

Research Article

Bio control Efficacy of Entomopathogenic Nematodes against Black Cutworms, *Agrotis ipsilon* (Hufnagel) (Noctuidae: Lepidoptera) in Potato

Sharmila Radhakrishnan^{1*}, Subramanian Shanmugam¹ and Rajesh Ramasamy²

¹Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu

²Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu

Abstract

The efficacy of entomopathogenic nematodes (EPN) against larvae of *Agrotis ipsilon* (Hufnagel) (Noctuidae: Lepidoptera) in potato was evaluated under laboratory and glasshouse conditions. Under laboratory and glass house conditions, larvae were highly susceptible to the two nematode species, *Heterorhabditis indica* and *Steinernema glaseri* (Nematoda: Rhabditidae) when used separately and the percentage mortality increased with increase the dose of nematodes. In laboratory studies, median lethal concentration and median lethal time of *S. glaseri* registered lowest LC₅₀ of 18.03 IJ/larva and LT₅₀ of 23.98 h/larva and for *H. indica* which recorded highest LC₅₀ 16.39 and LT₅₀ of 28.69 IJ /larva for *A. ipsilon*. Median lethal concentration and time of *S. glaseri* was less on *A. ipsilon* than *H. indica* under laboratory conditions. In potato, *S. glaseri* was effective on *A. ipsilon* under glass house conditions. *S. glaseri* @ 5×10⁹ IJ/ha caused 83.33 per cent larval mortality with 23.80 per cent tuber damage compared to 71.42 per cent tuber damage in control.

Keywords: Entomopathogenic nematodes, *H. indica*, *S. glaseri*, *A. ipsilon*, larval morality, tuber damage.

*Correspondence

Author: Sharmila Radhakrishnan
Email: sharminema@gmail.com

Introduction

Potato (*Solanum tuberosum* L.) is the fourth most important food crop in the world to play a major role in the agricultural economy of India. It is attacked and damaged by a number of insect pests including wireworms, white grub, aphids, cutworm and others as a result, the crop is adversely affected. Potato Cutworm and peach aphid are the two devastating insect pests in the spring crop [1]. Cutworms are polyphagous pests, Larvae of cutworms can damage 30 cultivated and 20 wild species of plants but the greatest damage was observed on potato and vegetable crops.

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* with their associated symbiotic bacteria (*Xenorhabdus* and *Photorhabdus*, respectively) are widely distributed in soils throughout the world. These nematode parasites of insects, killing them within 48h with the aid of their associated bacterial symbionts, and have a great potential for biological control of many insect pests. Continuous application of synthetic chemical insecticides resulted in numerous problematic situations including development of insect resistance, food hazard, ground water contamination and destruction of natural enemies. These disadvantages serve as an impetus for the development of alternate insect control measures. Particular attention, in recent years, has been focused on biological control agents, including certain entomopathogenic nematodes. These nematodes can be used as biological control agents to suppress a various number of economically important insect pests [2]. Soil is the substratum for these nematodes and hence application in soil results in successful control of various soil pests including cutworms [3].

The potato cutworm cause considerable damage to potato crop in hilly and sub-mountainous regions of Ooty. This paper presents the results of laboratory and glass house trials conducted on the efficacy of entomopathogenic nematodes against *A. ipsilon*.

Materials and Methods

White grub

The third and fourth instar larvae of *Agrotis ipsilon* were collected from infested potato fields at Horticultural Research Station, Woodhouse farm, Udthagamandalam. Larvae were used for laboratory and pot culture studies.

Nematodes

The nematodes viz., *H. indica* and *S. glaseri* were obtained from Sugarcane Breeding Institute, Coimbatore and mass cultured in *Corcyra cephalonica*. The insect larvae were reared on broken cumbu grains sterilized at 100°C for 30 minutes [4]. The third stage juveniles (IJs) were harvested from water surrounding White's trap within 10 days of emergence from their hosts. A stock suspension of the IJs in distilled water was stored at 20°C for 2 weeks before use in BOD incubator.

