## RESEARCH

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Field evaluation of whorl application of sand mixed entomopathogenic nematodes for the management of invasive fall armyworm, Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae) in sweet corn

B. Ratnakala<sup>1</sup>, C. M. Kalleshwaraswamy<sup>1\*</sup>, M. Rajkumar<sup>2</sup>, Sharanabasappa S. Deshmukh<sup>1</sup>, H. B. Mallikarjuna<sup>3</sup> and Lakshmi Narasimhaiah<sup>4</sup>

## Abstract

Background Fall armyworm (FAW) Spodoptera frugiperda, also called 'whorlworm' is a global pest threatening maize production. Larvae stay in the whorl of maize/sweet corn and efficient delivery of insecticides or bio-agents is the major issue for its successful management. Biological control of FAW through entomopathogenic nematodes (EPNs) offers promise, as EPNs are soil colonizers and commercially available in various formulations, soil or sand can ideally be exploited as natural substrate targeting FAW under field conditions.

**Results** In the present study, field evaluations were carried out using the aqueous suspension of *Steinernema car*pocapsae and Heterorhabditis indica in two cropping seasons. The Infective Juveniles (IJs) stages of EPNs mixed with sand at different doses were applied to the whorl region of sweet corn twice during cropping season, i.e. on 25th and 40th day after sowing. For comparison, a recommended insecticide, Chlorantraniliprole 18.5 SC mixed in sand was taken as check. S. carpocapsae @ 500 IJs, significantly reduced the larval population and leaf damage score. It was statistically on par with Chlorantraniliprole 18.5 SC whorl application. Principal component analysis revealed that there was a relationship between larval population and leaf damage score. Survival of S. carpocapsae was significantly greater than the *H. indica* in the three media tested. Soil was found to be the best media followed by sand and frass.

**Conclusions** Field study of whorl application of sand mixed EPNs supported by laboratory studies on the persistence connoted that EPNs could be used as an eco-friendly option through whorl application for the management of FAW.

Keywords Spodoptera frugiperda, Entomopathogenic nematodes, Whorl application, Persistence, Biological control, Aqueous suspension

## Background

Sweet corn (Zea mays var. saccharata. L), a close relative of maize and a member of the corn family, is divergent from other maize types by the presence of a gene which alters endosperm starch synthesis resulting in the plants being used as a vegetable (Dagla et al. 2014). It is prevalent among people as immature cobs are eaten fresh as a vegetable. The major sweet corn-growing states in

\*Correspondence:

C. M. Kalleshwaraswamy

kalleshwaraswamycm@uahs.edu.in Full list of author information is available at the end of the article



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India are Andhra Pradesh, Karnataka and Maharashtra (Dagla et al. 2014). Commercial value and demand for sweet corn are rising gradually in the peri-urban ecosystems due to its high market potential. Nonetheless, a significant economic pest is recently invaded FAW, which severely affects this crop, causing great yield loss.

Fall armyworm, Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae), an invasive pest, autochthonous to Central and South America, reportedly invaded the Indian subcontinent in the year 2018 (Sharanabasappa et al. 2018). Eventually, FAW has expanded its geographical range covering other regions of the country (Repalle et al. 2020), with the potentiality of competitively displacing other major stem borers and defoliators of the maize (Kalleshwaraswamy et al. 2023). With high host range and a high capacity for dispersal and adaptation, FAW holds the title of a polyphagous and potential pest in several countries (Montezano et al. 2018). In India, FAW became only the major pest causing economic loss in maize (Divya et al. 2021b). This is mainly attributed to cannibalistic and early habitat occupancy capabilities of FAW (Kalleshwaraswamy et al. 2023). The discernable and distinctive damage the pervasive "whorl worm" inflicts on the maize has made it the most discussed pest in India. FAW completes, on average, 12 generations in a year, causing damage to maize and, as a result, economic loss. This circumstance elicited the indiscriminate use of chemical insecticides by the farmers as a consequence, FAW developed resistance (Bolzan et al. 2019). Another feature of this pest, which has possibly given it fame among crop pests, is the process of divergence, that is, the crossing of biotypes (Vélez-Arango et al. 2008).

