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Table: 3, Figs: 3

Field efficacy of *Steinernema abbasi*-nematodes enriched bio-insecticidal powder to control termite in wheat and chickpea

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ABSTRACT

Entomopathogenic nematodes (EPNs) are well-known biocontrol agents to control both above- and below-ground insect pests. EPNs can also control plant parasitic nematodes by releasing allelochemicals through symbiotic bacteria present in their guts. This study aimed to evaluate the bio-efficacy of a novel powder formulation comprising of infective juveniles (IJs) of a heat-tolerant indigenous EPN species of India, *Steinernema abbasi*, against subterranean termites (*Odontotermes obesus*) in wheat and chickpea fields. The bioefficacy of test formulation in field study for three *rabi* seasons (2020-2023) in wheat and chickpea grown in field heavily infested with subterranean termites decreased the plant damage from termite attack, monitored as relative number of infested tillers in wheat and infested plants in chickpea fields. The reduced damage in test crops caused by termites increased growth, yield attributes and yield of test crops. This present research validated the prepared formulation's potential as a biopesticide ideal for organic farming and integrated pest management practices.

Keywords: Allelochemicals, biopesticides, EPN, infective juvenile, IPM, nematode, *Steinernema abbasi*, termites, wheat.

INTRODUCTION

Termites are destructive polyphagous pests that attack many crops, including sorghum, chickpeas, maize, bajra, rice and barley (22). Crops are attacked by termites from sowing till harvest (1). Usually, till the symptoms appear, it is too late to manage. Termite damage is generally more (20-25 %) in rain-fed crops than in irrigated ones (10 %) (18). Termites cause yield loss of 15-25 % in maize in India (31). Termite cause around 60 % yield losses in chickpeas (21). Out of its 16 species that damage the wheat crop in India, two major species, *Odontotermes obesus* Rambur (Isoptera: Termitidae) and *Microtermes obesi* Holmgren (Isoptera: Termitidae), cause drastic yield losses (43 to 80 %) from sowing to dough stage of wheat (8).

Termites cause damages from seedlings to maturity stages. They feed on seeds and reduce their germination rate. Symptoms in standing crops consist of distinctive semi-circular feeding marks on the leaf margin. It causes the cellulose in plant tissues to break down, which causes the outer leaves to yellow and dry up before the interior leaves do. The severely injured plants dry out. Patchy looks result from uneven plant spacing and seedling mortality. If roots are partially injured, plants begin to yellow (6). Termites are challenge to control because of their versatile ways of living, which cause significant damage to crops. Different cultural (flood irrigation, growing on ridges, application of green manures, etc.) and physical control measures (mechanical destruction by dismantling

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the termite king and queen from their mounds, use of physical barriers such as sand or gravel aggregates, metal mesh, etc.) are used to control termite (18). Nematicides are also used to control termites (23) such as seed treatment with fipronil 5 FS @ 6 g/ kg of seeds and seed dipping in 0.1 % imidacloprid 70 WS or 0.04 % chlorpyriphos 20 EC for 5 min (3).

Due to increasing concern about the harmful environmental effects of synthetic insecticides, the application of eco-friendly termite management approaches is preferred (7). Moreover, innovative biocontrol techniques may be possible as a result of the secondary metabolites (Allelochemicals) produced by plants, bacteria, fungi or insect parasitic nematodes that alter the metabolic pathway in insects. Allelochemicals have biopesticidal effects on different insect pests (20). Entomopathogenic nematodes exerts allelopathic effects to suppress plant parasitic nematodes (9,11). These are also well-established biocontrol agents to manage several below- as well as above-ground pests (14). The biocontrol potential of these bioagents may be enhanced by developing EPN-based biopesticides by using suitable carriers. Random choice of carriers often resulted in poor performance of EPN-based biopesticides (19). Therefore, we assessed the bioefficacy of an EPN bioagent [*Steinernema abbasi* (Rhabditida: Steinernematidae)] enriched bioinsecticidal powder formulation against subterranean termites (*Odontotermes obesus*) in wheat and chickpea under field conditions, taking Chlorpyriphos 20 % EC as a positive control. The experiments were conducted from 2020-2023 and the mean values of test parameters were used to compare the treatment effects.

