

Bioefficacy of different formulations of entomopathogenic nematode *Steinernema carpocapsae* against Diamond back moth (*Plutella xylostella*) infesting Cabbage (*Brassica oleracea* var. *capitata*)B.S. Sunanda¹, P. Jeyakumar¹ and V. V. Jacob²**ABSTRACT**

Diamondback moth (DBM); *Plutella xylostella* (L.) (Lepidoptera: Yuponomeutidae) is a major pest of cabbage throughout the world. Several bio-pesticides have been tested for the management of DBM in different parts of the country, but only few have shown promising results. Among different bio-agents, entomopathogenic nematodes were found comparatively better over others in suppressing the population of diamondback moth (DBM). Laboratory and field studies were carried out to evaluate the efficacy of an indigenous population of *Steinernema carpocapsae* against fourth instar larvae of diamondback moth. In laboratory conditions the efficacy of EPN was tested at four dosages viz., 250, 500, 750 and 1000 IJs/petri plate. The per cent mortality of DBM larvae after 72 hours was recorded, maximum 100 % with an inoculum level of 1000 IJs/petri plate followed by 85 % 76.17 %, and 71.84 % at 750, 500 and 250 IJs/ petri plate respectively at 72hrs. The comparative efficacy of different formulations of entomopathogenic nematode, *S. carpocapsae* was also tested against diamond back moth (*P. xylostella*) infesting cabbage in field conditions with different inoculum levels (doses) of 20 lakh and 30 lakh IJs /plot. The maximum (53.75) per cent mortality of DBM was recorded at 30 lakh IJs/ plot with an antidesiccant liquid paraffin 1% after 7th day of application in the field conditions.

MS History: 10.09.2014 (Received)-09.10.2014 (Revised)-19.10.2014 (Accepted)

Citation: B.S. Sunanda, P. Jeyakumar and V. V. Jacob. 2014. Bioefficacy of different formulations of entomopathogenic nematode *Steinernema carpocapsae* against Diamond back moth (*Plutella xylostella*) infesting Cabbage (*Brassica oleracea* var. *capitata*). *Journal of Biopesticides*, 7(2):209-214.

Key words: Comparative efficacy, Antidesiccants, DBM, Formulations, Inoculum level.

INTRODUCTION

In India, vegetables play an important role in nutritional security, economic viability and source of remunerative income and employment for many small and marginal farmers under intensive farming system. More than 60 kinds of vegetables are grown in tropical, subtropical and temperate agro-climates of the country. India is the largest producer of vegetables in the world, tops in production of okra and ranks 2nd in production of potato, onion, cabbage and cauliflower. During 2013-2014, India produced 162.19 million tonnes of vegetables and exported worth of Rs. 5462.93 crores (Indian Horticulture Database 2013). Among vegetables, cole crops are one of the most abundantly consumed vegetables all over the world. They belong to the genus *Brassica* of family Brassicaceae. This group includes a wide variety of vegetable crops. Cabbage (*Brassica oleracea* Var. *Capitata* L) is a leafy winter vegetable grown for its edible enlarged

terminal bud. In addition to several minerals such as P, K, Ca, Fe, it also contains high percentage of vitamin A, B and C. It is consumed either cooked or raw as salad.

Diamondback moth (*Plutella xylostella* L.) (DBM), is a major cosmopolitan pest. It is a defoliating caterpillar that hampers the successful cultivation of cabbage in the world. In India it was first recorded (Fletcher, 1914) on cruciferous vegetables. Now the pest has been noticed all over India on all the crops grown belonging to the family Brassicaceae (Devi *et al.*, 2004). This pest causes colossal loss to cabbage every year. It damages the crop by feeding on the foliage. Simultaneous attack by a large number of larvae hinders the growth of the plant leading to significant yield reduction. The crop loss due to DBM is estimated up to 52 per cent (Krishnamoorthy, 2004). According to Talekar (1992) the annual cost of managing this pest

globally is estimated to be one billion US dollar. The annual production of cabbage in the country is 8534000 tones from an area of 372000ha with a productivity of 22.9mt/ha (Indian Horticulture Database, 2013). The major caterpillars of the Diamond back moth (*P. xylostella*), the cabbage web worm (*Hellula undalis*) and cabbage aphids (*Brevicoryne brassicae*) are the most serious pests of cabbage.