In the recent past, entomopathogenic nematodes (EPNs) of the family, Steinernematidae and Heterorhabditidae have been used as traditional, conservational and augmentative biological control agents because they possess many of the attributes of competent biological control agents (Grewal et al. 2005). The EPNs are effectively used to control insect pests in agricultural and horticultural crops (Georgis et al. 2006). The susceptibility of FAW to EPNs has been reported (Acharya et al. 2020), and virulence of locally available strains have been tested (Patil et al. 2022). The characteristics of EPNs, such as wide host range, rapid speed of kill, host searching ability, easy mass production and compatibility with other conventional and eco-friendly methods, facilitated their utilization as a good candidate for integrated pest management and sustainable agriculture. The types of the carrier material, moisture, temperature and wind speed are the major extrinsic factors that determine the survival of EPNs under field conditions.

As the EPNs are soil-inhabiting, their locomotion and persistence in the soil are perhaps determined by various

factors such as soil texture, soil moisture and targeted host (Yadav 2012). Nonetheless, many laboratory studies have comprehended the effects of many of these factors (Nouh 2022). The ability of IJs to persist and disperse until host location, and infection is the primary factor determining the success of EPN application as a biological control agent. The EPNs dispersal and persistence depend on numerous intrinsic and extrinsic factors (*e.g.* soil moisture, temperatures, soil texture, pH, UV radiation).

Hitherto, researchers have used foliar spray to evaluate the efficacy of pests against *S. frugiperda;* however, EPNs, when sprayed, may exposed to harsh environmental conditions like high temperatures, wind speed, desiccation and UV radiations. EPNs are predominantly isolated from soil habitats and better survive in their niche. As FAW is also called 'whorl worm', its feeding is restricted to whorl region; we hypothesised that the direct application of EPNs, *Steinernema carpocapsae* (Weiser, 1955) and *Heterorhabditis indica* (Poinar, 1976), to the whorl with sand as carrier material may provide better results against larvae. EPNs could persist in the sand and frass of *S. frugiperda*, which may provide extra substantiation to the study.

#### Methods

At two locations, the field evaluation of EPNs against FAW was conducted during Kharif-2021 and Rabi 2021–22. First season field evaluation (Kharif-2021) was conducted at the College of Agriculture, KSNUAHS, Shivamogga (13° 58′ 15″ N, 75° 34′ 47″ E), and the second season field evaluation (Rabi 2021–22) was conducted at AHRS, Bavikere (13° 72′ 73″ N, 75° 71′ 53″ E). The randomized completely block design (RCBD) was laid out with six treatments and four replications. The crop was raised by adopting a standard package of practice except for plant protection measures.

Steinernema carpocapsae aqueous suspension was sourced from the South Canara Coconut Farmers Company, Vittal, Karnataka, and was used for evaluation against FAW. Before the imposition of treatments, IJ suspensions of S. carpocapsae were enumerated for various concentrations (200, 400, and 500 IJs per treatment). Treatments were imposed twice during the experimental period, 25 and 40 days after sowing. These two application periods correspond to the critical stages of sweet corn,  $V_6$  and  $V_{10}$ , which are vulnerable to S. frugiperda infestation. A known concentration of S. carpocapsae (200, 400, and 500 IJs per treatment), Chlorantraniliprole 18.5 SC (Coragen) at 0.4 ml, and H. indica commercially available as Soldier (Source: Multiplex Ltd, Bangalore, India) at 100 g were mixed with one kg of sand having approximately 11 per cent moisture. One treatment consisted of 0.4-ml Chlorantraniliprole 18.5 SC was used as a check against *S. frugiperda*. Each plant received five grams of sand in each of the treatments. Treatments were applied during evening hours (17.00–18.00 h) to create a congenial environment for nematode survival and infection.

The number of larvae per plant in each treatment plot was counted before and after the treatment application following the non-destructive sampling method. Pre-count of the larval population was conducted one day before treatment, and a post-count was undertaken seven and fourteen days after treatment application. To confirm the larval death by EPNs, the field-collected cadavers were brought to the laboratory of the Department of Entomology and were dissected under ZEISS stemi-508 stereo binocular microscope, and IJs were observed inside the cadaver of S. frugiperda. FAW leaf damage severity was recorded based on a 1-9 rating scale, described by Davis and Williams (1992) and modified by Prasanna et al. (2018), to indicate damage severity in each treatment plot. Number of cobs/plant, number of Kernels/plant and cob yield was recorded at the time of harvest. The data on the mean number of larvae per plant, damage score and cob yield were separated adopting Tukey's post hoc multiple rage test in SPSS (Version 18.0).

