biopreparations and protection of natural enemies of pests living in the environment, with other non-chemical and chemical methods. Unfortunately, entomopathogenic nematodes are sensitive to soil pollution with heavy metals (Jarmuł, Kamionek 2000) and oil derivatives (Gospodarek, Jaworska 2009, Jaworska, Gospodarek 2009). Although nematodes do not respond directly by elevated mortality, their ability to search and parasitize insects suffers. It was found that magnesium interaction with heavy metal ions positively affects the entomopathogenic activity of nematodes (Jaworska et al. 1999). The aim of the paper was to identify the influence of magnesium on the pathogenic abilities of entomopathogenic nematodes in soil contaminated with oil derivative substances.

MATERIAL AND METHODS

A representative soil sample collected for laboratory experiments was analyzed at the Chemical-Agricultural Station in Krakow. The soil parameters were determined as follows: soil acidity pH in KCl 6.7, i.e. neutral reaction, light soil, liming unnecessary, total nitrogen content 0.161%, and humus content 2.48%. The content of microelements was also assessed. The sample revealed a low concentration of potassium (9.3 mg 100 g^{-1} of soil) and a high content of magnesium and potassium (respectively 5.2 and 17.5 mg 100 g^{-1} of soil).

A Polish commercial preparation called Owinema SC, containing infective juveniles of *Steinernema feltiae* Filipjev (*Rhabditida: Steinernematidae*), was used to test the effect of oil derivatives on the pathogenicity and reproduction of entomopathogenic nematodes. The preparation is manufactured by Owiplant Ltd. Horticultural Enterprise in Poznań. Containers lined with filter paper discs and containing nematodes were prepared, to which the above soil was added and polluted with petrol, diesel oil and used engine oil; the control was soil unpolluted with oil derivatives.

The first test series made use of commercial Owinema. Half a packet (100 g) was divided into two parts, which were then added to 300 ml of distilled water (150 ml each). A 3-ml dose of the suspension was used, corresponding to *ca* 250 000 specimens of infective juveniles per container. In the second series, magnesium sulphate ($MgSO_4 \cdot H_2O$) was added to the other part, in the amount of 16 mg per 1 l of the suspension. Three oil derivatives (mentioned above) in concentrations of: 0.3 g, 0.6 g and 1.0 g were poured over the soil weighted portion. The control remained unchanged. Three replications were made.

Owinema was used again in the second experiment. The remaining half of the packet (100 g) was divided into two parts and each part was thoroughly stirred in 150 ml of distilled water. Neither the number of nematodes nor the amount of added magnesium sulphate changed. The same oil derivatives were applied but in double doses, i.e. 0.6 g, 1.2 g and 2.0 g. The control objects remained unchanged. Three replications were made.

Subsequently, bait insects such as *Tenebrio molitor* L. larvae, purchased in a zoological shop, were placed in the containers with the suspension. In order to prevent their escape from a container, the larvae were first put in “traps” specially prepared from cut drinking straws. Additionally, each straw was specially perforated in order to make easy the host body infestation by nematodes. When insect larvae were put in a trap, its ends were sealed with plasticine. Five traps with larvae were placed in each container. The next stage involved covering the larvae with a 100 g soil weighted portion and then the oil derivatives in three different concentrations were poured over. The average temperature in the laboratory during the experiment was ±31°C.

Bait insects were kept in the containers for 7 days, after which they were removed from the soil and taken from the traps in order to check their mortality. Live specimens were put separately on Petri dishes lined with filter paper and then kept under laboratory conditions. Mortality of *Tenebrio molitor* L. was checked over 7 consecutive days. Mortality was expressed as the percentage of dead specimens in relation to all larvae per replication. *Tenebrio molitor* L. larvae were selected for the tests because they are readily available and easy to culture. The pest is a polyphagous and may be encountered on all plant products. It is a comopolitan species, which prefers closed spaces and does not occur in high concentrations. Another important characteristic of this species is their sensitivity to the infestation with entomopathogenic nematodes, which is why they are often used as traps.

Furthermore, in order to test the reproduction rate of entomopathogenic nematodes, dead larvae were removed to “islands” previously prepared according to the Dutky method, i.e. dead larvae were placed on a watch glass covered with a filter paper disc, and a small amount of distilled water was added to prevent the drying up of larvae.

The suspension with multiplied nematodes was poured from a Petri dish into 100 ml plastic cups, which were next put into a refrigerator and kept at 4°C. This step was repeated several times over two weeks. At the subsequent stage, nematodes were counted under a microscope. The results were presented as the number of entomopathogenic nematodes per 1 dead larvae of *Tenebrio molitor* L.

The significance of differences between the results was tested by means of one-way Anova. The means were differentiated using the Duncan’s test at the significance level $p = 0.05$.

