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Bio efficacy of *Heterorhabditis bacteriophora* and *Oscheius chongmingensis* against *Helopeltis theivora* and *Andraca bipunctata*

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A B S T R A C T

Native isolates of EPNs, *Heterorhabditis bacteriophora* and *Oscheius chongmingensis* were isolated from the tea plantation crop of Jorhat district of Assam and were tested against tea mosquito bug *Helopeltis theivora* andbunch caterpillar, *Andraca bipunctata* through mortality test under laboratory condition. For that 5, 10, 50, 100,150, 200 and 250 infective juveniles of each isolate were inoculated per insect. The insect mortality was observed after 24, 48, 72 and 96h exposure. It was observed that 50, 60 and 70 per cent mortality of *H. theivora* were recorded by *H. bacteriophora* (150 IJs/insect) at 48 h, 72h and 96h of exposure. It recorded that with the increase in the exposure period to 96 h, *H. bacteriophora* could induce up to 90% mortality of the tested insect at the dose of 250 IJs. Similarly, mortality of *H.theivora* increased with increase in the dosage of *O.chongmingensis* and recorded 50, 60 and 70 per cent mortality at 200 IJs/insect at 48 h, 72 h and 96h. Bioassay of *H. bacteriophora* and *O. chongmingensis* against the 3rd instar larvae of *A. Bipunctata* indicated that at 250IJs/larva recorded 70 and 60% mortality in 48 hours of exposure, while 100 and 90% mortality of *A. bipunctata*was recorded at 96h.However, in the present investigation, probit analysis revealed that *H. bacteriophora* showed maximum mortality than *O. chongmingensis* against *H. theivora* and *A. bipunctata* at 48 h,72 h and 96h of exposure time.

Introduction

Tea (*Camellia sinensis* L.) belonging to the family Theaceae and tribe Gordonaceae is a long duration perennial crop grown under monoculture. Tea leaves are mostly plucked for the making of tea beverages like green tea and black tea and it contain the polyphenols such as catechins and epicatechins. Such compounds act as antioxidant and provide health benefits like reduction of weight loss, the risk of diabetes and cardiovascular disease.

The crop is extensively cultivated in 13 states of India out of which Assam, West Bengal, Tamil Nadu and Kerala are the largest producers. In Assam, it is cultivated on 765 tea gardens covering an area of 307.08 thousands hectares and more than 17%

workers of Assam are engaged in the tea industry (Anonymous, 2018).The total annual production of tea in Assam is 676.31 million kg in year 2017-18, which is more than 50 percent of India's total tea production (Anonymous, 2018). Crop loss in tea due to pests, diseases and weeds varies between 7-15% (Borthakur *et al.,* 1992). Pest infestation is a major problem associated with tea cultivation that caused reduction not only in the quantity but also in quality of tea. Tea plantation provides a permanent ecosystem for more than1034 arthropods (Chen and Chen, 1989). Das (1965) reported that 167 species of arthropods have been noted from the northeast India and cause 11.00-55.00% annual yield loss. Among them, tea mosquito bug, *Helopeltis theivora* (Waterhouse) (Hemiptera: Miridae) is one of them. *H.theivora* becomes the greatest enemies of tea planters in Africa and Asia causing 55% and 11-100% crop loss, respectively (Wilson and Clifford 1992; Anonymous, 1994; Sundararaju and Sundara Babu, 1999). In Assam *H. theivora* caused 15-20% crop loss (Hazarika *et al.,* 2009; Anonymous, 2010). This particular pest causes damage to tea plant throughout the year but the incidence is more severe during the months of May-September.

Bunch caterpillars, *Andraca bipunctata* Walk. (Lepidoptera: Bombycidae) is a well-known pest of tea (Watt and Mann, 1903) and mostly occurs in India, Indonesia, Taiwan, China, and Vietnam. In India, the pest is reported from Assam, Sikkim, and West Bengal. The hatched larvae feed on the shoot, bud, young leaves, and matured leaves in a group and completely defoliate the tea plant causing 15% yield loss in Assam (Anonymous, 2010).

Nematodes which are capable of killing, sterilizing or hampering the development of insect and completing at least one stage of their life cycle in the host are called entomopathogenic nematodes (EPNs). Entomopathogenic nematodes, *Steinernema* and *Heterorhaditis* in the family Steinernematidae and Heterorhabditidae of the order Rhabditida are obligate parasites of insect pests. The other genus *Oscheius*, in the family Rhabditidae, is also recently been isolated and found to be entomopathogenic.

