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# Management of cotton bollworms *Helicoverpa armigera* and *Earias vittella* by entomopathogenic nematodes

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## Abstract

**Background:** The bollworm complex consisting of *Helicoverpa armigera* and *Earias vittella* is a major threat in cotton production globally. The habit of developing resistance to many insecticides including *Bt* transgenic cotton necessitates the exploration of an alternate strategy to manage bollworms. The entomopathogenic nematodes (EPN) *Steinernema carpocapsae* strain APKS2 and *Heterorhabditis bacteriophora* strains KKMH1 and TRYH1 at different concentrations of  $1 \times 10^9$  infective juveniles (IJs)·hm<sup>-2</sup>,  $2 \times 10^9$  IJs·hm<sup>-2</sup>, and  $3 \times 10^9$  IJs·hm<sup>-2</sup> in 500 L of water were evaluated as a foliar spray in fields naturally infested with *H. armigera* and *E. vittella* located at Eastern Block and Cotton Research Farm of Tamil Nadu Agricultural University, Coimbatore, India during October 2010–February 2011 and October 2011–February 2012, respectively.

**Results:** In general, all three tested EPN strains reduced the larval population of *H. armigera* and *E. vittella*; reduced square and boll damage; and subsequently increased cotton yield compared with the untreated control. The *S. carpocapsae* APKS2 is most effective against *H. armigera* whereas both *S. carpocapsae* APKS2 and *H. bacteriophora* KKMH1 were equally effective against *E. vittella*. The higher dose of  $3 \times 10^9$  IJs·hm<sup>-2</sup> was highly significant in the reduction of *H. armigera* larvae. However, the doses  $2 \times 10^9$  IJs·hm<sup>-2</sup> and  $3 \times 10^9$  IJs·hm<sup>-2</sup> were equally effective for *E. vittella* control. The *S. carpocapsae* APKS2 at  $3 \times 10^9$  IJs·hm<sup>-2</sup> caused a 62.2% reduction of *H. armigera* larvae, 34% reduction of square damage, 58.5% reduction of boll damage, and yielded 45.5% more seed cotton than the untreated control plots. In *E. vittella* infested field, *S. carpocapsae* strain APKS2 and *H. bacteriophora* strain KKMH1 at  $2 \times 10^9$  IJs·hm<sup>-2</sup> resulted in 60.6%–62.4% larva reduction, 68.4%–70.7% square damage reduction, 66.6%–69.9% boll damage reduction and 45.9% yield increase over the untreated control. The effective EPN treatments were comparable to the chemical insecticide chlorpyrifos 20% emulsifiable concentrate spraying at 2 mL·L<sup>-1</sup>.

**Conclusions:** This study has shown that EPN have great potential in the management of the bollworm complex in cotton. Foliar spraying EPN strain *S. carpocapsae* (APKS2) at  $3 \times 10^9$  IJs·hm<sup>-2</sup> and *S. carpocapsae* (APKS2) or *H. bacteriophora* (KKMH1) at  $2 \times 10^9$  IJs·hm<sup>-2</sup> five times at 10 days intervals are the best for the management of *H. armigera* and *E. vittella*, respectively.

**Keywords:** Bollworms management, Cotton, EPN, Foliar application, *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*

## Background

Cotton (*Gossypium hirsutum* L.), popularly known as "white gold", is a natural fiber crop grown in more than 70 countries in the world (Nikam et al. 2017). It is a well-suited crop for both irrigated and dryland production

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systems. The prime cotton-producing countries are China, India, the USA, Pakistan, Uzbekistan, Turkey, Australia, Greece, Brazil, and Egypt, with total coverage of 34 million hectares (Khadi et al. 2010). Lint is the economic product from cotton plants, and serves as a high-quality fiber for textile manufacturing. Cotton seeds, the by-product from the lint industry, are used for making edible oil and protein-rich livestock feed. One prime challenge to attain high cotton production is the damage caused by insect pests. The nature of the cotton plant such as its succulent leaves, nectarines and fruit abundance is more attractive to many injurious species of insects. The pest spectrum of cotton is wide, and as many as 200 species of insects have been described to feed on cotton throughout the plant's life span (from germination to harvest). Among them, American bollworm *Helicoverpa armigera* Hüb. (Lepidoptera: Noctuidae) and spotted bollworm *Earias vittella* Fab. (Lepidoptera: Noctuidae) are the most important pests affecting the cotton plants from flowering to the fruiting period (Dhaliwal et al. 2010).

