The influence of pesticides on the viability and infectivity of entomopathogenic nematodes (Nematoda: Steinernematidae)

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Summary. The influence of pesticides on the viability and infectivity of EPNs *Steinernema feltiae*, *S. arenarium* and *S. kraussei* was determined in water solutions of 13 pesticides (clopyralid, fluoroxypyr, trifluralin, sodium2-methoxy-5-nitrophenol, captan, mancozeb, sulphur, propamocarb, chlorpyrifos, oxamyl, lambda-cyhalotrin, fenithrothion and propargite). The most toxic pesticides were those containing oxamyl and sulphur. Trifluralin has a high potential for decreasing viability and it seems that chlorpyrifos, lambda-cyhalotrin, captan and mancozeb can also cause higher mortality. The difference between the tested species was not significant except for *S. feltiae*, which was more sensitive to pesticides than the others. It was found that both the exposure time and concentration of pesticides had a significant influence on the degree to which they impacted viability. The highest negative influence on infectivity was seen in oxamyl, sulphur and also mancozeb and fenitrothion. Both treated and untreated *S. arenarium* has shown the lowest infectivity value whereas *S. feltiae* the highest one. The concentration had a significant influence on infectivity.

Key words: compatibility, integrated pest management, mortality, *Steinernema arenarium, S. feltiae, S. kraussei.*

Entomopathogenic nematodes (EPNs) are widely used in the biological control of insect pests. Even though they are considered relatively resistant to many chemical compounds, pesticides or fertilisers and growth regulators that are used in forestry and agriculture, some of these substances may have a significant influence on EPNs. The only free-living stage is non-feeding third-stage, called the infective juvenile (IJ). Their mouth and anus are closed and thus the only point of access is the cuticle. Species that have the second-stage cuticle fixed on the external surface of the third-stage should therefore be better protected. Nematode resistance to some pesticides can be enhanced and oxamyl resistant strains of *Heterorhabditis bacteriophora* have been found (Glazer *et al.*, 1997). The other advantage of nematodes is the presence of butirilcholinesterase in the synapses. Butirilcholinesterase protects acetylcholinesterase from acetylcholinesterase inhibitors (Selkirk *et al.*, 2001). It is therefore possible to observe different reactions to pesticides from the same group; one may be an acetylcholinesterase inhibitor but another not. But the influence of pesticides on infectivity or viability is not necessarily only negative. Some insecticides were found to enhance *Steinernema carpacapsae* activity (Ishibashi & Takii, 1993).

In agriculture in the Czech Republic herbicides, fungicides and insecticides are widely used. Herbicides do not usually have a marked influence on EPNs except in some specific cases, for example glyphosate. EPNs which live in soil treated with glyphosate are less infective (Gibb & Buhler, 1998). Fungicides do not, in general, have a marked influence on EPNs. For example the a.s. thiophanatemethyl has no negative influence on *Steinernema kushidai* (Fujiie *et al.,* 1993). Krishnayya & Grewal (2002) showed that azoxystrobin does not harm *S. feltiae* and it is possible to use them together.

The most widely tested group of pesticides is insecticides and nematocides. *Steinernema carpocasae* and *H. bacteriophora* are highly resistant to fipronil but *S. arenarium* showed a mortality rate of 94.6 % after 24 h exposure (Del Pino & Jové, 2005). The influence of this substance on infectivity in *Galleria mellonella* larvae is insignificant. However, some pesticides have an

influence both on viability and infectivity; for example, those containing the a.s. trichlorfon reduce the infectivity and pathogenicity of *S. feltiae* and *H. bacteriophora* (Alumai & Grewal, 2004). Nearly all authors agree that the most dangerous for EPNs group of pesticides are carbamates and organophosphates. These substances cause a decrease in reproduction (Hara & Kaya, 1982), cause the high mortality (Li *et al.,* 1994; Gordon *et al.,* 1996) or have a strong influence on the activity of EPNs (Ishibashi & Takii, 1993). Parathion, aldicarb, methomyl, flubenzimin, metham sodium and phenamifos are considered to be the most toxic of pesticides (Rovesti & Deseo, 1990). Only the influence of pesticides on the viability or mortality of EPNs is usually tested. This is very important, for example, for combinations in tank-mixes but in our opinion, it is also very important to observe the influence on infectivity, as we would like to use EPNs and pesticides in long-term cultures like orchards, because the survival and also effectiveness of EPNs is dependent on their infectivity. The present study examined the influence of pesticides that are frequently used in agriculture in the Czech Republic on the viability and infectivity of three EPNs species.

