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Infectivity of entomopathogenic nematode against the cabbage butterfly (*Pieris brassicae* L.) in polyhouse and in field condition

Preety Tomar¹, Neelam Thakur^{1*}  and Ambika Sharma²

Abstract

Background: Cabbage butterfly, *Pieris brassicae* Linnaeus (Lepidoptera: Pieridae), is a major insect pest affecting cole crops worldwide. Excessive applications of chemical-based insecticides have a devastating impression over the organisms and environment.

Results: In this study, entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* Poinar strain EUPT-S26 (local isolate) was evaluated for *Pieris brassicae* control under polyhouse and field conditions. Under the polyhouse conditions, the highest insect mortality 91.6 and 94.0% was observed in the plots treated with the nematodes suspension 1500 IJs/ml and 2000 IJs/ml, respectively. Based on the highest cabbage plant protection under polyhouse conditions, *H. bacteriophora* EUPT-S26 was also applied for field assays in the course of the crop's productive phase. Data demonstrated from the field treatments signify the highest concentration (2000 IJs/ml) showed the maximum larval mortality and least damage percentage $45 \pm 1.07\%$ that remained constant until harvesting; this resulted in the highest productivity in polyhouse and under field conditions.

Conclusion: According to assessed field conditions, it was suggested to perform 3 applications of EPNs during the vegetative phase and at the time of head formation to increase productivity and to reduce damage. The results approved that EPNs are an effective alternative of chemical-based insecticides to control the cabbage butterfly.

Keywords: Biocontrol, Damage, *Heterorhabditis bacteriophora*, *Pieris brassicae*, Productivity

Background

Pieris brassicae Linnaeus of family Pieridae is the key insect pests of cabbage. It generates significant losses as compared to costs of synthetic chemical insecticides for its control (Kasi et al. 2021). Cabbage butterfly is dispersed all over the world where brassicaceae are planted. Winter generations showed a longer life stages than those through the rainy and summer seasons. Temperature range (15.2–30 °C) was found ideal for its multiplication. Other different abiotic factors did not affect the life stages of insect (Thakur and Deka 1997). The gregariously

feeding insect larvae consumed the whole leaves, except veins of the cauliflower, cabbage and flower buds of broccoli (Boczek and Lewandowski 2016). Over 50% yield losses have been observed due to the infestation by these insect pests in cabbage annually (Abbas et al. 2021). Sood (2007) reported that cabbage butterfly caused extensive damage to the cabbage crop in the month of April (last week) and May in Himachal Pradesh, India.

Although the chemical synthetic insecticides has been proven most trustworthy for the control of these voraciously feeding larvae, the repercussions of the persistent use of these synthetic insecticides result in various environmental hazards, especially the chemical residues also affect the soil fertility, affects the wildlife, contaminate resources of ground water, developed resistance amongst insects along with re-emergence of huge insect

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population along with minor insect invasion (Thakur et al. 2021). At present, a huge amount of micro-biome-based biopesticides were developed from bacteria, fungi, protozoan and nematodes and are used against insect pests worldwide (Kumar and Singh 2015). EPNs application against cabbage butterfly is a biological management strategy (Abbas et al. 2021).

Earlier a laboratory experiment showed mortality 67–72% triggered by *Steinernema feltiae* and *H. bacteriophora* in 2nd larval instar of *P. brassicae* (Kumar et al. 2021). In field, mortality rates up to 100% have been achieved by using *H. bacteriophora* together with the *Steinernema glaseri* (Rhabditida: Heterorhabditidae) (Abbas et al. 2021). These nematodes were recognized as very successful biopesticides against several insect pests' species belonging to the order; Diptera, Isoptera, Lepidoptera, Hemiptera, Coleoptera and Hymenoptera (Labaude and Griffin 2018). EPNs are also compatible (tank-mixed) with other chemical fungicides, herbicides and insecticides (also with fungal or bacterial products) (Gupta et al. 2009). Nematodes biocontrol agents (BCAs) are also compatible with *Azadirachta indica* (Neem) products (Mahmoud et al. 2007) at lesser doses and at short-term exposures. Application of entomopathogens also encourages sustainable agriculture (Singh et al. 2020). The foliar application of these BCAs in field do not show any detrimental impact on the applicator and consumer (Damalas and Koutroubas 2018). To evaluate the role of EPNs in managing the population of insect pests, the present study on the application of *H. bacteriophora* EUPT-S26 was evaluated against *P. brassicae* in cabbage at polyhouse and under field conditions.

