https://dx.doi.org/10.4314/ejppri.v7i2.9

Egypt. J. Plant Prot. Res. Inst. (2024), 7 (2): 255–262

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Physiological effects of entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* on the adult grasshopper *Heteracris littoralis* (Acrididae: Orthoptera)

Salem, M. M. Hagar; Ayat, I. El-Kholy and Anwaar, M. Abaza

Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt.

ARTICLE INFO

Article History

Received: 22/4/2024

Accepted: 25/6/2024

Keywords

Heteracris littoralis, biological control, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora* and physiological characteristics.

Abstract

A current study was carried out to evaluate one of the important biological control methods in the management of grasshoppers, *Heteracris littoralis* (Rambur) (Acrididae: Orthoptera) which infesting many different plants through artificial infestation by two entomopathogenic nematodes (EPN); *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* (HP88) (Rhabditida: Heterorhabditidae) in the physiological laboratory, and noticing its physiological effects on the physiological characteristics of grass hopper *H. littoralis* adults. Whereas current laboratory experiment was carried out through four doses of two tested entomopathogenic nematodes species; *S. carpocapsae* and *H. bacteriophora*; 200, 300, 400, and 500 IJs/100 ml.H2O at 25°C and 60% humidity for 72 hrs. Results obtained indicated that as general effectiveness of *H. bacteriophora* was higher than the other *S. carpocapsae* on the physiological characteristics of the successive grasshopper *H. littoralis*. Also, the higher doses of both of the EPNs were more effective on these physiological characteristics than the lower doses. Physiological characteristics of grasshopper *H. littoralis* were examined stimulated in the most internal substances secreted by that insect; Total proteins, carbohydrates, total lipids, and important enzymes (Chitinase, lipase, phosphatase, kinase, alpha esterase, beta esterase, oxidation enzymes and digestive enzymes).

Introduction

Grasshopper *Heteracris littoralis* (Rambur) (Acrididae: Orthoptera) is one species of short-horned grasshopper in the family Acrididae, it is found in many areas such as Africa, Southern Europe and Asia and it is one of the most serious pests infesting different crops in north of America, Long *et al.* (2019) who also indicated that Grasshoppers *H. littoralis* were among the most dangerous agricultural pests which cause serious damages to many crops. Also, Lu *et al.* (2022) in China indicated that grasshoppers such as *H.*

littoralis are among the most dangerous agricultural pests of China however the monitoring prediction and control of grasshoppers are complex and difficult.

Steinernema carpocapsae (Weiser) (Rhabditida: Steinernematidae) is one of the most important entomopathogenic nematodes which is used in the integrated pest control, Nobuyoshi (1993) who indicated that the combinations of chemical or biological agents with steinernematid nematodes can increase the nematodes efficacy against insect pests, whereas author added that in particular field application of *S. carpocapsae* with a given chemical pesticide (Oxamyl, fenithrothion, diazinon, acephate, or permethion) yielded better results for control of soil and foliage insect pests than an application of either the nematode or pesticide alone.

Heterorhabditis bacteriophora (HP88) (Rhabditida: Heterorhabditidae) are also considered one of the most important entomopathogenic nematode which are used mutually with the enteric bacterium, *Photorhabdus luminescens* globally for the biological control operations of different insects, Todd (2007) who added also that EPN has dealt with applied aspects related to the biological control. Also, Kumar *et al.* (2012) studied biology of entomopathogenic nematodes *Heterorhabditis* sp. and *Steinernema* spp. and indicated that nematodes associated with insects are referred to as entomophagous nematodes and indicated that these entomopathogenic nematodes are highly potential biocontrol agents for several lepidopteran and coleopteran insect pests.

Current study was carried out to evaluate one of the important biological control methods on the management of grasshopper, *H. littoralis* which infesting many different plants by two entomopathogenic nematodes, *S. carpocapsaeb* and *H. bacteriophora* on the physiological characteristics of grasshopper *H. littoralis* adults.

Materials and methods

1. Locust rearing:

H. littoralis adult grasshoppers (♂ and ♀) were reared in the physiology lab using samples of field-grown lucerne from the Egyptian village of Abu Rawash. In hardwood cages of 40 by 40 by 30 centimeters, with a density of 30 to 60 adults per cage, insects were raised for almost three generations during their gregarious phase. Eggs hatch within 26-30 days in summer and a little longer time in winter. Grasshoppers have 5 instars five days each and 8 days in winter. The lifetime of grasshoppers ranges from 2.5 months in summer to 5 months in winter. A thread of sand in each cage was used by the locusts to lay their eggs. Cages were cleaned daily. The grasshopper was given fresh leaves of Lucerne.

