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Efficacy of the entomopathogenic nematode isolates against *Spodoptera littoralis* (Boisduval) and *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae)

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Abstract

Background: Entomopathogenic nematodes (EPNs), as biological control agents, have been isolated from many regions throughout the world. Local isolates of EPNs are usually more effective for controlling indigenous insect pests as they are adapted to the local environmental conditions and the insect pest species.

Results: In the present work, EPN isolates were searched in the soil under citrus and guava trees, and Egyptian clover at Noubaria region, Elbhaira governorate, Egypt, within two consecutive years. The EPNs were isolated from two positive soil samples of Egyptian clover (*Trifolium alexandrinum*) (TAN5) and guava trees (*Psidium guajava*) (PGN6), while the EPNs were not existent in the soil samples under citrus. Laboratory applications of the two EPNs isolates against the cotton leafworm, *Spodoptera littoralis* (Boisd.), and the black cutworm, *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae), were recorded. Nematodes naturally occurring in the soil were trapped by full-grown larvae of the greater wax moth (*Galleria mellonella* L.). Infected larvae turned from whitish beige to dark reddish color, proofed that these isolates belong to the genus *Heterorhabditis*. Laboratory results revealed that the mortality rate ranges from 24 to 100% with TAN5 while from 18 to 96% with PGN6 at *A. ipsilon* larvae. The LC_{50} values of TAN5 against *A. ipsilon* were 1285.527 and 1560.747 IJs/cup, while those values for *S. littoralis* were 1339.099 and 2531.605 IJs/cup in larvae and pupae, respectively. The 3rd instar larvae of *A. ipsilon* and *S. littoralis* were more sensitive than the pupae. Production of *Heterorhabditis* sp. strain TAN5 was the highest in the reproduction of infective juveniles than the strain PGN6 at all concentrations.

Conclusions: The EPNs isolated from the soil samples belonged to the genus *Heterorhabditis*. *Heterorhabditis* sp. strain (TAN5) collected from the soil under Egyptian clover at Noubaria region was the highest reproduction and the most effective against both tested pests, *A. ipsilon* and *S. littoralis* larvae and pupae. EPN species would serve as an alternative to chemical pesticides and fit well in an integrated pest management program against larvae as well as adults and pupae of many economic insect pests which inhabit the soil.

Keywords: Entomopathogenic nematodes, Biological control, *Heterorabditis* spp., *Galleria mellonella*, *Spodoptera littorals*, *Agrotis ipsilon*

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Background

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae with their associated symbiotic bacteria (*Xenorhabdus* and *Photorhabdus*, respectively) are widely distributed in soils throughout the world (Adams et al., 2006). These nematodes are pathogenic to insects, killing them within 48 hrs with the aid of their associated bacterial symbionts, and have great importance as biological control agents of many insect pests (Laznik et al., 2011). The bacteria multiply inside the host and release a number of virulence factors, including complexes of toxins, hydrolytic enzymes, hemolysins, and antimicrobial compounds (Eleftherianos et al., 2010), thus providing nutrients for the nematode development and reproduction within the insect cadaver. Nematodes are commercially available in many countries for the control of soil-inhabiting insects (Susurluk 2011). EPNs are found in a variety of habitats, and the various species and isolates exhibit considerable variation in their host range, reproduction, infectivity, and conditions for survival (Laznik and Trdan 2012). Environmental factors affect the presence of nematodes in the soil and their distribution and survival. Biotic and abiotic factors affect the distribution of EPNs to differ across different regions (Karthik Raja and Lakshminarayanan 2011). Major factors namely temperature, host availability, and percentage of moisture in the soil are thought to be important in determining the distribution of nematodes inside the soil (Susurluk 2011). *Heterorhabditis* and *Steinernema* are distinguished by the speed and ease of reproduction and infect many insect families, compatibility with chemical pesticides, and their ease in the application has made them easier for researchers to work on. To date, around 100 species of *Steinernema* and 21 species of *Heterorhabditis* were identified from different countries of the world. In Egypt, the initial research on EPN began in the 1970s when Dr. El-Kifl worked on the biological control potential of *Neoaplectana* (= *Steinernema*) *carpocapsae* against the cotton leafworm, *Spodoptera littoralis*, one of the most economically important insect pests in Egypt. The surveys carried out in Egyptian soils revealed that species of heterorhabditids were more prevalent than steinernematids; however, research regarding their use to control other insect species was done as well (Aashaq et al., 2020).