MATERIALS AND METHODS

Materials

The aqueous suspension of infective juveniles of the entomopathogenic nematode, *Steinernema abbasi* and the test insect, *Galleria mellonella* (L) (Lepidoptera: Pyralidae) 4th instar larvae were obtained from the Division of Nematology and Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India. The biopolymer (pH: 6.00, water absorption capacity: 60 g/g) was obtained from the local market, powdered and autoclaved before use. Three types of 2:1 type aluminosilicate clays were obtained from the local market and used as such without further modification. The synthetic insecticide, chlorpyriphos 20 % EC (Lethal 20 EC) was obtained from Insecticides (India) Limited, India. The test wheat variety, HD 3226, and chickpea variety, GNG 1581, were obtained from Institute of Maize Research and Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India.

Preparation of EPN-enriched bioinsecticidal powder

The details of the formulation composition are not revealed due to intellectual property interests (29). Briefly, a specific volume of an aqueous EPN solution with an IJ count of around 80,000-110000 IJs per mL was admixed with an optimized amount of biopolymer-clay blend to prepare the EPN bioinsecticidal powder. Three types of aluminosilicate clays were used in the blend. The prepared EPN-enriched bioinsecticidal powder (Figure 1A) was kept in BOD incubator at 25 °C for further experiments.

Mortality and virulence of EPN IJs in bioinsecticidal powder

The prepared powder formulation was tested upto 3 months for EPN IJ mortality. To do this, the nematodes first were released by mixing 250 mg of powdered material with 3 mL water. Then, using a stereo microscope, the released nematodes were examined. The nematodes were gently touched with a probe and those that did not move as a result of touch were deemed to be dead. Then, the percentage was calculated using the formula below:

$$\text{Percent nematode mortality} = \frac{\text{Dead nematode IJs observed per count}}{\text{Total nematode IJs observed per count}} \times 100$$

Three biological replicates with 3-technical replicates were taken each time. The mortality (%) study of EPN IJs was evaluated periodically up to 3-months of storage at monthly interval starting immediately after preparation of formulation.

For the virulence assessment of nematode IJs in the formulation, one perforated plastic box was filled with 100 g moist field soil (30), carefully mixed with 1.5-2.5 g of the formulation containing about 500 IJs to provide around 100 IJs per larvae (28). Five *Galleria mellonella* 4^{th} instar larvae were then placed over the soil. The box was then covered and left at room temperature for 72 h. For each box, the mortality (%) was computed and three boxes were taken to accomplish this experiment. The virulence of EPN IJs was assessed periodically up to 3 months of storage at monthly interval starting immediately after preparation of the formulation.

Field study

Field experiments were done at the experimental farm, Indian Agricultural Research Institute, New Delhi, India (latitude: 28^{O} 38’23” N, longitude: 77^{O} 09’27” E ., altitude: 228.61 m above MSL) during *rabi* season (November -March) from 2020 to 2023. The experimental plots selected for the present study had a history of termite infection.

The test wheat cultivar was ‘HD 3226’. Before sowing, the field was prepared according to the recommended agricultural practices. Sowing was done at seed rate of 100 kg/ ha with row spacing of 25 cm. Wheat was irrigated at 21, 40, 70, 90, and 115 days after sowing (DAS). NPK fertilizer (150:60:40 kg/ ha) was applied as a basal dosage before sowing.

The test chickpea cultivar was ‘GNG 1581’. The field was prepared with recommended agricultural practices before sowing. Using a ferti-seed drill machine, sowing was done at seed rate of 67.5 kg/ ha. The row-to-row spacing was 30 cm. The fertilizers (20 kg N and 40 kg P/ ha) were applied as basal dosage. The crop was irrigated during the pre-flowering and pod development periods.

To assess the bio-efficacy of prepared EPN bioagents enriched powder against termites in wheat and chickpea, there were three treatments:

T1 : Untreated control,
T2: Chlorpyriphos 20 % EC and
T3: EPN-enriched powder.