#### Persistence of EPNs

To know the persistence of tested EPNs in different media, soil, river sand and frass of S. frugiperda were used. Soil samples were collected from the experimental field plot, whereas frass of S. frugiperda was collected from the laboratory-reared FAW. All the three media were autoclaved for 12 h and air-dried for overnight. Each media of 500 g was then taken in a container and moistened, having approximately 11 per cent moisture. One-ml aqueous suspension of S. carpocapsae and H. indica consisting of 10,000 IJs were then inoculated to each media tested (soil, sand and frass). Isolation of EPNs from the different media was done on seven days after the EPN inoculation, following a standard "Insect-baiting" technique of Galleria mellonella (Tarasco et al. 2020). Five larvae of last instar G. mellonella were introduced to 100 g (representative samples) of each media in five replicated trials (Orozco et al. 2014). After two days, the dead G. mellonella larvae were collected and washed under tap water for two to three times and dissected under ZEISS stemi-508 stereo binocular microscope. Infective Juveniles emanating out of the cadaver body were counted. Similar procedure was repeated on 14th days after inoculation. Here, counting of live IJs (persistence) was done at seven and 14 days as in field conditions, the effect of EPNs were evaluated at seven and 14 days after the treatment application. The enumerated value of live IJs from 100 g (representative sample) was multiplied by 5 to get the total count of IJS in 500 g. Persistence of EPNs was represented in per cent live and active IJs per 500 g of the medium. Data on the per cent active IJs was arcsine transformed and subjected to Tukey's post hoc multiple range test in SPSS (Version 18.0).

#### Principal component analysis (PCA)

To find the relationship between the number of larvae on plants, crop season and percentage larval reduction and leaf damage scores, principal component analysis (PCA) was performed using SPSS (Version 20.0). Results are presented as triplot ordinations of PCA representing three major principal components (eigenvalues > 1.25).

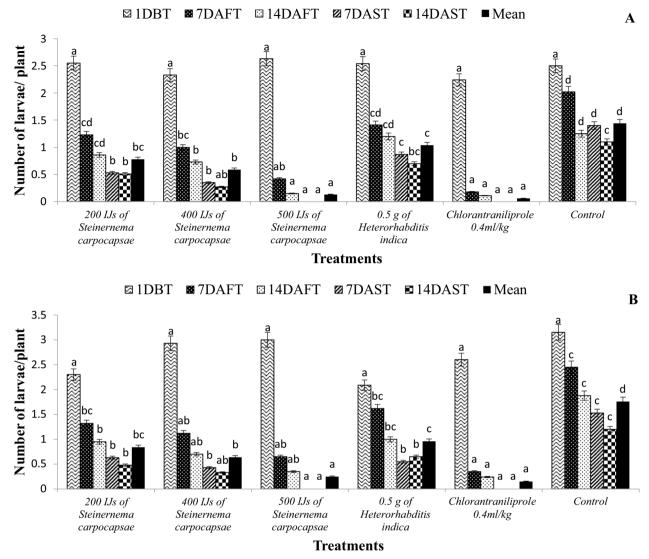
## Results

## Effect of EPNs on the larval population of Spodoptera frugiperda

There was a significant effect of the EPNs on the larval population of *S. frugiperda* during Kharif-2021 (F=90.38, df=5, 15, p <0.001) (Fig. 1A) and Rabi 2021–22 (F=57.62, df=5, 15, p <0.001) (Fig. 1B). Observations on the larval population recorded on the seventh and 14 days after the first and second application of treatments showed that *S. carpocapsae* applied @ 500 IJs/5 g of sand/plant proved to be the best treatment with effective reduction in the mean larval population of 0.13 and 0.25 larvae/plant during Kharif-2021 and Rabi 2021–22, respectively. This was statistically on par with check Chlorantraniliprole 18.5 SC treated at 0.4 ml/kg of sand with 0.06 during Kharif 2021 and 0.15 larvae/plant during Rabi 2021–22.

