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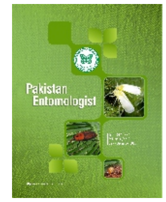

SUSCEPTIBILITY OF LARVAE OF WHEAT SAWFLY, *DOLERUS PUNCTICOLLIS* (TENTHREDINIDAE; HYMENOPTERA) TO AN ENTOMOPATHOGENIC NEMATODE, *STEINERNEMA CARPOCAPSAE* AND INDOXACARB

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ABSTRACT

Wheat sawfly larvae, *Dolerus puncticollis* (Tenthredinidae; Hymenoptera) were detected in wheat fields of Moghan (Ardebil province, Iran) for first time. A bioassay experiment was carried out to determine the susceptibility of *D. puncticollis* to an entomopathogenic nematode species *Steinernema carpocapsae* and indoxacarb. Probit analysis showed high susceptibility of *D. puncticollis* larvae to *S. carpocapsae* with LC_{20}, LC_{50} and LC_{80} values of 1.1, 4.5 and 18 infective juveniles per larva, respectively. The LC_{20}, LC_{50} and LC_{80} values of indoxacarb for *D. puncticollis* larvae were 0.1, 1.15 and 12.2 ppm, respectively. Due to the extremely low value of LC_{50}, this species is very sensitive to indoxacarb possibly reflecting the lack of chemical control history against the pest.

Keywords: Indoxacarb, LC_{50}, Leaf-feeding, *Steinernema carpocapsae,* Wheat sawfly

INTRODUCTION

Wheat is one of the most important crops. The percentage of total daily calories from wheat is 39% and the percentage of daily protein supply from wheat is 41.86% in Iran (Wheat Atlas, 2015). Thus controlling notorious pests of this crop is very critical for supporting food supply in many countries as well as Iran.

Two genera of graminivorus sawfly i.e., *Dolerus* and *Pachynematus* have been reported from winter wheat, *Triticum aestivum* L. spring barley, *Hordeum vulgare* L. and a range of grasses (Barker and Reynolds, 2004) causing sporadic crop damage in cereals in Europe (Heyer and Wetzel, 1988; Miczulski and Lipinska, 1988), America (Kamm, 1975; Corp and Fisher, 2003) and Asia (Xu and Chen, 1991). Weather and tillage appear to be significant factors influencing pest population. Cool and wet weather can delay or prevent spring cultivation, which usually would kill many of the larvae that overwinter in the soil (Corp and Fisher, 2003). Accumulated surface residue of plants on fields serves to shelter overwintering larvae through the winter. In the eastern U.S., outbreaks usually occur after an abnormally warm spring that creates ideal egg-laying conditions or mild winters which upset the natural biological control of the sawfly larvae (they are heavily parasitized by other wasps) (Corp and Fisher, 2003). None of these wheat sawfly genera have been reported from North West of Iran including Ardabil province.

Entomopathogenic nematodes (EPNs) (Rhabditida: Heterorhabditidae and Steinernematidae) are obligate and generalist pathogens of insects present in soils of many ecosystems around the world (Adams *et al*., 2006). Their pathogenicity is partly due to their symbiotic association with bacteria of the genera *Photorhabdus* and *Xenorhabdus* (Bedding *et al*., 1983; Forst *et al.,* 1997; da-Silva *et al*., 2000). Due to its wide host range and geographical distribution, *Steinernema carpocapsae* is the most studied, available, and versatile of all EPNs (Llacer *et al*., 2009).

Indoxacarb is a biorational broad-spectrum oxidiazine insecticides that is uniquely metabolically activated by decarboxylation in insects from a low-toxicity pro-insecticide to an active form which targets the voltage-gated sodium channel. The speed of action of the insecticide is varied (Wing *et al*., 2000; Lapied *et al*., 2001). Indoxacarb is taken up either through the cuticle or via ingestion by the insect (Wing *et al*., 2000).

