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Effect of temperature and soil moisture on the efficacy of indigenous and imported strains of the entomopathogenic nematode, *Heterorhabditis* sp. against the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera/Noctuidae)

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Abstract

Background: Biotic and abiotic factors influence survival, infectivity, development, reproduction, and activity of the entomopathogenic nematodes (EPNs). EPNs have been used to suppress the soil-inhabitant insects, which applied as a successful biological control agent against the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera/Noctuidae) larvae, and pupae.

Results: Studying the effect of different temperatures 20, 25, and 30°C ± 2, and the soil moisture content at 10, 15, and 25% on the susceptibility of two *Heterorhabditis* sp. strains, Egyptian strain (TAN5) and imported strain (Hb_{88}) against the *A. ipsilon* 4th and 6th larval instars and 3-day-old pupae was carried out under laboratory conditions. The tested concentrations of the two strains were 30, 60, 120, 240, and 480 IJs/cm^2 of soil surface. The mortality rate of 4th instar *A. ipsilon* larvae was high after *Heterorhabditis* strain TAN5 treatments at all concentrations, which ranged between (24 and 100%) and (6–100%) at 25°C and 30°C, respectively. At 20°C, *Heterorhabditis* strain Hb_{88} recorded higher mortality percentages for *A. ipsilon* 4th and 6th larval instars and pupae than the indigenous strain TAN5. The soil moisture content of 25% gave the highest mortality rates for the 4th instar larvae of *A. ipsilon* after treatments of the two strains. The Egyptian *Heterorhabditis* strain TAN5 was tolerant to the increase in temperature and more tolerant to the change in the water content of the soil than the imported strain Hb_{88} at all concentrations tested. In a semi-field experiment, *Heterorhabditis* strain TAN5 at concentrations between (1000–8000 IJs/cm^2 of soil surface) showed mortality rates (27–95%) for 4th instar *A. ipsilon* larvae and (19–81%) for the 3-day-old pupae, respectively.

Conclusions: *Heterorhabditis* strains TAN5 can was utilized against the black cutworm of *A. ipsilon* at the temperatures 25 and 30°C. *Heterorhabditis* strains TAN5 and Hb_{88} can be utilized against the black cutworm of *A. ipsilon* at the soil moisture content from 15 to 25%. In the semi-field experiment, *Heterorhabditis* TAN5 and Hb_{88} strains were effective against larvae and pupae of *A. ipsilon* at high concentrations of the nematodes.

Keywords: Entomopathogenic nematodes, *Heterorhabditis* sp., *Agrotis ipsilon*, Temperature, Soil moisture content

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Background

The black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera/Noctuidae), is an important pest that attacks several economically important crops in Egypt and worldwide (Sharaby and El-Nojiban 2015). *A. ipsilon* is difficult to combat due to larval hiding behavior during the day the insect remains buried in the ground, which obstructs their vision. Often used the chemical methods of control this pest (Devi 2020). *A. ipsilon* spends most of its survival in the soil where many microorganisms survive, including entomopathogenic nematodes (EPNs). EPNs are long utilized to control soil-inhabitant insects like cutworms and are effective biological control agents against *A. ipsilon* larvae (Yuksel and Ramazan 2018). EPNs are one of the most important biological control agents against many agricultural pests soil-inhabiting especially, lepidopteran larvae and pupae, because of their presence below ground (Vashisth et al. 2013). EPNs of *Steinernema* and *Heterorhabditis* with their associated symbiotic bacteria *Xenorhabdus* and *Photorhabdus*, respectively spread in soils (Grewal et al. 2005). When infected the host pest to nematodes, associated symbiotic bacteria are released from the nematode intestine and multiply rapidly in the host hemolymph, causing septicemia within 24–48 h. The nematodes feed upon the bacterial cells and the tissues of insects and mature, mate, and produce the new progeny of infective juveniles (IJs) emerge from the cadaver begin searching for new hosts (Gaugler 1988). EPNs have the search-ability of hosts and the possibility to survive in the soil. They have free-living infective juveniles (IJs) that can survive a long time without feeding (Koppenhöfer et al. 2000). The utilization of EPNs is prevailing in many countries and regions of the world and could be grown in vast numbers at comparatively low expenses (Mutegi et al. 2018). The survival, infectivity, development, and reproduction of EPNs are adversely affected when presented to unsuitable environmental conditions. Among the different environmental factors, temperature, moisture, and ultraviolet radiation play an important role in their effectiveness (Sharmila et al. 2018). Yuksel and Ramazan (2018) studied the effectiveness of two *Heterorhabditis* sp. against the fourth instar larvae of *A. ipsilon* at different concentrations under laboratory conditions. The most elevated mortality rate (100%) was attained within 2 days after inoculation. EPNs are potent and effective against *A. ipsilon*; therefore, they are used as biocontrol agents, environmentally safe instead of insecticides (Sobhy et al. 2020).