## Effect of EPNs on the leaf damage by *Spodoptera frugiperda* on sweet corn

A similar trend was observed in the case of leaf damage score (1-9), and significantly reduced leaf damage was observed in sweet corn plant, post-EPN and Chlorantraniliprole 18.5 SC application during Kharif-2021 (F=29.64, df=5, 15, p < 0.001) (Fig. 2A) and Rabi 2021– 22 (*F*=28.62, *df*=5, 15, *p*<0.001) (Fig. 2B). Observations on the leaf damage score recorded on the seventh and 14 days after the first and second application of treatments revealed that S. carpocapsae applied @ 500 IJs/5 g of sand/plant performed better and significantly feeding of FAW larvae on the sweet corn plant. This treatment recorded leaf damage score of 2.33 and 2.37 damage score (1–9) during Kharif 2021 and Rabi 2021–22, respectively. Likewise, in the case of leaf damage, this treatment was statistically on par with the check treatment proving its effectiveness.



**Fig. 1** Effect of whorl application of sand mixed EPNs and Chlorantraniliprole 18.5 SC on the larval population of *S. frugiperda* on sweet corn **A** Kharif 2021; **B** Rabi-2021–22. Bars with different letters indicate significant differences for different treatments (*P* < 0.05, Tukey's test); 1DBT: a day before the treatment; 7DAFT: 7 days after the first treatment; 14 DAFT: 14 days after the first treatment; 7DAST: 7 days after the second treatment; 14DAST: 14 days after second treatment; Mean number of larvae per plant

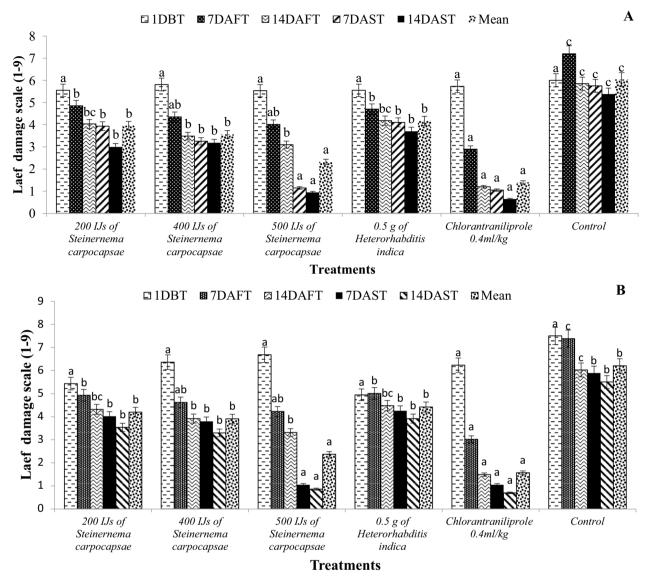
## Yield and cost economics

The number of cobs/plant (F=20.03, df=5, 15, p<0.001) (Fig. 3A), number of kernels/cob (F=17.26, df=5, 15, p<0.001) (Fig. 3B), cob yield (F=34.89, df=5, 15, p<0.001) (Fig. 3C), and yield economics of the sweet corn were significantly affected by EPN and Chlorantraniliprole 18.5 SC application. *Steinernema carpocapsae* treated at 500 IJs/5 g of sand/plant produced more yields (20.19 t/ha), cobs (1.90 cobs/plant) and kernels (462.52 kernels/cob). These treatments proved effective and on par with check Chlorantraniliprole 18.5 SC. Similarly, the cost–benefit ratio was relatively high for check

Chlorantraniliprole 18.5 SC applied at 0.4 ml mixed in one kg of sand (1:2.55) and net return (1,57,159.98 Rs./ ha) followed by *S. carpocapsae* treated at 500 IJs/5 g sand/ plant (1:2.34) with a net return of 1,41,456.22 Rs./ ha.

