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Sensitivity of imago and larvae of the lesser mealworm *Alphitobius diaperinus* (Panzer 1797) in a sawdust litter to selected species and strains of Steinernematidae and Heterorhabditidae under laboratory conditions

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Abstract: *Sensitivity of imago and larvae of the lesser mealworm Alphitobius diaperinus (Panzer 1797) in a sawdust litter to selected species and strains of Steinernematidae and Heterorhabditidae under laboratory conditions.* Sensitivity of imagines and larvae of the lesser mealworm to selected species and strains of entomopathogenic nematodes was studied in a pine sawdust litter on which chicken were kept from one to six weeks according to their production cycle (Niemiec 1998). The following nematode species and strains were used: *S. feltiae* from bioinsecticides Ovinema and Nemaplus, *S. affinis*, *S. carpocapsae* and *H. bacteriophora* strain Brecon. *S. feltiae* from biopreparation Ovinema appeared most invasive to the larvae and imagines of *A. diaperinus.*

Key words: lesser mealworm, pine sawdust, entomopathogenic nematodes, biological control

INTRODUCTION

The lesser mealworm can be found worldwide in Europe, Asia North and South America, Australia and Africa. The insect inhabits dark, wet and warm places (Arshad and Khattack 1984, Awaknavar and Rajasekhar 1999) feeding on groats, flour, cereal grains (Lorenzo 1990) or milk powder (Basak et al. 1991). First information on the presence of the lesser mealworm in broiler houses appeared in the 1950s in the USA (Gould and Moses 1951, Harding and Biasell 1958). The lesser mealworm is dangerous as a potential carrier of disease-causing organisms like: bacteria *Escherichia* sp. (McAllister et al. 1996), *Salmonella typhimurium*, *Bacillus*, *Streptococcus* (McAllister et al. 1994), viruses causing Marek's, Gumboro, Newcastle diseases and bird flu, parasites of the *Eimeria* sp., the larvae of tapeworms *Raillietina* sp. and *Choanotaenia* sp. (Eidson et al. 1965, Wilson et al. 1986, Avancini and Ueta 1990, Goodwin and Waltman 1996, Steelman 1996). The pathogens are transmitted when birds eat infected insects. A broiler may eat 450 larvae a day. Moreover, the insects bring substantial economic losses. They destroy polyurethane isolation of farm houses during their migration and pupation (Despins 1991, Steelman 1996).

The control of the lesser mealworm with insecticides does not bring expected results, moreover, the chemicals have a long waiting periods and insects quickly become immune to insecticides. Therefore, entomopathogenic nematodes (EPNs) seem to be an alternative as bioinsecticides in the population control of this pest (Geden et al. 1985, 1987a and b, Geden and Axtell 1988, Szczepanik 2000, Szalanski et al. 2004).

MATERIAL AND METHODS

Steinernema feltiae from biopreparations Ovinema and Nemaplus, *S. affinis*, *S. carpocapsae*, and *H. bacteriophora* (Brecon) were selected for experiments on the sensitivity of growth stages of the lesser mealworm in straw and litter. These nematodes were grown in the laboratory of the Department of Zoology, Warsaw University of Life Sciences – SGGW with the following method. Ten caterpillars of *Galleria mellonella* were placed in a Petri dish of a diameter of 9 cm lined with filter paper and then 1 ml of a suspension of 500 larvae of appropriate nematode species was poured over the dish. Caterpillar mortality was controlled every day. Dead insects were transferred onto small sponges immersed in water. After 7 to 14 days invasive larvae of nematodes started to transfer into water. After two-week keeping in a fridge the invasive larvae of nematodes were ready for experiment.

Experiments with the larvae and beetles of the lesser mealworm were performed in plastic boxes 9.5 cm long, 7.5 cm wide and 6.0 cm high. The boxes were filled with pine sawdust from a broiler house to a height of 3.0 cm. Ten adult insects or 10 larvae of the lesser mealworm were placed in the box together with pine sawdust from substratum on which chicken were bred. Broiler production lasted 6 weeks.

Litter used in experiments was taken one, two, three and four weeks after the broiler house was stocked with chicken. Since no effect of nematode activity was found in three- and four-week litter, the experiments with older litter were neglected. The dose of nematodes used in the experiment was $1 \cdot 10^6$ per m^2 in the variant with larvae and $1 \cdot 10^6$ per m^2 in that with beetles. Nematodes were introduced in 1 ml of water supplemented to 5 ml with distilled water. Experiments were carried out at 28°C in the SANYO chamber. Moisture was controlled every day. After 7 and 14 days insect mortality was counted and checked whether nematodes were the reason. Control variant consisted of insects in clean, moist pine sawdust sprayed with 5 ml of distilled water.

Tukey-Freeman test was used for statistical processing of results.