2. Entomopathogenic nematode species:

Two EPNs were reared and used in the experiments S. *carpocapsae* and *H. bacteriophora*, entomopathogenic nematode were reared in the Plant Protection Research Institute, Agricultural Research Centre for many generations. The two EPNs' infectious juveniles (IJs) were kept in the bigger wax moth Galleria mellonella's final instar larvae.

3. Bioassay test:

Studies were carried out against adult *H. littoralis* using doses of 200, 300, 400, and 500 IJs/100 ml. H2O for two tested EPN species at 25°C and 60% humidity for 72 hrs. at the Department of Pest Physiology, Plant Protection Institute, Agricultural Research Center, Egypt : In aerated plastic jars measuring 8 by 10 by 10 centimeters, 100 g of air-dried, sterilized sandy soil (60% sand, 20% clay, and 20% silt) was mixed with the previously indicated quantities of two applied EPNs. By adding distilled water, the soil's moisture content was maintained at 10% (v/w). The locusts were given freshly cleaned Lucerne leaves. Three adult/aerated plastic jars were used to expose the IJs of each nematode species. To find the fatality rate, at 72 hrs. check was conducted. For the tests, a total of two nematode species, four concentrations, and one species of grasshopper, were used. Water was the only thing that kept the adults in check. With the two nematode species, the test was conducted three times five replicates each.

4. Statistical analysis:

SPSS was used to analyses the lethal activity of EPN species in order to calculate the LC_{50}, lower bound, and upper bound (95% confidence limits). When examining the impact of EPNs on biochemical analyses and the reproduction rate of the tested EPN species, $P < 0.05$ indicated a significant difference between the groups (SAS Institute, 1988).

Results and discussion

A current study was carried out to evaluate one of the important biological control methods in the management of the serious grasshopper, *H. littoralis* which infests many different plants, through artificial infestation by two entomopathogenic nematodes, *S. carpocapsae* and *H. bacteriophora* in the physiological laboratory. Results obtained show that the LC_{50} values for the successive grasshopper *H. littoralis* adults when treated by the first *S. carpocapsae* delivered via the nematode-inoculated sand approach were (251.7 IJs/100 ml. H2O) according to that data tabulated at Table (1). Whereas the LC_{50} values for the other EPN treatment with *H. bacteriophora* utilizing the nematode-inoculated sand method was (348.9IJs/100 ml.H2O) according to that data also tabulated at Table (1).

Data obtained and tabulated in Table (1) also show that *S. carpocapsae* was more effective than *H. bacteriophora* on the death percentage % of the successive grasshopper *H. littoralis*. Statically analysis shows that there were significant differences between the death percentage % of the successive grasshopper *H. littoralis* treated by *H. bacteriophora* and *S. carpocapsae* compared to control (non-treated grasshopper).

Table (1): Lethal activity of two entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* against grasshopper *Heteracris littoralis* adults by nematode-inoculated Sand application at 25°C.

EPNs	LC50 (Ijs/100 ml. H2O)	95% Confidence limit (Ijs/100 ml. H2O) LB UB	Slope±S.E	Chi-square ($\chi 2$)
Steinernema carpocapsae	251.7	351 811	5.5±0.03	0.4
Heterorhabditis bacteriophora	348.9	348.9 1511.8	5.1±0.007	0.7

LC_{50}: lethal concentration brings out 50% mortality (LB, UB): Lower Bou

Effectiveness of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* on the physiological characteristics of grasshopper *Heteracris littoralis*:

Experiments were carried out to study the effectiveness of two entomopathogenic nematodes, *S. carpocapsae* and *H. bacteriophora* on the physiological characteristics of grasshopper *H. littoralis* utilizing four doses of the two tested EPN species; 200, 300, 400, and 500 IJs/100 ml. H2O Experiments were carried out at 25°C and 60% humidity for 72 hrs. three times 5 replicates each at the Department of Pest Physiology, Plant Protection Research Institute, Agricultural Research Center, Egypt. Physiological characteristics of grasshopper *H. littoralis* were examined; total proteins, carbohydrates, total lipids and important enzymes (Chitinase, lipase, phosphatase, kinase, alpha esterase, beta esterase, oxidation enzymes and digestive enzymes).