### Principal component analysis

The PCA extracted three major principal components (eigenvalues > 1.25) that together accounted for 99.25% of the variance (Fig. 4). Principal component 1 (PC1, *x*-axis) and principal component 2 (PC2, *y*-axis) explained 63.82% and 25.05% of the dataset variation, respectively. The major principal components were loaded positively

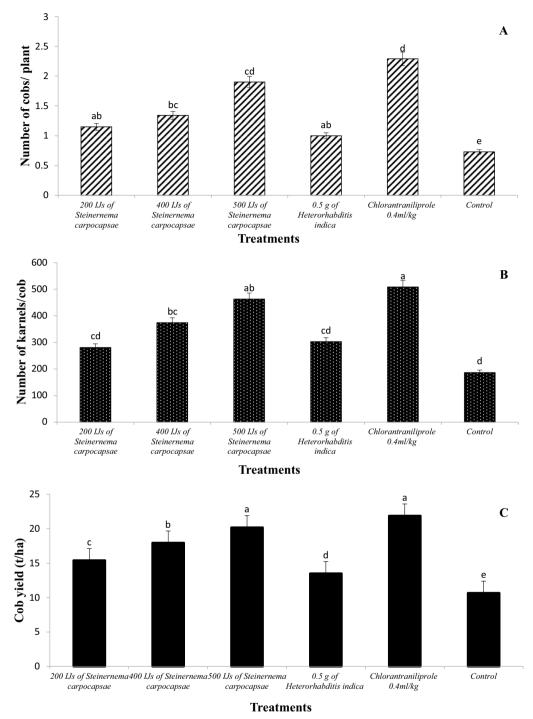


**Fig. 2** Effect of whorl application of sand mixed EPNs and Chlorantraniliprole 18.5 SC on the leaf damage by *S. frugiperda* on sweet corn **A** Kharif 2021; **B** Rabi-2021–22; Bars with different letters indicate significant differences for different treatments (P < 0.05, Tukey's test); 1DBT: a day before the treatment; 7DAFT: 7 days after the first treatment; 14 DAFT: 14 days after the first treatment; 7DAST: 7 days after the second treatment; 14DAST: Seven days after the second treatment; Mean number of larvae per plant

with the percentage reduction in *S. frugiperda* larvae of both seasons (RKS-Kharif season and RRS-Rabi season), the leaf damage score at a day before treatment in both seasons (LDKP and LDRP) and the pre-treatment larval population in the Kharif sown crop (PKP). The two major contributing components were also positively loaded with leaf damage scores of both seasons (LDRS and LDKS) and larval population (PKS and PRS) after the post-application in both seasons and the pre-treatment population during Rabi sown crop (PRP). Correlation analyses supported the PCA association findings as the percentage reduction, leaf damage score and larval population of *S. frugiperda* on ten plants after the second round of treatment were positively correlated between the Kharif and Rabi sown crops. In contrast, the pretreatment larval population and leaf damage score was positively related to the Kharif and Rabi sown crops, respectively.

#### Persistence of EPNs

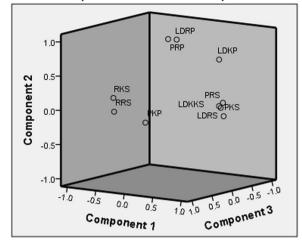
There was a significant difference between the survival of the two EPN species and the media used (F=41.36,



**Fig. 3** Effect of whorl application of sand mixed EPNs and Chlorantraniliprole 18.5 SC on the yield parameters of sweet corn **A** number of cobs/ plant; **B** the number of kernels/cob and **C** cob yield. Bars with different letters indicate significant differences for different treatments (P < 0.05, Tukey's test)

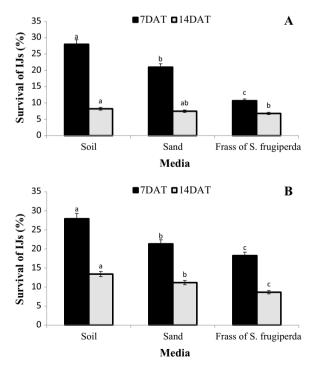
df=2, 30, p < 0.001). All the media positively supported the persistence of *H. indica* and *S. carpocapsae* until 14 days of post-EPN inoculation (Fig. 5). Among the three media, soil was proven to be best in supporting the

survival of the two EPNs nonetheless, the survival of *S. carpocapsae* (13.39%) was highest than *H. indica* (8.22%). Similarly, sand effectively contributed to the persistence of the EPNs, which followed a similar pattern to that of



