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Efficacy of entomopathogenic nematodes against the cabbage butterfly (*Pieris brassicae* (L.) (Lepidoptera: Pieridae) infesting cabbage under field conditions

Tarique Hassan Askary^{1*} and Mohammad Jamal Ahmad²

Abstract

Experiments were conducted to test the pathogenicity of entomopathogenic nematode, *Heterorhabditis pakistanensis* alone and in combination with the chemical insecticides, viz., Dichlorvos and Quinalphos and Neem product, viz., Azadirachtin against the cabbage butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae) infesting cabbage plants under laboratory and field conditions. In laboratory test, inoculum level of 200 infective juveniles (IJs) was most effective causing mortality to both 3rd and 4th instars larvae at 48 h. Nematode multiplication rate in insect cadaver was inversely proportional to the size of initial inoculum level of IJs, whereas IJs yield was directly proportional to the size of larva. The 4th instar larva resulted in a highest yield of IJs, followed by 3rd, 2nd, and 1st instars larvae at all the inoculum levels used in the study. IJs were found compatible with the chemical insecticides and Neem product at lower than half of the optimum single dose. The cumulative mean survival percentage of IJs recorded the highest (77.5%) in the combination of 100 IJs + Azadirachtin (0.25%) and lowest (70.6%) in 100 IJs + Dichlorvos 76 EC (0.05%). Under field conditions, *H. pakistanensis* (3.0 lakh IJs/m²) + Dichlorvos 76 EC (0.025%) resulted to the highest larval mortality (79.65%), followed by *H. pakistanensis* (3.0 lakh IJs/m²) + Azadirachtin (0.25%), where it was 76.22%. The larval mortality was the least (61.16%) in case of *H. pakistanensis* (3.0 lakh IJs/m²) when applied alone. *H. pakistanensis* + Dichlorvos resulted to the highest increase (31.42%) in cabbage yield over untreated control.

Keywords: Cabbage, *Pieris brassicae*, Entomopathogenic nematodes, Insecticides, Neem

Background

Cabbage (*Brassica oleraceae* L. var. *capitata*) is an important vegetable crop grown in almost all parts of India. Cabbage butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae), is one of the major limiting factors of cabbage production in Kashmir valley, causing severe losses to the crop by larval feeding on leaves. Young larvae scrap the leaf surface whereas older larvae consume leaves from the margins inward leaving only the main veins. In

case of heavy infestation, the entire plants are eaten up. Since past few decades, chemical insecticides have been a general practice used by cabbage growers for the management of *P. brassicae* in Kashmir valley. But the adverse effect of chemicals on the environment such as ground water contamination, pesticide resistance, toxicity hazards, and destruction of biodiversity of useful natural enemies demand an effective alternative method of crop insect pest management that should be eco-friendly and safe for non-target organisms. Entomopathogenic nematodes (EPNs) that belong to families, Steinernematidae and Heterorhabditidae, are lethal parasites of insect pests, safe to humans, other vertebrates,

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non-target organisms, easy to apply, and cause no hazardous effect on the environment (Askary and Abd-Elgawad 2017). Besides, they are compatible with many chemical insecticides (Gupta 2003; Priya and Subramanian 2008; Laznik and Trdan 2014) and Neem (*Azadirachta indica*) products (Pezowicz et al. 1997; Mahmoud 2007) at lower doses and short-term exposures. Therefore, can be tank mixed for applying together in the field against target crop insect pests.

Keeping in view the role of EPNs in managing the insect pests, the present study was undertaken for the first time in Kashmir valley by using nematodes in combination with chemical insecticides, viz., Dichlorvos 76 EC (Emulsified Concentrate), Quinalphos 25 EC, and Neem product, viz., Azadirachtin against the target pest cabbage butterfly under field conditions.

Materials and methods

Rearing of rice moth, *Corcyra cephalonica*

In order to multiply EPNs, the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) was used as a host. The insect was reared on artificial diet under controlled atmospheric conditions at Biological Control Laboratory of the Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar campus, Srinagar. Maize was used as artificial diet to rear *C. cephalonica*. The crushed maize grain was sterilized in an oven at 100 °C for 30 min. Two kilograms of the sterilized crushed maize was filled in a wooden box (15 × 30 × 45 cm) and 2000 eggs of *C. cephalonica* obtained from Biological Control Laboratory were spread on the crushed maize kept in the wooden box. The box was fitted with perforated lid. The larvae were ready for use after 45 days. Some of *C. cephalonica* larvae were left in the box to complete their life cycle and for the emergence of rice moths. Adults were collected and transferred to separate oviposition containers for egg laying. The eggs thus were collected from oviposition containers were added fresh on an artificial diet prepared according to Patel (2011).