The objective of this study was to evaluate the effect of the biotic and abiotic factors (the temperature and the soil moisture content) of an Egyptian and imported strain of the EPN, *Heterorhabditis* sp. against the black cutworm, *A. ipsilon* under laboratory and semi-field conditions.

Methods

Target insect

Rearing of Agrotis ipsilon

Agrotis ipsilon larvae used in this study were collected from a vegetable field at Giza Governorate, Egypt, latitude 31.01° north and height above the sea level 30 m. 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, emerging adult moths were fed by on 20% honey solution till mating and laying eggs. All rearing procedures were carried out at 25 ± 1°C and 75 ± 5% RH (Zhang et al. 2019). The newly hatched larvae were transferred into small plastic jars and provided daily with castor bean leaves (*Ricinus communis L.*) as a source of food.

Rearing of *Galleria mellonella*

The greater wax moth, *G. mellonella* (L.), larvae were collected from injured hives and put in jars (2 kg capacity) until the appearance of moths and reared according to the method illustrated by Birah et al. (2008); the media used are (wheat (130 g), wheat bran (130 g), milk powder (130 g), maize flour (97.5 g), yeast powder (97.5 g), wax (26 g), honey (195 ml) and glycerol (195 ml). Related components with different rates were modified the used media later by Huang et al. (2010).

Entomopathogenic nematode strains

Heterorhabditis strains: *Heterorhabditis bacteriophora* Pionar (Hb88 strain) was obtained from Randy Gaugler, Rutgers University, New Brunswick NJ, USA, and *Heterorhabditis* strain TAN5 that isolated by Nouh (2021) was collected from the Noubaria El-Bhaira governorate, Egypt, at Egyptian clover (*Trifolium alexandrinum*). Mass culturing of both nematode strains occurred in vivo using larvae of *G. mellonella* as a host (Woodring and Kaya 1988).

Effect of temperature

Infection of the EPNs was carried out to the 4th and 6th larval instars and 3-day-old pupae of *A. ipsilon* in plastic cups (30 cm^3) half-filled with moistened sterile sandy soil (agricultural sandy soil were obtained from Nubarya, Beheira Governorate, Egypt (latitude 31.03° north and height above the sea level 9 m) and used in all the laboratory experiments after being sterilized) and then covered with plastic lids. A 100g of castor bean leaves (*R.*

communis) were offered for daily food. Cups were treated with each strain of *Heterorhabditis* at 5 concentrations 30, 60, 120, 240, and 480 IJs/cm^2 of soil surface. Ten larvae and 3-day-old pupae were used/cup/5 replicates/concentration. The experiments were carried out at three temperatures 20, 25, and 30 ± 2°C and 55–60 ± 2% RH. Water content in the soil was 20% of the soil weight.

Effect of soil moisture content

Infection of the EPNs on 4th and 6th larval instars and 3-day-old pupae of *A. ipsilon* was the same as the previous described method with some changes in soil moisture and temperature. The experiment was conducted at 25 ± 2°C and 55–60 ± 2% RH, and three different water contents in the soil were 10, 15, and 25% of the soil weight. Cups were treated by the two *Heterorhabditis* strains (TAN5 and Hb_{88}) at five concentrations of 30, 60, 120, 240, and 480 IJs/cm^2 of soil surface. Ten of larvae or 3-day-old pupae were used/ cup/ 5 replicates/ concentration, with 100-g castor bean leaves (*R. communis*) as a means of daily nutrition.

Semi-field experiments

This experiment was carried out in large plastic boxes filled with 1-kg moistened sterile sandy soil. Nematode strains used each strain placed individually at concentrations of 1000, 2000, 4000, and 8000 IJs/cm^2 where the soil surface was applied by (100 ml) water and mixed with the sterile sandy soil and placed inside boxes. In this experiment, 25 individuals of the 4th instar larvae or 3-day-old pupae were placed in a large plastic box (30 × 15 × 15 cm), covered with plastic lids with the castor leaves (*R. communis*) as a means of daily nutrition. Larvae and pupae were used/ plastic box/ 4 replicates/ concentration. The experiment was carried out at the temperature of 25°C ± 2 open air in November inside the biological control Department and water content in the soil at 20% and 55–60% ± 2 RH.

