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RESEARCH REPORT

Effects of different pesticides on virulence and mortality of some entomopathogenic nematodes

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Abstract

Entomopathogenic nematodes (EPNs) have been used against especially soil borne insect pests. EPNs are feasible and attractive for biological control, because of their virulence against various insect pests, host seeking ability, being usable with standard equipment, and long-term efficacy. In addition EPNs can be applied simultaneously with some pesticides. These properties make EPNs ideal biocontrol agent in integrated pest management. In the present study, effects of 4 widely used pesticides (Glyphosate, Chlorpyrifos-ethyl, Captan, Fosetyl-al) on virulence and mortality of three EPN strains (*Heterorhabditis bacteriophora* Alman, *H. bacteriophora* HbH and *Steinernema carpocapsae* DD-136) were examined at 24 and 48 h periods. All strains were able to infect *Galleria mellonella* larvae averagely above 90 % rate, after 24 and 48 h treatments with all pesticides. However, some of the pesticides showed negative impact on the viability of the strains. Especially, DD-136 and Fosetyl-al seemed like incompatible, as the mortality rates were significantly higher than control for both 24 and 48 h. The results of the present study showed that it may be possible to use some EPN strains with some pesticides. It is expected that the results of the study will provide useful information for future integrated pest management programs.

Key Words: entomopathogenic nematodes; mortality; virulence; pesticides

Introduction

Biological control provides safe and environmentally friendly control of insects, diseases and weeds, and it is raising its popularity every day with new developments and technologies. One of the most important biocontrol agents is entomopathogenic nematodes (EPNs), belonging to the families Heterorhabditidae and Steinernematidae (Poinar, 1979). EPNs are soil-dwelling obligate endoparasitic organisms. They have control potential of many economic important insect pests (Peters, 1996; Susurluk *et al.*, 2011; Ulu *et al.*, 2014), while safe for non-target organisms and environment (Boemare *et al.*, 1996; Ehlers, 1996).They can be mass produced (Ehlers, 2001) for widespread commercial use. EPNs seek for their hosts, penetrates through natural openings or intersegmental membrane, and release symbiont bacteria to the hemocoel of the host. The host is killed within 36 - 48 h and free-living third-stage infective juveniles (IJs) emerge from host cadaver (Poinar, 1979; Akhurst and Boemare, 1990; Brown and Gaugler, 1997).

Although pesticides make pest control easier, they have negative impacts on the environment, human health and other various ecosystems because of the excessive use. Integrated pest management (IPM) arose as a solution to problems associated with the excessive use of chemical pesticides to control pests, diseases and weeds, more than 50 years ago (Hokkanen, 2015). With IPM, agricultural practices and control methods are used in a harmony and environmental risks are minimized. EPNs are environmentally safe organisms and they can be applied with standard pesticide sprayers or irrigation systems (Georgis, 1990; Wright *et al.*, 2005). EPNs are known as resistant to various agricultural chemicals (Rovesti and Deseö, 1990; Georgis and Kaya, 1998). Simultaneous applications with pesticides lead EPNs to become a possible option in IPM systems.

Pesticides are still the most convenient method for controlling pests in agriculture. However, the proportion of the other control methods such as biological, biotechnical, genetic, cultural, etc. are

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rising due to the negative effects of chemicals. Although it doesn't seem possible to take pesticides out of agriculture, it may be possible to lower their use or enhance their efficiency with some agricultural applications. EPNs are effective biocontrol agents, especially on soil-dwelling insects and they are known as resistant against several agricultural chemicals. The aim of the present study was to determine compatibility of different pesticides on commercial and a hybrid EPN strain. Thus, it may be possible to use EPN simultaneously with common pesticides and enhance controlling while reducing labor cost.

