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THE EFFECT OF VARIABLE SOIL FERTILISATION ON ENTOMOPATHOGENIC NEMATODES *STEINERNEMA CARPOCAPSAE* (WEISER 1955)

WPŁYW ZMIENNEGO NAWOŻENIA NA NICIENIE ENTOMOPATOGENICZNE *STEINERNEMA CARPOCAPSAE* (WEISER 1955)

Key words: entomopathogenic nematodes, *Steinernema carpocapsae*, fertilisation, NPK, manure.
Słowa kluczowe: nicienie entomopatogeniczne*, Steinernema carpocapsae,* nawożenie, NPK, obornik**.**

Summary

The aim of this study was to estimate the effect of variable fertilisation with mineral and organic fertilisers on entomopathogenic nematodes used in organic farming to control plant pests.

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Soil samples were taken from experimental plots in Chylice in 2007. Samples were divided into groups according to fertilisation protocol: NPK mineral fertiliser, ½ NPK + ½ cattle manure and a control non-fertilised soil. The experiment consisted of three stages. In the first stage caterpillars were placed directly after nematode application to soil, in the second it was done after 7 days and in the third stage – after 14 days since the application of invasive larvae. Only soil fertilised with manure positively affected nematodes. In other cases the invasiveness of nematodes decreased with time the nematodes spent in fertilised soil without host insects.

Streszczenie

Celem pracy było określenie wpływu zmiennego nawożenia nawozem mineralnym i organicznym na nicienie entomopatogeniczne, używane w rolnictwie ekologicznym do zwalczania szkodników upraw.
Glebę pobrano z poletek doświadczalnych w Chylicach w 2007 roku. Próbki zostały podzielone pod kątem sposobu nawożenia: obornik bydlęcy, nawóz mineralny NPK, ½ NPK + ½ obornik bydlęcy oraz próba kontrolna nie zawierająca żadnego nawozu. Doświadczenie podzielone było na trzy etapy. W pierwszym etapie gąsienice wkładano od razu po aplikacji nicieni na glebę, w drugim odczekano 7 dni, a w trzecim 14 dni od aplikacji larw inwazyjnych. Obornik, jako jedyny wpływał korzystnie na nicienie przetrzymywane w glebie z jego dodatkiem. W pozostałych przypadkach inwazyjność nicieni zmniejszała się wraz z czasem, w którym nicienie przebywały w nawożonej glebie bez owadów żywicieli.

1. INTRODUCTION

Organic farming has recently become a popular discipline and sometimes a fashion in many countries. Earlier, in the years of pesticide (DDT) and mineral fertilisers' boom, people lost awareness of how it might affect the environment. The effects of chemical treatments, which were not or couldn't be tested in the lab, often revealed many years later. Now, more and more popular becomes the application of organic fertilisers like manure which do not alter environmental equilibrium and are 100% environmental-friendly [Krzywy 2000; Lenart 2004; Mercik and Stępień 2006].

Much hope is also pinned up on the application of natural control of crop pests. Vertebrates like frogs or toads are used to control the populations of snails or millipedes and application of predatory larvae of ladybugs may limit the number of aphids grazing on crops. The use of entomopathogenic nematodes to control pests becomes also very popular. Best specialised in infecting insects are the species of the family *Steinernematidae* and *Heterorhabditidae* [Bednarek and Gaugler 1997; Ropek 2005; Mracek et al. 2005].

Representatives of these families are often successfully used as natural means of controlling pests like the horse-chestnut leafminer, thrips and dark-wing fungus gnats in greenhouses. One should, however, remember to provide nematodes with optimum growth conditions, to avoid using nematocides and apply optimum fertilisation. Organic or mineral fertilisation may have an important effect on the populations of entomophilous nematodes in soils. Balanced fertilisation with mineral fertilisers may positively affect the growth of soil microflora and consequently, the growth of entomopathogenic nematodes [Dmowska 1982; Sosnowska 2004]. The use of fertilisers with a high concentration of urea or ammonium saltpetre may cause the decline of nematode populations in soils [Witkowski 1972; Krzywy 2000; Mercik and Stępień 2006].