EPNs are considered as one of the most significant non-chemical alternatives to insect pest control due to their high reproductive potential, ease of mass production and their harmlessness to microbes, animals, humans and plants. Only the third juvenile stage is the infective juvenile that is free-living in the soil, non-feeding, encased in a double cuticle with closed mouth and anus and capable of surviving for several weeks in the soil, before infecting a new host individual.

The infective juveniles actively penetrate through the mid gut wall or tracheae into the insect body cavity (hemocoel) containing insect haemolymph. EPNs have a mutualistic partnership with gram-negative gamma-proteobacteria in the family Enterobacteriaceae. *Xenorhabdus* bacteria are associated with steinernematid nematodes while *Photorhabdus* are symbionts of heterorhabditids.

Photorhabdus and *Xenorhabdus*, are carrying in their intestines, whereas *Heterorhabditidoides* (=*Oscheius*) carry symbiotic bacterial strains of *Serratia* in both the intestine and the cuticle (Kaya and Gaugler, 1993; Torres-Barragan *et al.,* 2011; Zhang *et al.,* 2008, 2009, 2012).

Nematode and bacteria overcome the insect immune system and the host insect is killed within 24-48 hours post infection (Adams and Nguyen, 2002). The EPNs thrives in wide range of climatic conditions and distributed in natural and agricultural soils. They have

enormous potential as biocontrol agent against a wide range of insect pests but their pathogencity varied from species to species and /or strain to strain. The application of native isolates found to be a prime importance for the management of insect pest of various crops because they share common niche with their host. The present study was undertaken to evaluate the best native isolates of EPN against tea pestlike *H. theivora* and *A.bipunctata* under *in-vitro* condition through bio efficacy test.

Materials and Methods

Collection and preparation of insects

Helopeltis theivora and *Andraca bipunctata* were collected from the sites in tea (*Camellia sinensis*) plantation areas (26.714910″N 94.204440″E) of AAU, Jorhat, Assam, India. The collected insects used in the experiment were kept in large plastic containers in laboratory at room temperature (25-30°C). The adults of *H. theivora*, having the average weight of 6.48±0.13mg and 3rdstage larvae of *A. bipunctata* average weight of 519.70±2.70mg were separated and used for infectivity test.

Maintenance of nematode culture

The entomopathogenic nematodes *viz*., *Heterorhabditis bacteriophora* and *Oscheius chongmingensis* were isolated from tea through *Galleria* bating technique and were continuously multiply on larva of greater wax moth, *G. mellonella*. The insect larvae were exposed to the nematode as per the method described by Woodring and Kaya (1988).

About ten larvae of *G. mellonella* were released into a Petri dish over two Whatman No.1 filter papers inoculated with infective juveniles stored in sterile distilled water (1 ml suspension containing 200 infective juveniles). The Petri dishes were sealed with cling film and stored in polythene bags to conserve moisture. The infective juveniles of nematodes were harvested by using White Traps as described by White (1927). The nematode multiplications in *G. mellonella* were continued in batches and the infective juveniles (IJs) were collected up to 12 days of inoculation. These juveniles were washed and rinsed several times with sterile distilled water. The collected IJs were preserved in a beaker at 10°C and utilized within two days for the study.

Infectivity assay

The infectivity tests were carried out in cavity block (55mm x 55mm) lined with a wet filter paper. One larva/adult of test insect was placed in each cavity block. Infective juveniles of *H. bacteriophora* and *O. chongmingensis* were inoculated at 0 (control), 10, 50, 100,150, 200 and 250 /insect. In control the insects were not exposed to the nematodes. The cavity blocks were wrapped with tiny perforated polyethylene bag and kept at room temperature in a dark room (30±2°C) and replicated 10 times along with control.

Insect mortality was observed after 24, 48, 72 and 96h exposure. Dead insects were dissected and the hemolymph of each one was observed under a stereoscopic microscope. When nematodes in different stages of development were detected, we considered they were the cause of death.

The mortality data (expressed in percentage) were transformed to arc sine and subjected to analysis of variance. The means were separated by Duncan's Multiple Range Test (SPSS, 2007). The corrected per cent mortality was computed after considering kill in control treatment. The LD_{50} was calculated using probit analysis and all comparisons

were made at $P = 0.05$ level of significance (Hewlett and Placket, 1979).

Results and Discussion

In infectivity study, indigenous isolates of EPNs, viz., *H. bacteriophora* and *O. chongmingensis* tested in the laboratory bioassay caused mortality of *H. theivora* and *A. bipunctata* (Table 1). The data revealed that *H. theivora* exposed to *H. bacteriophora* and *O. chongmingensis* exhibited increase in mortality with increase in exposure time coupled with inoculation rate.