The objective of this study was to evaluate the efficacy of indigenous EPNs isolates, collected in different soils at Nubaria region, Elbhaira governorate, Egypt. Laboratory studies targeted two economic insect pests: *S. littoralis* and *A. ipsilon*.

Methods

Galleria mellonella

The greater wax moth (*G. mellonella* L.) larvae were obtained from infested hives and reared in jars (2 kg capacity) until the emergence of moths according to the technique described by Birah et al. (2008). Similar constituents, but with different proportions, were tried later by Huang et al. (2010). Fully grown larvae of *G. mellonella* were also used for isolation and production of entomopathogenic nematodes.

Target insects

Rearing of Spodoptera littoralis

A field strain of *S. littoralis* was obtained from an open field of the vegetable farm at Giza Governorate, Egypt, transferred to the laboratory, and reared at 25 °C ± 2 °C and 65–75 RH%. *S. littoralis* larvae were placed in glass jars and fed on castor leaves (*Ricinus communis* L.) (Zhang et al. 2019a). The jars were provided daily with castor leaves as a source of food for the larvae. The pupae were transferred to suitable cages for mating; the emerging adults were fed on 20% honey solution even lay eggs (Ibrahim 1974).

Rearing of Agrotis ipsilon

The *A. ipsilon* larvae used in this study were collected from an open field of a vegetable farm at Giza Governorate, Egypt. The 4th instar larvae were reared singly inside plastic tubes (1.5 cm in diameter, 15 cm in height) or in small groups in plastic jars to avoid cannibalism until the last instar of the larvae developed to the pupal stage. All pupae were transferred into suitable cages for mating, the emerging adult moths were fed by a on 20% honey solution till laying eggs. All of the rearing procedures for *A. ipsilon* were carried out at 25 ± 1 °C and 75 ± 5% RH (Zhang et al., 2019b). The newly hatched larvae were transferred into small plastic jars and provided daily with castor leaves as a source of food.

Soil sampling

Soil samples were collected from Nubarya Elbhaira governorate, Egypt, in the years 2018 and 2019 every 15 days under citrus trees (*Citrus sinensis* and *Citrus tangerine*), under guava trees (*Psidium guajava*), and under Egyptian clover (*Trifolium alexandrinum*). Sandy soil was chosen for the soil samples' collection. About 1 kg of soil/sample was collected, 10–15 cm below the soil surface. Samples were kept in plastic sacs labeled with the necessary information. The soil sacs were then placed in an icebox and transferred to the laboratory. In the laboratory, soil samples were placed in cups and fully grown larvae of (*G. mellonella*) were added onto the soil samples.

Isolating nematodes

G. mellonella baiting technique modified after Bedding and Akhurst (1975). Soil sample of each sac was thoroughly mixed and then divided into 10 plastic cups (about 100 ml volume). Five *G. mellonella* late instar

larvae were placed onto the soil surface/cup, and the cups were covered by their lids, turned upside–down every 24 hrs to mix in the soil and kept at the temperature of 25 ± 2 °C. The cups were examined daily and throughout 6–10 days later, dead larvae, suspected to be nematode–infected, were picked up carefully, rinsed several times in distilled water, and incubated at 25 °C in extraction White traps. Each White trap contained only one dead larva, using the method originally described by Dutky et al. (1964). Infective juveniles of nematode were harvested daily from the White traps by receiving them in 0.1% formaldehyde solution and stored at 15 °C (White 1927). Effectiveness of the infective juveniles of nematode was tested against larvae and pupae.

Laboratory experiments

Infectivity of the two strains of *Heterorhabditis* sp. to 3rd instar larvae and 1-day-old pupae at 25 °C ± 2 of the *S. littoralis* and *A. ipsilon* was tested. Ten 3rd instar larvae or 10 1-day-old pupae were placed at 1 cm depth from the surface and treated with each of the tested nematodes strain. The infection took place in plastic cups (100 cc capacity), filled with 1 cc sterile sandy soil, and covered with plastic lids. The nematode suspension was poured in the vials and mixed with the soil at 5 concentrations 500, 1000, 2000, 4000, and 8000 IJs/cup. Five replicates were used for each concentration. The numbers of dead larvae or pupae were recorded after 6 days of treatment. Water content in the soil was always kept at 20% of the soil weight. The control treatment was carried out using distilled water.