MATERIAL AND METHODS

Pesticides and species of entomopathogenic nematodes. Thirteen pesticides (Table 1) and three species of entomopathogenic nematodes were tested, the nematodes being *S. feltiae* topotype (from Ustinov, Russia), *S. arenarium* (from Malacky, Slovakia) and *S. kraussei* (from Vitosha, Bulgaria).

Testing the influence of pesticides on the viability of EPN. The influence of each pesticide on the viability of EPNs was tested in an aqueous solution (10 ml) on 9 cm diam. Petri dishes in the dark at a temperature of 14°C. Four concentrations were tested (2×, 1×, 0,5× and 0,25× the recommended concentration). All experiments were repeated twice at four time intervals (24, 48, 72 and 96 h). Each Petri dish held 10 ml of solution of pesticide and distilled water, in which 1000 infective juveniles (IJs) were placed. After 24, 48, 72 and 96 h live and dead IJs were counted. This was done under a stereo microscope. IJs were counted in 12 drops of 10 µl of solution.

The influence of pesticides on the infectivity of EPN. For these experiments the same experimental scheme as for the testing of the influence of the pesticides on the viability of EPNs was used, the difference being that a moist filter paper was placed on the bottom of the 9 cm Petri dishes and one larva of *Galleria mellonella* was placed on the paper. Then 100 IJs were added. IJs were obtained from live IJs from the previous experiment. These dishes were stored in the dark at a temperature of 14°C for 5 days. Dead *G. mellonella* larvae were then dissected and the number of adult EPN were counted.

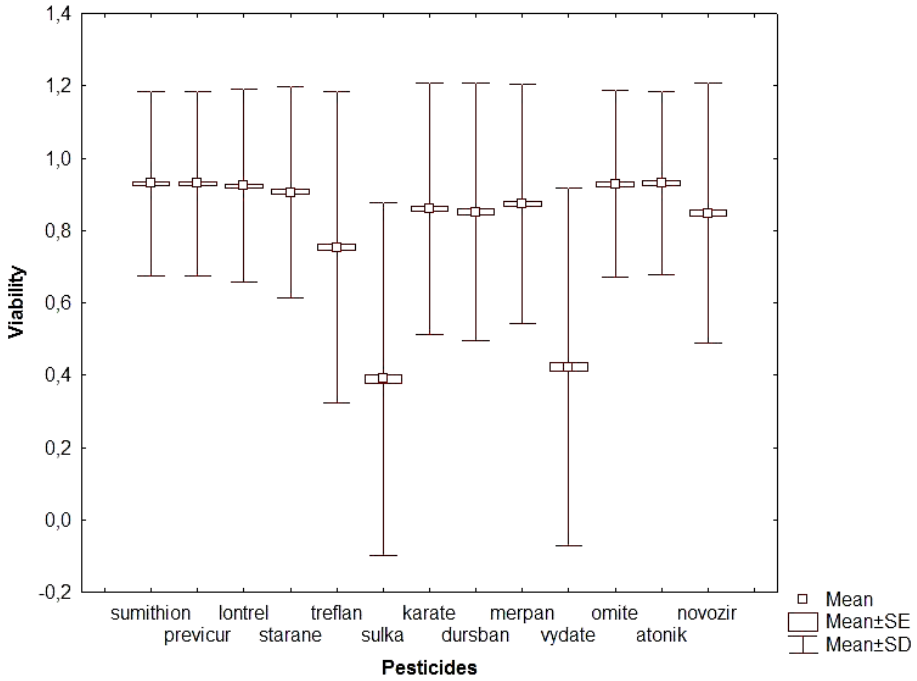

Fig. 1. The influence of pesticides on the viability of entomopathogenic nematodes (mean of all tested species).

