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THE EFFECT OF OIL DERIVATIVES ON THE ABILITY
OF ENTOMOPATHOGENIC NEMATODE Steinernema feltiae
TO FIND HOST

Abstract: The aim of the research was to evaluate the effect of oil derivatives on entomopathogenic nematode Steinernema feltiae. The effect of unleaded petrol, diesel oil and used engine oil on the ability of S. feltiae infective juveniles to locate test insect was investigated. The experiments were carried out in laboratory conditions in four replications. In the first experiment the effect of oil derivatives on behavior of infective juveniles on agar medium in the presence of test insects was investigated. Oil derivatives were added to the medium at the rate of: 2000, 4000, 6000 and 8000 mm³/dm³. Infective juveniles did not dispersed towards test insects on agar medium contaminated with oil derivatives. The application of high doses of oil derivatives had also negative effect on nematodes activity and mortality. In the second experiment infective juveniles were applied into contaminated soil. Soil was polluted with following oil derivatives: unleaded petrol, diesel oil and used engine oil at the rate of: 2000, 4000, 6000 i 8000 mg/kg. In soil contaminated with oil derivatives test insect mortality was significantly lower than in uncontaminated control. It was also revealed that less extensive infestation of test insects with nematodes was observed in contaminated soil. Reaction of S. feltiae to particular oil derivatives was diverse. The most toxic effect on nematode ability to find host was observed in soil polluted with unleaded petrol. The applied oil derivatives had also an adverse effect on the female/male ratio of nematodes infesting host insect.

Keywords: Steinernema feltiae, oil derivatives

Pollution with oil derivatives is one of the most important threat to the environment. The most devastating disasters are usually in marine environment, but on land there are also many accidents ie car accidents, which causes environment pollution. Oil derivatives cause substantial changes in soil environment affecting all living organisms [1]. However there are not many studies concerning the effect of oil derivatives on soil fauna [2] and only a few concerning nematodes [3]. Nematodes are used in soil ecotoxicology both in field and laboratory studies [4]. Raymond et al [5] investigated presence of wild nematodes in soil in the USA during

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degradation process of various oil kinds. Similar study was carried out by Prihonen and Huhta [3] in forest soil in Finland. More studies were done on the effect of oil derivatives on aqueous invertebrate [6–10]. It was revealed that oil derivatives has severe and long-lasting toxicological effects, primarily aromatic hydrocarbons.

An important group of nematodes are entomopathogenic ones, which are widely used in biological plant protection [11]. They naturally occur in soil environment and are regarded as possible indicators of the environment contamination [12]. Entomopathogenic nematodes were used to evaluate the effect of soil pollution with heavy metals [13]. Pathogenicity of entomopathogenic nematodes was significantly affected by heavy metal ions [14].

The aim of the research was to evaluate the effect of oil derivatives on ability of entomopathogenic nematode *Steinernema feltiae* to locate and kill host insect.

**Material and methods**

In the first experiment the effect of oil derivatives on behavior of infective juveniles (IJs) on agar medium was investigated. Oil derivatives were added to the agar medium at the rate of: 2000, 4000, 6000 and 8000 mm$^3$ · dm$^{-3}$. In the control unpolluted medium was applied on Petri dishes (90 mm diameter). At the edge of the dish a drop containing 100 IJs was applied. The excess of water was removed by filter paper to facilitate movement of the IJs. In the center of the dish a larvae of test insect (*Tenebrio molitor*) wrapped in perforated aluminum foil was put. The distance from the IJs application point to the test insect was 4 cm. The movement of IJs was observed under stereomicroscope. After 3 hours the position of IJs was marked. Number of migrating IJs from application point and the number of IJs which were able to reach test insect were counted. IJs which had characteristic straight posture were recognized as dead. Migrating IJs and those that did not left application point but were moving were recognized as active. The experiment was carried out in 22 °C in four replications.

Soil was polluted with following oil derivatives: unleaded petrol, diesel oil and used engine oil. Unpolluted soil was used as control. In the conducted experiments sensitivity to oil derivatives was tested for of *Steinernema feltiae* (Nemasys) infective juveniles (IJs). Experiment was carried out in 120 cm$^3$ containers. Each container held 50 g of soil dry mass. Doses of 2000, 4000, 6000 and 8000 mg of oil derivatives per 1 kg soil d.m. were applied. Distilled water was used in the control. Entomopathogenic nematodes were applied at the bottom of the container. After that the container was filled with soil. The depth of soil in the container was about 5 cm, that was the distance the IJs had to the surface, where 10 test insects (*Tenebrio molitor* larvae) were put after 24 hours. The larvae were covered with perforated foil to avoid direct contact of insect with polluted soil and enable nematodes penetration inside the host. The experiment was carried out in 22 °C. The larval mortality was checked every 24 hours. Dead larvae were removed into sterile Petri dishes and stored at 25 °C for 48 hours. After two days since the test insect death they were dissected under a binocular magnifying glass to count the number of nematodes able to actively penetrate into their bodies. Rate of male and female of nematodes entering insect body was also calculated.
The results were analysed statistically using the Statistica programme. ANOVA analysis was conducted and the Newman-Keuls critical intervals were computed. The value of the final step was used for differentiating means at the significance level $p < 0.05$.