Laboratory conditions

Heterorhabditis indica and *S. glaseri* were used for testing virulence against *A. ipsilon*. Dose and time mortality relationship tests were conducted in 9 cm diameter Petri dishes lined at the bottom with a Whatman No. 1 filter paper and moistened with 1ml sterile distilled water. Infective juveniles were evenly applied over the filter paper. The dosages used were 0, 50, 100, 150, 200, 250 and 300 infective juveniles per larva, with 10 larvae of *A. ipsilon* per insect per replicate and four replicates for each level.

Glasshouse conditions

Two pot culture experiments were conducted for testing the bioefficacy of entomopathogenic nematodes against larvae of *A. ipsilon* on potato under glasshouse conditions at Horticultural Research Station, Udthagamandalam. Potato tubers (var: Kufri Jyoti) were surface sterilized and washed in water. They were planted in earthen pots of 5 kg capacity and two tubers per pot were sown. After germination and establishment of the seedling to inoculate *A. ipsilon* larvae collected in the potato field were inoculated and starved for one week to increase host adaptation suitability of larvae. The nematode treatments were given as *H. indica* @ 1.25, 2.5 and 5×10^9 IJ/ha and *S. glaseri* @ 1.25, 2.5 and 5×10^9 IJ/ha.

The treatments were replicated thrice in a Completely Randomized Design (CRD). The nematodes were inoculated in soil for each treatment. Insect mortality counts were taken at every 24 h upto 96 h after application. The number of dead larvae were counted and confirmed for the presence of nematodes inside the cadavers. Damaged tubers due to the larvae were also recorded in all the treatments.

Statistical analysis

The observations recorded were statistically analysed for the experiments. Means of all experiments were used to compare the efficacy of treatments. Per cent insect mortality data were analysed by multifactor ANOVA followed by Duncan's multiple range test ($P > 0.05$) for separation of means.

Results

Laboratory experiment

The virulence of two species of EPNs, viz., *H. indica* and *S. glaseri* were tested against larvae of *A. ipsilon* under laboratory conditions. The nematode species *H. indica* caused 50 per cent larval mortality of *A. ipsilon* after 28.69 h/ larva and 16.39 IJ/larva respectively, which consumed more time and dose for causing maximum mortality period **Figure 1**. *Steinernema glaseri* was more virulent to larvae of *A. ipsilon*. The lowest LC_{50} of 18.03 IJ/larva and LT_{50} of 23.98 h/larva were observed for *A. ipsilon* **Tables 1** and **2** respectively.



Figure 1 Virulence of entomopathogenic nematodes against certain insect pest

Table 1 LC₅₀ Values calculated from dosage response assays conducted with different nematodes species and last instar larvae of *A. ipsilon*

Nematode species	Incubation period (h)	LC ₅₀	Fiducial limit (95 %)	
			UL	LL
<i>H. indica</i>	24	6.81	4.98	9.32
	48	6.92	5.05	9.47
	72	16.39	12.80	20.98
	96	29.77	22.39	39.58
<i>S. glaseri</i>	24	8.45	6.05	11.81
	48	9.01	6.18	11.91
	72	18.03	12.70	25.91
	96	21.48	15.62	29.54

Table 2 LT₅₀ Values calculated from dosage response assays conducted with different nematodes species and last instar larvae of *A. ipsilon*

Nematode species	Incubation period (h)	LT ₅₀	Fiducial limit (95 %)	
			LL	UL
<i>H. indica</i>	24	23.42	19.98	27.44
	48	23.42	20.57	27.83
	72	28.69	25.46	32.33
	96	38.66	33.66	43.31
<i>S. glaseri</i>	24	16.27	13.51	19.60
	48	17.06	14.17	20.55
	72	23.98	20.61	27.90
	96	36.93	32.61	41.83

Pot culture experiment

The aim of this study was to evaluate the efficacy of *H. indica* and *S. glaseri* against larvae of *A. ipsilon*, a pest of potato. A pot culture experiment was conducted under glass house condition to test the efficacy of entomopathogenic nematodes against *A. ipsilon* on potato **Figure 2**.