No insect mortality was recorded at 24 h exposure with these nematodes. Fifty, 60 and 70 per cent mortality of *H. theivora* at 150 IJs/insect was observed at 48 h, 72 h and 96h respectively. With the increase in the exposure period to 96 h, *H. bacteriophora* could induce up to 90% mortality of the tested insect at the dose of 250 IJs. Similarly, mortality of *H. theivora* increased with increase in the dosage of *O. chongmingensis* and recorded 50, 60 and 70 per cent mortality at 200 IJs/insect at 48 h, 72 h and 96h.However, there was no significant difference between the dose of IJs, i.e., 150, 200 and 250 for mortality of *H. theivora* adult when they were treated with *H. bacteriophora* and *O.chongmingensis.* Bioassay of *H. bacteriophora* and *O. chongmingensis* against the 3rd instar larvae of *A. Bipunctata* indicated that at 250IJs/larva recorded 70 and 60% mortality in 48 hours of exposure, while 100 and 90% mortality of *A. bipunctata* was recorded at 96h, respectively (Table 2). However, there was no significant difference in the dose of IJs, i.e., 100,150,200,250 for mortality of *A. bipunctata* larvae when they were treated with *H. bacteriophora*. Similarly, no significant difference was found in the dose of IJs, *i.e.* 150,200,250/larvae for mortality of *A. bipunctata* when they were treated with *O. chongmingensis*.

The LD_{50} values determined from different dosage levels for both EPN species against *H. theivora* and *A. bipunctata* indicated that *H. bacteriophora* was superior to *O.chongmingensis* with the lowest LD_{50}at 48 h, 72 h and 96 h exposure (Table 3). The LD_{50} values for *H. bacteriophora* were 150.61(95% FL 76.58-221.58), 101.92 (95% FL 44.23-144.44) and 38.23 (95% FL 9.63-101.28) at 48, 72 and 96 h of exposures on *H. theivora*. The LD_{50} values decreased when exposure period was increased (Table 4).

The LD_{50} values for *O.chongmingensis* were 205.31 (95% FL 98.67-458.90), 150.92 (95% FL 84.43-241.23) and 87.02 (95% FL 48.61-155.77) at 48, 72 and 96 h of exposure on *H. theivora.* The LD_{50} values for *H. bacteriophora* were 150.61 (95% FL 76.58-221.58), 95.65 (95% FL 51.97-176.04) and 45.49 (95% FL 19.48-105.68) at 48, 72 and 96 h of exposures on larvae of *A. bipunctata*, respectively. The LD_{50} values for *O. chongmingensis* were 201.65 (95% FL 82.82-248.75), 133.87 (95% FL 66.76-248.65) and 77.96 (95% FL 31.13-195.23) at 48, 72 and 96 h of exposures on *A.bipunctata.* Thus, the present study confirmed the biocontrol potential of indigenous entomopathogenic nematodes *H. bacteriophora* and *O. chongmingensis* against *H. theivora* and *A. bipunctata.*

Pathogenicity of two indigenous species of entomopathogenic nematodes (EPNs), *viz*., *H. bacteriophora* and *O. chongmingensis* to *H. theivora* and *A. bipunctata* was studied under laboratory conditions with bioassay techniques.

Mortality of *H. theivora* and *A. bipunctata* increased with increase in dosage. The adult mortality of *H. theivora* was 90% and 80% per cent in 96 h exposure period at the dose of 250 infective juveniles (IJs) of *H. bacteriophora* and *O. chongmingensis,* respectively.

It was least at 5 IJs/adult. Similarly, the larval mortality of *A. bipunctata* was 100% and 90% per cent in 96 h exposure period at the dose of 250 infective juveniles (IJs) of *H. bacteriophora* and *O. chongmingensis*, respectively. It was least at 10 IJs/larva of all EPNs tested. Of the two isolates tested, *H. bacteriophora* were more effective against both of the insect than*O. chongmingensis*. The present record on LC_{50} of both the EPNs indicated that *H. bacteriophora* required less number of IJs (150.61,101.92,38.23) than *O. chongmingensis* (205.31,150.92,87.02) toresult 50% mortality of adult of *H. theivora* at 48 h, 72 h and 96h indicating higher pathogenicity of *H. bacteriophora* compared to *O. chongmingensis.* Similarly, LC_{50} of both the EPNs recorded that *H. bacteriophora* required a smaller number of IJs (150.61,95.65,45.49) than *O. chongmingensis* (201.65,133.87,77. 96) toresult 50% mortality of larvae of *A. bipunctata* at 48h, 72h and 96h.