Compatibility of the pesticides with EPNs has been investigated by researchers for more than 25 years (Rovesti *et al.*, 1988; Rovesti and Deseo, 1990; Patel and Wright, 1996; Alumai and Grewal, 2004; Laznik and Trdan, 2014; Baimey *et al.*, 2015). Although there are many studies conducted, more investigations are still necessary as it has been showed that EPN-pesticide compatibility can be also strain-specific (De Nardo and Grewal, 2003; García-del-Pino and Jové, 2005; Gutiérrez *et al.*, 2008; Laznik *et al.*, 2012; Atwa *et al.*, 2013).

In the present study, effects of 4 different pesticides (Fosetyl-al, Glyphosate, Captan and Chlorpyrifos-ethyl) on virulence and mortality of *Heterorhabditis bacteriophora* Alman, *H. bacteriophora* HbH and *Steinernema carpocapsae* DD-136 strains were examined. With the results of the study, reduction of application cost, effective control of pests with simultaneous use of chemical and biological control, and introducing new biological control agents for IPM are expected.

Materials and Methods

Nematode strains

Three EPN strains, *H. bacteriophora* Alman, *H. bacteriophora* HbH and *S. carpocapsae* DD-136, were used in the experiments. HbH was obtained by hybridization of two native EPN strains from Turkey. The nematodes were produced in last instar larvae of greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae) according to White Trap method as described by White (1927). The IJs were harvested from White Trap and stored in flasks in Ringer's Solution at 4 °C for a week before trials.

Pesticides

Especially, pesticides applied to soil were preferred as EPNs generally live under the soil and are effectively used against soil-borne pests. Chlorpyrifos-ethyl usually is used against soil-borne pests as insecticide. Fosetyl-al and Captan are used for soil-borne pathogenic fungi, and Glyphosate is for soil-borne pathogenic fungi, and Glyphosate is applied against weed on fruit and vegetable fields in Turkey. Therefore, these active ingredients (Table 1) were used in order to determine their toxicity on IJs of HbH, Alman and DD-136 strains.

Toxicity tests

The 100 IJ/10µl solutions of IJs were prepared in Ringer's solution and transferred in each well of 24-well plate for each nematode strain. One ml pesticides prepared at field doses (Table 1) were added on nematodes in wells. The plates were incubated in shaker at 25 °C, 150 rpm and %70 RH, in order to prevent settling of pesticide+nematode mixture. The experiment was replicated four times. Tap water used for control. Dead and alive EPNs in each well were counted after 24 and 48 h, and their mortality rates were assessed (Rovesti *et al*., 1988; Patel and Wright, 1996).

Virulence tests

Harvested nematodes from white trap were transferred into 100 ml Erlen flasks with density of 2000 IJs/ml and field doses of the prepared pesticides were added on the EPNs. Flasks were incubated in shaker at 25 °C. Firstly, the nematodes were separated from the pesticide solutions with micro sieve and suspended in pure water for 2 h (Hara and Kaya, 1983). Subsample was taken from pure water plus nematode solution for counting, afterwards a new suspension was prepared which has 50 IJs/larva, according to commercial dose. Nematode virulence after 24 and 48 h of exposure to pesticides was tested against the last instar *G. mellonella*. One larva were placed for each well of the plates, the wells were filled with 10 % moist sterile silver sand (particle size 300 - 400 µm). The plates were sealed with parafilm and incubated at 25 °C. After four days, death larvae were collected to determine nematode infectivity. The death larvae were dissected under stereomicroscope in order to prove whether the larva killed by nematodes. Tap water used for control. The virulence experiment was replicated 3 times with 10 larvae used per replicate.

Statistical analyses

Before analysis, all treatment data were corrected based on the control mortality (< 5 %) using Abbott's (1925) formula. Toxicity and virulence test data were analyzed using one-way ANOVA (analysis of variance) with JMP®7.0 software. LSD (Least Significant Differences) test (0.05 significance level) was used to determine the difference between applications.