Preparation of entomopathogenic nematode culture

EPN *Heterorhabditis pakistanensis* used in the study was obtained from its clay powder formulation supplied by National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, India. It was maintained in the laboratory at Division of Entomology, SKUAST-K, Shalimar campus, Srinagar, India. The nematode was cultured in the last instar larvae of *C. cephalonica*. Hundred larvae were kept in 50 cm diameter Petri dish, lined with a filter paper, and inoculated with approximately 10,000 infective juveniles (IJs) of *H. pakistanensis* contained in 1 ml of sterilized distilled water. The Petri

dishes were placed in Bio-oxygen demand (BOD) incubator at 20 °C (Dutky et al. 1964). After incubation at 20 °C for 3 days, the infected *C. cephalonica* larvae were taken out from the Petri dishes and the cadavers were placed on white traps (White 1927). White trap was placed in BOD incubator at 20 °C. After 7-10 days, IJs moved from the *C. cephalonica* cadavers to the water reservoir of white trap. IJs were collected in a clean beaker, allowed to settle for 1 h, and the supernatant was decanted. The beaker was refilled by distilled water, and the process was repeated 3 times until a clean suspension was obtained.

Storage of the entomopathogenic nematode

The IJs collected were washed with 0.05% formaldehyde and stored in the sterile distilled water in tissue culture flasks in BOD at 10 °C. The nematode concentration was kept in the range of 10,000 IJs/ml of sterile distilled water. IJs were acclimatized at room temperature (22-25 °C) for an hour before use.

Maintenance of cabbage butterfly in laboratory

Larvae of the cabbage butterfly, *P. brassicae*, were collected from the cabbage growing fields at SKUAST-K, Srinagar. The larvae were brought to the laboratory, placed in rearing cages (20 cm × 12 cm) and maintained at room temperature. Fresh unsprayed leaves of cabbage were collected from the field for the rearing of larvae. The feed was changed at every 24 h and the cages were simultaneously cleaned thoroughly to prevent any contamination. The neonate larvae were allowed to develop by feeding on the cabbage leaves until they reach the full grown. The appropriate instar of *P. brassicae* was harvested and placed in sterilized plastic containers with a mesh for ventilation ready to be used in the bioassay. Dead *P. brassicae* larvae were removed from the rearing cage daily and the fecal pellets were cleaned with moist cotton wool.

Laboratory test of entomopathogenic nematode against cabbage butterfly

IJs of *H. pakistanensis* were evaluated against the larvae of *P. brassicae* but prior to the evaluation viability of the IJs were ascertained after examining them under a stereoscopic microscope. Only live and active IJs were considered for the experiment. Four different concentrations (50, 100, 150, and 200) of IJs/200 µl of distilled water were prepared. Bioassay was performed in 6-well plates. Each well was lined by a Whatman no. 1 filter paper. One surface sterilized *P. brassicae* was placed in each well of a single 6-well plate and required concentration of IJs was added into each well. For each instar larva of *P. brassicae*, one single 6-well plate was used. Thus, each treatment was replicated 6 times. After

placing the larva and adding IJs into it, the 6-well plates were covered by their respective lids, labeled and incubated into BOD at $20 \pm 2^\circ\text{C}$. While incubating, the 6 well plates were kept in plastic bags to conserve moisture. The plates were observed regularly upto 4 days for the mortality of *P. brassicae* larvae. The infected larvae were lightly rinsed in sterilized distill water and placed on a white trap for the emergence of IJs.

Compatibility test

This experiment was carried out to assess the survival ability of EPN, *H. pakistanensis* in combination with insecticides/neem product. Two chemical insecticides, viz., Dichlorvos 76 EC and Quinalphos 25 EC and one neem (*Azadirachta indica*) product, viz., Azadirachtin 50,000 ppm were used in the study. One hundred IJs were kept in combinations at 3 different concentrations of Dichlorvos 76 EC, Quinalphos 25 EC, and Azadirachtin 50,000 ppm, i.e., one at recommended concentration and 2 at lower than recommended concentrations. For each combination of IJs + insecticide/neem product, single 6-well plate was used. The percentage survival and mortality of IJs were assessed at 24, 48, and 72 h interval.