*H. armigera* is the major threat to cotton production in countries of central and southern Europe, China, India, Pakistan, Nepal, Bangladesh, Africa, Australia, and New Zealand (Karim 2000). It is a polyphagous feeder and attacks corn, sorghum, soybean, pigeon pea, chickpea, groundnut, tomato, vegetables, and fruits. Outbreaks of *H. armigera* may cause extreme foliage damage (Dastjerdi et al. 2008). The early instars of the larvae feed on foliage and late instars on squares and bolls. The larvae feeding on squares and bolls causes symptoms of regular circular boreholes with faecal pellets outside the holes. This insect has a high reproductive potential, facultative diapause and a tendency to develop resistance to many chemical pesticides (Razmjou et al. 2014). It causes an estimated annual loss of 2 billion US dollars in different crops around the globe (Paramasiva et al. 2014). The spotted bollworm *E. vittella* is also one of the serious pests of cotton in countries/regions like India, Pakistan, China, South East Asia, Africa, Iraq, Israel, and Australia (Gajmer et al. 2002). The caterpillars start feeding in the vegetative stage and the affected plants show the symptoms of wither, drop and drying of shoots. Later, the caterpillars feed inside the buds, flowers and fruits, which cause premature opening and shedding of squares and young bolls. It leads to loss of quality and quantity of cotton. The heavy infestation of *E. vittella* could cause up to 50% cotton yield loss (Ahmad and Iqbal Arif 2009).

Synthetic pyrethroids and organophosphate insecticides have been used to manage bollworms during the past two decades. However, the chemical control has failed as the cotton bollworms have developed resistance to many insecticide groups (Tabashnik and Carrière

2019). Chemical control was also a concern due to the health risk of the applicator, devastation of natural enemies, the resurgence of minor pests and environmental pollution (Seenivasan and Murugan 2011; Akhtar and Farooq 2019). In recent years, the focus has been on the use of genetically modified *Bt* (*Bacillus thuringiensis*) cotton. *Bt* cotton has been proven to be effective against bollworms. However, resistance to the insecticidal crystal proteins of *B. thuringiensis* in cotton bollworms has been documented (Akhurst et al. 2003; Wu et al. 2004) and the sustainable use of *Bt* cotton for bollworm management is becoming highly questionable. In this situation, the introduction of an alternate bio-control agent for cotton bollworm management will be a promising approach.

In recent years, entomopathogenic nematodes (EPN) *Steinernema* spp. (Steinernerematidae) and *Heterorhabditis* spp. (Heterorhabditidae) are emerging as a potential biological control agent for the management of insect pests. They have symbiotic bacteria *Xenorhabdus* (Steinernerematidae) and *Photorhabdus* (Heterorhabditidae) in their gut and release the bacteria into the insect haemocoel after invasion. Most biocontrol agents require days or weeks to kill the pest, but entomopathogenic nematodes with their symbiotic bacteria can kill insects in 12~24 h. EPN also have many desirable attributes such as high reproduction potential, easy to mass produce, safety to humans and other invertebrates, ability to reach insects in cryptic habitats, compatible with many insecticides, easy to deliver through the irrigation system to manage soil insects or as a spray for foliar pests (Koppenhofer et al. 2000). EPN are used to control a wide variety of economically important insect pests (Shapiro-Ilan et al. 2006). Many cotton pests like bollworm *Helicoverpa zea* (Boddie), fall armyworm *Spodoptera frugiperda* (Smith), beet armyworm *Spodoptera exigua* (Hübner), cabbage looper *Trichoplusia ni* (Hübner), tobacco budworm *Heliothis virescens* (Fabricius) and the pink bollworm *Pectinophora gossypiella* (Saunders) are susceptible to entomopathogenic nematodes (Gaugler 2001). The infectivity of EPN species *Steinernema carpocapsae* (Weiser), *Steinernema riobrave* (Cabanillas and Poinar) and *Steinernema feltiae* (Weiser) on *H. armigera* and *Earias insulana* was established earlier (Glazer 1997). Gassmann et al. (2006) demonstrated the successful field control of pink bollworm using an EPN species *S. riobrave* on cotton. However, the information on field control of the American bollworm *H. armigera* and spotted bollworm *E. vittella* by EPN is scanty. Preliminarily, 27 strains of EPN comprising of 16 *S. carpocapsae*, 3 *Steinernema siamkayai* (Stock, Somsok and Reid), 1 *Steinernema monticolum* (Stock, Choo and Kaya) and 7 *Heterorhabditis bacteriophora* (Poinar) strains were isolated from the cotton ecosystem (Seenivasan et al. 2012; Seenivasan and Sivakumar 2012).

Among them, the strains of KKMH1 (*H. bacteriophora*), APKS2 (*S. carpocapsae*), and TRYH1 (*H. bacteriophora*) showed the advantages such as more virulence against *H. armigera* and *E. vittella*, high reproduction potential, tolerance of heat and desiccation under laboratory conditions (Seenivasan and Sivakumar 2014; Nagachandrabose 2021). The objective of this study is to test the bio-efficacy of EPN strains stored at the Department of Nematology, Tamil Nadu Agricultural University, India such as KKMH1 (*H. bacteriophora*), APKS2 (*S. carpocapsae*), and TRYH1 (*H. bacteriophora*) against *H. armigera* and *E. vittella* under field conditions.