Production of entomopathogenic nematode

Ten larvae were used for each concentration. Full-grown larvae of *G. mellonella* were confined, individually, in plastic cups (100 cc capacity) lined with filter paper and covered with plastic lids. The infection took place using five concentrations of 1, 2, 4, 8, and 16 IJs/cup for each of the 2 strains of *Heterorhabditis* sp. Infective juveniles were harvested daily using White traps according to White (1927). The whole number of IJs produced/larvae was estimated. All experiments were carried out in a conditioned laboratory at 26–27 °C and 50–60% R.H.

Statistical analysis

Mortality rates were corrected according to Abbott's formula (Abbott 1925); the toxicity lines and LC_{50} values were calculated according to Finney (1971). The mass production of the two strains was statistically analyzed by ANOVA, and the mean values were separated by the least significant difference (L.S.D.) procedure (Snedecor and Cochran, 1980). Also, *t* test between the average production of the two strains at different concentrations or at each one was calculated.

Results

Nematode isolates

Two EPNs isolates, named TAN5 collected from the soil samples under Egyptian clover (*Trifolium alexandrinum*) and PGN6 collected from the soil samples under guava trees (*Psidium guajava*) while the EPNs was not existent in the soil samples under citrus. *Galleria* larvae were used for nematode isolation. All the infected *Galleria* larvae by the two EPN isolates turned to a dark reddish color indicating that the nematodes belong to the genus *Heterorhabditis*.

Laboratory experiments

Data in Table 1 shows the percentage mortality of the 3rd instar larvae and 1-day-old pupae of *A. ipsilon* and *S. littoralis* after 5 days of treatment with *Heterorhabditis* sp. (strain TAN5 and PGN6) at different concentrations. Data showed a comparison of the EPN infectivity where *A. ipsilon* was the most sensitive. The percent mortality increased as the concentration of infective juveniles (IJs) increased. Strain TAN5 revealed high mortality rates.

Table 1 Mortality percentage by different concentrations and comparative toxicity of *Heterorhabditis* sp. strains (TAN5 and PGN6) after 5 days of treatments against larvae and pupae of *Spodoptera littoralis* and *Agrotis ipsilon*

Con./ cup (IJs/ cup)	*Spodoptera littoralis*				*Agrotis ipsilon*			
	Larvae		Pupae		Larvae		Pupae	
	TAN5	PGN6	TAN5	PGN6	TAN5	PGN6	TAN5	PGN6
500	20	16	10	6	24	18	16	14
1000	40	32	26	22	44	40	32	26
2000	62	54	48	38	62	56	48	40
4000	84	76	60	56	100	76	66	62
8000	100	92	76	70	100	96	80	72
Average	61.2	54	44	38.4	66	57.2	48.4	42.8
Slope	2.0055	1.8860	1.4390	1.4406	1.6753	1.7360	1.5069	1.4108
LC_{50}	1339.099	1731.973	2531.605	3312.720	1285.527	1560.747	2156.3955	2831.470

Table 2 Production of different *Heterorhabditis* sp. strains (TAN5 and PGN6) in full-grown *Galleria mellonella* larvae

Con./ larvae (IJs/ larvae)		Production		*t* test values	Significance level < 0.05
		TAN5	PGN6		
1	Mean ± S.E. (IJs ± S.E.)	30,947 ± 1817.89	28,242 ± 2072.97	0.9794	N.S.
2		47,455 ± 2271.51	43,328 ± 2546.44	1.2076	N.S.
4		65,912 ± 3403.01	64,900 ± 2753.66	0.2308	N.S.
8		89,696 ± 3021.32	84,740 ± 3027.05	1.1568	N.S.
16		140,402 ± 7920.63	136,360 ± 8259.02	0.3526	N.S.