Field efficacy of entomopathogenic nematode against cabbage butterfly

The efficacy of *H. pakistanensis* was tested under field conditions against *P. brassicae* during 2017-2018 season. Cabbage cv. Golden Acre seedlings were obtained from vegetable nursery of SKUAST-K. Seedlings were 28 days old, and were transplanted at a farmer's field located at Ganderbal, Kashmir. The experiment was laid out with 5 treatments and 4 replications, following randomized block design (RBD). The size of each plot was 4 m^2 ($2 \times 2\text{ m}$). Spacing was maintained ($45\text{ cm} \times 45\text{ cm}$) among the rows and plants. Treatments were applied by the onset of insect incidence with the help of watering can provided with a nozzle having small holes to break up the stream of water into small droplets. The field temperature in the morning hours during nematode application was $22 \pm 2^\circ\text{C}$. The percentage of mortality of insect larvae was recorded at 24, 48, and 72 h. The dead insect larvae (cadavers) were collected on daily basis in order to check the pathogenicity of the cadavers by placing them on a white trap for the release of IJs.

Statistical analysis

Statistical analysis was performed by the SPSS (version 16.0) statistical software package. The percentage of insect mortality data were analyzed by multifactor ANOVA and means of all experiments were used to compare the efficacy of treatments ($P < 0.05$).

Results and discussion

Laboratory tests

Larval mortality was found negatively correlated with increase of IJs inoculum level of *H. pakistanensis*. In case of 50 IJs inoculum level, the mortality recorded at 45 and 48 h for 1st and 2nd instar larvae of *P. brassicae*, respectively, whereas inoculum level of 150 and 200 IJs were at par causing mortality at 24 h to 1st and 2nd instar larvae (Table 1).

Inoculum level of 200 IJs was the most effective rate that caused mortality to both 3rd and 4th instars larvae at 48 h. However, 50 and 100 IJs consumed maximum time causing mortality to 3rd instar larvae at 70 h and 4th instar larvae at 91 and 95 h, respectively. Thus, increase in inoculum level of IJs resulted in high larval mortality of *P. brassicae* at quicker rate than at lower inoculum level. It was also observed that the time consumed by IJs to kill the larva depends upon the size of the larva. At a given inoculum level, the 2nd and 3rd instar larvae were killed at a short interval of time than the 4th instar. Obtained results confirm the findings of Mantoo and Zaki (2014) who observed that with the increase in inoculum level of IJs, time consumed for larval mortality decreased but when the larval size increased the time consumed in larval mortality also increased. Similar findings were also reported by other research workers (Lalramliana 2009; Gorgadze et al. 2018).

Nematode production in larvae of cabbage butterfly

The nematode production was significantly affected by the size of *P. brassicae* larvae. The multiplication of nematode in different instars of insect cadavers at one particular inoculum level was significantly different ($P \leq 0.05$) from each other. The maximum emergence of IJs was recorded from 4th instar larva, followed by 3rd, 2nd, and 1st instars larvae (Fig. 1).

Nematode multiplication rate in insect cadaver was inversely proportional to the size of initial inoculum level of IJs. The multiplication rate was maximum by inoculum level of 50, followed by 100, 150 and 200 IJs. In case of 1st instar cadaver, the production of IJs was maximum (18, 340) at 50 IJs and minimum (12, 665) at 100 IJs inoculum level and both were significantly different ($P \leq 0.05$) from each other; however, there were not much difference in IJs production between inoculum level of 100 and 150 IJs and statistically not different from each other. IJs recovered from 3rd instar cadaver was maximum (1, 05, 328) with inoculum level of 50 IJs, followed by 100 IJs (98, 180), 150 IJs (94, 375) and 200 IJs (81, 140) and they were significantly different ($P \leq 0.05$) from each other. Similarly, IJs recovered from 4th instar cadaver was maximum by 50 IJs (1, 61, 672), followed by 100 IJs (1, 40, 118), 150 IJs (1, 38, 466), and

Table 1 Correlation between different concentration of infective juveniles (IJ_s) of *Heterorhabditis pakistanensis* and period of larval mortality of different instars of *Pieris brassicae* under laboratory conditions

Number of IJs/ larva	100% larval mortality (h)			
	1st instar larva	2nd instar larva	3rd instar larva	4th instar larva
50	45	48	70	95
100	41	44	70	91
150	24	24	66	70
200	24	24	48	48
<i>r</i> =	- 0.92*	- 0.93*	- 0.86*	- 0.96*