RESULTS

The mean mortality of *Tenebrio molitor* L. larvae (%) is presented in Figure 1.

Significant differences between objects were demonstrated by analyses. The lowest mortality, about 10%, was recorded in the objects where petrol and engine oil were applied at the lowest concentration of 0.3 g. In the control, the mortality reached almost 70% and did not differ significantly from the sample where a dose of 1 g of diesel oil was used. The other objects did not differ markedly between each other, with the mortality fluctuating at around 30%. In the second part of the experiment, when magnesium supplement was applied, the average mortality was higher. The lowest value was

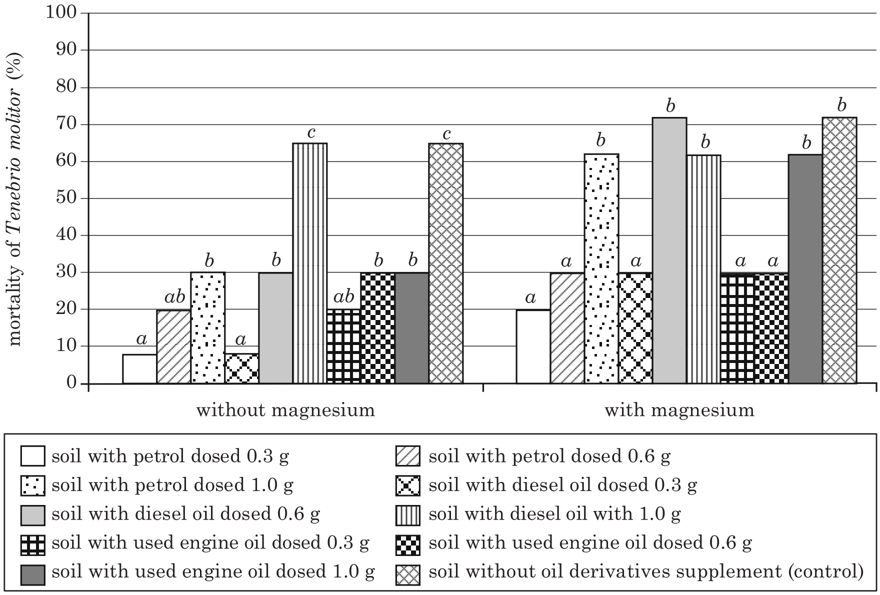

Fig.1. Mean accumulated mortality of *Tenebrio molitor* L. test larvae (%)

noted in the objects where oil derivatives at the lowest concentration (0.3 g) were applied, even though the mortality rate in these treatments increased to over 30%, in which they differed markedly from the other objects. In the control soil with magnesium ions, the mortality of the insects grew to about 80%. Figure 2 shows the results of nematode reproduction in the bodies of dead test larvae.

In the present experiment, the highest reproduction of nematodes in the insect larvae was observed in the control sample, where it reached about 5600 nematodes per 1 insect larva. The lowest value was noted in the object where the highest dose of used engine oil was applied, which differed significantly from the objects where petrol was added in the concentrations of 0.3 and 0.6 g and from the control. The analyses did not show any differentiation in comparison with the other objects. Similarly, after the application of magnesium supplement, the highest number of produced nematodes was observed in the control, where there were *circa* 6040 nematode larvae per insect. The reproduction rate of nematodes observed in the other objects was not significantly higher, despite the applied magnesium.

Figure 3 below shows the mean accumulated mortality in the second experiment in which the commercial preparation Owinema, containing *S. feliate* nematode strain, was used together with a double dose of oil derivatives

In this experiment, considerably increased mortality was observed in comparison with the previous series. A disadvantageous effect of oil derivatives on the test organisms was noticeable. In the variant where no magnesium was added, the lowest mortality was observed in the object with petrol added in the dose of 0.6 g, whereas the highest one was in the treatment