During present study, with different dose of IJs of *H.bacteriophora* and *O. chongmingensis*, *H. theivora* and *A.bipunctata* are recorded as good host. However, there was no significant difference between the dose of IJs, i.e., 100, 150, 200 and 250 for mortality of *H. theivora* adult and *A. bipunctata* larvae when they were treated with *H. bacteriophora* and *O. chongmingensis*, respectively. A similar result was reported by Leite *et al.,* (2007) according to whom *H. indica* (IBCB-n05), applied in concentrations of 5.7 and 22.6 IJ/cm^2, the concentrations did not influence the virulence of the tested EPN strains as did not present any statistical difference, reaching efficiency of 75% and 85%, respectively, to control *Bradysia mabiusi* larvae and by Batista *et al.*, (2011) who applied three different concentrations of EPNs on *M. fimbriolata* in Petri dishes and noticed that this did not influence nymph mortality.

Vyas and Yadav (1992) found ina laboratory bioassay of *S. glaseri* against *Agrotisipsilon* and *S. litura*, cent per cent larval mortality at 16 and 32 IJs/ g of soil after 72 h of exposure period respectively. Gupta *et al.,* (1987) reported 66 per cent cutworm, *S. litura* mortality in tobacco. Baweja and Sehgal (1997) also observed 80 per cent mortality of *S. Litura* due to *H. bacteriophora.* Similar results were also reported by Kondo and Ishibashi (1986) and, Ricci *et al.,* (1995) in *S. litura*. Rajkumar *et al.,* (2003) The LD_{50} values for prepupa of *S. litura* at different exposure periods (12, 24, 36 and 48 hours) were 55.73, 33.44, 16.68and 10.49 (IJs/larva). Hussaini *et al.,* (2002) obtained centpercent mortality of *L. orbonalis* with *S. carpocapsae* with 25 IJs / larva but only after 96 hrs.

Stokwe and Malan, (2010) in a laboratory bioassay showed that both adult and intermediate life stages of *Pseudococcus viburni* (Signoret) were most susceptible to *H. zealandica* and *S. yirgalemense* with 78%and 76% mortality respectively. *P. citri*, the citrus mealy bug was susceptible to *H. zealandica* (with 91% mortality) and *S. yirgalemense* (with 97% mortality). Both species were able to complete their life cycles within the insect host, while *S. yirgalemense* proved to be faster at locating and infecting *P. citri*than *H. zealandica* (Van Niekerk, 2012). Bioassays were done on Petri dishes infested with females of *P. citri*, which were sprayed with juveniles of 18 numbers of different EPN strains. The strain with larger pathogenicity and virulence in laboratory was *H. bacteriophora* RS33 (from 69.0% to 92.2% of mortality) (Negrisoli *et al.,* 2013).

In a laboratory bioassay test conducted by Zaki *et al.,* 2000, recorded that *H bacteriophora* caused 5, 40, 80, and 100% mortality of *Corcyra cephalonica* within 24, 48, 72 and 96 hours at 15 IJs per larva .

Similarly, *S. carpocapsae* resulted in 40, 75 and 100% mortality of *C. cephalonica* after 48, 72 and 96 hours of application at same inoculums level. *H. bacteriophora* and *S. carpocapsae* caused 40 and 48% mortality of *Bombyx mori* after 48 h. Cent per cent mortality of 4th instar larva of *A. ipsilon* was brought about by *H.bacteriophora and S. carpocapsae* at the rate of 25 IJs within 77 and 96 hours respectively.

Efficacy of *Oscheius* sp. against spindle bug and red ant in areca nut (Mohandas and Rajamma, 2005; Mohandas *et al.*, 2005), rice yellow stem borer (Gururaj Katti *et al.*, 2003; Prasad *et al.*, 2003; Padmakumari *et al.*, 2005; Pudmakumari *et al.*, 2007) have been documented in India.Ratnasinghe and Hague (1995) have reported LD_{50} value of 3.6 IJ/larva of *S. feltiae* against *Plutellaxylostella.* Glazer and Navon (1990) recorded a higher LD_{50} value of 54 IJ/larva of *S. feltiae* against *Helicoverpaarmigera,* however, a lower LD_{50} value of 10.51 IJ/larva and LT_{50} value of 47.54 h were reported for *S. glaseri* against *H. armigera.*

Saravanapriya (2005) also reported lower LC_{50} values of 9.4, 7.3, 5.5and 2.0 IJs/larva of *H. indica* compared to those of *S. carpocapsae* with 10.5, 9.0, 5.2 and 2.5 IJs/larva against fifth instar larvae of *Heliothis armigera*., *S. litura, Cnaphalocrocis medinalis* and *P. xylostella,* respectively (Fig. 1–7).