**Materials and methods**

**Experimental sites**

Two field experiments were conducted during 2010–2012 at Tamil Nadu Agricultural University, Coimbatore, India. Experiment I was conducted from October 2010 to February 2011 at Eastern Block which lies 11° 12' N and 77° 03' E at an altitude of 426.74 m (Location I). Soil texture was a clay loam (33% clay, 22% silt, 30% sand), pH 8.4, cation exchange capacity (CEC) 11.1 cmol (p<sup>+</sup>) kg<sup>-1</sup>, organic carbon 34 g·kg<sup>-1</sup>, electrical conductivity 0.17 dS·m<sup>-1</sup>, and available N, P, and K in the soil were 225, 19.9, and 570 kg·hm<sup>-2</sup>, with Ca, Mg, and Zn at 38, 14.6, and 1.29 µg·g<sup>-1</sup>, respectively. The site had a history of *H. armigera* infestation and the larval population was maintained by planting cotton cv. MCU5 in the field without insecticide spray before experimenting. Experiment II was conducted from October 2011 to February 2012 at the Cotton Research Farm which lies 11° 50' N and 77° 50' E at an altitude of 426.72 m above mean sea level (Location II). Soil texture was a sandy clay loam

(24% clay, 32% silt, 48% sand), pH 7.8, CEC 10.6 cmol (p<sup>+</sup>) kg<sup>-1</sup>, organic carbon 3.4 g·kg<sup>-1</sup>, electrical conductivity 0.24 dS·m<sup>-1</sup>, and available N, P, and K in the soil were 180, 19, and 485 kg·hm<sup>-2</sup>, with Ca, Mg, and Zn at 32, 16, and 6 µg·g<sup>-1</sup>, respectively. The site of the experiment II had a history of *E. vittella* infestation, and the cotton cv. MCU5 was used to maintain the larval population without insecticide spray before the experimental year. Weather parameters like monthly mean rainfall, monthly rainy days, maximum and minimum temperature, monthly average relative humidity, and monthly mean evaporation prevailed in the fields during experimental period were retrieved from the Department of Agro Climate Research Centre, Coimbatore, Tamil Nadu, India and summarized in Table 1.

**EPN culture**

Three EPN strains KKMH1 (*H. bacteriophora*), APKS2 (*S. carpocapsae*), and TRYH1 (*H. bacteriophora*), earlier isolated by baiting soil samples from different cotton fields of Tamil Nadu, India, were acquired from the Department of Nematology, TNAU, Coimbatore, India. The EPN were cultured in the laboratory on the last instar larvae of the rice grain moth, *Corcyra cephalonica* (Lepidoptera: Pyralidae) at (27 ± 4) °C as described by Kaya and Stock (1997). The infective juveniles (IJs) released from *C. cephalonica* larval cadavers were collected in sterile distilled water using a modified White's trap (Kaya and Stock 1997), sterilized in 0.05% formalin (v/v) solution and maintained on aerated sterile water in plastic tissue-culture flasks at 15 °C. The IJs younger than

**Table 1** Weather parameters recorded from the Observatory located 300 and 500 m distance from experimental field locations I and II during the trial execution periods

Month and year	Mean rainfall/ mm	Monthly rainy days	Mean temperature /°C		Mean relative humidity /%		Mean evaporation per day /mm
			Max	Min	Morning	Evening	
<i>Field experiment I</i>							
October, 2010	156.4	9.0	31.1	22.2	91	62	4.7
November, 2010	311.1	15.0	28.1	21.3	95	70	2.5
December, 2010	35.0	2.0	28.3	19.3	93	61	2.9
January, 2011	100.4	5.0	30.1	19.0	89	44	3.7
February, 2011	3.5	3.0	31.6	18.4	89	40	4.6
<i>Field experiment II</i>							
October, 2011	305.3	14	31.6	22.6	91	59	4.3
November, 2011	243.1	10	28.7	20.8	90	61	3.1
December, 2011	11.6	1	29.3	19.1	89	52	3.3
January, 2012	1.0	–	29.7	18.4	89	46	3.7
February, 2012	0	–	32.3	19.4	83	35	4.8

two weeks old were acclimatized at room temperature for 6 h before field application.

### Experimental design

Experiments I and II consisted of three EPN strains APKS2, KKMHI, and TRYH1 applied at three rates of  $1 \times 10^9$ ,  $2 \times 10^9$ , and  $3 \times 10^9$  IJs·hm<sup>-2</sup>. A positive control was a recommended chemical control practice for bollworm management as per Crop Production Guide such as chlorpyrifos 20% emulsifiable concentrate (EC) at 2 mL·L<sup>-1</sup> (Anonymous 2021) and an untreated control were also maintained. The experiments were laid out in a randomized complete block design with four replicates for each treatment. Each plot was 20 m<sup>2</sup> (5 × 4 m) with 96 plants. Plots within blocks were separated by 1-m buffers and blocks were separated by 2-m buffers. Cotton (*Gossypium hirsutum* var. MCU 5, an *H. armigera* and *E. vittella* susceptible variety) was planted on 15 October 2010 in location I and 30 October 2011 in location II in rows at 30 × 60 cm spacing. Each plot comprised six rows with 16 plants in each row. The EPN were applied 5 times at 10 days intervals, at a rate of  $1 \times 10^9$ ,  $2 \times 10^9$ , and  $3 \times 10^9$  IJs·hm<sup>-2</sup> in 500 L of water, using a knapsack sprayer (diaphragm pump, cone nozzle) with an operating pressure of 300 kPa. Glycerin 1 mL·L<sup>-1</sup> was added as an anti-desiccant to EPN treatments 1–9 at 0.1% (v/v). Additionally, in order to lower the risk of desiccation, EPN was applied between 5:00 pm and 7:00 pm. The positive control chemical pesticide chlorpyrifos at 2 mL·L<sup>-1</sup> was also applied five times like EPN spray. The first sprayings of EPN strains and chemical pesticides were started 40 days after planting (DAP). Separate sprayers were used for the EPN spraying and chemical spraying. In addition to the foliar application, the soil below the plant was also drenched with EPN suspension and chlorpyrifos at 100 mL·m<sup>-2</sup>, so that final instar larvae and pre-pupal stages of the insect were also exposed to nematode as well as chlorpyrifos suspension (pupation is reported to take place in the soil). Uniform foliar applications were made to ensure that the whole plant was saturated with the EPN suspension. The topsoil (to a depth of 1 cm) of the root zone area of the plant (up to 10 cm diameter) was also uniformly wetted with the suspension from the sprayer. The field was irrigated 24 h before spraying, to increase the activity of the EPN.