Methods

Biological material

The nematodes (*H. bacteriophora* EUPT-S26) were procured from rhizospheric soil of fruit orchards of district Sirmour of Himachal Pradesh, India, via soil baiting technique. The isolated nematodes were identified as *H. bacteriophora* EUPT-S26 on the basis of morphological observations and identification keys. To attain pathogenic and viable infective juveniles (IJs), they were replicated through in vivo using last instar of *Galleria mellonella* L. (Lepidoptera: Pyralidae) via larvae contagion (Kaya and Stock 1997). Infective juveniles (IJs) were kept at 15 °C in sterilized sponge foams. The seeds of *Brassica oleraceae* (Brassicales: Brassicaceae) were purchased from local market and grown at polyhouse to establish breeding stock at the University biological control polyhouse (average temperature: 17–20 °C; average relative humidity: 75–81%; light regime: 12 h light/12 h dark). Cabbage plants after 27 days of post-germination were transplanted into the plastic pot (20 × 20 × 14 cm), filled

with rice husk and soil. Watering was done daily and plants were fertilized with Agrobium's® (NPK-8:25:25, 2 g/plant). Initiation of breeding stock of *P. brassicae* was maintained by transferring the *P. brassicae* larvae on cabbage plant (28 days from seedling stage) 5 larvae/plant, which were then placed in entomological cages (40 cm³) for confinement. The *P. brassicae* life cycle lasted approximately 27 days on average under polyhouse conditions.

Polyhouse phase

Heterorhabditis bacteriophora EUPT-S₂₆ application

Pieris brassicae susceptibility to *H. bacteriophora* EUPT-S26 infection was determined by conducting an experiment on cabbage plants at different vegetative stages. The experiment was conducted under the polyhouse (44 × 36 m) where the area of the experiment was conducted in area of 22 × 11 m, further divided into 2 blocks containing 13 plots on each side (2 m²). The cabbage plants were transplanted at 12 plants per plot with a planting distance of 16 cm. To this laboratory reared, 4th instar larvae were stationed in each plant (5 larvae/plant) along with 260 *P. brassicae* adults, 8 h before the test (experiment). Different treatments containing IJs were suspended in the distilled water with the 0.3% Polysorbate 20. These suspensions with varied juvenile concentrations were sprayed via pressure sprayer over the cabbage leaves (abaxial and adaxial). The result variables were the leaf damage percentage and larvae mortality percentage were assessed at every 24 h for the nine days. Plant damage level was mainly evaluated through graphical method by using scale with 4 levels, namely 1, 2, 3 and 4 with differences in degrees of infestation ranging from 1 to 10%: level 1, 10–40%; 2, 40–70%; 3 and 70–100%: level 4 (Carballo et al. 1989).

Insect pest mortality was determined by yellow-ochre cadavers when placed into the white traps up to 9 days to authenticate the presence of IJs (Kaya and Stock 1997). A completely randomized experimental design was implemented, where nematodes dose factor had 5 levels, IJs (0, 500, 1000, 1500 and 2000 IJs/ml of water). For statistical analyses of damage progress, area under the patches was observed. Results' variable such as damage percentage of plants and mortality percentage in larvae was calculated. Statistical differences were determined by ANOVA using OPSTAT software. The experiment was repeated twice for the 2 year during the cropping season along with 5 replicates.