The mortality rate of *A. ipsilon* ranged from 24 to 100% in larvae while it ranged from 16 to 80% in pupae. Results of the same strain against *S. littoralis* showed mortality rate ranged from 20 to 100% in larvae while it recorded 10 to 76% in pupae. The LC_{50} values (the highest mortality rate with the lowest LC_{50} values) of TAN5 and PGN6 against *A. ipsilon* larvae were 1285.527 and 1560.747 IJs/cup, respectively. While LC_{50} values of TAN5 against *S. littoralis* were 1339.099 and 2531.605 IJs/cup in larvae and pupae, respectively. *A. ipsilon* and *S. littoralis* 3rd instar larvae were more sensitive than the pupae.

Production of entomopathogenic nematode

Table 2 shows the average nematode production at different concentrations of *Heterorhabditis* sp. strains TAN5 and PGN6. The tested full-grown larvae of *G. mellonella* were used individually for each concentration of the two strains (*Heterorhabditis* sp.). The results showed production of 30,947 IJs/larva at the lowest concentration of (1 IJs/larva) *Heterorhabditis* sp. strain TAN5, while the production rate increased to 140,402 IJs/larva at the highest concentration of (16 IJs/larvae). *Heterorhabditis* sp. strain PGN6 produced 28,242 and 136,360 IJs/larva at concentrations 1 and 16 IJs/larva, respectively. These concluded that strain TAN5 was higher in reproductive EPN juveniles than strain PGN6 at all concentrations. A non-significant difference was found among the production averages at different treated concentrations for the two strains (level < 0.05).

Discussion

Abdel-Razek et al. (2018) recorded new EPN isolates from soil samples collected from 13 different Egyptian Governorates as Giza, Behera, Alexandria, Sohag, Qalubeia, El-Sadat City, Sharkia, Bani-Suif, North Sinai (El-Arish), and South Sinai (Ras-Seder), Tanta, Fayoum, and Suez. One isolate coded IB was isolated from Sharkia (Belbies), identified as *Heterorhabditis indica*, based on its morphometric. Ebubekir and Ramazan (2018) used the EPNs to combat the insects that live in the soil like cutworms and are a successful biological control agent against *A. ipsilon* larvae. The extreme mortality rate (100%) was reached within 2 days after treatment by *Heterorhabditis bacteriophora* (FLH-4-H) and *H. indica* (216-H) isolates at concentrations of 50 and 100 IJs/cm^2, in laboratory experiments. The results showed that all native EPN isolates can be used in biological control against *A. ipsilon*.

(Hassan et al., 2020) was assessed the effectiveness of the EPNs against the larvae of *S. littoralis* and *A. ipsilon*. The sensitivity of both larval species to the EPN species, *Steinernema monticolum* and *H. bacteriophora* was estimated under laboratory conditions. Disagree with results in the results Hassan et al., (2020) the larvae of *S. littoralis* were more sensitive to *H. bacteriophora* than the larvae of *A. ipsilon*. While the results are compatible with results strain TAN5 in the highest concentration was the most efficient concentration for all larval instars of the tested pests where the mortality percentage reached 100%.

Nouh and Abo Abdalla (2016) studied the productivity of EPNs at different concentrations. A single full-grown larva of *G. mellonella* treated with *H. bacteriophora* produced 98,136 IJs /larva, at the highest concentration, 80 IJs/larva.

Conclusion

The EPNs were isolated from two soil samples under Egyptian clover and guava trees at Nubaria region Elbhaira governorate, named as TAN5 and PGN6, respectively, whereas the infected *Galleria* larvae turned from whitish beige to a dark reddish color, which indicates that these EPN isolates belong to the genus *Heterorhabditis*. *Heterorhabditis* sp. strain TAN5 was the highest reproduction and the most effective, where the recorded mortality rate on both *A. ipsilon* and *S. littoralis* larvae and pupae was the highest. It was concluded that the EPNs are of great importance to use in biological control. It is possible to rely on the results of this research in control of *A. ipsilon* and *S. littoralis* for use in the field application of pests. EPNs species would serve as an alternative to chemical pesticides and fit well in

integrated pest management program against larvae as well as pupae and adults of many economic insect pests which inhabit the soil.

Abbreviations

EPNs: Entomopathogenic nematodes; TAN5: *Trifolium alexandrinum* Nubaria; PGN6: *Psidium guajava* Nubaria; IJs: Infective juveniles; ANOVA: Analysis of variance; L.S.D.: Least significant difference

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