**Results and discussion**

IJs of nematodes can actively search for potential host. Some substances eg fertilizers and pesticides may impair their ability to locate potential host insects. In the present studies an adverse effect of oil derivatives on IJs of *S. feltiae* applied on contaminated agar medium was noted (Fig. 1). Infective juveniles did not migrated from application point on agar medium contaminated with high doses of oil derivatives. The most adverse effect was noted when petrol was added into the medium. Low doses of oil derivatives also negatively affected nematode migration, but to a lesser extent. On control medium (without oil derivatives) IJs were able to move towards test insects, and almost 90% of them left application point. During 3 hours experiment almost 20% of applied IJs were able to reach the test insect (Fig. 2). On medium contaminated with diesel oil the migration of IJs was very low even when low doses of pollutants were used and only few percent of IJs were able to reach test insect when low doses of diesel oil and used engine oil were used. The application of high doses of oil derivatives had negative effect on nematodes mortality (Fig. 3). On agar medium contaminated with petrol high mortality rate of IJs was observed. Not all IJs that did not left application point were dead. Basing on the IJs posture of body they were recognized as dead, active or inactive. In uncontaminated control all IJs were active after 3 hours since the time

Fig. 1. Migration of *S. feltiae* infective juveniles (IJs) on Petri dish with agar containing oil derivatives in the presence of test insect (*T. molitor* larvae). C – control, UP – unleaded petrol, DO – diesel oil, UEO – used engine oil, 2000–8000 – concentration in mm$^3$·dm$^{-3}$
they were applied (Fig. 4). On agar medium contaminated with oil derivatives most of IJs were dead or inactive. The most toxic effect was observed when petrol was added to the medium. Oil derivatives added to the agar medium significantly affected nematodes.
ability to locate and reach potential host. This method may be useful to assess the toxicity of some substances on entomopathogenic nematodes.

The mortality of test insects in soil contaminated with oil derivatives are presented in Fig. 4. The highest mortality was observed in control (unpolluted soil). The comparison of test larvae mortality revealed that the highest one was observed in soil contaminated with unleaded petrol. The adverse effect of oil derivatives was most visible at higher doses of pollutants. Diesel oil at the concentration of 2000 and 4000 mg kg\(^{-1}\) d.m. of soil did not significantly affected the pathogenicity of \(S. \text{feltiae}\). In soil contaminated with high doses of unleaded petrol (6000 and 8000 mg kg\(^{-1}\) d.m. of soil) mortality of test insects caused by nematodes was very low: 2.5–5 %.

Infective juveniles of entomopathogenic nematodes are able to find and infest insects in soil environment [15]. Pollutants such as heavy metals may affect nematodes activity and their ability to find host [16]. In soil contaminated with heavy metals nematodes needed more time to locate and invade potential host. Similar result were obtained in present study (Fig. 5). Nematode infective juveniles were placed on the bottom of the containers filled with contaminated soil. In order to reach their victim the larvae had to cover a distance of about 5 cm through contaminated soil. Average time needed to locate and kill test insect was significantly longer in soil contaminated with oil derivatives than in unpolluted control (Fig. 6). Oil derivatives differ in their physical and physiological properties. Petrol is mobile and readily volatile liquid and usually deeply penetrates into soil. Diesel oil is much less volatile than petrol and used engine oil characterize with very low volatility. Diesel oil and used engine oil envelops organisms with thin films, which may be reason of nematodes inability to find test insects.
Oil derivatives impact on the nematode activity has been reflected by the extensive-ness of the test insect infestation (Fig. 7). Even low doses of unleaded petrol, diesel oil
and used engine oil caused significant reduction of IJs, which were able to invade test insects. Not only number of IJs was reduced but also the ratio female/male was affected by applied contaminants (Fig. 8). In contaminated soil number of male nematodes...
isolated from insects was very low. In soil contaminated with high doses of unleaded petrol only female nematodes were able to infest test insects. It suggest that IJs which develop inside insects into males are more sensitive to oil derivatives than the larvae developing into females.

Entomopathogenic nematodes are important group of soil mesofauna, which may be used as indicators of soil pollution ie with heavy metals [13]. Present studies shows that these group of invertebrates are sensitive to pollution with oil derivatives and nematode *S. feltiae* can be useful as indicator of soil contamination with such substances.

**Conclusions**

1. Oil derivatives impaired nematode ability to migrate towards test insect on agar medium, affecting also their activity and mortality. Petrol revealed the most toxic effect on the tested nematode *S. feltiae* among all three applied pollutants

2. In soil contaminated with oil derivatives the ability of IJs to infect test insects was impaired. *S. feltiae* revealed the greatest sensitivity to soil contamination with petrol at the dose of 8,000 mg · kg$^{-1}$ soil d.m.

3. The applied oil derivatives had an adverse effect on the female/male ratio of nematodes infesting host insect.

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**References**


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