2. MATERIAL AND METHODS

Study material consisted of soil samples collected three times in 2007 from experimental plots in Chylice fertilised with mineral fertiliser and/or manure. The following fertilisation was applied in the year of soil sampling:

- manure – 250 kg,
- NPK – 1.25 kg of ammonium saltpetre, 0.9 kg superphosphate and 2.5 kg of 60% potassium salt per 62.5 m^2 plot,
- ½ NPK + ½ manure – 0.62 kg of ammonium saltpetre (34% N), 0.45 kg of double superphosphate (46% P_2O_5), 1.25 kg of potassium salt (60% K_2O) + 125 kg of manure.

The number and invasiveness of nematodes *S. carpocapsae* were determined using the caterpillars of the greater wax moth (*Galleria mellonella*) from the own culture of the Department of Zoology which are excellent trap insects. Soil with NPK, manure, ½ manure and ½ NPK and control (non-fertilised) soils were placed in boxes. Soil samples were heated in a drying oven to get rid of microflora that might falsify the results. Relatively large caterpillars of the greater wax moth and a solution containing minimum 5000 invasive larvae of *S. carpocapsae* were placed in each box. Entomopathogenic nematodes were placed in boxes which received the first caterpillars of *G. mellonella* directly after nematode application and after 7 and 14 days.

Boxes with soil and invasive larvae of *S. carpocapsae* were then incubated at 25^{o}C. Controls were performed every second day for 21 days. Dead caterpillars were transferred on Petri dishes lined with wet filter paper. Dead caterpillars were replaced by live ones. Males and females of entomopathogenic nematodes in dissected caterpillars were counted every second day. ANOVA and SPSS programme were used to assess the effect of variable fertilisation on nematodes *S. carpocapsae*.

3. RESULTS

The use of biopreparation with invasive larvae of entomophilous nematodes and application of mineral fertilisers decreased the ability of these animals to search for host and to infect it in soil. NPK, however, markedly decreased these abilities but only when nematodes spent 14 days in soil without the larvae of the greater wax moth (Tab. 1).

Table 1. The effect of fertilisation type on the number of entomopathogenic nematodes
Tabela 1. Wpływ rodzaju nawożenia na nicienie entomopatogeniczne

Variable soil fertilisation	Invasive larvae application directly with *G. mellonella*	Invasive larvae application to soil or 7 days later	Invasive larvae application to soil or 14 days later
Manure	13.1	4.5	4.5
NPK	19.1	14.6	14.4
½ NPK + ½ manure	22.5	9.3	9.3
Control	22.2	15.2	15.2

When nematodes had an access to caterpillars in the moment of their application to soil or 7 days later, many of them attacked the moths but after 14 days the number of nematodes infecting the host (both males and females) decreased over two times. Hence, one should consider the effect of time the nematodes spend in NPK fertilised soil on their infection abilities.

The same situation was observed with respect to NPK+ ½ manure. After preliminary increase of invasiveness the number of nematodes systematically decreased with time of their exposition to fertiliser. The effect pertained only to females since the number of males, if only nematodes had an access to caterpillars, was the same in soils treated with various fertilisers (Tab. 1).

Bonferroni test showed that the number of females in the control group was significantly ($p<0.01$) higher ($M = 11.94$, $SD = 22.22$) than in samples of soil fertilised with manure ($M = 7.31$, $SD = 15.4$). The number of females in samples of soil fertilised with a mixture of manure and NPK ($M = 11$, $SD = 26.89$) was significantly ($p < 0.05$) higher than in manure fertilised soil ($M = 7.31$, $SD = 15.4$). LSD test showed additionally the differences between the group of NPK fertilised soil samples and the control group. The latter had significantly ($p < 0.05$) more females than the NPK fertilised group ($M = 9.18$, $SD = 20.76$).