### Crop husbandry

Cultural practices were followed as per Crop Production Techniques of Agricultural Crops (Anonymous 2021). NPK fertilizers at 120, 80, and 50 kg·hm<sup>-2</sup> were applied as recommended. Half amount of the nitrogen was applied at planting and the other half at 30 DAP, and all P and K were applied only at planting. The crop was

irrigated at the rate of 4~5 L·m<sup>-2</sup>·day<sup>-1</sup> of water by drip irrigation with 1 bar pressure. Since there was no severe incidence of any disease or other pests, no fungicides or other insecticides were applied as plant protection measures.

### Assessment of larva, damage and yield

The *H. armigera* was the major bollworm in location I whereas in location II *E. vittella* was the major bollworm present. Data on the number of live larvae of each pest at seven days after each spray were recorded from 10 randomly tagged plants per experimental plot and means were reported. The damage to squares and bolls was assessed by counting the diseased and the total number of squares and bolls. Based on the numbers of damaged and undamaged squares/bolls, the percentages of damaged squares and bolls at each observation were calculated and the pooled mean was reported. Harvesting was done by handpicking. Seed cotton yield (kg·hm<sup>-2</sup>) per plot was recorded from each harvest and pooled.

### Statistical analysis

The data were analyzed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Shapiro–Wilk test was performed to test the normality of data. To maintain homogeneity, larval population values were transformed to square root and cotton square damage/boll damages were converted to arcsine for analysis. The data were subjected to analysis of variance and means were separated by performing Tukey–Kramer test at  $P < 0.05$ . The transformed data were back-transformed for presentation.

## Results

### Field experiment I

The population density of *H. armigera* in the cotton crop before treatments ranged 9.7~10.3 larvae per 10 plants (Table 2). Spraying three EPN strains at three doses effectively reduced the population of *H. armigera*. There was a significant difference in the number of bollworms per 10 plants among the three strains (APKS2, KKMHI, and TRYH1) and three doses ( $1 \times 10^9$  IJs·hm<sup>-2</sup>,  $2 \times 10^9$  IJs·hm<sup>-2</sup>, and  $3 \times 10^9$  IJs·hm<sup>-2</sup>) tested ( $P < 0.05$ ). The larval population of *H. armigera* was significantly ( $P < 0.05$ ) lower in plots sprayed with APKS2 whereas the untreated control plots supported more larval populations. Among three EPN dosages tested, EPN sprayed at  $3 \times 10^9$  IJs·hm<sup>-2</sup> in 500 L of water recorded significantly fewer *H. armigera* larval populations. When considering both strain and dosages, *S. carpocapsae* APKS2 at  $3 \times 10^9$  IJs·hm<sup>-2</sup> was significantly effective in reducing the *H. armigera* population by 62.2% compared with the control

**Table 2** Effect of EPN strains at three application rates on *Helicoverpa armigera* population, square and boll damage of cotton—Field experiment I