Toxicity tests

Results showed that, among all pesticides, Fosetyl-al was the most toxic chemical for all strains at 24 and 48 h exposure times. Fosetyl-al caused significant mortality rates on every strain ranging between 9.78 - 82.92 %, at both 24 and 48 h counts ($p < 0.05$) comparing with control. Besides Fosetyl-al, other pesticides also showed significant toxic effect on different strains at different exposure times. As seen from Table 2, pesticides showed significant effects on mortality of the strains ($p < 0.05$). DD-136 strain showed significantly higher mortality than Alman and HbH in all chemicals at both exposure times. Mortality of Alman and HbH strains also differed within pesticides ($p < 0.05$). Statistical summary of the tests indicated in Table 3.

Table 1 Information of the pesticides

Active Ingredient	Classification	Commercial Product	Manufacturer	Recommended Field Dose
Fosety-al	Fungicide	Placate	Platin Chemistry	250 g/100 l water
Glyhosate	Herbicide	Roundup	Monsanto	300 g/100 l water
Chlorpyrifos-ethyl	Insecticide	Dursban 4	Dow Agro	200 ml/100 l water
Captan	Fungicide	Captan	Koruma	300 g/100 l water

Table 2 Corrected mortality rates of EPN strains exposed to different pesticides

Strains	Time	Mortality (%)				
		Control	Fosetyl-al	Glyphosate	Captan	Chlorpyrifos-ethyl
Alman	24h	0^{bA}	9.78^{aC}	11.47^{aB}	0.67^{bB}	2.44^{bB}
DD-136		0^{dA}	52.97^{aA}	27.30^{bA}	25.72^{bA}	13.40^{cA}
HbH		0^{bA}	18.78^{aB}	1.21^{bC}	2.76^{bB}	0.93^{bB}
Alman	48h	0^{dA}	16.09^{aC}	12.14^{bB}	1.10^{dC}	7.53^{cB}
DD-136		0^{dA}	82.92^{aA}	32.14^{bA}	26.40^{bA}	16.63^{cA}
HbH		0^{cA}	65.30^{aB}	3.88^{bcC}	12.22^{bB}	2.23^{cC}

Different lowercase letters indicate statistical significance between treatments for each strain at 24 and 48 h separately ($p < 0.05$).
Different uppercase letters indicate statistical significance between strains for each treatment at 24 and 48 h separately ($p < 0.05$).

Results and Discussion

Virulence tests

The results of the virulence tests were not similar with toxicity, as the pesticides didn't affect virulence of the strains as much as they did in mortality experiment. For 24 h, there were no significant difference on the virulence of Alman and HbH strains exposed to the pesticides comparing with control ($p > 0.05$). DD-136 showed the lowest mortality and all pesticides significantly affected the virulence of the strain ($p < 0.05$). For 48 h, Captan and Chlorpyrifos-ethyl had a negative impact on the virulence of HbH strain, and Fosetyl-al had a negative impact on DD-136. For both toxicity and virulence results, DD-136 strain was the most affected one among others (Table 4).

The results of the present study indicated that the most toxic pesticide was Fosetyl-al for all strains. Toxic effects of the other pesticides varied among strains and exposure time, but the least-toxic pesticide was Chlorpyrifos-ethyl, which is one of the most widely used organophosphate insecticides all around the world, according to United States Environmental Protection Agency. From another sight, the weakest strain was *S. carpocapsae* DD-136 and *H. bacteriophora* strains were more tolerant than DD-136. According to Baimey *et al.* (2015), all tested *Heterorhabditis* species were more tolerant to glyphosate and fipronil than the *Steinernema* species, which was a similar result with the present study.