Field phase

Heterorhabditis bacteriophora EUPT-S26 treatment

Field phase was carried out in the agricultural farm, located at (30.7537° N, 77.2965° E, 1900 m altitude), having mean temperature between 15 and 20 °C and a RH

between 50 to 64%. For planting a 100 m² area was pre-fabricated first, soil was allowed to recover strength and then plowing was done. The planting area was fertilized with Agrobium's NPK (8:25:25). The experimental area was then partitioned into 2 equal blocks (50 m²). Each block was distributed into the 13 plots (3 m²). In the each plot, 26-day post-germinated cabbage plants (16 plants/plot) were transplanted with a planting distance of 18 cm. After 3–4 weeks of cabbage transplantation, 780 pupae and 260 *P. brassicae* adults were released in the entire field, homogeneously spreading 390 pupae and 130 adults per block. Treatments of nematode juvenile mixed with 0.3% Polysorbate 20 were applied in each plot along with complete control.

Throughout the crop growth or development, 3 applications of EPNs were used, according to the variation in the population dynamic of *P. brassicae* and cabbage plant's phenological stage. The 1st application was applied 1 week of post-inoculation of insects containing 5 larvae/ plant at vegetative stage. The 2nd application was sprayed 1 week after the first application (4 larvae/ plant) also at vegetative stage. At the end, the 3rd application was applied 1 week after the 2nd application (during reproductive stage at the completion of head formation). These applications were performed throughout the crop cycle up to harvesting according to *P. brassicae* population fluctuation.

A completely randomized block experiment was carried out where the result variables, i.e., productivity and damage percentage, were calculated. Percentage of damage was determined according to formula (Carballo et al. 1989) scales. Data were obtained weekly from the time of pest released; the damaged area was also calculated to examine progress and consequently performed statistical analyses. Insect mortality and cabbage productivity was also noted.

Statistical analyses

For statistical analyses, the used treatments were congregated to perform one-way ANOVA. Each 5 treatments included absolute controls. The treatments correspond to the 4 applications of *H. bacteriophora* EUPT-S26 along with absolute control. Pearson's correlation analysis among damage percentage and productivity were also calculated.

Results

Polyhouse phase

Heterorhabditis bacteriophora EUPT-S26 application

Cabbage plants treated with *H. bacteriophora* EUPT-S26 IJs revealed a significant decline in the damage percentage than the non-treated plants ($F=3.4$, $DF=4$, $P<0.05$). The minimum damage (injury) percentage was noticed

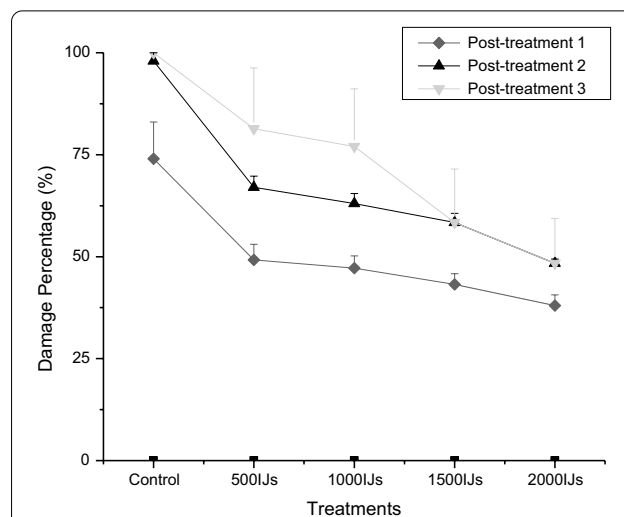


Fig. 1 Leaf damage percentage by *Pieris brassicae* larvae after three constitutive foliar applications of entomopathogenic nematodes under greenhouse conditions

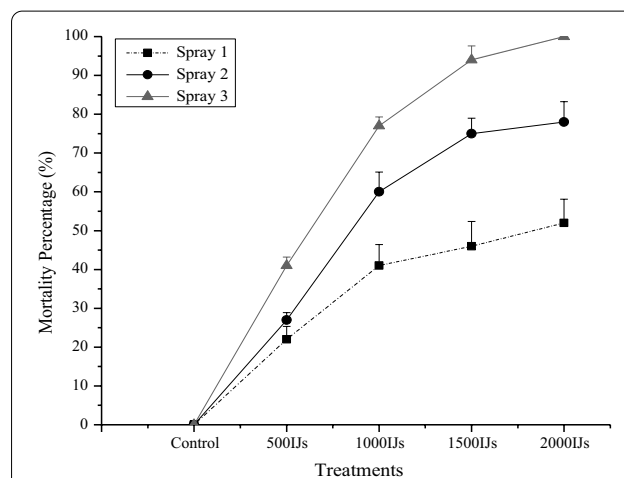


Fig. 2 Mortality percentage in *Pieris brassicae* larvae after three constitutive foliar applications of entomopathogenic nematodes under greenhouse conditions