Treatments	Pre-treatment population/10 plants	Post-treatment population/10 plants (polled mean of five sprays)	Square damage /%	Boll damage /%
<i>EPN strains at three doses</i>				
APKS2 at $1 \times 10^9$	10.2 a	6.4 e	17.1 c	12.7 d
APKS2 at $2 \times 10^9$	10.0 a	4.6 g	13.1 f	9.7 g
APKS2 at $3 \times 10^9$	10.0 a	4.3 g	11.5 h	8.0 h
KKMH1 at $1 \times 10^9$	10.0 a	7.2 c	18.4 b	13.7 c
KKMH1 at $2 \times 10^9$	10.3 a	6.8 d	13.6 e	10.4 f
KKMH1 at $3 \times 10^9$	10.3 a	5.1 f	12.3 g	9.3 g
TRYH1 at $1 \times 10^9$	10.0 a	11.3 a	21.0 a	17.0 b
TRYH1 at $2 \times 10^9$	9.7 a	9.0 b	15.4 d	12.6 d
TRYH1 at $3 \times 10^9$	10.0 a	7.3 c	14.1 e	11.4 e
Chlorpyrifos	10.0 a	3.2 h	9.7 i	7.1 i
Control	9.7 a	11.4 a	21.1 a	19.3 a
<i>EPN strains</i>				
APKS2	10.0 a	5.1 d	13.9 d	10.1 d
KKMH1	10.2 a	6.4 c	14.8 c	11.1 c
TRYH1	9.9 a	9.3 b	16.8 b	13.7 b
Chlorpyrifos	10.0 a	3.2 e	9.7 e	7.1 e
Control	9.7 a	11.4 a	21.1 a	19.3 a
<i>EPN dosages</i>				
$1 \times 10^9$ IJs·hm <sup>-2</sup>	10.0 a	8.3 b	19.0 b	14.5 b
$2 \times 10^9$ IJs·hm <sup>-2</sup>	10.0 a	6.8 c	14.0 c	10.9 c
$3 \times 10^9$ IJs·hm <sup>-2</sup>	10.1 a	5.6 d	12.8 d	9.6 d
Chlorpyrifos	10.0 a	3.2 e	9.7 e	7.1 e
Control	9.7 a	11.4 a	21.1 a	19.3 a

Means followed by the same letter in columns are not significantly different at  $P < 0.05$  (Tukey–Kramer test)

( $P < 0.05$ ). In the chlorpyrifos treated plot, the number of larvae decreased by 72.0% compared with the control.

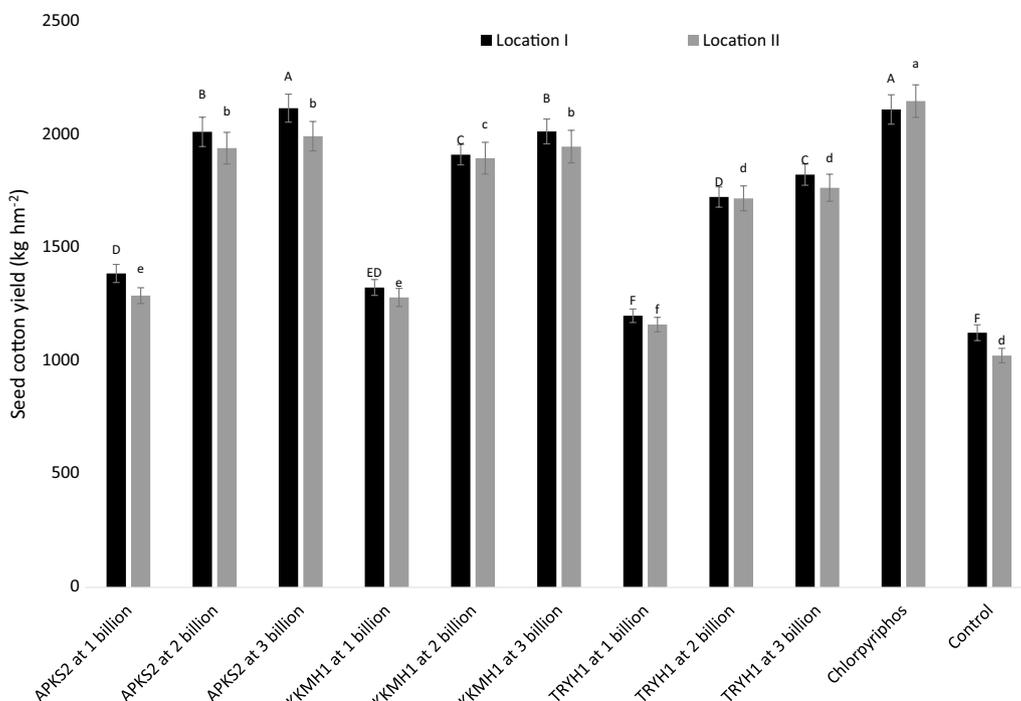
Another method to determine the bio-efficacy of EPN on the bollworm was by counting the damaged squares and bolls in the trial plots. Table 2 showed that both chlorpyrifos and EPN decreased the injury of *H. armigera* on the plants compared with the untreated control but there was a significant difference among EPN strains and dosages ( $P < 0.05$ ). Among EPN strains, APKS2 reduced square and boll damage compared with KKMHI and TRYH1. The higher rate of  $3 \times 10^9$  IJs·hm<sup>-2</sup> had significantly less square and boll damage than the lower rates. Square and boll injury were significantly low in Chlorpyrifos followed by APKS2 at  $3 \times 10^9$  IJs·hm<sup>-2</sup> ( $P < 0.05$ ).