Table.1 Per cent mortality of *H. theivora* induced by *H. bacteriophora* and *O. chongmingensis* at different dose and different time exposure

Nematode	Dose/insect	Mortality (%) after			
		24h	48h	72h	96h
Heterorhabditis bacteriophora	5	0	10(09.18)	20(18.14)	30(27.09)
	10	0	20(18.14)	30(27.09)	40(36.04)
	50	0	30(27.09)	40(36.04)	50(45.00)
	100	0	40(36.04)	50(45.00)	60(53.95)
	150	0	50(45.00)	60(53.95)	70(62.90)
	200	0	60(53.95)	70(62.90)	80(71.86)
	250	0	70(62.90)	80(71.86)	90(80.81)
Oscheius chongmingensis	5	0	0(0.23)	10(9.18)	30(27.09)
	10	0	10(9.18)	20(18.14)	40(36.04)
	50	0	20(18.14)	30(27.09)	40(36.04)
	100	0	30(27.09)	40(36.04)	50(45.00)
	150	0	40(36.04)	50(45.00)	60(53.95)
	200	0	50(45.00)	60(53.95)	70(62.90)
	250	0	60(53.95)	70(62.90)	80(71.86)
Control		0	0 (0.23)	10(9.18)	20(18.14)
Mean		0	32.66(29.47)	42.66(38.43)	54.00(48.57)
CD(0.05)			(34.37)	(36.74)	(37.66)

Mean of ten replicates with one insect per replication. Figures in parentheses are arc sin transformed values

Table.2 Per cent mortality of *A. bipunctata* induced by *H. bacteriophora* and *O. chongmingensis* at different dose and different time exposure

Nematode	dose/insect	Mortality (%) after			
		24h	48h	72h	96h
Heterorhabditis bacteriophora	5	0	10(9.18)	20(18.14)	40(36.04)
	10	0	20(18.14)	30(27.09)	50(45.00)
	50	0	30(27.09)	40(36.04)	60(53.95)
	100	0	40(36.04)	50(45.00)	70(62.90)
	150	0	50(45.00)	60(53.95)	80(71.86)
	200	0	60(53.95)	70(62.90)	90(80.81)
	250	0	70(62.90)	80(71.86)	100(89.76)
Oscheius chongmingensis	5	0	0(0.23)	10(9.18)	30(27.09)
	10	0	10(9.18)	20(18.14)	40(36.04)
	50	0	20(18.14)	30(27.09)	50(45.00)
	100	0	30(27.09)	40(36.04)	60(53.95)
	150	0	40(36.04)	60(53.95)	70(62.90)
	200	0	50(45.00)	70(62.90)	80(71.86)
	250	0	60(53.95)	80(71.86)	90(80.81)
Control		0	0(0.23)	0(0.23)	10(9.18)
Mean		0	32.66(29.47)	44.00(39.62)	61.33(55.14)
CD(0.05)			(34.37)	(35.61)	(34.96)

Mean of ten replicates with one insect per replication**.** Figures in parentheses are arc sin transformed values

Table.3 Dose response mortality of *H. theivora* by *H. bacteriophora* and *O. chongmingensis*

Exposure time	LD_{50}	Fiducial limits (95%)	Slope	$\chi 2$	Regression equation (Y)	R^2
H. bacteriophora						
48h	150.61	76.58-221.58	0.93	0.85	0.93x+3.05	1
72h	101.92	44.23-144.44	0.98	1.29	0.98x+3.39	1
96h	38.23	9.63-101.28	1.09	2.67	1.09x+3.36	1
O. chongmingensis						
48h	205.31	98.67-458.90	1.09	0.65	1.09x+2.44	1
72h	150.92	84.43-241.23	1.84	1.69	1.84x+0.97	1
96h	87.02	48.61-155.77	1.39	1.67	1.39x+2.29	1

Table.4 Dose response mortality of *A. bipunctata* by *H. bacteriophora* and *O. Chongmingensis*

Exposure time	LD_{50}	Fiducial limits (95%)	Slope	$\chi 2$	Regression equation (Y)	R^2
H. bacteriophora						
48h	150.61	76.58-221.58	0.93	0.85	0.93x+3.05	1
72h	95.65	51.97-176.04	1.31	0.46	1.31x+2.39	1
96h	45.49	19.48-105.68	1.08	2.06	1.08x+3.16	1
O. chongmingensis						
48h	201.65	82.82-248.75	1.10	0.60	1.10x+2.44	1
72h	133.87	66.76-248.65	1.19	0.45	1.19x+2.45	1
96h	77.96	31.13-195.23	0.90	1.18	0.90x+3.28	1