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Larvae of wheat sawfly, *Dolerus puncticollis* (Tenthredinidae; Hymenoptera) with leaf-feeding activity on wheat, was observed in the wheat fields in Moghan (Ardebil province, Iran) during April, May and June, 2015, for first time. In present study, susceptibility of *D. puncticollis* to *S. carpocapsae* and indoxacarb was evaluated. Wheat sawflies are not economic pests of wheat in the world (Barker and Reynolds, 2004), however risk of their outbreaks makes investigation on their integrated management items necessary. There is not any published investigation carried out on efficacy of entomopathogenic nematodes against *Dolerus* species. Here in, *D. puncticollis* is introduced as a new susceptible physiological host for *S. carpocapsae* for first time.

MATERIALS AND METHODS

Nematode

An Iranian isolate of *Steinernema carpocapsae*, provided by Dr. M. Nikdel (Center of Agricultural Research and Natural Resources of East Azarbaijan, Tabriz, Iran) was cultured using the last instar greater wax moth larvae, *Galleria mellonella*. Infective juveniles (IJs) were stored in distilled water at 5 °C and used in all experiments within 30 days of emerging from the host. Before starting the experiments, nematodes were kept at 25 °C for 20-30 min.

Insects

Dolerus puncticollis

Larvae of grass sawfly, *D. puncticollis* were collected from wheat fields in Moghan (Ardebil province) during April, May and June, 2015 and were stored in plastic boxes (23 cm length, 17 width, 10 cm height; 20 larvae per box) with ventilated lids (26 ± 2°C, 55 ± 5 % RH and 16:8 (L:D) photoperiod). Fresh wheat leaves were provided daily. One day after collecting, the last instar larvae weighing 0.10±0.02 g were used in bioassay experiments.

Galleria mellonella

G. mellonella was reared under the same environmental conditions and in similar boxes as for *D. puncticollis* larvae, but on an artificial diet (1200 g wheat flour, 120 g beeswax, 300 g dried yeast, 600 g honey and 500 ml glycerol 99 %).

Insecticide

Indoxacarb (Avaunt® 150 SC, DuPont) was used in insecticide bioassays.

Bioassay

Larvae of *D. puncticollis* were exposed to infective juveniles of *S. carpocapsae* in petri dishes (9 cm in diameter) lined with filter paper previously wetted with distilled water (1.5 ml) before adding nematodes. Small pieces of wheat leaf (3 cm × 1 cm) were used as food resource. The nematodes were used at rates ranging between 2 and 16 IJs per larva (i.e., 2, 4, 8 and 16 IJs per larva) in 0.5 ml distilled water. Distilled water (0.5 ml) was used as control. Preliminary experiments showed that the larval mortality occurred by 24 h post infection, therefore, the mortality was recorded 24 h post infection in all experiment replications. For each concentration, 12-15 larvae were used and the experiment was replicated three times in different time points using insect larvae from different cohorts in each time point. Three days later, the dead insects were dissected to ensure the presence of the nematodes.

Residual bioassay using glass petri dishes (9 cm in diameter) were performed for indoxacarb bioassay. Serial dilutions as ppm of the active ingredient of the test compound were prepared using distilled water. Preliminary dose-response experiments were carried out to determine the range of indoxacarb concentrations which caused mortality ranging from 20 to 80 percent by 24 h. Finally, certain concentrations of the indoxacarb (i.e., 0.5, 1, 3, 6 and 20 ppm from trade product) were used in bioassay. Distilled water was used as control. For each concentration 12-15 larvae were used and the experiment was replicated three times in different time points using insect larvae from different cohorts in each time point. Mortality was recorded 24 h post treatment.

For both bioassay experiments incubation conditions were 26±2° C, 55 ± 5% RH, and 16:8 (L: D) photoperiod.

Statistical analysis

Lethal experimental data were transformed into square root of (x+1) before analysis. Data were analyzed by analysis of variance and means for mortality were evaluated by Duncan's multiple-range test (SAS Institute, 2004). LC_{20}, LC_{50} and LC_{80} values were computed by Probit analysis using SAS software (SAS Institute, 2004).