at 1500 and 2000 IJs/ml concentrations (Figs. 1 and 5). The damage percentage were also high (58.4 and 48.4%, respectively, at 5-day post-application) after 1st spray, which was reduced after 2nd spray and infestation level was very low after 3rd spray. A significant control result was detected at infestation evaluated level 5 larvae/plant, beyond economic threshold level established for *P. brassicae* (1 larva/plant). Larvae mortality was also demonstrated a considerable difference amongst the IJs applied concentrations ($F=131.21$, $DF=4$, $P<0.05$) with the mortality percentage 38.0, 76.4, 91.6 and 94.0% at 5-day post-nematodes application after the 3rd spray (Fig. 2). In

this first period of evaluation under the polyhouse conditions, it was determined that *H. bacteriophora* 2000 IJs/ml concentrations produced the highest protection to cabbage plants. The larval mortality by *H. bacteriophora* EUPT-S26 was confirmed by collecting the dead cadavers from the polyhouse and kept into the laboratory using the white trap. The emergence of nematode juvenile was observed.

Field phase

Application of *H. bacteriophora* EUPT-S26

Application of *H. bacteriophora* EUPT-S₂₆ presented significant variances in the percent damage ($F=3.2$, $DF=4$, $P<0.05$) (Figs. 3 and 5). Latterly treatments detected damage percentage maximum in control (100%). In the lowest concentrations of EPNs, damage percentage ranged from $84 \pm 1.07\%$ in the treatment concentrations of 500 IJs/ml and $70 \pm 1.07\%$ in concentrations of 1000 IJs/ml after 3rd application of nematodes. Although mortality data were recorded after each spray, the mortality percentage was quite low in the initial application. Maximum larval control was observed after 3rd application of EPNs. At the highest concentration, the damage percentage was $45 \pm 1.07\%$ retained constant until harvesting, established the biological pest control by EPNs. For the confirmation of larval mortality by *H. bacteriophora* EUPT-S26, the dead cadavers were brought to the laboratory and kept over the white trap. The emergence of nematode juvenile was recorded.

Regarding the yield response variable, characterized by cabbage head weight was observed the highest in *H. bacteriophora* EUPT-S26 (2000 IJs/ml) with high

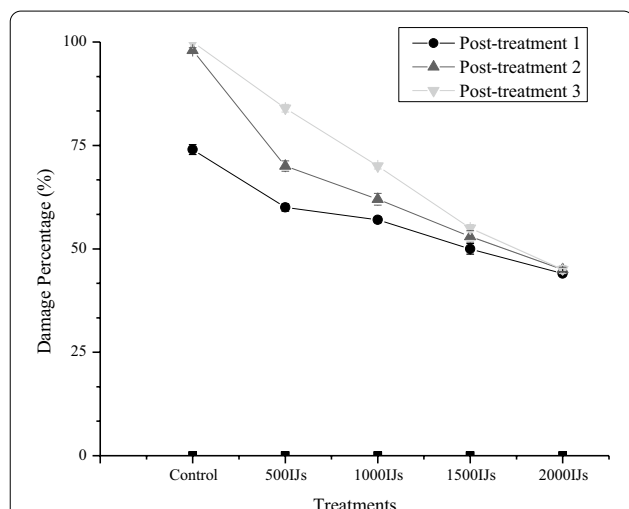


Fig. 3 Leaf damage percentage by *Pieris brassicae* larvae after three constitutive foliar applications of entomopathogenic nematodes under field conditions