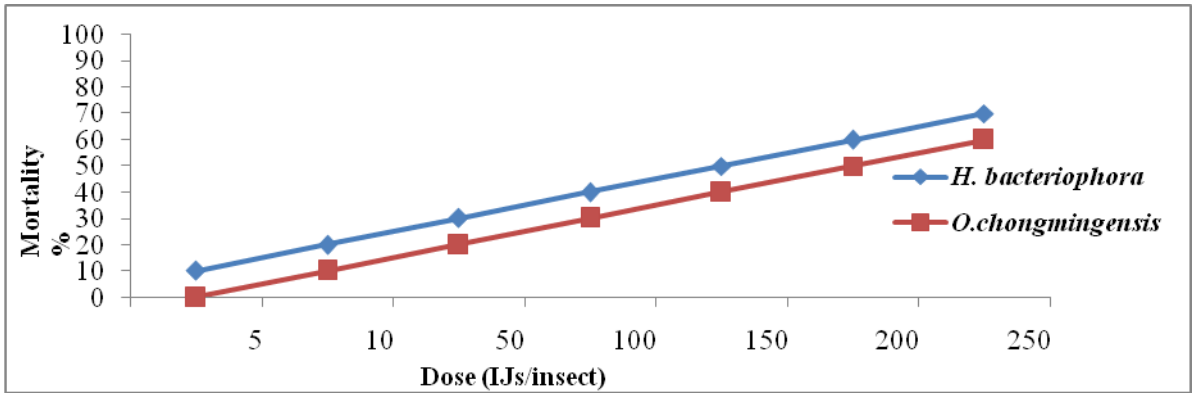

Figure.1 Per cent mortality of *H. theivora* induced by *H. bacteriophora* and *O. chongmingensis* at different concentrations of EPNs at 48h exposure period

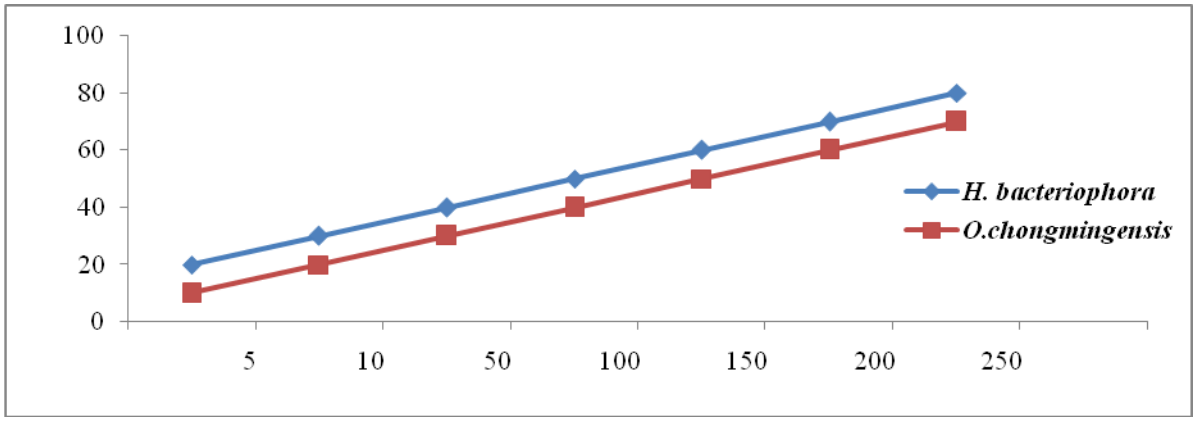

Figure.2 Per cent mortality of *H. theivora* induced by *H. bacteriophora* and *O. chongmingensis* at different concentrations of EPNs at 72h exposure period