200 IJs (1, 27, 645) and they were statistically different from each other.

Thus, overall results indicated that nematode reproduction rate inside the insect cadaver was inversely proportional to the size of nematode initial inoculum level but directly proportional to the size of insect larva. The reproduction rate of nematode at lowest initial inoculum level was highest in an insect cadaver; however, progeny production increased with the increase in larval size. This confirms that when the food is plenty the reproduction capacity of nematode increases. Since the EPNs have a mutualistic association with its symbiotic bacteria, which lead to the conversion of insect host into a nutrient soup to which the nematodes feed (Askary 2010). Thus, the progeny production solely depends on the total nutrition available. In the large sized larvae, the total quantity of nutrient soup available for the nematodes is relatively more, which supports maximum production of nematodes and consequently no competition.

The findings confirm the report of Bulla Jr et al. (1975) that insect larvae in a later stage of bacterial infection are under greater nutritional stress than those in an earlier stage and that this may influence the production of nematode progeny. The increased production may be the result of bacterial infection initiating the breakdown of complex food storage molecules, allowing quicker utilization by the nematode and thereby contributing to enhanced nematode development (Mantoo 2011).

Compatibility of nematode with chemical insecticides/ neem product

The cumulative mean of highest survival percentage of IJs after 72 h was noticed at the combination of 100 IJs + Azadirachtin @ 0.125% (81.8%), followed by 100 IJs + Quinalphos 25 EC @ 0.0175% (80.9%), and 100 IJs + Dichlorvos 76 EC @ 0.025% (80.3%) (Table 2).

The cumulative mean of least survival percentage of IJs after 72 h was observed at 100 IJs + Dichlorvos 76 EC

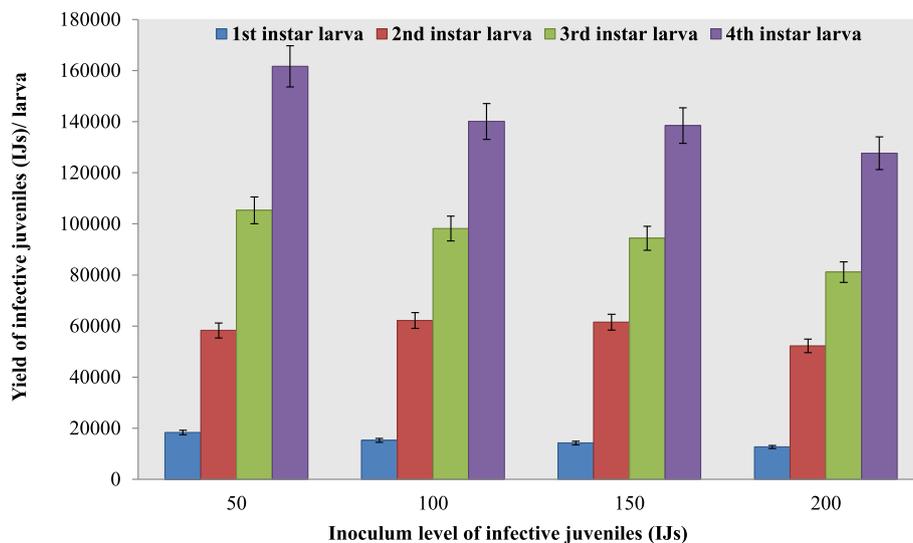


Fig. 1 Average yield of *Heterorhabditis pakistanensis* obtained from the cadavers of cabbage butterfly *Pieris brassicae* at different inoculum level under laboratory conditions

Table 2 Compatibility of infective juveniles (IJs) of *Heterorhabditis pakistanensis* with different insecticides and neem product under laboratory conditions