Cotton yield was enhanced by spraying EPN and chlorpyrifos, but there were significant differences in yield among EPN strains and dosages. APKS2 at  $3 \times 10^9$  IJs·hm<sup>-2</sup> supported the highest seed cotton yield compared with the control ( $P < 0.05$ ) (Fig. 1). The yield was significantly higher in APKS2 followed by KKMHI than

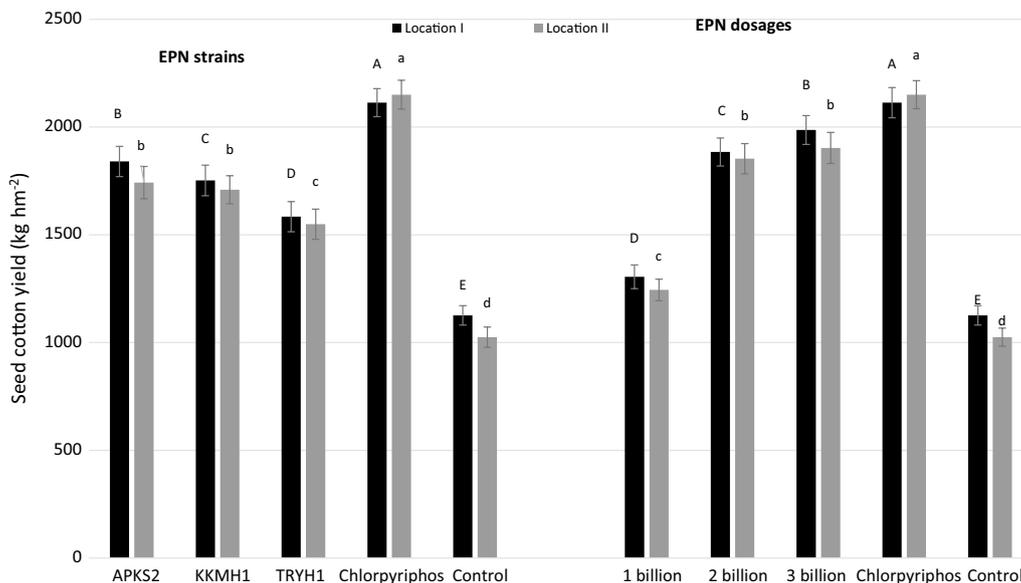
TRYH1 (Fig. 2). EPN strains applied at  $3 \times 10^9$  IJs·hm<sup>-2</sup> recorded significantly higher yields than those applied at  $2 \times 10^9$  IJs·hm<sup>-2</sup> and  $1 \times 10^9$  IJs·hm<sup>-2</sup> (Fig. 2). Results demonstrated that foliar spraying of EPN strain APKS2 at  $3 \times 10^9$  IJs·hm<sup>-2</sup> was considered a prominent eco-friendly treatment for the control of *H. armigera* in cotton.

#### Field experiment II

Results of the field trial showed that the EPN strains reduced the *E. vittella* larval populations, but there were differences in the efficacy among the EPN and rates tested (Table 3). The population of *E. vittella* was significantly reduced in the plots sprayed with APKS2 and KKMHI compared with TRYH1. Among three EPN dosages test, EPN spray at  $3 \times 10^9$  IJs·hm<sup>-2</sup> and  $2 \times 10^9$  IJs·hm<sup>-2</sup> recorded significantly fewer *E. vittella* larval populations whereas in low dosage ( $1 \times 10^9$  IJs·hm<sup>-2</sup>) larval populations were higher. When considering both strain and dosage, the APKS2 and KKMHI strains applied at the two highest rates were similarly effective



**Fig. 1** Effect of EPN strains at three application rates on yield of cotton infested with *Helicoverpa armigera* (location 1) and *Earias vittella* (location II). Bars with the same letter do not differ significantly according to Tukey–Kramer test at  $P < 0.05$ . Capital letter represents field experiment I and small letter denotes field experiment II



**Fig. 2** Comparative performance of EPN strains as well as overall effect of three different concentrations of EPN on yield of cotton infested with *Helicoverpa armigera* (location 1) and *Earias vittella* (location II). Bars with the same letter do not differ significantly according to Tukey–Kramer test at  $P < 0.05$ . Capital letter represents field experiment I and small letter denotes field experiment II

**Table 3** Effect of EPN strains at three application rates on *Earias vittella* population, square and boll damage of cotton—Field experiment II

Treatments	Pre-treatment population/10 plants	Post-treatment population/10 plants (polled mean of five sprays)	Square damage /%	Boll damage /%
<i>EPN strains at three doses</i>				
APKS2 at $1 \times 10^9$	15.0 a	8.9 c	12.3 c	8.8 dc
APKS2 at $2 \times 10^9$	15.0 a	6.5 d	9.0 de	6.4 e
APKS2 at $3 \times 10^9$	15.3 a	6.1 d	8.5 f	6.0 fe
KKMH1 at $1 \times 10^9$	15.3 a	9.5 c	13.4 c	9.7 c
KKMH1 at $2 \times 10^9$	15.0 a	6.8 d	9.5 e	6.9 e
KKMH1 at $3 \times 10^9$	15.3 a	6.4 d	9.0 ef	6.5 e
TRYH1 at $1 \times 10^9$	15.3 a	12.9 b	17.9 b	13.0 b
TRYH1 at $2 \times 10^9$	15.0 a	9.2 c	12.7 c	9.2 c
TRYH1 at $3 \times 10^9$	15.3 a	8.6 c	12.0 dc	8.7 dc
Chlorpyriphos	15.0 a	5.3 e	7.8 g	5.0 g
Control	15.3 a	17.3 a	30.1 a	20.7 a
<i>EPN strains</i>				
APKS2	15.1 a	7.1 c	9.9 c	7.0 d
KKMH1	15.2 a	7.5 c	10.6 c	7.7 c
TRYH1	15.2 a	10.2 b	14.2 b	10.3 b
Chlorpyriphos	15.0 a	5.3 d	7.8 d	5.0 e
Control	15.3 a	17.3 a	30.1 a	20.7 a
<i>EPN dosages</i>				
$1 \times 10^9$ IJs·hm <sup>-2</sup>	15.2 a	10.4 b	14.5 b	10.5 b
$2 \times 10^9$ IJs·hm <sup>-2</sup>	15.0 a	7.5 c	10.4 c	7.5 c
$3 \times 10^9$ IJs·hm <sup>-2</sup>	15.3 a	7.0 c	9.8 d	7.0 c
Chlorpyriphos	15.0 a	5.3 d	7.8 g	5.0 d
Control	15.3 a	17.3 a	30.1 a	20.7 a