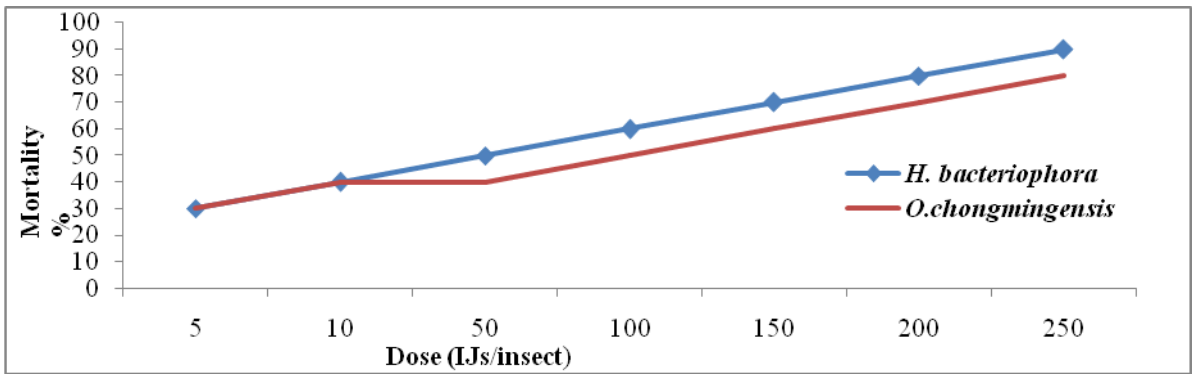

Figure.3 Per cent mortality of *H. theivora* induced by *H. bacteriophora* and *O. chongmingensis* at different concentrations of EPNs at 96h exposure period

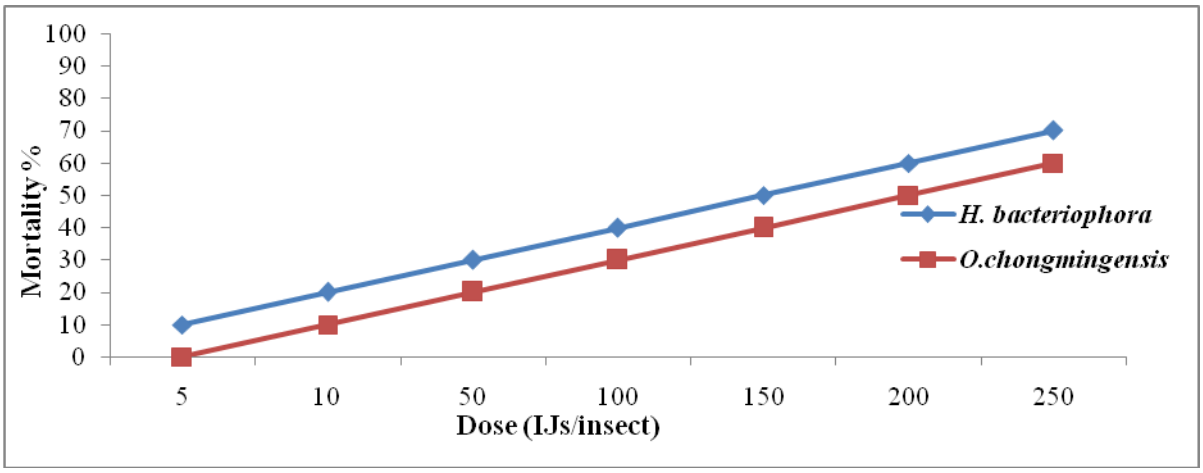

Figure.4 Per cent mortality of *A. bipunctata* larvae induced by *H. bacteriophora* and *O. chongmingensis* at different concentrations of EPNs at 48h of exposure period

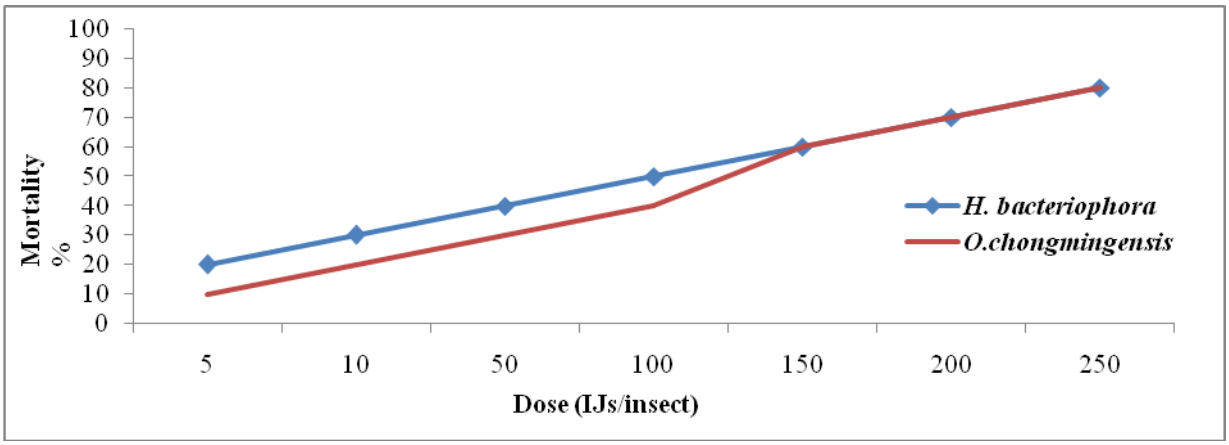

Figure.5 Per cent mortality of *A. bipunctata* larvae induced by *H. bacteriophora* and *O. chongmingensis* at different concentrations of EPNs at 72h exposure period