Figure 2 Efficacy of entomopathogenic nematodes against *A. ipsilon*

Entomopathogenic nematodes were found to control *A. ipsilon* effectively on potato at all dosage levels tested. A positive correlation existed between larval mortality, dosage levels and exposure time. The highest larval mortality was observed at 83.33 per cent after 72h for *S. glaseri* @ 5×10^9 IJ/ha. *S. glaseri* @ 2.5×10^9 IJ/ha and *H. indica* @ 5×10^9 IJ/ha were on par with each other with larval mortalities of 75.00 per cent and 66.66 per cent respectively. The least mortality was observed with *H. indica* @ 1.25×10^9 IJ/ha (38.33 %). The plant damage was highest in control plants (71.42 %). The least tuber damage was observed with *S. glaseri* @ 5×10^9 IJ/ha. In other treatments, tuber damage ranged from 23.80 to 47.61 per cent **Table 3**.

Table 3 Bioefficacy of entomopathogenic nematodes against *A. ipsilon* on potato under pot culture conditions

Treatments	Per cent insect mortality (hr after treatment)			Per cent Tuber damage
	24	48	72	
T ₁ - <i>H. indica</i> @ 1.25×10^9 IJs/ha	6.66 ^b (14.75)	13.33 ^d (21.33)	38.33 ^c (38.24)	61.90 ^b (51.96)
T ₂ - <i>H. indica</i> @ 2.5×10^9 IJs/ha	8.33 ^b (16.59)	23.33 ^c (28.78)	46.66 ^d (43.08)	47.61 ^{cd} (42.62)
T ₃ - <i>H. indica</i> @ 5×10^9 IJs/ha	25.00 ^a (29.92)	38.33 ^b (38.24)	66.66 ^c (54.75)	33.33 ^{de} (38.03)
T ₄ - <i>S. glaseri</i> @ 1.25×10^9 IJs/ha	6.66 ^b (14.75)	28.33 ^c (32.01)	71.66 ^{bc} (57.86)	61.98 ^{bc} (49.10)
T ₅ - <i>S. glaseri</i> @ 2.5×10^9 IJs/ha	8.33 ^b (16.59)	43.33 ^b (41.15)	75.00 ^b (60.07)	33.33 ^{ef} (32.31)
T ₆ - <i>S. glaseri</i> @ 5×10^9 IJs/ha	25.00 ^a (29.92)	61.66 ^a (51.75)	83.33 ^a (65.95)	23.80 ^f (25.57)
T ₇ - Control	0 (0.28)	0 (0.28)	0 (0.28)	71.42 ^a (61.05)
CD (p=0.05)	5.23	5.23	3.50	7.82

Figures in parentheses are arc sine transformed values

Column figures followed by different letters are significantly different from each other

Discussion

Laboratory conditions

The present investigation indicated that *H. indica* and *S. glaseri* were more virulent to insect species, *A. ipsilon*. The findings showed that LC₅₀ and LT₅₀ of *S. glaseri* were low for *A. ipsilon* and high for *H. indica*. [5] reported that cent per cent larval mortality in a laboratory bioassay of *S. glaseri* against *A. ipsilon* and *S. litura*. *S. glaseri* was effective