Table 1. Pesticides used for testing of influence on the viability and infectivity of entomopathogenic nematodes

Trade name	Pesticide classification	Active substance	Content of active substance	Recommended concentration
Lontrel 300®	herbicide	clopyralid	300 g l^{-1}	1 g l^{-1}
Starane 250 EC®	herbicide	fluoroxypyr	250 g l^{-1}	3 g l^{-1}
Treflan 48 EC®	herbicide	trifluralin	480 g l^{-1}	4 g l^{-1}
Sumithion super®	insecticide	fenitrothion	1000 g l^{-1}	0.1 %
Previcur 607 SL®	fungicide	propamocarb	607 g l^{-1}	0.2 %
Merpan 80 WG®	fungicide	captan	80%	0.2 %
Karate 2,5 EC®	insecticide	lambda cyhalotrin	25 g l^{-1}	0.1 %
Sulka ®	fungicide/acaricide	sulphur	14%	4%
Novozir MN 80®	fungicide	mancozeb	80%	0.2 %
Atonik®	growth regulator	sodium 2-methoxy-5-nitrophenol	1 g l^{-1}, 2 g l^{-1}	0.04 %
Omite 570 EW®	insecticide	propargite	570 g l^{-1}	0.1 %
Vydate®	nematocide/insecticide/ acaricide	oxamyl	24%	0.25 %
Dursban 10 G®	insecticide	chlopyrifos	10%	20 kg ha^{-1}

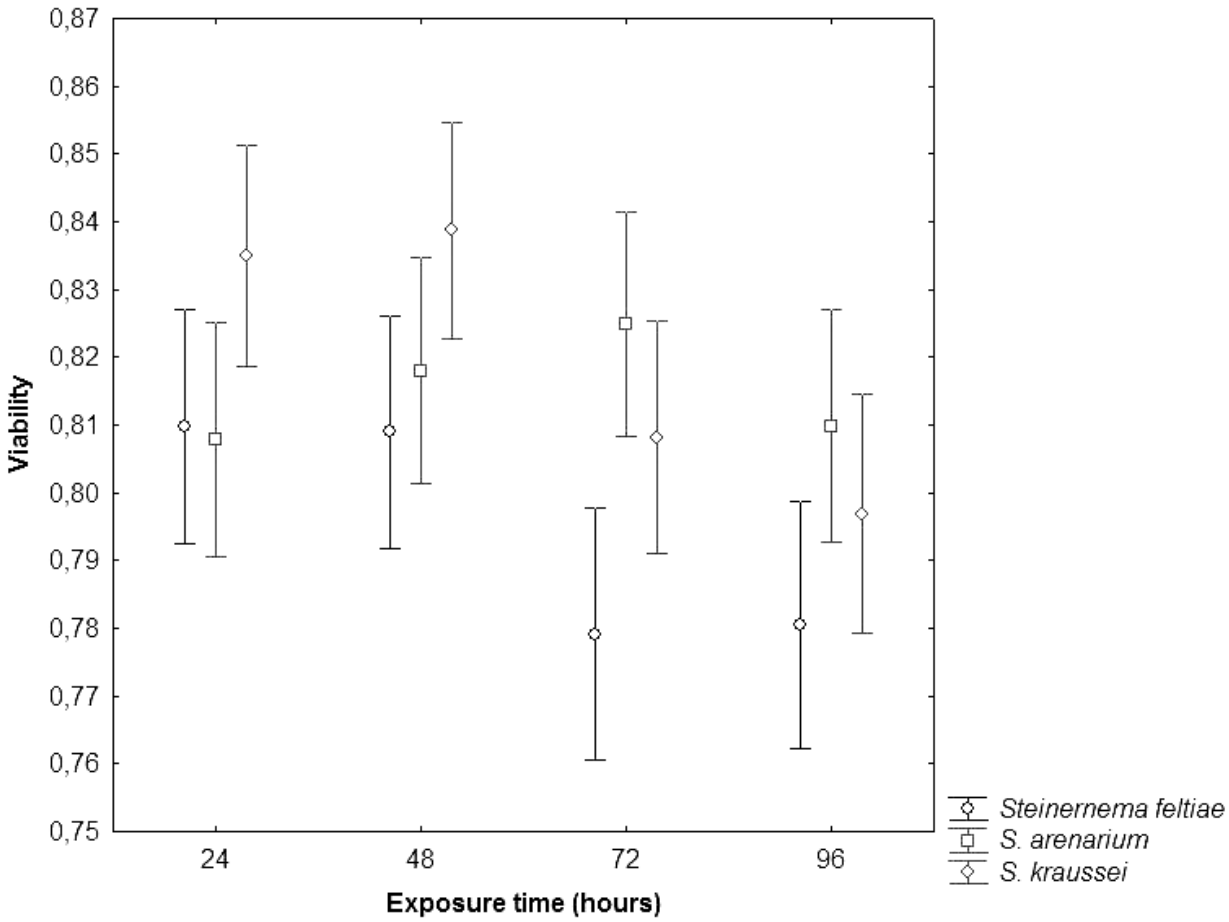

Fig. 2. The influence of exposure time on the viability of different species of entomopathogenic nematodes (mean of all tested pesticides).