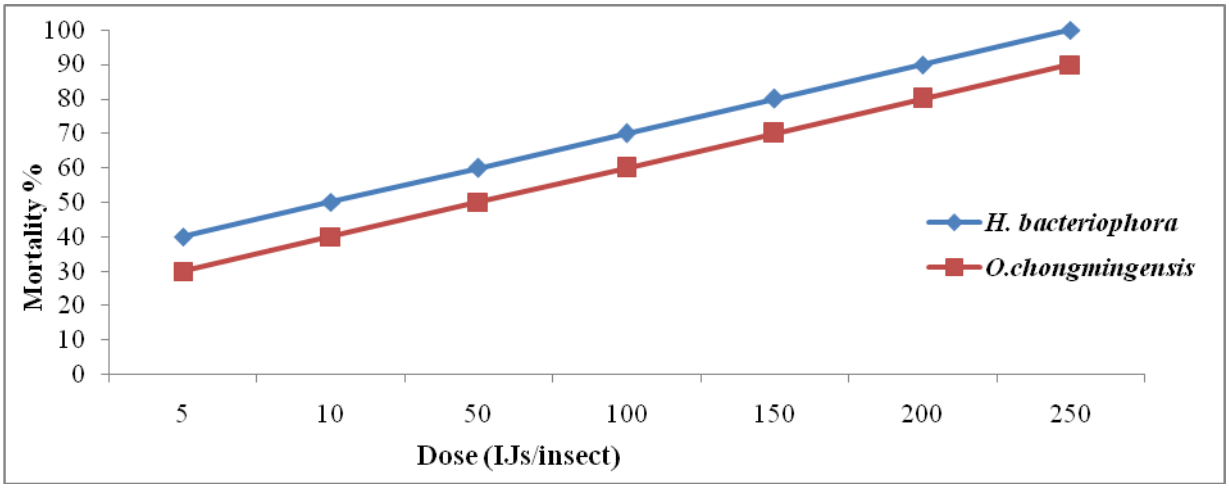

Figure.6 Per cent mortality of *A. bipunctata* induced by *H. bacteriophora* and *O. chongmingensis* larvae at different concentrations of EPNs at 96h exposure period

Lower LC_{50} values of *H. indica* (200, 34 and31 IJs/larva) compared to those of *Steinernema* sp. (275IJs/larva), *S. bicornutum* (64 IJs/larva) and *S.carpocapsae* (34 IJs/larva) against *Chilopartellu* (PDBC, 2000), *A. ipsilon* (Hussaini *et al.,* 2002) and *P. xylostella* (Hussaini, 2003), respectively was also recorded. Shinde and Singh (2000) tested eight entomopathogenic nematode species/strains against the final instar larvae of *P. xylostella.* They found that all nematodes species were pathogenic. However, *H. bacteriophora* was the most pathogenic amongst the tested nematodes on the basis of $LC_{50}$and LT_{50}.

Due to the associated bacteria, *Photorhabdus luminescens* and *Serratia nematodiphila* also reacted differently when in contact with insect hosts (Zhang *et al.,* 2009). Moreover, *H. bacteriophora* is a cruiser forager that actively looks for its prey (Ciche, 2007).

EPNs demonstrated great variation in infectivity in different hosts, and none of the species/strains of EPNs may infect all the tested insect species or some species or strains may be highly host specific (Gaugler *et al.,* 1989;Georgisand Manweiler 1994).

Such variation in the insect mortality by the two nematode species may be attributed, to the host preference by nematodes or host recognition and penetration or nematode's compatibility/virulence with its host, and to the vulnerability of insect pests (stages as well as among the species, the behavioral, morphological and physiological defense strategies of insects (Bedding *et al.,* 1983;Fuxa *et al.,* 1988; Glazer and Navon, 1990;Glazer, *et al.,* 1992; Berry *et al.,* 1997;Glazer and Lewis, 2000;Shapiro-Ilan and Cottrell, 2005).

Moreover, the faster invasion rate was recorded with smaller insects for all nematodes strains. Thus the LC_{50} and LT_{50}increase in proportion to the size of insect (Bedding *et al.,* 1983). The time taken for mortality and percent mortality is also greatly influenced by temperature. This was evident from the delayed mortality of *G. melonella* by *Steiner nemathermophilum* at 10 and 20°C whereas mortality was faster at 25-35°C (Ganguly and Singh, 2001). Therefore, laboratory screening of EPNs for infectivity is an important component for developing a biological control programme for a particular pest (Ricci *et al.,* 1996).

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