In all experiments, mortality rates of 4th and 6th larval instars or 3-day-old pupae of *A. ipsilon* were transferred after 4 days of treatment to White traps to make sure that they were infected with nematodes (White 1927). The third-stage juveniles (IJs) were harvested from the water surrounding White's trap within 10–14 days of emergence from their hosts. The control remediation was carried out utilizing distilled water.

Statistical analysis

Mortality rates were corrected according to Abbott's formula (1925). Mortality rates of *A. ipsilon* larvae and pupae by *Heterorhabditis* strains TAN5 and Hb88 were statistically analyzed by ANOVA one way. T test was calculated between the mortality percentage of treated larval and pupal *A. ipsilon* (Snedecor and Cochran 1980).

Results

Effect of temperature

Data in Table 1) showed mortality percentages of *A. ipsilon,* 4 days after applying different concentrations of *Heterorhabditis* strains TAN5 and Hb_{88} at different temperatures. *Heterorhabditis* strain TAN5 had higher mortality rate than *Heterorhabditis* strain Hb_{88} at 25 and 30°C at the tested concentrations. The percent mortality increased as the concentration of infective juveniles (IJs) increased. The mortality rate of *A. ipsilon* in 4th instar larvae was high with *Heterorhabditis* strain TAN5 treatments in all concentrations, where they ranged from 24 to 100% and from 6 to 100% at 25°C and 30°C, respectively. Mortality rate decreased from 6 to 80% at 20°C, while *Heterorhabditis* strain Hb_{88} at 20°C had higher mortality percentage of *A. ipsilon* in 4th and 6th larval instars and pupae than *Heterorhabditis* strain TAN5. The 4th instar larvae were more susceptible than the 6th ones and pupae at all temperatures. The highest mortality rate by the two strains was recorded at 25°C. Eventually, the biocontrol efficacy of the EPNs against insect pests is impacted greatly by temperature. Table 1 shows the effect of three temperatures on the two *Heterorhabditis* strains against *A. ipsilon* 4th and 6th larval instars and pupae. Average mortality of *Heterorhabditis* strains in larvae and pupae differed non-significantly between the different concentrations at the three tested degrees of temperature 20, 25, and 30°C ($F = 0.6114$ and 0.3525), ($F = 0.0907$ and 0.0732) and ($F = 0.2007$ and 0.1761), respectively.

Effect of moisture

Soil moisture is also an influential factor for nematode movement and survival but increase moisture in the soil may cause death to nematodes due to lack of oxygen. Table 2 shows mortality percentages of *A. ipsilon* 4 days after applying different concentrations of *Heterorhabditis* strains TAN5 and Hb_{88} at different moisture. *Heterorhabditis* strains TAN5 and Hb_{88} were affected by a loss of soil moisture when the water content of the soil to 10 and 15%, which led to a decrease in the mortality rates of *A. ipsilon* at the tested concentrations. The mortality rate by *Heterorhabditis* strain TAN5 ranged between 8 to 64% and 14 to 90%, at soil moisture 10 and 15%, respectively, while for *Heterorhabditis* strain Hb_{88} was between 6 to 58% and 12 to 90%, on the 4th instar *A. ipsilon* larvae at the same moisture contents, respectively. The soil moisture content of 25% gave the highest mortality rate at the 4th instar larvae of *A. ipsilon* with the two strains. *Heterorhabditis* strain TAN5 caused mortality rate from 20 to 100%, while *Heterorhabditis* strain Hb_{88} mortality

Table 1 Mortality percentages of *Agrotis ipsilon* (4th and 6th larval instars and 3-day-old pupae) at different concentrations of *Heterorhabditis* strains TAN5 and Hb_{88} at temperature 20, 25, and 30 ± 2°C and water content in the soil 20%

Con. IJs/cm^2 of soil surface	% Mortality of *Agrotis ipsilon*					
	Heterorhabditis strains TAN5			*Heterorhabditis* strains Hb_{88}		
	4th instar larvae	6th instar larvae	3-day-old pupae	4th instar larvae	6th instar larvae	3-day-old pupae
20 ± 2 °C						
30	6	4	2	8	6	4
60	24	20	14	26	24	20
120	48	36	26	56	38	34
240	62	54	38	68	54	50
480	80	68	52	84	72	64
Average	44	36.4	26.4	48.4	38.8	34.4
F-value*	0.6114			0.3525		
25 ± 2 °C						
30	24	20	16	18	16	14
60	44	40	32	38	34	30
120	62	60	54	60	56	52
240	80	80	74	78	76	70
480	100	100	94	100	96	90
Average	62	60	54	58.8	55.6	51.2
F-value*	0.0907			0.0732		
30 ± 2 °C						
30	16	14	10	14	12	10
60	36	34	28	34	32	26
120	58	52	46	56	50	44
240	82	78	64	76	72	62
480	100	96	82	100	92	80
Average	58.4	54.8	46	56	51.6	44.4
F-value*	0.2007			0.1761		