RESULTS

Analysis of variance revealed significant differences between mortality of *D. puncticollis* larvae treated with different doses of *S. carpocapsae* (F =387.99; *df* = 5; P<0.0001). All nematode treatments caused greater mortality than the control ($p < 0.05$) and the highest concentration of *S. carpocapsae* elicited the great mortality. Linear Probit mortality analysis revealed a significant relationship between log dose and insect mortality ($p < 0.05$, Fig. 1 & Table 1). Because goodness-of-fit χ^2 values were not significant for Probit analysis, no heterogeneity factor was used in the calculation of confidence limits and LC_{20}, LC_{50} and LC_{80} values. Dissection of dead larvae at three days post treatment revealed adult nematodes appearance inside the insect hemocoel. Dissection of dead larvae at three days post treatment revealed adult nematodes appearance inside the insect hemocoel.

The results of dose-response bioassay for indoxacarb using *D. puncticollis* larvae are summarized in Table 1 and Fig. 1. The values of LC_{20}, LC_{50} and LC_{80} were 0.11, 1.15 and 12.17 ppm from trade product, respectively (Table 1).

DISCUSSION

Generally, the concentrations of nematodes found to cause mortality in this study were very low since 4.5 IJ per larvae lead to 50 percent mortality of *D. puncticollis* larvae. Nematode reproduction was observed within dead dissected insects which reveals that probably the insect have not effective defensive mechanism against nematode infection.

On the other hand, nematode recycling is an important factor in determining the persistence of nematodes after application as biological control agents. Therefore, nematode reproduction in at least some cadavers will contribute to their success.

Dissection of dead larvae at three days post treatment revealed appearance of the adult nematodes inside the insect hemocoel. As mentioned before, mortality of the larvae was observed 24 h post treatment; most insects die within 48 h post treatment with entomopathogenic nematodes (Kaya and Gaugler, 1993). The symbiotic bacteria are responsible for septicemia and death of arthropod hosts of the entomopathogenic nematodes (Adams and Nguyen, 2002). Probably larvae of *D. puncticollis* are highly susceptible to symbiotic bacteria of *S. carpocapsae*. High efficacy of the Iranian isolate of *S. carpocapsae* has been reported against some other pests including *Leptinotarsa decemlineata* (Ebrahimi *et al*., 2014 a, b) and *Helicoverpa armigera* (Ebrahimi and Niknam, 2011).

As mentioned before, wheat sawflies are not economic pests of wheat, however because of the risk of their outbreaks, scouting their abundance in the fields is necessary. Barker and Reynolds (2004) identified three factors that appear to have key roles in determining patterns of sawfly abundance: insecticide use, differences in host-suitability of different cereals and grasses and the vulnerability of overwintering sawflies to soil cultivation. Previous studies showed that *Dolerus* larvae are very susceptible to broad-spectrum insecticide use (Sotherton 1990; Moreby *et al*. 2001) and populations may take as long as 7 years to recover from a single direct application of insecticide (Aebischer, 1990). Therefore, high susceptibility of Iranian population of *D. puncticollis* to indoxacarb was expected and is consistent with previous investigations.

Entomopathogenic nematode species can recycle and persist in the environment; can play an indirect role in improving soil quality and are compatible with a wide range of chemical and biological pesticides used in IPM programs (Lacey and Georgis, 2012). Using the nematodes in pest management will decrease the amount of chemical insecticides used for pest management. *Dolerus* species overwintering inside the soil (Barker and Reynolds, 2004), on the other hand, the infective juveniles of the entomopathogenic nematodes have capable of seeking out and infecting the hosts in the soil (Burnell and Stock, 2000).