Pest infestation normally leads to reduction in market value and in some cases total crop failure. Farmers in India have been applying various synthetic pesticides to reduce damage caused by these pests, at different growth stages of the crop. These pesticides have been reported to cause toxicological and environmental problems (toxic residues in food, soil, water bodies and elimination of non-target organisms) as well as the development of resistant strains of pests (Ninsin, 1997). The improper use of pesticides is an issue of much concern.

Entomopathogenic nematodes (EPNs) are beneficial nematodes parasiting insect pests and are being effectively used as a bio pesticide against a wide variety of insect pests. The impressive attributes of EPN have stimulated strong commercial interest in nematodes as biological insecticides and are perceived as viable alternative to chemicals in integrated pest management (IPM) programme. EPN have many attributes, which make them a good and promising bio control agent (Ahmad *et al.*, 2005). They often behave like insecticide or other plant protection chemicals and they can be easily incorporated as a component of IPM programme. The EPNs can easily be cultured and applied using standard defined methods and application equipment's. They are compatible with many chemical pesticides and can be incorporated as an important component in the Integrated Pest Management (IPM).

Entomopathogenic nematodes mainly belong to Steinernematidae and Heterorhabditidae family. They have symbiotic association with gram positive bacteria (*Xenorhabdus* spp. with Steinernematids and *Photorhabdus* spp. with Heterorhabditids). These EPNs normally enter the host through natural

openings and release the symbiotic bacteria in the insect haemocoel. The bacteria in turn kills host insect by causing septicemia within 24-48 hrs. Cabbage is the most sought vegetable commodity in urban areas. The trend among rural areas is also shifting more towards cabbage and cauliflower with changing food habit. However, these crops are cultivated under frequent use of heavy dosages of pesticides to get the quality produce. These pesticides residue is causing a major health hazard and already awareness among the public is increasing. The urban dwellers prefer pesticide free or organically produced vegetables than regularly produced vegetables. In order to meet this growing demand for low pesticide or no pesticide residue vegetables, there is a need to develop candidate bio control agents for insect pest management. The current experiment was undertaken with a hypothesis of employing entamopathogenic nematodes (EPNs) for management of diamondback moth in cabbage. Both laboratory and field studies were conducted to derive the better conclusion in deciding the role of EPNs for management of DBM in cabbage.

MATERIALS AND METHODS

Laboratory conditions

The EPN suspension consisting of IJs stored in sterile distilled water was first examined under stereoscopic microscope to check the activity of the juveniles and diluted with a known quantity of sterile distilled water for making the suspension according to the required number of IJs. Ten larvae of diamondback moth (*Plutella xylostella*) were placed on Whatman filter paper No. 1 in 9 cm sized glass petri plates together with IJs of *Steinernema carpocapsae* at 250, 500, 750, and 1000 IJs / petri plate. The treatments were replicated four times.

Field conditions

The efficacy of *S. carpocapsae* was tested in field conditions against *P. xylostella* during 2013-14. Commercial cabbage Hybrid F1 seedling nursery was raised and 28 days old seedlings were transplanted in main research field of NIPHM. The experiment was laid out with 13 treatments and four replications following completely Randomized Block Design (RBD). Each plot measures 24sqmt (6x4 / length x width). Spacing of 45cm between the

rows and 30 cm between the plants was maintained. Treatments were imposed immediately on onset of insect incidence. Three formulations *viz.*, F1. Calcium gel alginate capsule (CGAC), F2. Sponge bits and F3. Water Dispersible Granules (WDG) were employed with two dosages of EPNs D1. 20lakh IJs/plot and D2. 30lakh IJs/plot with a control and two different antidesiccants t1. Liquid paraffin and t2. Tween 80 1%.

The IJs were sprayed on infested crop according to the treatments using Knap sack sprayer. Other nutritional and plant protection practices such as weed management and disease management were undertaken following recommended package of practices. The per cent mortality of insect larvae was recorded on first, second, third, fourth, fifth, sixth, and seventh day after application at inoculum levels of 20 lakh and 30lakh IJs/plot. The observations were recorded on the mortality of host larvae every day up to seven days from the first day of inoculation of IJs. The dead insect larvae were collected and kept for the release of IJs on White trap to check the pathogenicity. All the treatments were replicated four times

Statistical analysis

The observations recorded were statistically analysed and significance of results was tested. For above experiments, completely randomized design and randomized block design were followed. 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 tests ($P < 0.05$) for separation of means. Figures in parentheses are arc sin transformed values.