Before sowing, the seeds were treated with Chlorpyriphos 20 % EC for overnight at recommended dose (4 ml/kg wheat seeds, and 30 ml/kg chickpea seeds). A 3-months-old

EPN-enriched powder formulation was used in all field experiments. The EPN-enriched powder formulation @ 10^9 EPN IJs/ ha was applied on the day of sowing through broadcasting after mixing with the field soil in ratio of 1:5 (EPN-enriched powder formulation: field soil, w/w) for uniform coverage of the formulation throughout the experimental plot. Any ratio below 1:5 did not result in the development of free-flowing mixture to be broadcasted. The wheat was sown on 14.11.2020, 08.11.2021 and 13.11.2022 standard meteorological weeks of 2020-21, 2021-22, and 2022-23, respectively. Chickpea, was sown on 02.11.2020, 03.11.2021, and 07.11.2022 standard meteorological weeks of 2020-21, 2021-22, and 2022-23, respectively. The plot size was 10m × 2.5m, 15m × 3.5m in wheat and chickpea, respectively. From each plot, three subplots 1 m^2 area were selected to monitor termite infestation from germination to harvest. The termite infestation was recorded at harvest in fields, therefore the test parameters (the number of infested tillers and infested plants in wheat and chickpea respectively) were recorded at harvest. At maturity 10-tillers/plants from each plot of wheat/chickpea were removed for recording growth and yield attributes. For yield, two border rows were left and the net plot yield of each treatment was recorded and expressed in q/ha. To measure the test weight, 1000-seeds from harvested seeds in each wheat plot and hundreds of seeds from harvested seeds in each chickpea plot were counted thrice and the mean weight was recorded and expressed as g/1000 seeds for wheat and g/100 seeds for chickpea. The field experiments were done during the *rabi* season of three consecutive years (2020-2023) and the 3-years' mean value ± standard deviation (SD) of all the test parameters were used to compare the treatment effects.

Effect of field soil samples on *Galleria* mortality (*in-vitro*)

At 30 DAS, soil samples were taken from each treatment plot, and the infectivity of the field soils against *Galleria mellonella* 4^{th} instar larvae was assessed using the bait method. For this, 100 g field-collected soil was initially put into each plastic box. Three insect larvae were then spread out across the soil. To check the test insect mortality, the boxes were covered and left at room temperature for 72 h. For each box, the mortality (%) was computed. For every treatment plot's soil, three boxes were taken. The average mortality (%) of three boxes was considered as the mean mortality (%) corresponding to each treatment plot's soil.

Statistical analysis

Three years' mean data for each treatment were analysed using one-way analysis of variance (ANOVA) using R software (24). The treatment means were separated using Fisher's least significant difference (LSD) at 5 % level of significance (10).

RESULTS AND DISCUSSION

Mortality and virulence of EPN IJs in bioinsecticidal powder

After 3 months of storage, the powder-formulated nematode IJs reported 0 % mortality, hence 100 % survival at 25 °C storage temperature. Moreover, the 3-month-old nematode IJs resulted in 100 % mortality of the test insect, *Galleria mellonella* 4^{th} instar larvae (Figure 1B).

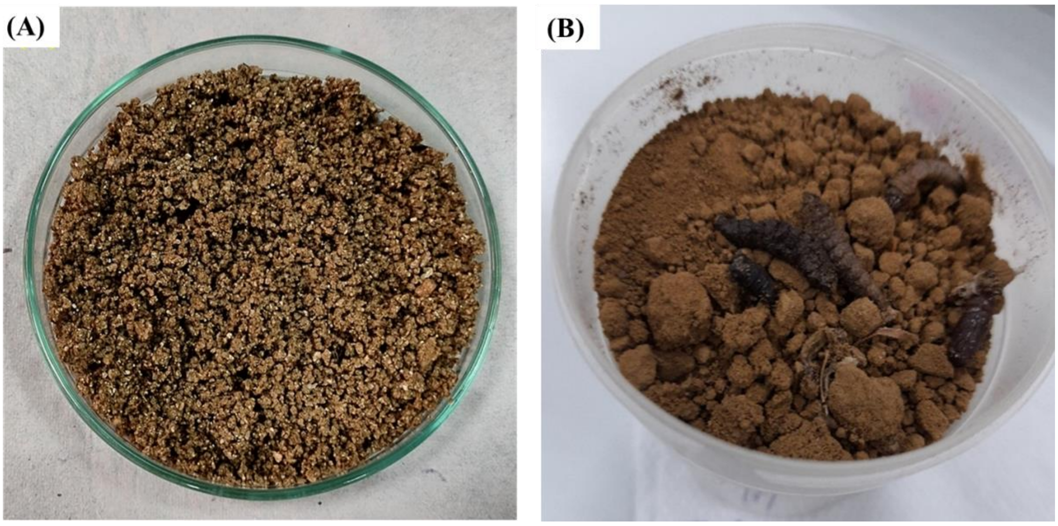

Figure 1. EPN bioinsecticidal formulation (A), and virulence of powder-formulated EPN IJs against *Galleria mellonella* 4th instar larvae (B)