Means followed by the same letter in columns are not significantly different at  $P < 0.05$  (Tukey–Kramer test)

at reducing *E. vittella* numbers. Square damage was significantly reduced in APKS2 and KKMHI (Table 2). Considering the different rates of application of EPN,  $3 \times 10^9$  IJs·hm<sup>-2</sup> had significantly less square and boll damage than the  $1 \times 10^9$  IJs·hm<sup>-2</sup> and  $2 \times 10^9$  IJs·hm<sup>-2</sup> rates. However, the standard chemical spray (chlorpyriphos) recorded the lower larval population and square damage percentage compared with all the EPN strains at all three dosages sprayed. A similar trend was observed for boll damage percent. The yield was significantly high in APKS2 and KKMHI over TRYH1 and the untreated control. EPN strains applied at  $2 \times 10^9$  IJs·hm<sup>-2</sup> and  $3 \times 10^9$  IJs·hm<sup>-2</sup> recorded significantly higher yield than the low rate applied at  $1 \times 10^9$  IJs·hm<sup>-2</sup> and the untreated control. Yield followed a similar pattern as seen with the square and boll damage. Results showed that foliar spraying of EPN strains APKS2 or KKMHI at  $2 \times 10^9$  IJs·hm<sup>-2</sup> and  $3 \times 10^9$  IJs·hm<sup>-2</sup> were considered prominent eco-friendly treatments for the control of *E. vittella* in cotton.

## Discussion

Results of this study demonstrated the biocontrol potential of EPN to attack cotton bollworms *H. armigera* and *E. vittella* when applied as foliar spray under field conditions. Field application of EPN for insect pest control has already been attempted in several crops on multiple insect genera (Odendaal et al. 2016a, b; Goettig and Herz 2018; Helmberger et al. 2018; Platt et al. 2019; Steyn et al. 2019a, b; Jaffuel et al. 2020; Kim et al. 2021). Seenivasan and Sivakumar (2014) conducted virulence assays wherein they have established that *H. armigera* and *E. vittella* are hosts for EPN. They reported 92%~94% mortality of *H. armigera* and 93% mortality of *E. vittella* under laboratory conditions by EPN. Other scientists have also reported the bio-efficacy of different *Steinernema* spp. and *Heterorhabditis* spp against *H. armigera* and *E. vittella* (Ali et al. 2007; Seenivasan et al. 2012; Seenivasan 2017). In our field study, the biocontrol potential of native *S. carpocapsae* and *H. bacteriophora* strains was confirmed against *H. armigera* and *E. vittella* when applied as a foliar spray.

In our field experiments, foliar application of all EPN reduced larval populations of *H. armigera* and *E. vittella* and provided control of square or boll damage caused by these insects. Our research findings add to the earlier reports on cotton against pink bollworm, *P. gossypiella* (Gassmann et al. 2006). The control of *H. armigera* and *E. vittella* larval due to EPN application was also reported by several investigators in crops such as beans (Glazer and Novan 1990), chickpea (Ali et al. 2008), corn (Ali et al. 2007), and pigeon pea (Vyas et al. 2002).

This study showed a 62.2% and 64.7% reduction of *H. armigera* and *E. vittella*, respectively, due to EPN application. In general, EPN efficacy was reported to be highly variable with low (<10%) to high (>60%) in field situations (Arthurs et al. 2004). Field application of EPN was not reported as promising in many experiments due to several factors such as lack of tolerance of EPN IJs to extreme temperature, UV radiation, desiccation, and relative humidity (Nyasani et al. 2008). Seenivasan and Sivakumar (2014) have established that the EPN strains used in this study namely KKM1 (*H. bacteriophora*), APKS2 (*S. carpocapsae*), and TRYH1 (*H. bacteriophora*) have the potential to tolerate heat up to 40 °C for 2 h and to tolerate rapid and slow desiccation pressure to some extent. The adjuvant used in this study glycerol has been reported to reduce the negative effect of desiccation (Prabhuraj et al. 2005). Spraying EPN in the evening was demonstrated to minimize the effects of UV radiation (Ali et al. 2008). Hence, the improvement of field efficacy reported in this study was attributed to the use of thermo and desiccation-tolerant strains, the use of anti-desiccation adjuvant in spray liquid and judicious time of field application.