Data obtained and tabulated in Tables (2 and 3) show concentrations of the important internal substances secreted by grasshopper *H. littoralis* adults; total proteins, carbohydrates, total lipids, and important enzymes (Chitinase, lipase, phosphatase, kinase, alpha esterase, beta esterase, oxidation enzymes and digestive enzymes) in the grasshopper adults which were treated by both *S. carpocapsae* and *H. bacteriophora* compared to control (Untreated grasshopper).

Data obtained and tabulated in Tables (2 and 3) show that the effectiveness of treatment of grasshopper *H. littoralis* adults with both *S. carpocapsae* and *H. bacteriophora* on the important internal substances secreted by that grasshopper were arranged in descending order as follows; 500, 400, 300, 200, 100 IJs /100 ml water respectively. Whereas treatment of the successive grasshopper *H. littoralis* by both entomopathogenic nematodes, *S. carpocapsae* and *H. bacteriophora* with concentration of 200 ml./H2O was slightly effective on the concentrations of the successive internal substances secreted by that grasshopper compared to control, and there were non-significant differences between these concentrations on the grasshoppers when treated by the two EPNs compared to control. While treatment by the two EPNs with the concentration of 300 IJs/ 100 ml H2O had more effect on the concentration of the successive internal components secreted by grasshopper than 200 IJs / 100 ml H2O concentration compared to control.

On treatment of the successive grasshopper *H. littoralis* by both of the entomopathogenic nematodes with concentration 400 IJs/100ml H2O there was a higher effect on the concentrations of the successive internal components secreted by that grasshopper compared to control compared to the concentration of 300 IJs/100ml H2O. There were significant differences between most of these concentrations on the grasshoppers treated by the two EPNs compared to the control and non-significant differences on a few of these substances secreted by that grasshopper.

Finally, on treatment of the successive grasshopper *H. littoralis* by both of the entomopathogenic nematodes with a concentration 500 IJs/100ml H2O there was a higher effect on the concentrations of the successive internal components secreted by that grasshopper

compared to the control than the 400IJs/100mlH2O concentration, and there were significant differences between all of these concentrations on the grasshoppers treated by the two EPNs compared to control.

These results agree with those obtained by Jessica *et al.* (2012) in Hawaii who studied the effectiveness of *S. carpocapsae* against *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae) in the physiological laboratory and indicated the serious effect of the entomopathogenic nematodes *S. carpocapsae* on many physiological processes in that insect. Jose *et al.* (2016) in Argentina studied nematodes (Mermithidae) parasitizing on grasshoppers and indicated that nematodes cause serious physiological effects on grasshoppers.

Also, Wahid *et al.* (2020) in America studied the control of pest grasshoppers in North America and indicated that grasshopper population outbreaks occur frequently and cause serious damage to different crops and authors also indicated that the main method of controlling grasshopper outbreaks with chemical insecticides but this way had negative effects on humans and the environment so authors indicated that biological control organisms such as treatment by entomopathogenic nematodes *S. carpocapsae* was more specific to pest grasshoppers and less environmentally hazardous alternative to traditional insecticides. Halloran and Burnell (2003) tested insect responses to insect parasitic nematode *H. bacteriophora* and indicated that entomopathogenic nematodes have serious effects on many physiological processes in the examined insects. Alper and Ehlers (2008) studied field persistence of the entomopathogenic nematode *H. bacteriophora* in different crops and indicated that EPN causes many serious effects on some physiological characteristics in the tested insects. Yavuz *et al.* (2018) studied new applications for entomopathogenic nematodes *H. bacteriophora* against grasshopper *Locusta migratoria* and indicated that EPN is being used as biocontrol agents against many soil-borne insect pests in agriculture.

A current study was carried out to evaluate one of the important biological control methods in the management of grasshopper, *H.littoralis* through artificial infestation by both of entomopathogenic nematodes, S. *carpocapsae* and *H. bacteriophora* in the physiological laboratory. The results obtained indicated that as general effectiveness of *H. bacteriophora* was higher than the other *S. carpocapsae* on the physiological characteristics of the successive grasshopper *H. littoralis*. Also, the higher doses of both of the EPNs were more effective on these physiological characteristics than the lower doses. Physiological characteristics of grasshopper *H. littoralis* were examined; total proteins, carbohydrates, total lipids and important enzymes (Chitinase, lipase, phosphatase, kinase, alpha esterase, beta esterase, oxidation enzymes and digestive enzymes).