*Statistical analysis confirmed that the mortality rate between 4th, 6th larval instars and pupae for each of the treated EPN was non-significant

rate was from 18 to 100% at soil moisture 25%, respectively. Table 2 shows the effect of three soil moisture contents on two *Heterorhabditis* strains against *A. ipsilon* 4th and 6th larval instars and the pupae. The average mortality of *Heterorhabditis* strains in larvae and pupae differed non-significantly among the different concentrations at three soil moistures content 10, 15, and 25% ($F = 2.0687$ and 2.1778), ($F = 0.1268$ and 0.1888) and ($F = 0.1398$ and 0.2235), respectively.

Semi-field experiments

In the semi-field experiment, the respective mortality rates of 4th instar larvae and pupae of *A. ipsilon* were from 27 to 95%, and from 19 to 81%, respectively, when used the Egyptian *Heterorhabditis* strain TAN5. While when using the highest concentration of the imported *Heterorhabditis* strain Hb_{88}, mortality rates were 89 and 75% for larvae and pupae, respectively. The mortality rates in the lowest concentration were 21 and 13% in larvae and pupae, respectively. *Heterorhabditis* strain TAN5 and Hb_{88} were effective against larvae and pupae of *A. ipsilon*, especially in the highest concentrations of the two tested nematodes. Table 3 shows the mortality for the two *Heterorhabditis* strains against the 4th instar larvae and pupae of *A. ipsilon.* Non-significant differences among the mortality rates at different concentrations of the two strains were recorded between larvae and pupae.

Discussion

Morton and Garcia-del-pino (2009) and Sharmila et al. (2018) studied the optimum temperature for infection and reproduction of different nematode strains from *Heterorhabditis*. Infection with *Heterorhabditis* at temperatures under 15°C was not effective, while at temperatures between 15 to 35°C, its infectivity reached to the optimum and gave high mortality rates. The highest infection

Table 2 Mortality percentages of *Agrotis ipsilon* (4th and 6th larval instars and 3-day-old pupae) at different concentrations of *Heterorhabditis* strains TAN5 and Hb_{88} with three different water content in the soil 10%, 15%, and 25% at temperature 25 ± 2°C

Con IJs/cm^2 of soil surface	% Mortality of *Agrotis ipsilon*					
	Heterorhabditis strains TAN5			*Heterorhabditis* strains Hb_{88}		
	4th instar larvae	6th instar larvae	3-day-old pupae	4th instar larvae	6th instar larvae	3-day-old pupae
Soil moisture content 10%						
30	8	6	0	6	2	0
60	22	16	6	20	10	6
120	36	26	18	32	22	12
240	50	38	20	46	32	18
480	64	48	26	58	44	20
Average	36	26	14	32.4	22	11.2
F-value*	2.0687			2.1778		
Soil moisture content 15%						
30	14	10	6	12	8	6
60	30	28	24	32	28	22
120	52	50	42	50	46	40
240	70	68	60	70	66	56
480	90	88	78	90	80	74
Average	51.2	48.8	42	50.8	45.6	39.6
F-value*	0.1268			0.1888		
Soil moisture content 25%						
30	20	16	14	18	14	12
60	42	38	32	36	34	28
120	60	56	50	58	52	46
240	78	74	68	80	74	62
480	100	94	86	100	94	80
Average	60	55.6	50	58.4	53.6	45.6
F-value*	0.1398			0.2235		

*Statistical analysis confirmed that the mortality rate between 4th, 6th larval instars and pupae for each of the treated EPN was non-significant

Table 3 Mortality percentages of *Agrotis ipsilon* (4th instar larvae and 3-day-old pupae) at different concentrations of *Heterorhabditis* strains TAN5 and Hb_{88} in the semi-field application at 25°C and water content in the soil 20%

Con IJs/cm^2 of soil surface	% Mortality of *Agrotis ipsilon* 4th instar larvae			% Mortality of *A. ipsilon* 3-day-old pupae		
	Egyptian *Heterorhabditis* strain TAN5	Imported *Heterorhabditis* strain Hb_{88}	*T* test	Egyptian *Heterorhabditis* strain TAN5	Imported *Heterorhabditis* strain Hb_{88}	*T* test
1000	27	21	N.S. (0.3264)	19	13	N.S. (0.34557)
2000	50	43		41	33	
4000	73	65		60	54	
8000	95	89		81	75	
Average	61.25	54.5		50.25	43.75	

and pest mortality rates by *Heterorhabditidae* sp. were recorded at 20°C. The effects of some *Heterorhabditidae* species showed at the optimum temperature of 25°C. Hassan et al. (2016) studied *H. bacteriophora* Poinar (Hb_{88} strain) against the 4th and 6th larval instars of *A. ipsilon* at 25°C, which indicated that the 4th instar larvae were the most infected instars than the 6th instar. After 4 days of infection, mortality rates ranged from