Termite damage in wheat and chickpea under field conditions

The effects of different treatments on termite damage in wheat and chickpea has been shown in Table 1. The representative images of termite infestation in different treatment plots of wheat are shown in Figure 2. In wheat, the EPN-enriched powder showed that it was at par with the Chlorpyriphos 20 % EC treatment in terms of percent infested tillers at harvest. The infested tillers (%) was lowest in treatment T3 i.e. EPN-enriched Powder (24.58 ± 4.04 %), suggesting its superior bio-insecticidal potential as compared to the untreated control (40.10 ± 5.14 %).

Table 1. Effect of different treatments on termite damage in wheat and chickpea under field conditions

Treatments	Wheat	Chickpea
	Infested tillers (%) at harvest	**Infested plants (%) at harvest**
T1	40.10 ± 5.14^{a}	19.39 ± 1.75^{a}
T2	28.10 ± 9.84^{ab}	15.37 ± 5.47^{ab}
T3	24.58 ± 4.04^{b}	8.70 ± 1.77^{b}
LSD (P=0.05)	13.63	6.93

(T1: Untreated control, T2: Chlorpyriphos 20 % EC, T3: EPN-enriched Powder, applied during sowing; The results are shown as the mean ± standard deviation of mean over three years; Means with dissimilar superscript letters within a column are significantly different as per Fisher's LSD at P=0.05)

In chickpea, the percent-infested plants at harvest were lowest in EPN bioagent-enriched powder formulation (T3) (8.70 ± 1.77 %) and highest in untreated control (19.39 ± 1.75 %). Similar to wheat, EPN-enriched powder in chickpea was statistically at par with bioinsecticidal potential of chemical insecticide (Chlorpyriphos 20 % EC) treatment.

Sustainable termite control measures consider several strategies including monitoring, chemical, and non-chemical measures (1). In India, the most common synthetic insecticide used to control subterranean termites is Chlorpyriphos (25). This study is also in line with the previous report showing significantly less termite damage in Chlorpyriphos 20 % EC-treatment plots in both wheat and chickpea than untreated control. However, different studies reported the potential of other chemical insecticides in termite control. For instance, Bhagawati *et al*. (4) studied field efficacy of insecticides (Thiamethoxam 25 WG, Imidacloprid 17.8 SL, Acephate 50 % + Imidacloprid 1.8 % SP, Thiamethoxam 35 FS, Imidacloprid 600 FS, and Clothianidin 50 WDG) against *O. obesus* in preserved setts of sugarcane and found Clothianidin 50 WDG was the most effective. In another study, Kaur and Pathania (15) reported that the Thiamethoxam seed treatment @ 1g/kg seeds of wheat resulted in significantly lower termite damage than other test insecticides. However, sole reliance on chemical insecticides in crop pest control is discouraged globally due to their environmental and human health concerns. Rather, the application of biopesticides is preferred to maintain agricultural as well as environmental sustainability (27). Several EPN species control different termite species under laboratory conditions but there are few studies reported these biocontrol agents' termite control potential under field conditions (16). The present study tried to eliminate this research gap by reporting the biocontrol potential of *S. abbasi* IJs-enriched powder formulation against subterranean termites (*O. obesus*) under field conditions in wheat and chickpea crops for consecutive 3-years. Moreover, the infestation (%) obtained in the EPN powder formulation treated plots was statistically at par with Chlorpyriphos 20 % EC-treated plots,