Table (2): Concentrations of the internal contents secreted by grasshopper *Heteracris littoralis* adults on treatment by both *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* (200, 300 IJs/100ml) compared to control.

Adjectives mg/100g	Control	200 IJs/100mlH2O				300 IJs/ 100mlH2O			
		H.	S.	F(0.05)	LSD	H.	S.	F(0.05)	LSD
Total proteins	27.25^{a}	27.0^{a}	26.15^{b}	1.05*	9.78	26.11^{b}	25.75^{c}	1.03**	8.99
Carbohydrates	10.72^{a}	10.3^{a}	9.44^{b}	1.03*	7.35	9.85^{b}	9.0^{c}	1.04**	7.23
Total Lipids	15.33^{ns}	15.14^{ns}	15.0^{ns}	ns	ns	15.0^{a}	14.25^{a}	ns	Ns
Amino acids	12.25^{a}	12.0^{a}	11.45^{b}	1.05*	8.55	11.32^{b}	10.75^{c}	1.03**	9.41
Total phenols	17.33^{ns}	17.12^{ns}	17.0^{ns}	ns	ns	16.83^{b}	16.0^{b}	1.07*	7.22
Tannins	8.25^{ns}	8.0^{ns}	7.25^{ns}	ns	ns	7.33^{b}	7.0^{b}	1.02**	8.34
Flavonoids	10.42^{ns}	10.23^{ns}	10.0^{ns}	ns	ns	9.85^{b}	8.77^{c}	1.08**	9.76
Chitinase Enzyme	16.85^{a}	16.11^{a}	15.75^{b}	ns	ns	16.0^{a}	15.11^{a}	ns	Ns
Lipase Enzyme	29.75^{ns}	29.20^{ns}	29.0^{ns}	ns	ns	28.85^{b}	28.0^{b}	1.05*	8.41
Phosphatase Enzyme	25.11^{a}	24.33^{a}	24.0^{b}	1.03*	9.11	24.11^{b}	23.25^{c}	1.03**	10.31
Kinase Enzyme	19.75^{ns}	19.11^{ns}	19.0^{ns}	ns	ns	18.25^{b}	18.0^{b}	1.05*	8.71
Alpha Esterase	15.22^{a}	14.61^{b}	14.0^{b}	1.02*	8.56	14.25^{a}	13.85^{b}	1.07*	7.33
Beta Esterase	13.71^{ns}	13.22^{ns}	13.0^{ns}	ns	ns	12.85^{a}	12.0^{a}	ns	Ns
Oxidation enzymes	9.25^{a}	9.0^{a}	8.15^{b}	1.04*	10.35	9.0^{a}	8.0^{b}	1.01*	9.21
Digestive enzymes	8.73^{ns}	8.25^{ns}	8.0^{ns}	ns	ns	7.95^{a}	7.0^{a}	ns	Ns

Means within columns bearing different subscripts are significantly different (P> 0.05)
(ns) non significant - (*) significant -(**) significant
(***) high significant (S.) *S. carpocapsae* (H.) *H. bacteriophora*

Table (3): Concentrations of the internal contents secreted by grasshopper *Heteracris littoralis* adults on treatment by *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* with concentrations (400, 500 IJs/ 100mlH2O) compared to control.