**Component Plot in Rotated Space** 

**Fig. 4** Principal component analysis (based on the correlation matrix, eigenvalues > 1.25) of different parameters, such as the percentage reduction of *S. frugiperda* in Kharif (RKS) and Rabi (RRS) sown crops. PCA on the larval population of *S. frugiperda* on ten plants at pre and post-treatment in Kharif (PKP, PKS) and Rabi (PRP, PRS) sown crops and leaf damage score caused by *S. frugiperda* at pre- and post-treatment in Kharif (LDKP, LDKS) and Rabi (LDRP, LDRS) sown crops



**Fig. 5** Persistence of EPNs in soil, sand and frass of *S. frugiperda* **A** *S. carpocapsae* **B** *H. indica;* Bars with different letters indicate significant differences for different treatments (P < 0.05, Tukey's test)

the soil. Frass of *S. frugiperda* sustained the survival of the EPNs (11.15 and 7.48% for *H. indica* and *S. carpocapsae*, respectively) for 14-day post-inoculation with the highest per cent persistence of *S. carpocapsae* (8.63%) and *H. indica* (6.80%).

#### Discussion

Application of Steinernema carpocapsae at 500 IJs/5 g sand/plant significantly reduced the larval population and leaf damage than the control plots. The same treatment was statistically on par with the commonly used effective insecticide Chlorantraniliprole 18.5 SC @ 0.4 ml (Kalleshwaraswamy et al. 2022). Previously, sand mixed insecticide application has been demonstrated as more effective than the spray application (Divya et al. 2021a). Hence, for effective delivery of insecticide or bio-agents, whorl application could be an alternative technique than spray. Previous studies on the field efficacy of the EPNs against FAW were limited to the foliar application (Garcia et al. 2008) using advanced spraying equipment that produced electric charges to the spraying mix or those using hydraulic and rotary nozzle tips with a defined mesh size of filtering elements. Though spraying of EPNs using equipment was advantageous in reducing the larval population of S. frugiperda on the maize plant, this process may thrive EPNs to desiccation (Georgis et al. 2006). It may expose them to UV radiation, air movement, sunlight and low relative humidity as they are susceptible (Higginbotham 2021). Spraying of EPNs using equipment may result in damage to the IJs resulting from continuous agitation, which was must in spraying because EPNs tend to settle at the bottom of the spray tank.

There are various constraints faced in applying EPNs using sprayers as to selecting appropriate spraying equipment with the required mesh size. When EPNs were sprayed with XR8001 tips (100 mesh filters), there was a decrease in the concentration and viability (Garcia et al. 2008). However, applying spray volumes higher than 800 L per ha with boom sprayers can be higher than those used in chemical control, which may render the biological control using EPNs under study impractical. Adding adjuvants like tensoactive agents do not negatively influence the effectiveness of the EPNs (Garcia et al. 2008) but may impart extra cost burdens on the farmers.

Glazer and Navon (1990) tested *S. feltiae* mixed with two different solutions of antidesiccants, glycerol or folicote, against *H. armigera* and obtained 75 and 95% control. The erstwhile studies on the virulence of *S. carpocapsae* and *H. indica* to *S. frugiperda* in laboratory assays proved the effectiveness of both the EPNs (Patil et al. 2022). Regardless of this, in the field evaluation, only *S. carpocapsae* was effective against *S. frugiperda*, probably because of the difference in the formulation of EPNs and the suitability of certain abiotic factors between the two tested EPNs species (Shapiro-Ilan et al. 2004). The foraging behaviour of EPNs probably influences the capacity of a particular EPN to control the specific host. Ambusher, like S. carpocapsae was found to infect highly active insects, whereas cruiser, like H. indica, was more effective against relatively sessile insects (Allahverdipour & Karimi 2021). The spatial distribution of *S. frugiperda* larvae in the whorl region added a remarkable advantage to the whorl application of sand-mixed EPNs because EPNs tend to eventually have patchy distribution even if applied uniformly (Wilson et al. 2012). Hence, the application technique was developed by considering the spatio-temporal occurrence of the insect pest and the EPNs. Work carried out by earlier researchers have shown that the direct application of EPNs to the host niche resulted in higher mortality than the foliar spray of aqueous suspension (Shapiro-Ilan et al. 2006) that eventually resulted in higher nematode dispersal, infectivity and survival (Vicente-Diez et al. 2021). Applying EPNs with sand and applying them to the whorl region facilitates a better control of the pest compared to the foliar application. The comprehensive studies on the abiotic factors influencing the survivability and infectivity of EPNs under field conditions need dire attention in order to prove the field efficacy of EPNs against FAW.