4. DISCUSSION

Agro-technical measures (e.g. pesticide application) may limit or enhance the growth of entomopathogenic nematodes or their ability to infect insects. Apart from the type of fertiliser, its amounts applied to soil and short- *versus* long-term fertilisation is also important

[Krzywy 2000; Mercik et al. 2006]. Additional problems are associated with other biotic and abiotic factors that may directly or indirectly affect the number of nematodes, including entomopathogenic ones. Ropek [2005] demonstrated that entomopathogenic nematodes subjected to mineral fertilisers might have limited pathogenicity. It is noteworthy, however, that the decreased number of nematodes in NPK fertilised soil was observed in comparison with those found in sol fertilised with manure or with a mixture of manure and NPK. This might suggest a modifying effect of manure which under such circumstances favourably affected the number of nematodes (Tab. 1). Jarmuł et al. [2009] in his study on the effect of a long-term fertilisation on entomophilous nematodes observed that the soils fertilised with NPK or a combination of ½ NPK + ½ manure had no effect on the ability of infection of *G. mellonella* insects by soil nematodes. It was shown in the experiment that the number of males and females markedly decreased with the time they spent in soil with these fertilisers. This was because elemental composition, mainly nitrogen content, resulted in more abundant plant growth. Larger plants are more attractive for their pests – hosts for nematodes. If the former become more numerous that one may expect the higher number of their natural enemies.

Kozłowska [1977] concluded that NPK exerts such an indirect effect on entomophilous nematodes. Equilibrated fertilisation with NPK and manure introduces mineral components to soils in forms more available to plants [Sienkiewicz 2003]. According to Bednarek [1990] mineral fertilisers may have a negative effect on nematodes, mainly those of the families *Steinernematidae* and *Heterorhabditidae* in the host's body. Together with Gaugler they performed a study in 1997 which showed that NPK fertilisers might differently affect representatives of these families. A higher ability to infect insect host and an increased abundance was found in *S. feltiae* as opposed to *H. bacteriophora* whose larvae showed a decrease of both features. Bednarek and Gaugler [1997] demonstrated a positive effect of manure, thanks to which nematode larvae can survive in soil. On the other hand, Ishibashi and Kondo [1986] found that manure negatively affected the number of entomophilous nematodes. My results would rather support the conclusions of Bednarek and Gaugler [1997], though not directly. At simultaneous application of nematodes and moths, the number of nematodes that attacked the caterpillars was low and lower than in the control. When nematodes had to survive 7 days in soil, their number decreased further on but after 14 days it increased significantly and overcame the number of nematodes in samples of soil fertilised with NPK (Tab. 1).

Jarmuł et al. [2009] in his study on the effect of long-term fertilisation on nematodes arrived at the conclusion that the application of manure and mineral fertiliser (in 1:1 ratio) could increase the number of soil nematodes. Bednarek [1990] studied the effect of the MIS – 3 fertiliser on entomopathogenic nematodes in a short-term exposure and found an increase of larval invasiveness. When the exposure prolonged in time he observed a decrease of nematode invasiveness. Bednarek [1990] found also the increased number of nematodes in soil with manure as compared with soils not fertilised with this organic fertiliser. Ropek [2005] showed that the number and condition of entomopathogenic nematodes was indirectly affected by entomophilous fungi which could earlier find and kill the host before nematodes and that their spores hampered the finding and infecting the host by invasive larvae.

Season, atmospheric conditions and cultivated plant species play an important role under field conditions. Fertilisation of soils may affect soil animals not only directly; indirect effects of fertilisation are equally important. Fertilisation by changing the soil structure may interfere in nematode habitats. Long-term mineral and organic fertilisation significantly alters soil microstructure [Lenart 2004]. In this author's opinion, manure may favourably change the soil by affecting its aggregate structure. The aggregate soil structure determines many physical processes like water and air transport or porosity. Such structure may exert beneficial effect on the development of root zone of plants and on the growth of soil organisms [Hassink et al. 1994, Mercik and Stępień 2006].

5. CONCLUSIONS

1. The number of nematodes, both males and females, significantly decreased with time they spent in soil fertilised with mineral NPK or a combination of ½ NPK + ½ manure.
2. The greatest decline of nematode population was observed in samples of soil fertilised with NPK as opposed to that fertilised with manure, which exerted favourable effect on nematodes.

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