Adjectives mg/100g	Control	400 IJs/ 100mlH2O				500 IJs/ 100mlH2O			
		H.	S.	F(0.05)	LSD	H.	S.	F(0.05)	LSD
Total proteins	27.25 ^{a}	26.0^{b}	25.15^{c}	1.08**	10.22	25.11 ^{b}	24.0 ^{c}	1.21***	9.45
Carbohydrates	10.72 ^{a}	9.11^{b}	8.76^{c}	1.09***	8.75	9.0 ^{b}	8.0 ^{c}	1.34***	10.21
Total Lipids	15.33^{a}	14.22^{b}	14.0^{b}	1.03*	6.21	14.0^{b}	12.33^{c}	1.08**	8.77
Amino acids	12.25 ^{a}	11.0^{b}	10.22^{c}	1.08***	9.85	10.25 ^{b}	9.75 ^{c}	1.73***	11.25
Total phenols	17.33^{a}	16.11^{b}	15.21^{c}	1.06**	11.34	16.0 ^{b}	14.23^{c}	1.25***	7.52
Tannins	8.25^{a}	7.11^{b}	6.25^{c}	1.02***	10.21	7.0 ^{b}	5.52^{c}	1.65***	8.95
Flavonoids	10.42^{a}	9.13^{b}	8.54^{c}	1.05**	8.74	9.0 ^{b}	8.0^{c}	1.09**	11.26
Chitinase Enzyme	16.85 ^{a}	15.99 ^{a}	15.0^{a}	ns	ns	15.0^{b}	14.25^{b}	1.07*	7.32
Lipase Enzyme	29.75^{a}	28.44^{b}	27.95^{b}	1.02*	9.45	28.12 ^{b}	27.41 ^{b}	1.06*	9.41
Phosphatase Enzyme	25.11 ^{a}	24.0^{b}	23.0^{c}	1.07**	9.11	23.11 ^{b}	22.75 ^{c}	1.09**	8.91
Kinase Enzyme	19.75^{a}	18.0^{b}	16.75^{c}	1.12**	10.23	17.55 ^{b}	16.0^{c}	1.21**	10.73
Alpha Esterase	15.22 ^{a}	14.11 ^{b}	13.12^{c}	1.09**	8.56	14.0^{b}	12.85^{c}	1.34**	9.27
Beta Esterase	13.71^{a}	12.77^{a}	11.97^{b}	1.02*	7.23	11.75^{b}	10.3^{c}	1.09**	10.33
Oxidation enzymes	9.25 ^{a}	8.99^{b}	7.95 ^{b}	1.08*	10.35	8.11^{b}	6.63^{c}	1.25***	11.45
Digestive enzymes	8.73 ^{ns}	7.93 ^{ns}	7.0 ^{ns}	ns	ns	7.0^{b}	6.45^{b}	1.03*	7.35

Means within columns bearing different subscripts are significantly different (P> 0.05)
(ns) non significant - (*) significant -(**) significant
(***) high significant (S.) *S. carpocapsae* (H.) *H. bacteriophora*

References

Alper, S. and Ehlers, R. (2008): Field persistence of the entomopathogenic nematode *Heterorhabditis bacteriophora* in different crops. Bio Control, 53 (2): 627-641.

Halloran, D. and Burnell, A. (2003): An investigation of chemotaxis in the insect parasitic nematode *Heterorhabditis bacteriophora*. Parasitology, 127 (4): 375-385.

Jessica, L.; Robert, G. and Roxana, Y. (2012): Potential of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) against *Hypothenemus hampei* (Coleoptera: Curculionidae) in Hawaii. Florida Entomologist, 95 (4): 1194-1197.

Jose, M.; Nora, B. and Maria, F. (2016): Nematodes (Mermithidae) parasitizing grasshoppers (Orthoptera: Acarididae) in the Pampean region, Argentina. Brazilian Journal of Biology, 77 (3): 12-15.

Kumar, S.; Muthulak, M. and Subrama, S. (2012): Biology of entomo pathogenic nematodes *Heterorhabditis sp.* and *Steinernema spp.* Journal of Biopesticides, 5(2): 60-67.

Long, Z.; Michel, L.; Alexandre, L. and David, H. (2019): Locust and grasshopper management. Annual review of entomology, 64 (3); 15-34.

Lu, L.; Kong, W. and Sun, H. (2022): Detecting key factors of grasshoppers occurrence in typical steppe and meadow steppe by integrating machine learning model and remote sensing data. Insects, 13(10): 880–894.

Nobuyoshi, I. (1993): Integrated control of insect pests by *Steinernema carpocapsae*. Nematodes and biological control of insect pests, 105-113.

SAS Institute (1988): SAS/STAT User Guide, Ver. 6. 03. SAS Institute Inc., Cary, North Carolina.

Todd, C. (2007): The biology and genome of *Heterorhabditis bacteriophora*. Worm Book, The Online Review of C. elegans Biology.

Wahid, H.; Stefan, T. and Scott, S. (2020): Control of pest grasshoppers in North America. Insecta, 11(9): 566- 569.

Yavuz, S.; Ahcen, B.; Tufan, C. and Alper, S. (2018): New application method for entomopathogenic nematode *Heterorhabditis bacteriophora* (Poinar, 1967) (Rhbditida: Heterorhabditidae) HBH strain against *locusta migratoria* (Linnaeus). Turkish Journal of Entomology, 42(4): 305-312.