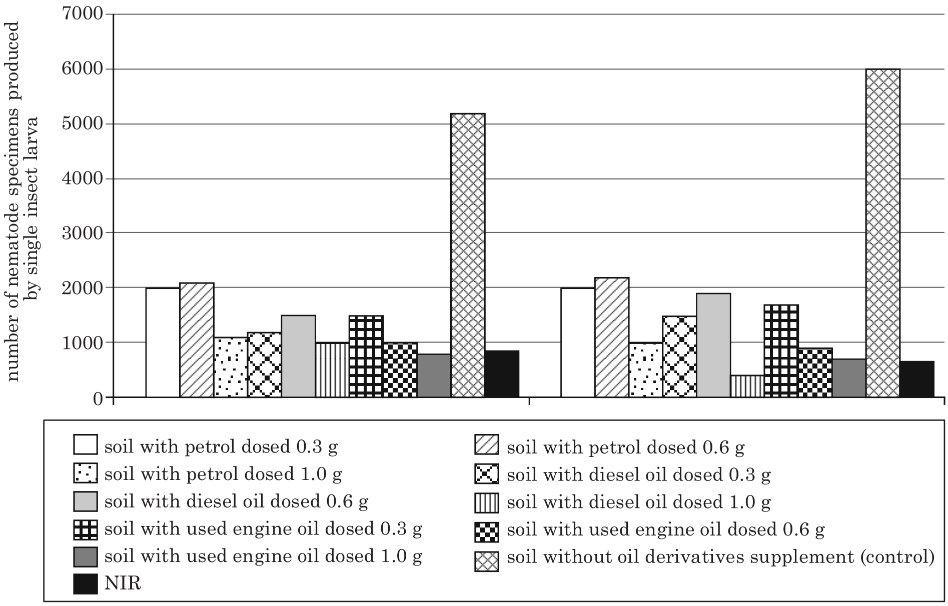

Fig.2. Reproduction of *Steinernema feltiae* (Filipjev) nematodes in the organisms of dead test insects (*T. molitor* L. larvae) kept in the analyzed objects

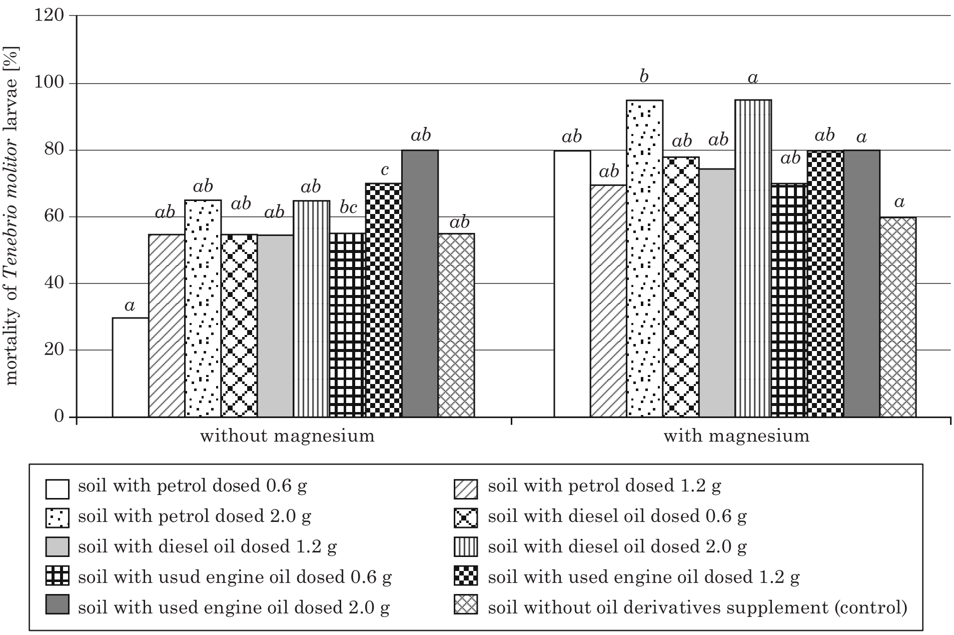

Fig. 3. Mean accumulated mortality of *Tenebrio molitor* L. test larvae (%) under a double level of pollution with oil derivatives

with used engine oil dosed at 2.0 g. In the second part of this experiment, the supplied magnesium additionally raised the mortality of *Tenebrio molitor* L., causing an increase in all average mortality values in all the combinations. The lowest mortality rate was in the control.

Figure 4 presents the results on the nematode reproduction in bodies of dead insect larvae kept in the analyzed objects, in the second series of the experiment, in which *S. feltiae* nematodes from the commercial Owinema preparation were applied. Additionally, the doses of oil derivatives were doubled.