Treatments	Mean mortality (%) of IJs at different intervals (h)			Cumulative mean	
	24	48	72	% IJs mortality	% IJs survival
100 IJs + Quinalphos 25 EC @ 0.0175%	14.8 (21.1)*	20.5 (26.8)	22.0 (27.8)	19.1	80.9
100 IJs + Quinalphos 25 EC @ 0.035%	18.5 (25.4)	25.1 (30.0)	29.3 (32.7)	24.3	75.7
100 IJs + Quinalphos 25 EC @ 0.07%	30.0 (33.1)	40.3 (39.3)	47.6 (43.6)	39.3	60.7
100 IJs + Dichlorvos 76 EC @ 0.025%	15.0 (22.7)	20.8 (27.0)	23.3 (28.7)	19.7	80.3
100 IJs + Dichlorvos 76 EC @ 0.05%	20.1 (26.5)	31.0 (33.7)	37.1 (37.5)	29.4	70.6
100 IJs + Dichlorvos 76 EC @ 0.1%	34.8 (36.1)	50.3 (45.1)	61.1 (50.9)	48.7	51.3
100 IJs + Azadirachtin @ 0.125%	14.5 (22.3)	19.3 (26.0)	21.0 (27.1)	18.2	81.8
100 IJs + Azadirachtin @ 0.25%	16.3 (23.7)	23.6 (29.0)	27.8 (31.7)	22.5	77.5
100 IJs + Azadirachtin @ 0.5%	28.5 (32.1)	37.0 (37.4)	42.3 (40.5)	35.9	64.1
Untreated control (100 IJs only)	4.1 (11.6)	4.1 (11.6)	4.1 (11.6)	4.1	95.9

CD ($P \leq 0.05$): Treatment: 1.73; Duration: 0.94; Treatment \times Duration: 2.99; Design: CRD; Replication: six

*Figures in parenthesis are Arc sine transformed value of mean percent mortality

@ 0.1% (51.3%), followed by 100 IJs + Quinalphos 25 EC @ 0.07% (60.7%), and 100 IJs + Azadirachtin @ 0.5% (64.1%). The high survival percentage of IJs showed a high compatibility and this may be due to very less concentration of chemical insecticides/neem product and so caused least effect on nematodes, whereas the low survival percentage of IJs is an indication of incompatibility, which may be due to toxic effect of insecticide/neem product on nematodes. The combinations where the cumulative mean survival percentage of IJs was recorded around 70% were 100 IJs + Azadirachtin @ 0.25% (77.5%), 100 IJs + Quinalphos 25 EC @ 0.035% (75.7%), and 100 IJs + Dichlorvos 76 EC @ 0.05% (70.6%), which indicates the compatibility of nematodes with chemical insecticides/neem product (Table 2). The findings agree with the report of several other workers that at lower doses and short term exposure, EPNs are compatible with quinalphos (Karunakar et al. 2002; Easwaramoorthy and Sankaranarayanan 2003), dichlorvos (Zhang et al. 1994), and Azadirachtin (Ali et al. 2006).

Field efficacy of nematode against cabbage butterfly

H. pakistanensis (3.0 lakh IJs/m²) + Dichlorvos 76 EC (0.025%) resulted to the highest larval mortality rates at 24, 48, and 72 h, which was 69.36, 77.20, and 79.65%, respectively, followed by *H. pakistanensis* (3.0 lakh IJs/m²) + Azadirachtin @ 0.25%, 66.17, 74.26, and 76.22%, respectively, *H. pakistanensis* @ 3.0 lakh IJs/m² + Quinalphos 25 EC @ 0.035%, 64.48, 73.36, and 74.53%, respectively and the least with *H. pakistanensis* @ 3.0 lakh IJs/m² applied alone, where it was 52.42, 59.70, and 61.16%, respectively (Table 3). Among the treatments, larval mortality rate at 24 h interval was significantly different ($P \leq 0.05$) with the mortality resulted at 48 h. However, at 48 and 72 h, *H. pakistanensis* (3.0 lakh IJs/m²) + Azadirachtin @ 0.25% and *H. pakistanensis* (3.0 lakh IJs/m²) + Quinalphos 25 EC were not significantly different from each other.

The results are similar to the findings of Le Vieux and Malan (2015) who reported synergistic effects between EPN, *H. bacteriophora* and chemical insecticide, imidacloprid and the combination significantly increased the

Table 3 Efficacy of *Heterorhabditis pakistanensis* alone and in combination with insecticides and neem product against *Pieris brassicae* infesting cabbage under field condition

Treatments	Mean mortality (%) of <i>Pieris brassicae</i> larvae at different intervals (h)		
	24	48	72
<i>Heterorhabditis pakistanensis</i> @ 3.0 lakh IJs/m ²	52.42	59.70	61.16
<i>H. pakistanensis</i> @ 3.0 lakh IJs/m ² . + Quinalphos 25 EC @ 0.035%	64.48	73.36	74.53
<i>H. pakistanensis</i> @ 3.0 lakh IJs/m ² + Dichlorvos 76 EC @ 0.025%	69.36	77.20	79.65
<i>H. pakistanensis</i> @ 3.0 lakh IJs/m ² + Azadirachtin @ 0.25%	66.17	74.26	76.22
Untreated control	3.30	4.0	4.0