Statistical analysis. The calculation was done with the Statistica program (Statsoft, Inc., 2004).

GLM for a binominal distribution with a logit link function was used to analyse data acquired from testing the influence of pesticides on the viability of EPNs. The interactions which were not significant were removed from the statistical analysis.

GLM for a normal distribution with an identity link function was used to analyse data acquired from testing the influence of pesticides on the infectivity of EPNs. To count the number of nematodes in cadavers, log transformation (log (number + 0.5)) was used. The interactions which were not significant, were removed from the statistical analysis.

RESULTS

Viability. The results of these experiments show that interspecific differences are small, only *S. feltiae* being marginally more sensitive to pesticides than *S. arenarium* and *S. kraussei* (Fig. 2). Interestingly, we found that the mortality of some nematodes increased sharply after 48 h (Fig. 2). Our analyses show that the most toxic pesticides are Vydate® (oxamyl) and Sulka® (sulphur). High mortality is also caused by herbicide containing trifluralin, fungicides containing captan and mancozeb, and insecticides containing chlorpyrifos and lambda-cyhalotrin (Fig.1). The influence of con

Table 2. Results of GLM for binominal distribution with logarithmic link function used to analyse the influence of pesticides on the viability of entomopathogenic nematodes.

	Degrees of freedom	Chi-Square	p
Pesticide	12	1334.406	0.001
Species	2	24.723	0.001
Time	3	37.555	0.001
Concentration	4	675.336	0.001
Species * time	6	15.691	0.015
Pesticide * concentration	48	975.472	0.001

Table 3. Results of GLM for normal distribution with identity link function, used to analyse the influence of pesticides on the infectivity of entomopathogenic nematodes.

	Degrees of freedom	Chi-Square	p
Pesticide	12	861.896	0.001
Species	2	273.760	0.001
Time	3	7.470	0.058
Concentration	4	485.415	0.001
Pesticide * species	24	130.024	0.001
Pesticide * time	36	146.405	0.001
Species * time	6	17.851	0.006
Pesticide * concentration	48	383.684	0.001
Species * concentration	8	19.137	0.014