productivity values in treatments with the 3 applications (Fig. 4). The data of productivity in different concentrations (2000 IJs/ml) showed a cabbage head weight between 320 and 410 g (Fig. 4). Regarding damage percentage at harvesting time, it was revealed that the lowest damage percentage (52.7–44%) correspond to the treatment concentrations of (1500 IJs/ml and 2000 IJs/ml), which were significantly differ than control (100%). Additionally, a negative correlation was also observed among the damage percentage and productivity. In this investigation correlation coefficient was reported high but were statistical significant (Pearson correlation, $r=0.98$ for productivity and $r=0.92$ for damage) that suggested foliage consumption by *P. brassicae* showed effect on the head formation (Fig. 5).

Discussion

Cruciferous or Brassicaceous crops like cabbage are extensively dispersed in India, signifying an economically important source to the farmers. Quality and productivity limitations are mostly due to *P. brassicae* cabbage butterfly insect pest. *P. brassicae* resistant to several chemical insecticides, predominant in all regions, where cabbage is planted. The absence of the other biocontrol agents of cabbage crops allows cabbage butterfly for promptly establishment in the producing regions. Moreover, cabbage butterfly migrates to the new areas that increased its reproductive potential. Due to the great threat *P. brassicae* represents in India's cruciferous crops; curiosity has developed on adopting friendly alternative for the control of insect pest. In biocontrol agents by employing the natural enemies, including EPNs, whose application resulted in the reduction in larvae plant damage and reflected in the better productivity per hectare.

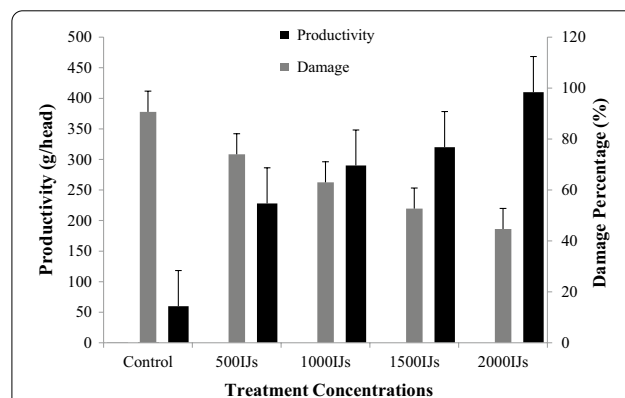


Fig. 4 Damage percentage and cabbage productivity after three applications of *Heterorhabditis bacteriophora* EUPT-S26 against *Pieris brassicae* under field conditions



Fig. 5 Application of *Heterorhabditis bacteriophora* against *Pieris brassicae* in the laboratory and field condition **a** *H. bacteriophora* population; **b** Mass multiplication in the laboratory; **c** Multiplied nematodes; **d, e** Stored nematodes; **f** *P. brassicae* larvae; **g, h** *P. brassicae* larvae infesting cabbage; **i** *P. brassicae* pupae; **j** Polyhouse trial; **k** Field trial; **l** application of *H. bacteriophora*; **m, n** Dead cadavers of *P. brassicae* collected from the polyhouse and field

Treatment time required by *H. bacteriophora* EUP-T-S26 to generate mortality in cabbage butterfly larvae, reducing cabbage damage percentage may be because of its foliar applications. In the present study, it is probably

IJs mobility and viability was abridged due to drying of leaves, temperature fluctuations and low relative humidity (Bueno-Pallero et al. 2018). This data showed the damage percentage reduced with concentration acceleration

and the mortality percentage not resulted in any significant differences as the concentration increase. The possibility of searching a host increased as the contact of IJs over leaf surface increases (Correa-Cuadros et al. 2014). Similarly, IJs were applied in the 5:00–5:30 pm that allowed greater time to establish them over leaf surface and contributed in maximum larvae infection. In contrast, at morning or noon, leaves dry out. Furthermore, adjuvant usage favors the IJs mobility, also increasing area of contact and its adherence to the leaf surface (Mahmoud 2014). The highest *P. brassicae* mortality percentage was noticed by *H. bacteriophora* at 2000 IJs/ml concentration (Abbas et al. 2021). At this time, used surfactant prevents the evaporation and facilitating IJs to penetrate into insect cuticle. The above-mentioned is the acceptable clarification how at low concentration it can generate a high mortality percentage in cabbage butterfly under polyhouse conditions.