CD ($P \leq 0.05$) Treatment: 2.45; Duration: NS (non-significant); Treatment \times Duration: 4.25; Variety: Golden Acre; Plot size: 2 \times 2 m; Design: RBD; Replication: four

Table 4 Effect on yield of cabbage after application of *Heterorhabditis pakistanensis* alone and in combination with insecticides and neem product against *Pieris brassicae* infesting cabbage under field conditions

Treatments	Yield of cabbage (q/4 m ² plot)	Yield of cabbage (q/hect)	% increase in yield over untreated control
<i>Heterorhabditis pakistanensis</i> @ 3.0 lakh IJs/m ²	0.081	202.5	15.71
<i>H. pakistanensis</i> @ 3.0 lakh IJs/m ² + Quinalphos 25 EC @ 0.035%	0.086	215.0	22.85
<i>H. pakistanensis</i> @ 3.0 lakh IJs/m ² + Dichlorvos 76 EC @ 0.025%	0.092	230.0	31.42
<i>H. pakistanensis</i> @ 3.0 lakh IJs/m ² + Azadirachtin @ 0.25%	0.089	222.5	27.14
Untreated control	0.070	175.0	
CD ($P \leq 0.05$)	0.004	7.37	

pathogenicity of *H. bacteriophora* against vine mealy bug, *Planococcus ficus*, in South African vineyards. Sabry et al. (2016) also reported that *S. carpocapsae* All in combination with the half recommended dose of chemical insecticides was much effective against tomato leaf miner, *Tuta absoluta*, than the nematode applied alone.

Cabbage yield

The yield of cabbage was recorded highest (0.092 q (quintal)/4 m²) in the plot treated with *H. pakistanensis* (3.0 lakh IJs/m²) + Dichlorvos 76 EC (0.025%) and it was significantly different ($P \leq 0.05$) from the treatment *H. pakistanensis* @ 3.0 lakh IJs/m² + Quinalphos 25 EC @ 0.035% (0.086 q/4 m²) and *H. pakistanensis* @ 3.0 lakh IJs/m² (0.081 q/4 m²) used alone but not significantly different from *H. pakistanensis* (3.0 lakh IJs/m²) + Azadirachtin @ 0.25% (0.089 q/4 m²) (Table 4).

The percentage increase in cabbage yield/plot over untreated plot was highest (23.91%) with the treatment with *H. pakistanensis* (3.0 lakh IJs/m²) + Dichlorvos 76 EC (0.025%) and it was closely followed by *H. pakistanensis* (3.0 lakh IJs/m²) + Azadirachtin @ 0.25% (21.34%), *H. pakistanensis* @ 3.0 lakh IJs/m² + Quinalphos 25 EC @ 0.035% (18.60%), and *H. pakistanensis* @ 3.0 lakh IJs/m² alone (13.58%) (Table 4). Similar results were found by Saba (2019) who prepared 3 combinations of EPNs and chemical insecticide, i.e., *H. bacteriophora* MK256378 + Imidacloprid 17.8 SL, *H. bacteriophora* MK256378 + Dimethoate 30 EC, and *H. bacteriophora* MK256378 + Chlorpyrifos 20 EC and applied against diamondback moth, *Plutella xylostella*, infesting cabbage in field. The results indicated significant higher mortality rates of insect larvae where nematode was applied in combination with chemical insecticides than where the nematode was applied alone.

Conclusion

The EPN, *H. pakistanensis*, appeared as a promising bio-control agent against *P. brassicae* and exhibited good

compatibility with the chemical insecticides. The combined applications of EPN + insecticides resulted in significant high larval mortality rates of *P. brassicae* and higher crop yield than the treatment with the nematode alone. Multi-locational field trials in farmers' fields should be conducted prior to the implementation of this IPM module.

Abbreviations

NBAIR: National Bureau of Agricultural Insect Resources; SKUAST-K: Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir; EC: Emulsified concentrate; IJs: Infective juveniles; EPN: Entomopathogenic nematode; BOD: Bio-oxygen demand; RBD: Randomized block design

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Authors' contributions

The first author, THA carried out the experiment and analyzed the data and was the major contributor. The second author, MJA reared the *Corcyra cephalonica* larvae for the multiplication of entomopathogenic nematodes and also made available the formulation of *Heterorhabditis pakistanensis* for the experiment. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

We, the authors do not have competing interests.

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