The efficacy of whorl application of sand mixed *S. carpocapsae* treated at 500 IJs per five grams of sand per plant was comparatively high in the first season, i.e. Kharif-2021 compared to Rabi-2021–22. Patil et al. (2022) reported higher efficacy of EPN *H. indica* and *S. carpocapsae* during Kharif compared to Rabi. The cob yield and the B: C ratio were comparatively high for *S. carpocapsae* at 500 IJs. Patil et al. (2022) demonstrated that cob yield of maize was significantly higher in EPN *S. carpocapsae* treated plots than in the untreated control plots.

Laboratory evaluation of the persistence of EPNs in different media proved that soil, sand and even the frass of the S. frugiperda effectively sustained the survival of S. carpocapsae and H. indica. Soil being the natural habitat of EPNs, apparently, supported their survival; nonetheless, a pivotal finding of this research is that even the frass of S. frugiperda acts as habitat for the EPNs. This study is the first laboratory study that throws limelight on the hypothesis that EPNs can persist and survive in the frass of S. frugiperda, giving additional evidence to the field evaluation results. Previous studies on the invasion efficiency and reproduction rate of EPNs within the larval host of S. frugiperda (Acharya et al. 2020) emphasized the fact that larval cadavers in the whorl region of sweet corn can serve as an inoculum. Likewise, when the new whorl leaf emerges, sand applied to the whorl would be pushed out, so the persistence of EPNs in the *S. frugiperda* frass and the presence of larval cadavers in the whorl give a long-term protection against FAW. Besides acting as the best habitat for EPN survival, sand also has a supplementary effect on the FAW larvae when applied to the whorl region. Sand abrades the cuticle of larvae feeding inside the whorl as it tries to escape (Babendreier et al. 2020). This laboratory experiment supported our idea of mixing EPN with sand applied to the whorl as there was better survival.

There are very few studies that examined quantitatively how EPNs might cascade to affect the plant biomass or yield. Nonetheless, increased production or yield, in essence, is the conclusive goal of research attempting for enhanced pest management. Agricultural ecosystems can be manipulated by reducing insect damage through an eco-friendly approach, thus allowing broad-scale pest suppression and improving crop biomass and yield using augmentative EPN release.

#### Conclusions

Based on the findings, the whorl application of sandmixed EPN, S. carpocapsae significantly reduced the FAW larval population and also reduced leaf damage. Among the two EPNs species tested, S. carpocapsae was found to be superior in reducing the FAW damage as compared to H. indica. Treatment with S. carpocapsae was statistically proven to be on par with the chemical insecticide Chlorantraniliprole applied as sand mixed. Using EPNs twice during cropping reduced the S. frugiperda larval population considerably. The laboratory analysis of EPN persistence in different media demonstrated EPNs could survive in soil, river sand and in the frass of S. frugiperda. Our findings emphasise that for effective management of S. frugiperda under field conditions, sand or soil serve as a pivotal carrier material for EPNs whorl application.

#### Abbreviations

EPNs	Entomopathogenic nematodes
IJs	Infective juveniles
FAW	Fall armyworm
h	Hours
et al.	Et alia
cm <sup>-2</sup>	Per square centimetre
RH	Relative humidity
%	Percentage
sp.	Species
DAT	Days after treatment
DBT	Days before treatment

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#### Author contributions

BR and CMK (equal first authors) designed and carried out the experiments, recorded the data interpreted the results and wrote the manuscript. BR, MR, SSD, HBM and LN analysed the data. All the authors read and approved the final manuscript.

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#### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

#### Declarations

**Ethics approval and consent to participate** Not applicable.

#### Consent for publication

All the authors are consented for publication.

#### **Competing interests**

The authors declare that they have no competing interests.

#### Author details

<sup>1</sup>Department of Entomology, College of Agriculture, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga 577 204, India. <sup>2</sup>Division of Plant Protection, Indian Council of Agricultural Research-Central Plantation Crops Research Institute, Head Quarters, Kudlu PO, Kasargod 671 124, Kerala, India. <sup>3</sup>Department of Agricultural Statistics, College of Agriculture, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga 577 204, India. <sup>4</sup>Department of Agricultural Statistics, College of Forestry, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga 577 204, India.

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