35.71 to 78.57% for the 4th instar larvae of *A. ipsilon,* while ranged from 6.67 to 53.33% for the 6th instar larvae. These results were consistent with the obtained ones where the 4th instar larvae was more sensitive to nematode infection than the 6th instar and contradict with mortality percentages of results in the present study. Also, in agreement with the present findings, Shapiro-Ilan et al. (2009) reported that the optimum temperature was from 25 to 30°C, which recorded high mortality rates. Sharmila et al. (2017) studied the activity of EPNs against *A. ipsilon* larvae in laboratory conditions. It was found that larvae were highly susceptible to the nematode, *Heterorhabditis* sp. and the percentage mortality increased with the increase in nematode concentrations. Yuksel and Ramazan (2018) studied the efficacy of local EPN isolates against the 4th instar larvae of *A. ipsilon* under laboratory conditions at 25 ± 1°C. The mortality rates increased with an increase in the tested concentrations. The highest mortality rate reached (100%) when treated with local *Heterorhabditis bacteriophora.* The results showed that all local EPN isolates can be used successfully in controlling *A. ipsilon* larvae. This research was concordant with the results under study, where the mortality rate increased by increasing the concentration. The local strain was more effective than the imported strain, and the mortality rate reached 100% at the highest concentration. Sobhy et al. (2020) evaluated the efficacy of the EPNs, *Heterorhabditis bacteriophora* against the larval stage of the black cutworm, *A. ipsilon* under laboratory conditions at 25 ± 2°C. The data indicated that 200 IJs/dish was the most effective concentration against the tested larval instars from the 2nd to 6th instars of *A. ipsilon* after 72 h because the mortality percentage was 100%, while in the current study, the highest mortality rate of 100% was obtained at the highest concentration of 320 IJs/cup against the 4th and 6th larval instars of the cutworm after 4 days of infection.

Alekseev et al. (2006) studied the effect of soil moisture on the movement and persistence of EPNs. While natural habitats of nematodes are within the soil, many agricultural pests spending their life or part of their life cycle in the soil as well. Therefore, consideration of the effect of the soil moisture on nematode activity is a prerequisite for the successful use of EPNs. Rohde et al. (2010) and Sharmila et al. (2018) observed that soil moisture was also an important factor for nematode mobility and survival, where EPNs require an adequate soil moisture for survival and movement. *Heterorhabditis* sp. has a low potential of being alive in case of desiccation. Soil moisture levels varying between 10 and 30% had a significant effect on nematode survival. That is consistent with the present study, where the best mortality rates were at 20–25% soil moisture. Chandel et al. (2021) studied the effect of EPNs such as *H. bacteriophora* to control cutworms, those are hidden in the soil during the day, and the effectiveness of nematodes was related to the soil moisture. It was found that these findings agree the current study where the soil moisture is an important factor for the nematode movement, infection and reproduction.

Conclusions

It was concluded that the Egyptian *Heterorhabditis* strain TAN5 was tolerant to temperature changes, especially the increase in temperature and more tolerant to the change in water content of the soil than the imported *Heterorhabditis* strain Hb_{88}. In the semi-field experiment, *Heterorhabditis* strain TAN5 and Hb_{88} were effective against larvae and pupae of *A. ipsilon*, especially in the high concentrations of the two tested nematodes. Eventually, the efficacy of the EPNs against insect pests is impacted greatly by temperature and soil moisture. The two strains can be efficiently used in the biological control of the black cutworm, *A. ipsilon*, and it can be included in the integrated control programs.

Abbreviations

EPNs: Entomopathogenic nematodes; IJs: Infective juveniles; TAN5: *Trifolium alexandrinum* Nubaria.

Authors' contributions

Author conceived the research. Author conducted laboratory experiments and conducted Semi-Field Experiments. Author contributed provided material. Author analyzed data and conducted statistical analyses. Author wrote the manuscript. Author provided funding. The author read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are openly available.

Declarations

Ethics approval and consent to participate

I agree to remain by the ethics of publishing in the journal and do all the experiments and review the research.

Consent for publication

I agree to publish the research in the journal Egyptian Journal of Biological Pest Control.

Competing interests

The author declares that I have no conflict of interest.

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