against sedentary pests [6]. The dosage mortality response against different stages of four lepidopteran insect reported by [7]. The 90 per cent mortality in *S. exigua* by *S. pakistanense* after 24h exposure period. [9] Observed that 50 per cent mortality in the least (20.27 h) exposure time to *H. indica* than *S. kari* (38.12 h) in diamond back moth (DBM) larvae. [10] reported that virulence is considered as an important factor in determining the time and dose of subsequent entomopathogenic nematodes application, which may be useful in reducing the cost of entomopathogenic nematode application in the field. A positive relation was evident between percent mortality and nematode concentration. In other words, mortality was concentration dependant. Laboratory bioassay proved that the entomopathogenic nematodes (EPN) were highly virulent to *A. ipsilon*, the virulence of nematodes species different greatly among associated symbiotic bacteria [11]. Overall, higher virulence of *Heterorhabditis* and a few *Steinernema* can be attributed to the cruiser foraging strategy. Lower mortality of pupae may be due to their sedentary nature and increase in exposure time which may increase the mortality percentages.

Glass house conditions

In the present study, larval mortality was the highest after 72h for *S. glaseri* @ 5×10^9 IJ/ ha. *S. glaseri* @ 2.5×10^9 IJ/ha and *H. indica* @ 5×10^9 IJ/ha which were on par with each other with larval mortality of 75.00 per cent and 66.66 per cent respectively. The least mortality was observed with *H. indica* @ 1.25×10^9 IJ/ha (38.33%). Similarly to [12] who compared the efficacy of *S. carpocapsae* against *A. ipsilon* and found that all the strains infected the cutworm larvae. Similar findings were also reported by [13] who found *S. carpocapsae*, *S. abbasi* and *H. indica* causing 60-80 per cent mortality against cutworms. The nematode isolates differ in their rank for virulence between the stages of the insect and also among the species of *Agrotis* tested as observed by [14].

In the existing soil environment, insect pests are well adapted and respond actively against any adverse situation. It was observed that 3rd and 4th instar *A. ipsilon* moved rapidly when entomopathogenic nematodes touch or cling to them, and this rapid movement seemed to be an attempt to avoid nematodes [15]. [16] Observed enhanced mortality of *A. ipsilon* with increased in time of exposure and soil types. Combination of *S. carpocapsae* and *H. indica* has an additive effect over their individual population. Performance of *Heterorhabditids* was significantly less effective than other *S. glaseri*. [17] Reported that the *Heterorhabditis* strain FL 2122 combination with insecticides provided synergistic effects in controlling *A. ipsilon*. *Heterorhabditis* strain FL 2122 caused 50 per cent mortality of 4th instar larva of *A. ipsilon*. [18] Reported that black cut worm was susceptible to *S. carpocapsae* in both laboratory and field trails.

Conclusion

To concluded that the efficacy of entomopathogenic nematodes *S. glaseri* effectively control *A. ipsilon* under laboratory and pot culture conditions. *S. glaseri* @ 5×10^9 IJ/ha caused 83.33 per cent larval mortality with 23.80 per cent tuber damage compared to 71.42 per cent tuber damage in control.

Acknowledgements

The authors express their gratitude to University Grants Commission, New Delhi for financial support by providing fellowship during 2013-15 to carry out the research work.

References

- [1] Sing, H. Household and Kitchen-Garden Pest, 2002, Kalyani publisher-Ne, Delhi. 420 pp.
- [2] Grewal, P., R.U. Ehlers and D.I. Shapiro Iian (Eds.). Nematodes as Biocontrol Agents, 2005, CABI Publishing, Wallingford, UK.
- [3] Morris, O.N., V. Converse and J. Harding. The Canadian Entomologist, 1990, 122, 309-319.
- [4] Kaya, H.K, and Gaugler, R. Entomopathogenic nematodes. Annual review of Entomology, 1993, 38: 181-206.
- [5] Vyas, R.V. and D.N. Yadav. Infectivity of entomopathogenic nematodes to the two soil dwelling lepidopterans. Annals of Biology, 1992, 8: 59-63.