RESULTS AND DISCUSSION

As seen in Table 1 the highest efficiency in all types of substratum was found in *S. feltiae* from Ovinema biopreparation. The extensity of infection of *A. diaperinus* larvae was 60% in clean pine sawdust and decreased to 42% in one-week pine sawdust and to 34% in two-week sawdust. The older was the litter the more hen faeces and ammonia it contained. Therefore, nematodes lost their invasiveness to the lesser mealworm larvae in such a habitat. In three-week litter the effect of nematodes was the same as in the control (Table 1). Entomopathogenic nematodes are the animals very resistant to various unfavourable external conditions. For example, most pesticides are not harmful to invasive larvae of these nematodes (Kaya 1990, Kamionek 1992) even at doses larger than the recommended. Some pesticides may even stimulate nematodes' movements (Vainio 1993). Only fungicides and herbicides, including ureal ones, decrease

TABLE 1. Comparison of the extensity of infection (%) of the lesser mealworm larvae after their contact with selected species/strains of Steinernematidae and Heterorhabditidae nematodes in substratum made of pine sawdust

Nematode species	Percentage of dead larvae of *A. diaperinus* in which nematodes were noted			
	Sawdust without faeces	One-week sawdust	Two-week sawdust	Three-week sawdust
S. feltiae (Ovinema)	60 d	42 c	34 bc	2 a
S. feltiae (Nemaplus)	46 c	36 c	20 b	2 a
S. affinis	32 bc	30 bc	20 b	4 a
H. bacteriophora (Brecon)	20 b	20 b	8 a	4 a
S. carpocapsae	32 bc	28 bc	16 a	2 a
Control	4 a	4 a	2 a	2 a

Different letters denote significant differences in the extensity at $p < 0.05$.

the pathogenicity of nematodes (Das and Divakar 1987, Kamionek 1992). The effect of organic fertilisation was studied by Bednarek and Gaugler (1997) who demonstrated the increased effectiveness of entomopathogenic nematodes after the contact with that fertiliser. Poultry faeces are also organic fertilisers but apart from N, P and K it contains large amounts of ammonia which is slowly released to the environment (Oudedag and Luesink 1998). This is probably the reason why most nematode species lose their pathogenic properties.

Mortality of adult insects of the lesser mealworm in various substrata is presented in Table 2. Most efficient were *S. feltiae* from Ovinema biopreparation. These nematodes caused 20% mortality in adult insects after two weeks of experiment, 24% mortality in one-week litter and 4% mortality in three-week litter. *S. affinis* caused 26% mortality in adult insects kept in clean sawdust and 20% mortality in one-week litter. Comparison of the Tables 1 and 2 shows that *S. feltiae* from Ovinema biopreparation were most efficient against both growth stages of the insect. Geden et al. (1985) obtained 35.8% infection in larvae and 10.2% infection in adult forms of *A. diaperinus* with nematodes *S. feltiae* in a substratum composed of pine sawdust, food remains and faeces. Szalanski et al. (2004) obtained similar results using a pine-cedar substratum and nematode strains *S. carpocapsae* Mexican and *S. feltiae* Pye at a dose corresponding to 200 invasive larvae per insect. Pezowicz (2006) studied the invasiveness of entomopathogenic nematodes in straw and in litter from a broiler house and obtained lower extensity than in this study.

Not until recently attempts have been undertaken to improve entomopathogenic nematodes' ability of finding hosts, to increase their invasiveness and in general to increase their effectiveness in insect control. Gaugler (1987) was a promoter of studies on artificial selection, genetic engineering and strain hybridization. Nematodes from Ovinema biopreparation are an outcome of these studies (Tomalak 1994 and 1998).

TABLE 2. Comparison of the extensity of infection (%) of the lesser mealworm imagines after their contact with selected species/strains of Steinernematidae and Heterorhabditidae nematodes in substratum made of pine sawdust

Nematode species	Percentage of dead imagines of *A. diaperinus* in which nematodes were noted			
	Clean sawdust	One-week sawdust	Two-week sawdust	Three-week sawdust
S. affinis	26 c	20 bc	18 b	6 a
S. feltiae (Ovinema)	36 d	24 c	20 bc	4 a
S. feltiae (Nemaplus)	24 b	18 a	14 a	4 a
H. bacteriophora (Brecon)	28 bc	20 b	16 a	2 a
Control	4 a	2 a	2 a	4 a

Different letters denote significant differences in the extensity at $p < 0.05$.

CONCLUSIONS

1. The invasiveness of entomopathogenic nematodes decreases with increasing concentration of ammonia from chicken faeces.
2. *Steinernema feltiae* from the biopreparation Ovinema appeared most effective in controlling larva and imagines of the lesser mealworm.
3. Pine sawdust is a better substratum than litter to control the lesser mealworm in broiler houses.

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Streszczenie: *Wrażliwość imago i larw pleśniakowca lśniącego Alphitobius diaperinus (Panzer 1797) w ściółce z trocin na wybrane gatunki i szczepy nicieni Steinernematidae i Heterorhabditidae w warunkach laboratoryjnych.* Celem badań było określenie wpływu nicieni entomopatogenicznych na śmiertelność larw i postaci imaginalnych pleśniakowca lśniącego *Alphitobius diaperinus* (Panzer 1797) w ściółce z trocin sosnowych. Zbadano przeżywalność stadiów rozwojowych owadów w cyklu produkcyjnym hodowli kurcząt. Stwierdzono, że ze wzrostem stężenia amoniaku pochodzącym z odchodów kurcząt maleje inwazyjność nicieni entomopatogenicznych. Nicienie żyły tylko do trzeciego tygodnia cyklu produkcyjnego kurcząt. Najbardziej skutecznym gatunkiem w stosunku do larw i imago pleśniakowca okazał się *S. feltiae* z biopreparatu Ovinema. Trociny sosnowe są lepszym podłożem niż ściółka słomiana do zwalczania pleśniakowca lśniącego w brojlerniach.

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