RESULTS AND DISCUSSION

Laboratory conditions

Maximum 100.0% mortality was observed at an inoculum level of 1000 IJs/ Petri plate followed by 90.00% at 750 and 82.50% at 500 75.00 % at 250 IJs/Petri plate respectively at 72 hrs. Similar studies in this regard were conducted by Vyas *et al.* (2002) who reported that the infective juveniles of *S. carpocapsae* provided a possible control of DBM by 27.8% at 400 IJs/pot. The current experiment reveals that to attain 100% mortality the dosage of EPNs should be 1000IJs/plate. The time taken for

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mortality was varying in observations made by many workers; however, the dosage of 1000IJs/plate could give 100% mortality within 72 hrs which is most essentially required in a candidate biocontrol agent. However, the mortality time may vary in some other species. Nyasani *et al.* (2008) conducted laboratory bioassays and recorded 86.7% mortality of DBM larvae at 200IJs/mL of *S. weiseri* within 72 hrs. Ganguly *et al.* (2004) recorded 100% mortality of DBM within 48 hrs of infection, with *Steinernema thermophilum*. These findings of experiments performed under Indian conditions and the results of the experiment are in agreement with results obtained by other workers (Belair *et al.*, 2003, Mahar *et al.*, 2004; Somvanshi *et al.*, 2006) who have reported the efficacy of different *Steinernema* spp. against DBM. A linear increase in the percentage mortality of DBM was observed with an increase in exposure time at 24 hrs, the percentage mortality of the DBM larvae was lowest for all the EPN inoculum levels and highest at 72 hrs. This may be because of time required for entry of IJs in to host insect and bacterial infection starting after the penetration of the nematodes into the body cavity of the insects. Therefore, infective nematode juveniles must locate the insect host and gain entry into the haemocoel.

Field conditions

In field conditions, no mortality of diamond back moth was found on first and second day after application. However, on third day maximum (17.50 %) per cent mortality of DBM larvae at 30 lakh IJs/plot in WDG formulation with liquid paraffin 1% was noticed. On 4th day maximum (30.00 %) mortality of DBM was observed in WDG as foliar application at 30, lakh IJs/plot as compared to other formulations. While minimum (17.50 %) mortality was recorded in CGAC at 20 lakh IJs/plot with liquid paraffin 1% as adjuvant. Observations on 5th day revealed that WDG formulation of *S. carpocapsae* at 30 lakh IJs / plot could bring maximum (42.50 %) mortality of DBM followed by 20 lakh IJs/pot (32.50 %) which differed significantly with each other. On sixth day maximum (50.00 %) mortality was recorded at 30 lakh IJs/plot in WDG with liquid paraffin 1% followed by (47.50 %) in sponge bit and (42.50 %) and CGAC formulation at 20lakh IJs/plot with

Tween-80 1%. On final observation on 7th day revealed maximum (60.00 %) mortality of DBM at 30 lakh IJs/plot in WDG with liquid paraffin 1% as foliar application followed by (53.75 %) at 30 lakh IJs/plot with Tween 80 1% in sponge bit and (51.25 %) in CGAC formulation at 30 lakh IJs/plot with liquid paraffin 1%.

Similar studies were conducted by Amos *et al.* (2002) who had and used improved calcium alginate gel formulation of *S. carpocapsae* and tested against *Spodoptera littoralis* and *Helicoverpa armigera* larvae. Hundred per cent mortality in 4th instar

larvae of the two insects was achieved by feeding them on 1000 IJ g⁻¹ of *Steinernema carpocapsae* (All strain) in the gel for 24 h. Exposing 2nd to 5th instars of *H. armigera* and 3rd to 6th of *S. littoralis* to 500 IJ g⁻¹ of *S. carpocapsae* (All strain) resulted in 70-100% larval mortality. Mature larvae were less susceptible to the nematodes. Mortality of larvae exposed to 500 IJ g⁻¹ of *S. carpocapsae* (All strain) ranged from about 45-55% at 4h to 90-95% at 48 h. Fourth instar larvae fed for 24 h with 250 IJ g⁻¹ of nematode strains in gel showed in *S. littoralis*.