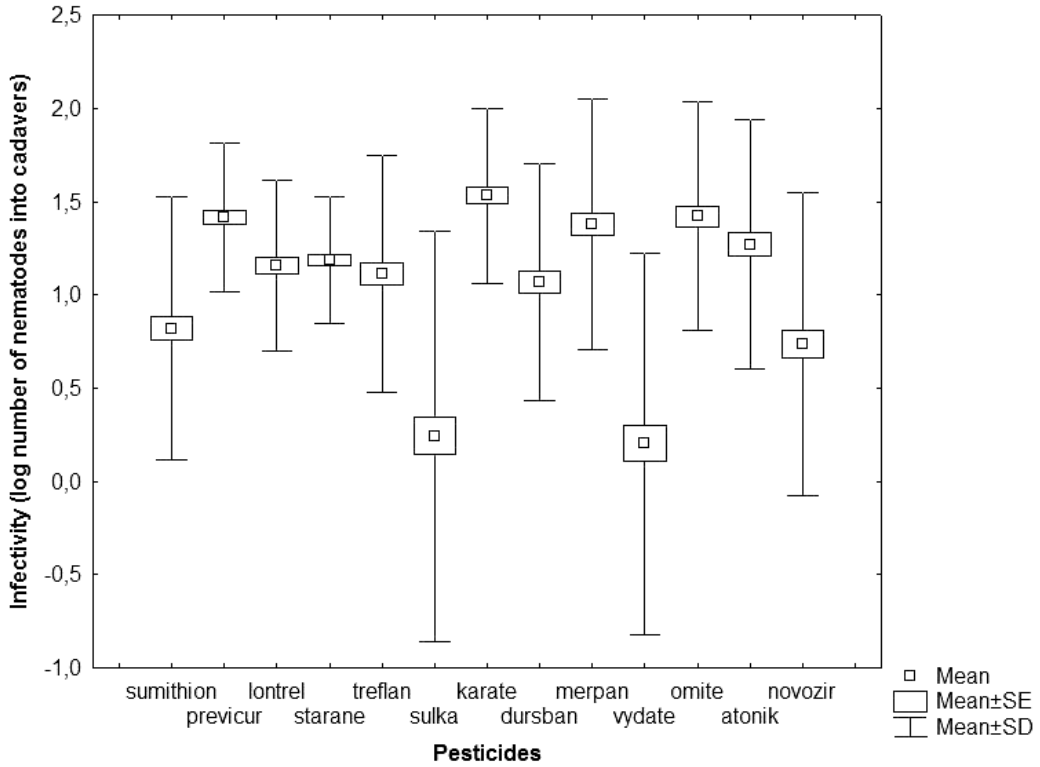

Fig. 3.. The influence of pesticides on the infectivity of entomopathogenic nematodes (mean of all tested species).

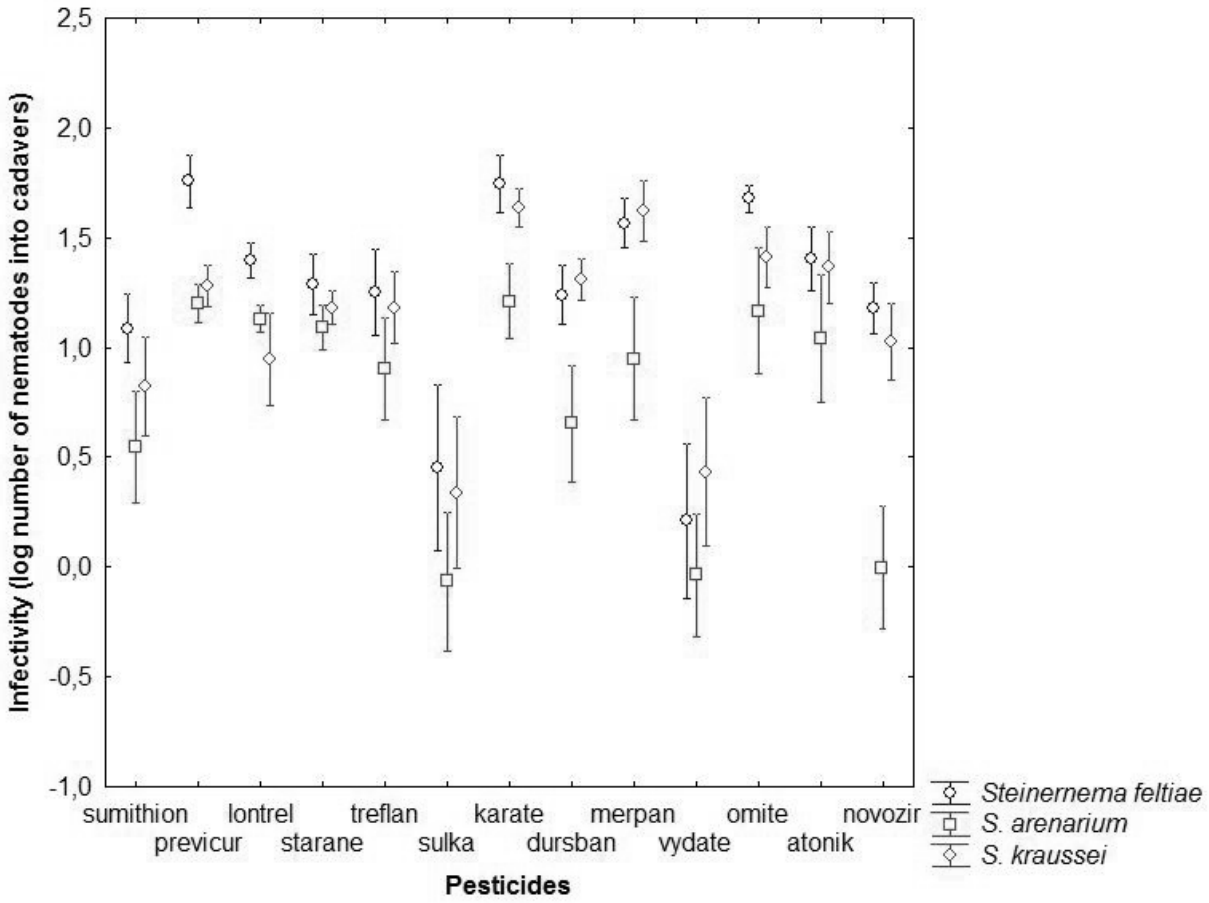

Fig. 4. The influence of pesticides on the infectivity of different species of entomopathogenic nematodes.

centration on the reduction of viability is also highly evidential ($P < 0.05$) (Table 2).