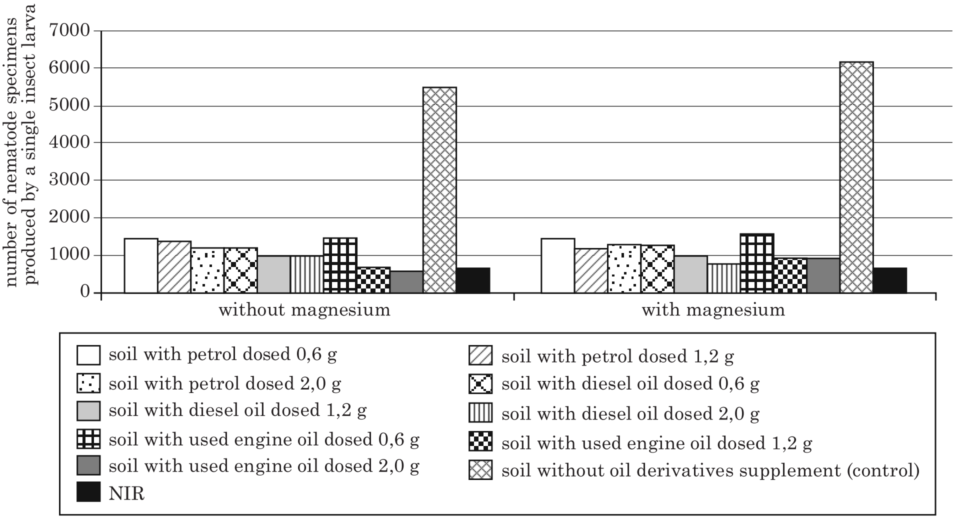

Fig. 4. Reproduction of *Steinernema feltiae* (Filipjev) in the organisms of dead test insects (*T. molitor* L. larvae) kept in the analyzed objects

The only object which significantly differed from the others was the control without any oil derivative (Figure 4). The number of infective juveniles of *S. feltiae* (Filipjev) nematodes isolated from dead *T. molitor* L. larvae bodies at that time in this object without magnesium reached on average 5503 infective juveniles per one dead test insect and 6066 larvae in the sample with magnesium. In the objects polluted with oil derivatives, the highest reproduction (about 2000 infective juveniles) was observed in both cases in the sample with petrol added in doses of 0.3 and 0.6 g. Thus, magnesium did not increase nematode reproduction under oil derivative pollution.

DISCUSSION

Entomopathogenic nematodes, as well as mutualistic bacteria with which they co-exist have been an interesting subject of research for many years. The interest is stimulated by the on-going development of integrated plant protection programmes. Biological methods which take advantage of the phenomenon of parasitism and predation among animal species in the environment play a crucial role in these programmes. Because they are safe in use, biological methods are recommended mainly in organic farming and in integrated production systems (Tomalak 2005).Owing to their ability to seek a host actively, nematodes have gained a considerable advantage over chemical preparations. Such active search for a host organism is the most important characteristic of these organisms, although it looks differently in various strains and species. Factors that affect nematodes may be divided into biotic and abiotic ones. The temperature is an abiotic factor. For the development of nematodes the temperature within the 13-20°C range seems optimal (Ropek 2005). The temperatures during the present experiments were much higher, which might have affected the results. Another possible abiotic factor is the pollution which people creeate in the environment, by consciously or unconsciously. Environmental pollution is one of the crucial issues currently addressed by many scientists. In their investigations, Pezowicz (2002) and Dzięgielewska (2008) demonstrated that environmental pollution poses a serious barrier to the occurrence and spread of nematodes, although these animals may appear even in polluted soils or in urban soils, where they can also be used for biological plant protection. The condition for their durability in the environment is the presence and availability of host insects (Jarmuł, Kamionek 2003). However, the effectiveness of nematode use in programmes of integrated control may be affected by many factors, including soil pollution with oil derivatives. In the research by Jaworska and Gospodarek (2009), soil contamination with oil derivatives caused a significant decline of both pathogenicity and reproductive abilities of entomopathogenic nematodes. Similar results were presented in the paper by Gospodarek and Jaworska (2009). They were also manifested in the present investigations. A negative effect of oil derivatives on the pathogenicity and reproduction of nematodes was observed in the presented research. Pathogenicity of nematodes was defined through the deetrmination of the mortality rate of the test insect larvae, i.e. *Tenebrio molitor* L. Their mortality observed in the experiments might have been affected by the high temperature in the laboratory, which did not favour either the insect larvae or the reproduction of nematodes. Still, the nematodes used for the experiment and the oil derivatives had the strongest influence.

The effect of heavy metals on nematodes depends among others on ionic concentrations and interaction with other ions, but the main effect is produced by the type of ion. Among the ions investigated so far, Cd and Pb ions proved most toxic. In such a case, the mortality of infective juveniles was affected both by the time of contact of nematodes with pollutants (*S.carpocapsae*) and their concentration. The situation looks different for magnesium ions. Instead of being toxic to nematodes, magnesium ison can act synergically, by protecting them against the unfavourable impact of other heavy metals (Jaworska et al. 1999). This dependence was observed also in the present experiment. A supplement of magnesium sulphate positively influenced nematodes' pathogenicity, even under heavy pollution with oil derivatives, although the reproduction rate was not observedd to be higher.

Crude oil and its derivatives negatively affect the environment in various ways (Surygała, Śliwka 1999). Different techniques and technologies of removing contamination with oil derivatives from ground and groundwaters are being developed. Here, at the Department of Agricultural Environment Protection, we have been completed several projects focused on finding out how these pollutants could be removed from the ground by microbiological methods, using entomopathogenic nematodes for checking the efficiency of biocleaning.

CONCLUSION

Magnesium added do the soil contaminated with oil-derivatives increases pathogenic abilities of entomopathogenic nematodes.

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