Table 1. Bioefficacy of *S. carpocapsae* against Diamond back moth (*P. xylostella*) in the laboratory conditions.

Treatments (IJs/Petri plate)	Mean Mortality (%) at Different Intervals (hrs.)					
	12	24	36	48	60	72
T1 (250IJs)	0.00	2.50	39.86	50.80	59.36	71.84
T2 (500IJs)	0.00	6.91	42.05	55.32	60.94	76.17
T3 (750IJs)	0.00	16.13	45.08	54.53	65.92	85.39
T4 (1000IJs)	0.00	19.45	47.57	60.94	68.97	90.0
T5(Control)	0.00	0.00	0.00	0.00	0.00	11.52
SEm±	0.00	3.560	0.904	3.175	2.860	2.993
CD at 5%	0.00	5.366	6.866	3.303	5.201	6.911

* Each value is the mean of four replications,

Similarly Divya *et al.* (2011) developed six formulations (sawdust, hydrogel, coirdust, talc, sponge and water) and evaluated survival of *Heterorhabditis indica* at 27 ± 2°C and their pathogenicity against cotton bollworm, *Helicoverpa armigera*. Sawdust (95%) and hydrogel (85%) formulations had enhanced highest survival than the other formulations coirdust (80%), talc (75%), water (70%) and sponge (65%) till 5th week period. At the end, a maximum shelf-life of more than 75 days was achieved in a water dispersible hydrogel formulation with 65% survival than sawdust (15%) formulation. Accomplishment of *Heterorhabditis indica* survival and virulence under formulation caused 85% and 70% pathogenicity on *Helicoverpa armigera* in hydrogel and sawdust respectively, exposed for 48 h treated at 100 IJs /larva. The current research also showed similar observations. The water dispersible granules gave maximum mortality of DBM than rest of the two formulations.

Patel and Vyas (1995) have observed that application of liquid formulations of *Steinernema glaseri* at 3000 and 4000 IJ/20 ml water gave 11.97 and 24.58 per cent mortality of *H. armigera* infecting chickpea, Vyas *et al.* (2002) reported that spray of liquid formulation of *Heterorhabditis* sp. at 10,000 IJ/m² caused 87 per cent mortality of *H. armigera* in pigeon pea and observed increased crop yield. Similarly *Steinernema riobrave* was reported to cause 95 – 100 per cent mortality of *H. Helicoverpa zea* on corn at 2 × 10⁹ IJs/ha (Cabanillas and Raulston, 1995). In general the per cent insect mortality was lower in micro-plots compared to glass house conditions.

Similar findings were also reported by Sezhian *et al.* (1996) and Hussaini *et al.* (2002), where insect mortality was lower under field conditions compared to laboratory bioassays and in trials under glasshouse conditions.

Table 2. Comparative efficacy of different formulations against DBM (*P. xylostella*) under field conditions during the year 2013-14.

Treatments (Combinations)	Mean mortality (%) at different intervals (days)						
	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
T ₁ = F ₁ D ₁ t ₁	0.00	0.00	14.84	21.48	28.23	34.68	39.78
T ₂ = F ₁ D ₁ t ₂	–	–	11.52	14.57	26.38	32.30	38.20
T ₃ = F ₁ D ₂ t ₁	–	–	16.42	24.08	30.72	37.73	42.84
T ₄ = F ₁ D ₂ t ₂	–	–	15.86	22.23	29.70	35.88	41.39
T ₅ = F ₂ D ₁ t ₁	–	–	11.52	11.25	22.23	31.55	37.62
T ₆ = F ₂ D ₁ t ₂	–	–	16.13	10.23	23.52	29.70	36.11
T ₇ = F ₂ D ₂ t ₁	–	–	21.21	16.69	28.23	34.64	40.64
T ₈ = F ₂ D ₂ t ₂	–	–	16.87	15.86	24.72	32.79	39.17
T ₉ = F ₃ D ₁ t ₁	–	–	11.52	22.50	31.55	37.62	43.56
T ₁₀ = F ₃ D ₁ t ₂	–	–	11.52	19.18	29.36	36.11	42.12
T ₁₁ = F ₃ D ₂ t ₁	–	–	17.71	25.18	34.72	40.67	47.16
T ₁₂ = F ₃ D ₂ t ₂	–	–	14.57	23.70	33.13	37.62	45.00
T ₁₃ = Control	–	–	0.00	0.00	0.00	0.00	0.00
SEm ±	–	–	3.493	3.198	1.927	1.775	1.524
CD at 5%	–	–	9.849	9.015	5.433	5.005	4.298