- [6] Alm, S.R., T. Yeh, L.L. Hanula and R. Georgis. Biological control of Japanese Oriental and black turfgrass ataenius beetle (Coleoptera: Scarabaeidae) larvae with Entomopathogenic nematodes (Nematoda : Steinernematidae, Heterorhabditidae). *Journal of Economic Entomology*, 1992, 85:1660-1665.
- [7] Saravanapriya, B. and S. Subramanian. Pathogenicity of Entomopathogenic nematode to certain foliar insect pests. *Annals of Plant Protection Science*, 2007, 15: 219-222.
- [8] Shahina. F. and A. K. Tabassum. Virulence of *Steinernema pakistanense* against different insect species in laboratory condition. *Pakistan Journal of Nematology*, 2010, 28: 279-284.
- [9] Nyasani, J.O., J.W. Kimenju, F.M. Olubayo and M.J. Wilson. Potential of using entomopathogenic nematodes in the management of diamondback moth *Plutella xylostella* (L.) (Lepidoptera : Plutellidae) in Kenya. *African Crop Science Conference Proceedings*, 2007, 8: 1063-1066.
- [10] Lalramliana, A.K. Soil moisture effects on the activity of three entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) isolated from Meghalaya, India *Journal of Parasitology*, 2012, 36: 94-98.
- [11] Dunphy, G.B., T.A. Rutherford and J.M. Webster. Growth and virulence of *Steinernema glaseri* influenced by different subspecies of *Xenorhabdus nematophilus*. *Journal of Nematology*, 1985, 17: 476-482.
- [12] Baur, M.E., H.K. Kaya and B.E. Tabashnik. Efficacy of a dehydrated steinernematid nematode against black cutworm (Lepidoptera : Noctuidae) and diamond back moth (Lepidoptera : Plutellidae). *Journal of Economic Entomology*, 1997, 90:1200-1206.
- [13] Hussaini, S.S., S.P. Singh and V. Shakeela. 2003. Variable efficacy of different formulations of Entomopathogenic nematodes against black cutworm, *Agrotis ipsilon*. In: *Biological Control of Lepidopteran Pests*.
- [14] Tandon, P.L.C.R. Ballal, S.K. Jalali and R.J. Rabindra (Eds). *Proceedings of the Symposium of Biological Control of Lepidopteran Pests*. July 17-18, 2002, Bangalore, India. 193-197 pp.
- [15] Glazer, L. and A. Navon. *Journal of Economic Entomology*, 83, 1990: 1795-1800.
- [16] Selvan, S., P.S. Grewal, R. Gaugler and M. Tomalak. Evaluation of Steinernematid nematodes against *Popillia japonica* (Coleoptera : Scarabaeidae) larvae: species, strains and rinse after application. *Journal of Economic Entomology*, 87, 1994: 605-609.
- [17] Hussaini, S.S., S.P. Singh, R.P. Parthasarathy and V. Shakeela. Virulence of native entomopathogenic nematodes against black cut worms *Agrotis ipsilon* (Hufnagel) and *A. segetum* (Noctuidae : Lepidoptera). *Indian Journal of Nematology*, 2000, 30: 103-105.
- [18] Seal, D.R., K.J. Vivek and T.X. Liu. 2010. Potential of various strains of Entomopathogenic nematodes in combination with insecticides for suppression of blackcutworm, *Agrotis ipsilon* (Lepidoptera : Noctuidae). *Annals of Plant Protection Sciences*, 9: 77-80.
- [19] Cabanillas, H.E. and J.R. Raulston. 1996. Evaluation of *Steinernema riobravis* and *S. carpocapsae* irrigation timing for the control of corn earworm, *Helicoverpa zea*. *Journal of Nematology*, 28: 75-82.

© 2017, by the Authors. The articles published from this journal are distributed to the public under “**Creative Commons Attribution License**” (<http://creativecommons.org/licenses/by/3.0/>). Therefore, upon proper citation of the original work, all the articles can be used without any restriction or can be distributed in any medium in any form.

Publication History

Received	03 rd Feb 2017
Revised	15 th Feb 2017
Accepted	16 th Feb 2017
Online	28 th Feb 2017