Pest management using *H. bacteriophora* EUPT-S26 was done with the infestation level (5 larvae/plant) that surpasses economic threshold reported earlier (1 larva/plant) (Londoño 2001). Cabbage butterfly control would begin from vegetative phase onward to elude the infestation levels 5 larvae/plant during primordium formation (flower) and starting of head formation, as foliage damage on these times indirectly disturbs the head formation and development (Mpumi et al. 2020). Schroer and Ehlers (2005) recorded that the highest attack by *P. brassicae* was done between 1 and 5 h of post-spray. Gupta et al. (2009) assessed the effectiveness of *H. indica*, *S. carpocapsae* strain PDBC and strain JMU against *P. brassicae* in field conditions. They reported that *S. carpocapsae* (JMU) @ 2 billion IJs/ha resulted in the highest mortality. The recorded larval mortality was 7.5% after first day of application that further increased and reached to 41.8% after 13 days of post-application. Leaf damage by pests alters the plant photosynthetic rate that initiating an imbalance between plant respiration and photosynthesis, reduced photosynthetic synthesis compulsory for the new organ formation plus development as head (Martínez-Jaime et al. 2016). Cabbage butterfly controlled by EPNs' applications permitted complementarily between the biocontrol agents at the field environments to cause infections, where diverse the theory can be concluded. Nematode's IJs penetrations to the insect larvae suppressed the immune system of insect and liberated their bacterial symbiont. Immune response by host might not be distinguished the bacterial infection and speedily leading to the septicaemia (Askary and Ahmad 2020).

Under field conditions, nematodes IJs application enhanced pest mortality resulted in lightened percent damage than the control, as short-term use of EPNs resulted in a rapid action against pest control. The

utilization of EPNs, as a biological control agent, functioned in a complimentary and successful manner and they had the capability of potentiating the larval mortality to decrease plant damages (Gozel and Gozel 2016). Wang and Li (1987) also assessed the *Steinernema* spp. in the conditions against cabbage butterfly and recorded 90% larval mortality after 15 hrs of nematode application. Abbas et al. (2022) applied *H. bacteriophora*, *S. glaseri*, combination of *H. bacteriophora* + *S. glaseri*, at the rate of 1500 infective juvenile/ml, *Xenorhabdus* spp., and *Photorhabdus* spp. at the rate of 4×10^{12} CFU, against cabbage butterfly in the greenhouse and field conditions. They recorded the maximum 90.43% larval mortality in the combined treatment of EPNs after 7 days of application.

Conclusions

The present study included foliar applications of locally isolated bioagent, EPNs strain (*H. bacteriophora* EUPT-S26) under the polyhouse and field conditions against *P. brassicae* larvae. The results demonstrated that the application of *H. bacteriophora* EUPT-S26 resulted in less damage percentage throughout the cabbage's phenological phases and the most crop productivity was obtained. This suggested nematodes as an effective bio-insecticide against *P. brassicae*. Therefore, to decrease the damage percentage and to enhance the productivity in assessed field environments, it is better to perform the applications of nematode (EPNs 2000IJs/ml). The nematode applications at the developmental stages of cabbage are an efficient alternative to chemical insecticides, in order to obtain significant cabbage butterfly control. By this way, farmers should have an effective and eco-friendly strategy that may be incorporated in cabbage integrated pest control management also.

Abbreviations

H. bacteriophora: *Heterorhabditis bacteriophora*; *P. brassicae*: *Pieris brassicae*; IJs: Infective juveniles; EPNs: Entomopathogenic nematodes; BCAs: Biocontrol agents; %: Percent.

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Author contributions

NT gave the concept. PT performed the experiment, wrote the manuscript and did the statistical analysis. AS looked into the language part mainly grammar section of the manuscript.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Competing interest

The authors declare that there is no conflict of interest.

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