Due to the extremely low value of LC_{50} in dose-response bioassay for indoxacarb using *D. poncticollis* larvae, which is around 400 times less than the recommended dose (500 ppm), this species is very sensitive to indoxacarb. This sensitivity is probably due to lack of chemical control history against the pest in previous generations. The coefficient of the determination of dose–response lines (R^2, Table 1) showed a high correlation between insecticide concentrations and response of the population, which reveals that the test population was homogenized. This fact is in consistent with lack of exposing the pest to indoxacarb in previous generations.

There is not any published work on efficacy of entomopathogenic nematodes against *Dolerous* species. In our study, *D. puncticollis* was introduced as a new susceptible physiological host for *S. carpocapsae*, however, field application of the nematode and recovery of the nematode from dead insects is needed for confirm the host-relationship.

Wheat sawflies are not economic pests of wheat in the world (Barker and Reynolds, 2004), however risk of their outbreaks makes investigation on their integrated management items necessary. In conclusion Probit analysis showed high susceptibility of the larvae of *D. puncticollis* to *S. carpocapsae* and indoxacarb. This is the first report on efficacy of entomopathogenic nematodes against *Dolerus* species. Using entomopathogenic nematodes is an environmentally benign method for pest controlling; however, economic considerations need to be notice for each agro-ecosystem.

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Table 1

LC_{20}, LC_{50} and LC_{80} values for *Steinernema carpocapsae* and indoxacarb against *Dolerus puncticollis* larvae.

Name	Slope ± SE	Chi-Square	LC_{20} (95% CL)	LC_{50} (95% CL)	LC_{80} (95% CL)	R^2	N
S. carpocapsae	1.39±۰.۳۱	۱.۸۰	۱.۱۲ (۰.۳۰-1.96)	4.49 (2.94-6.30)	18 (11.3-50.8)	0.92	164
Indoxacarb	0.82±۰.۱۸	1.31	0.11 (0.01-0.31)	1.15 (0.45-2.02)	12.17 (6.15-52.9)	0.95	190

LC = lethal concentration values based on ppm of trade product and IJ per larvae for indoxacarb and *S. carpocapsae,* respectively; CL = Confidence limits; R^2 = Coefficient of determination

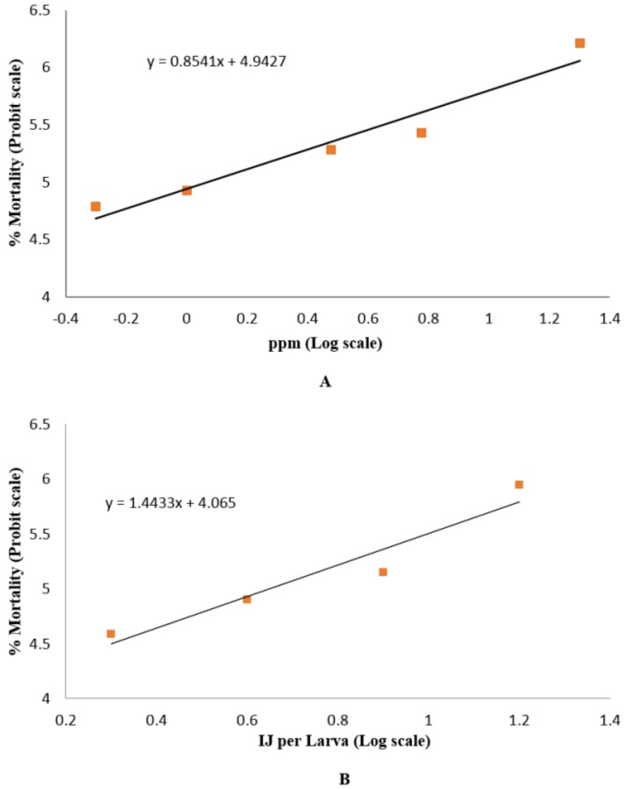

Fig. 1

Mortality (probit-transformed) of *Dolerus puncticollis* larvae treated with different doses of indoxacarb (A) and *Steinernema carpocapsae* (B)

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