Infectivity. A very surprising result of this analysis was that exposure time does not have a significant influence on the level of infectivity of EPNs ($P = 0.058$). However, an interspecific difference was found. The highest observed level of infectivity was in *S. feltiae*, the lowest in *S. arenarium* (Fig. 4). All of the tested pesticides caused a decrease in infectivity, but the most toxic were preparations containing oxamyl (Vydate®), sulphur (Sulka®) and mancozeb (Novozir®) or fenithrothion (Sumithion®) (Fig. 3). The influence of concentration on the reduction of infectivity is also highly evidential ($P < 0.05$) (Table 3).

DISCUSSION

Majority of studies on compatibility of EPNs and pesticides targeted only one specific group of pesticides, usually pesticides which are used against one specific pest (Head *et al.,* 2000; Negrisoli *et al.,* 2010), pesticides that belong to the same chemical group, e.g. carbamates (Gordon *et al.,* 1996) or have the same biological activity, e.g. nematicides (Hara & Kaya, 1982). Unfortunately, EPNs are found in locations where many other pesticides or growth regulators are used and therefore one of the main assets of this work is the wide range of tested pesticides, which represent all widely used groups of pesticides. In this our research is similar to some other previous studies (Rovesti *et al.,* 1988; Zang *et al.,* 1994; Rovesti & Deseo, 1991). But this does not mean that this work is the same; we tested new pesticides with different a.s. or different compounds.

The dataset was enlarged by using three different EPNs species, *S. arenarium*, *S. kraussei*, *S. feltiae*. Both elements, 13 pesticides and three species provide the basis for a substantial analysis of the influence of pesticides on viability and infectivity under the different conditions produced by interactions between particular pesticides, species,

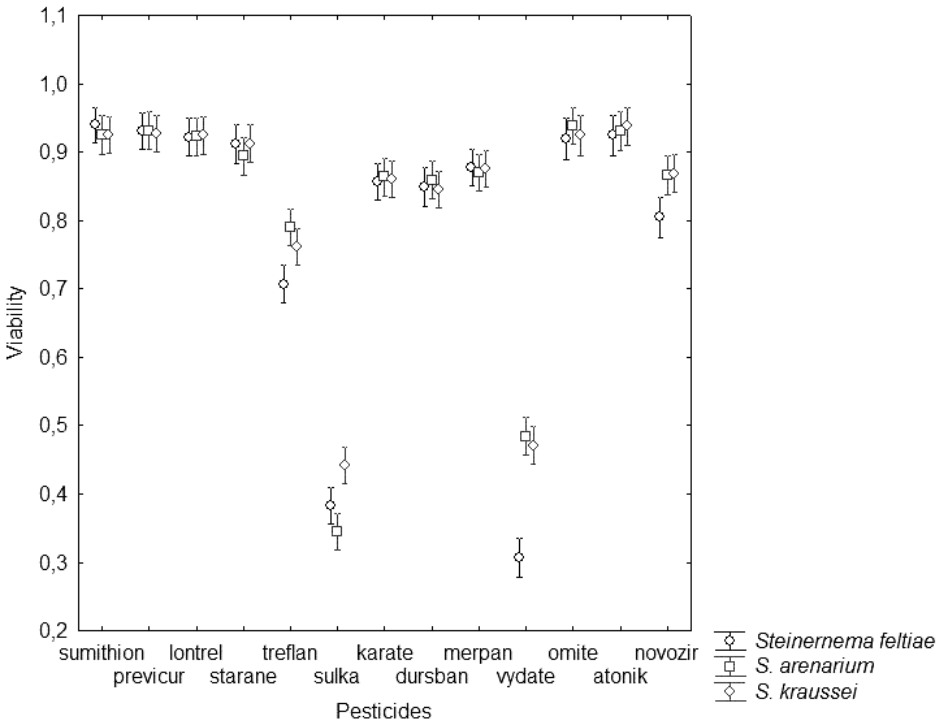

Fig. 5. The influence of pesticides on the viability of different species of entomopathogenic nematodes.

exposure times and concentrations of a pesticide. We did not therefore use any of the standard, widely used methods of statistical analyses (Rovesti & Deseo, 1990; Head *et al.*, 2000; Negrisoli *et al.*, 2010) but used GLM that also allowed us to test interactions, including differences between three EPNs species or 13 pesticides and other factors.