Table 3 Statistical summary of the toxicity tests

Toxic effects of the pesticides by strain		
	24h	48h
Fosetyl-al	*F*=161.33, df=2;9, *P*<0.0001	*F*=56.08, df=2;9, *p*<0.0001
Glyphosate	*F*=45.38, df=2;9, *P*<0.0001	*F*=36.03, df=2;9, *p*<0.0001
Captan	*F*=149.40, df=2;9, *P*<0.0001	*F*=40.24, df=2;9, *p*<0.0001
Chlorpyrifos-ethyl	*F*=42.71, df=2;9, *P*<0.0001	*F*=25.12, df=2;9, *p*<0.0001
Mortality rates of the strains by pesticide		
	24h	48h
Alman	*F*=36.81, df=4;15, *P*<0.0001	*F*=49.63, df=4;15, *p*<0.0001
DD-136	*F*=113.67, df=4;15, *P*<0.0001	*F*=104.30, df=4;15, *p*<0.0001
HbH	*F*=42.60, df=4; 15, *P*<0.0001	*F*=104.30, df=4;15, *p*<0.0001

Table 4 Virulence of pesticide-exposed EPN strains on *G. mellonella* larva

Strain	Time	Virulence (%)				
		Control	**Fosetyl-al**	**Glyphosate**	**Captan**	**Chlorpyrifos-ethyl**
Alman	24h	100^{a}	96.6^{a}	100^{a}	96.6^{a}	100^{a}
DD-136		100^{a}	83.3^{bc}	90^{b}	86.6^{b}	76.6^{c}
HbH		100^{a}	96.6^{a}	100^{a}	96.6^{a}	96.6^{a}
Alman	48h	100^{a}	93.3^{a}	100^{a}	96.6^{a}	93.3^{a}
DD-136		100^{a}	70^{b}	93.3^{a}	83.3^{ab}	96.6^{a}
HbH		100^{a}	100^{a}	93.3^{a}	76.6^{b}	73.3^{b}

Different letters indicate statistical significance between treatments for each strain at 24 and 48 h separately ($p < 0.05$).

study. Likewise, results of the study conducted by Negrisoli *et al.* (2010) indicated that, Lorsban^{TM} (Chlorpyrifos-ethyl) caused the lowest mortality among other 18 insecticides. Gutiérrez *et al.* (2008) conducted a study to determine effects of different pesticides, including Glyphosate and Chlorpyrifos-ethyl, on *S. feltiae*, and they stated that herbicides were more toxic then insecticides. Unlikely, most toxic pesticide of the present study was Fosetyl-al, which is a fungicide, and it is followed by Glyphosate. Their strain was *S. feltiae* and they did not use fungicides, this discordant result may be explained with strain and pesticide selection and interaction. According to another study performed by García-del-Pino and Jové (2005), *H. bacteriophora* and *S. carpocapsae* were both tolerant to fipronil while *S. arenarium* was sensitive. Mortality rate were increased with the dose and exposure time, as expected.

Another aim of the study was to determine the effects of pesticides on virulence of the EPN strains. *Steinernema carpocapsae* DD-136 strain was the weakest strain, while Alman didn't show significant differences between pesticide treatments and control. Virulence experiments were conducted with living IJs after exposing to the pesticides, and virulence results of the pesticides-exposed populations mostly didn't show significant differences between control populations. It can be stated that pesticides didn't affect the virulence of the strains. However, it must be taken into account that simultaneous applications of EPNs with these pesticides may lower effectiveness due to the toxic effect and mortality of strains. Negrisoli *et al.* (2010) tried the effects of 18 insecticides on infectivity of three EPN species. Their results were significantly different from control; however, highest infectivity was recorded with Lorsban^{TM} (Chlorpyrifos-ethyl) and Match^{TM} (Lufenuron), which can be stated as similar with the present study, as the highest virulence results were recorded for Chlorpyrifos-ethyl and Glyphosate. The study of Gutiérrez *et al.* (2008) showed that virulence of *S. felitae* was not seriously affected by the chemicals, likewise in the present study.

As it can be understood from former studies, there are lots of factors such as chemical type, strain, exposure time, different environmental conditions and etc., which have significant role on virulence, viability or other biological characters of EPNs. Based on our findings, it may possible to apply EPNs with compatible pesticides within IPM programs in future, which will reduce application time and labor cost. However, there are incompatible pesticides that may need to be applied alone. Further investigations may reveal new data for new pesticides and new species or strains.

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