In this study *S. carpocapsae* strain APKS2 caused a significantly higher reduction of *H. armigera* suggesting that *H. armigera* is more susceptible to *S. carpocapsae* than *H. bacteriophora*. Similar results were also reported in other lab studies (Glazer 1992; Ali et al. 2007). Both *S. carpocapsae* strain APKS2 and *H. bacteriophora* strain KKM1 controlled *E. vittella* equally effectively. The finding is in accordance with the earlier laboratory assay in which both strains caused 92.7% mortality of *E. vittella* (Seenivasan and Sivakumar 2014). EPN, even strains of the same EPN species, differ in their pathogenicity to different insect species (Nyasani et al. 2008). In this study, *H. bacteriophora* strain TRYH1 caused significantly more bollworm numbers with more boll and square damages than the *H. bacteriophora* strain KKM1, which might be due to the different geographical origins and environmental adaptations of the two strains.

Biological control of *H. armigera* and *E. vittella* by EPN was not only influenced by the nematode species/strains, but was also affected by the dose applied. For *H.*

*armigera* control, an increase of dose from 1 to  $3 \times 10^9$  IJs·hm<sup>-2</sup> caused a decrease of *H. armigera* larva, its damage and increased yield in cotton plants. An increase in EPN concentration led to an increased *H. armigera* control, validating the results of previous studies (Ebssa et al. 2001, 2004). This study also demonstrated that a high concentration of  $3 \times 10^9$  IJs·hm<sup>-2</sup> is needed to achieve better control of *H. armigera*. This finding is supported by earlier workers who have also shown the better performance of *Steinernema mashoodi* (Ahmad et al. 2015) and *S. carpocapsae* (Hussain et al. 2014) against *H. armigera* on chickpea; *S. carpocapsae* and *S. feltiae* against codling moth, *Cydia pomonella* on apple (Lacey et al. 2006); *Steinernema wesieri* against *Plutella xylostella* on cabbage (Nyasani et al. 2008); and *H. indica* against flush worm, *Laspeyresia bipunctata* on tea (Devrajan et al. 2010) at the dose of  $3 \times 10^9$  IJs·hm<sup>-2</sup>. Higher doses of EPN generally yield better insect control (Chen et al. 2003). Field doses of more than  $2.5 \times 10^9$  IJs·hm<sup>-2</sup> are commonly applied to ensure that a sufficient number of IJs is exposed to the target host insect for providing better control (Chen et al. 2003). For *E. vittella* control the dose of  $2 \times 10^9$  IJs·hm<sup>-2</sup> was equally effective as that of  $3 \times 10^9$  IJs·hm<sup>-2</sup>, which indicates that the dose of  $2 \times 10^9$  IJs·hm<sup>-2</sup> is optimum to get the required *E. vittella* control. Most probably, the smaller body size of *E. vittella* than *H. armigera* larvae is the reason that such low concentrations of IJs are required to obtain high control. Ebssa et al. (2001) reported similar results of differences in application dose with different sizes of target insects.

## Conclusion

A foliar spray of *S. carpocapsae* strain APKS2 at  $3 \times 10^9$  IJs·hm<sup>-2</sup> is efficacious in controlling *H. armigera* up to 62.2% and reducing square (34.1%) and boll damage (58.5%) in cotton under field conditions. However, in *E. vittella* infested fields sprayed with *S. carpocapsae* strain APKS2 or *H. bacteriophora* strain KKM1 at  $2 \times 10^9$  IJs·hm<sup>-2</sup> is recommended to achieve 60.6%~62.4% larva reduction, 68.4%~70.7% and 66.6%~69.9% reduction in square and boll damage, respectively. The spray application of EPN was proved to be useful in controlling *H. armigera* or *E. vittella* and increasing cotton yield 45.5%~45.9% higher than the untreated control. Despite the limitations associated with using EPN in the foliar arena, it was very encouraging that the effect of *H. armigera* or *E. vittella* control we obtained was comparable to that obtained using chemical insecticide. *S. carpocapsae* strain APKS2 and *H. bacteriophora* strain KKM1 appear from our result to have the most potential, but future studies should focus on the performance of these EPN isolates under different climatic zones. In addition, the impact of these EPN strains on natural enemies

prevails on the cotton ecosystem and the appropriate low-cost mass production technology of these strains is warranted.

### Abbreviations

Bt: *Bacillus thuringiensis*; Ca: Calcium; CEC: Cation exchange capacity; cmol: Centimole; DAP: Days after planting; EPN: Entomopathogenic nematodes; IJs: Infective juveniles; K: Potash; Mg: Magnesium; MSL: Mean sea level; N: Nitrogen; P: Phosphorus; UV: Ultraviolet; Zn: Zinc.

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### Authors' contributions

Nagachandrabose S: Plan of experiment, execution, analysis and writing. The author read and approved the final manuscript.

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### Availability of data and materials

The data will be available on request to the author.

### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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