The results of this work showed that nematodes were very resistant to the tested pesticides. The most toxic pesticides were Vydate® (oxamyl – carbamate) and Sulka® (sulphur). However, the Previcur® (propamocarb), for example, which is also a carbamate, was not very toxic for steinernematids. This corresponds with Gordon *et al.*, 1996 claiming that not all carbamates are highly toxic to nematodes. Also, pyrethroids have a strong influence on infectivity but not on viability (Head *et al.*, 2000). In this study lambda-cyhalotrin was tested but our results were different; its influence on both mortality and infectivity was very low. Many reports indicate that organophosphates are very toxic to nematodes (Nishimatsu & Jackson, 1998; Li *et al.*, 1994). Fenithrothion (Sumithion Super®) and chlorpyrifos (Dursban 10 G®) were tested in this study. They caused the marginally lower infectivity and higher mortality, respectively, in comparison with the other tested pesticides. On the contrary, in this study the mankozeb (Novozir MN 80®), a fungicide supposedly with minor effects on EPNs, has shown causing the higher mortality and lower infectivity compared with other tested pesticides. Trifluralin (Treflan 48 EC®) has also caused the high mortality, although it may be the result of the strong organic solvent and methanol contained in this herbicide. Most studies on the influence of herbicides on EPNs indicate that herbicides have no significant influence on EPNs (Gibb & Buhler, 1998 or Fujiie *et al.*, 1993). The results of the present study agrees with this (preparations Starane 250 EC® or Lontrel 300®); there are only very small or no differences in viability among tested EPNs species.

The infectivity of EPNs is affected by the concentration of a preparation but not by exposure time (del Pino & Jové, 2005). It is difficult to answer why exposure time does not significantly affect infectivity and more experiments are needed to address this aspect. The lowest infectivity of all tested species was observed, again, in treatments with oxamyl and sulphur. A negative influence on infectivity was shown especially by pyrethroids, carbamates and organophosphates (Nishimatsu & Jackson, 1998; Ishibashi & Takii, 1993; Head *et al.*, 2000), for example, oxamyl, mancozeb or fenitrothion. According to the results it seems that the most sensitive species is *S. arenarium* and the most resistant is *S. feltiae*.

The conclusion is that all tested pesticides are safe to use in integrated pest management except for the a.s. sulphur and oxamyl. The a.s. mancozeb,

fenitrothion and trifluralin should be used at lesser extent because in comparison with the other tested pesticides they can cause higher mortality or decreasing infectivity, which could have an influence on the survival of populations of EPNs in treated localities.

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J. Nermuť, Z. Mráček. Воздействие пестицидов на жизнеспособность и инвазионность энтомопатогенных нематод (Nematoda: Steinernematidae).

Резюме. Исследовали воздействие водных растворов 13 различных пестицидов (хлорпиралид, флюоксипир, трифуралин, Натрий-2-метокси-5-нитрофенол, каптан, манкозеб, сульфур, пропамокарб, хлорпирифос, оксамил, лямбда-цигалотрин, фенитротион и пропаргит) на жизнеспособность и инвазионность энтомопатогенных нематод видов *Steinernema feltiae*, *S. arenarium* и *S. kraussei*. К наиболее токсичным пестицидам относились оксамил и сульфур. Трифуралин существенно снижал жизнеспособность, также как хлорпирифос, лямбда-цигалотрин, каптан и манкозеб. Различия между видами не были статистически достоверными, за исключением нематод вида *S. feltiae*, которые оказались более чувствительными к пестицидам, чем другие виды. Показано, что как продолжительность воздействия, так и концентрация пестицидов оказывают статистически достоверное влияние на снижение жизнеспособности нематод. Наиболее выраженное отрицательное воздействие на инвазионность оказывали оксамил, сульфур, а также манкозеб и фенитротион. Продолжительность воздействия, в отличие от концентрации пестицидов, не показывала достоверного влияния на инвазионную способность.