F₁ = Sponge bit; D₁ = 20lakh IJs/ plot; t₁ = Liquid Paraffin 1%; F₂ = Calcium gel alginate capsule (CGAC); D₂ = 30 lakhIJs/ Plot

t₂ = Tween 80 1%; F₃ = Water dispersible granules (WDG); **Data in parenthesis are retransferred percent values

This may be due to the unfavorable environmental conditions, such as increased temperature, low relative humidity and UV radiation from direct sunlight, prevailing in the field. The results conclude that the EPNs could be engaged with WDG formulation with liquid paraffin as adjuvant. The EPNs could also become a best candidate bio control agent in IPM of DMB pest in cabbage, as most of the cabbage is grown in cold conditions and cooler climatic places.

ACKNOWLEDGEMENTS

The authors express their gratitude to Department of Science and Technology (DST) New Delhi (SERB/F/2832/2013-14 Dated: 08-08-2013) for support by providing grant as Fast Track Young Scientist Award 2013-14 to carry out these experiments

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Biopesticide formulations based on *Beauveria bassiana* and *Metarhizium anisopliae*, a wide host range insect pathogenic fungi, are being marketed and used in insect pest management [8,9]. These entomopathogenic hyphomycete fungi have great potential as biological control agents against insects and are an important component within integrated pest management systems. Determination of medium lethal time of *Steinernema yirgalemense* strain HI and Differences in virulence of entomopathogenic nematode species and isolates have

20. Ganguly S, Somvanshi VS (2007) Efficacy of foliar applications of entomopathogenic nematodes against the crucifer diamondback moth, *Plutella xylostella*-a review. *Nematol Medit* 35: 5-14. The diamondback moth (*Plutella xylostella*) is a major pest of broccoli worldwide. It mainly causes leaf defoliation and generates annual losses of 80%. In this study we evaluated the susceptibility of *P. xylostella* to entomopathogens *Heterorhabditis bacteriophora* HNI0100, *Beauveria bassiana* Bb9205 and *Metarhizium anisopliae* Ma9236. This study was aimed at determining the occurrence of entomopathogenic nematodes (EPNs) in different agroecosystems and their potential as biocontrol agents in the management of DBM. Soil samples were taken from a planted forest, pasture, a coffee field and a vegetable garden. EPNs were isolated from the soil using *Galleria mellonella* as the bait insect. The comparative efficacy of different formulations of entomopathogenic nematode, *S. carpocapsae* was also tested against diamond back moth (*P. xylostella*) infesting cabbage in field conditions with different inoculum levels (doses) of 20 lakh and 30 lakh IJs /plot. The maximum (53.75) per cent mortality of DBM was recorded at 30 lakh IJs/ plot with an antidesiccant liquid paraffin 1% after 7th day of application in the field conditions. Entomopathogenic nematode formulation technology has made significant progress in past 15 years. more.

Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* (Nematoda: Rhabditida) are extraordinarily lethal to more. Nowadays, the use of entomopathogenic nematodes as a biological control agent is a key component in IPM system. Infectivity and biocontrol potential of entomopathogenic nematodes against winter moths (*Operophtera brumata* and *O. fagata*) pupating in the soil were examined in laboratory, semi-field and field conditions. A pilot experiment conducted in the field showed that *Steinernema feltiae* was completely ineffective against pupae of these moths in the soil. The entomopathogenic nematode *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) is used to control diapausing codling moth larvae. The objective of this study was to define environmental conditions favouring the performance of the nematodes. Coccooned larvae were more susceptible than non-coccooned larvae. Susceptibility of pupae was low. To determine the influence of decreasing water activity (aw-value) on the activity of the nematodes, mortality of codling moth larvae and *Galleria mellonella* L. were tested in sand-sodium-polyacrylate mixtures of variable water activity. *S. feltiae* was able to infect both insects at aw-values >0.9. Coccooned larvae of both insects died at lower aw-values than non-coccooned larvae.