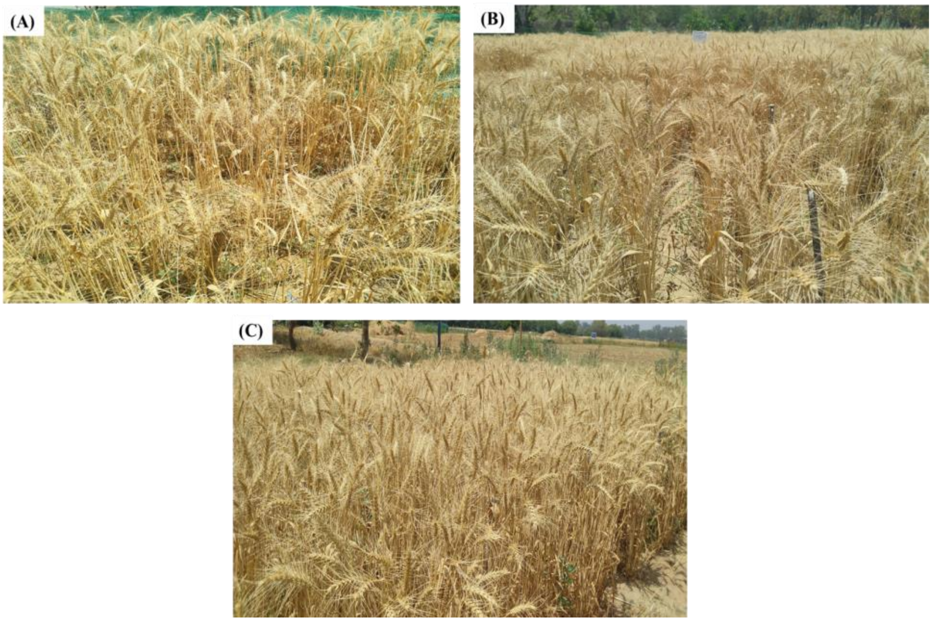

Figure 2. Termite infestation in wheat: Untreated control (A), Chlorpyriphos 20 % EC (B), EPN-enriched Powder (C)

suggesting the developed bioinsecticidal powder as an alternative to the synthetic insecticide for termite control. Further studies are required to use this EPN powder formulation as an integral component of integrated termite management programs.

Agronomical parameters of wheat and chickpea

The effects of different treatments on test agronomical parameters of wheat and chickpea are depicted in Table 2. In wheat, the application of powder formulation improved test agronomic parameters (plant height, number of spikelets/spike and weight of spike) than untreated control. The highest plant height was observed in T3 (EPN bioagent-enriched powder formulation) with a nominal value of 95.50 ± 4.14 cm. The number of spikelets/spike showed the pattern of T3 (19.20 ± 1.56) > T2 (18.87 ± 2.01) > T1 (17.07 ± 2.41). The spike weight in all treatments ranged between 2.47 ± 0.37 (T1) -3.46 ± 0.31 g (T2).

Table 2. Effect of different treatments on agronomic parameters of wheat and chickpea

Treatments	Wheat			Chickpea		
	Plant height (cm)	**No. of spikelets/ spike**	**Weight of spike (g)**	**No. of branches/ plant**	**No. of pods/ plant**	**Pod yield per plant (g)**
T1	87.87 ± 12.72^{a}	17.07 ± 2.41^{a}	2.47 ± 0.37^{b}	10.67 ± 0.53^{a}	52.87 ± 2.72^{b}	14.57 ± 1.07^{b}
T2	92.27 ± 6.77^{a}	18.87 ± 2.01^{a}	3.46 ± 0.31^{a}	11.93 ± 1.65^{a}	61.72 ± 5.10^{a}	16.45 ± 1.45^{b}
T3	95.50 ± 4.14^{a}	19.20 ± 1.56^{a}	3.46 ± 0.26^{a}	12.88 ± 1.54^{a}	62.13 ± 1.86^{a}	19.33 ± 1.42^{a}
LSD (P=0.05)	NS	NS	0.64	NS	7.00	2.65

(T1: Untreated control, T2: Chlorpyriphos 20 % EC, T3: EPN-enriched Powder, applied during sowing; The results are shown as the mean ± standard deviation of mean over three years; Means with dissimilar superscript letters within a column are significantly different as per Fisher's LSD at P=0.05; NS: non-significant)

In chickpea, the application of powder formulation significantly improved test agronomic parameters (no. of pods/ plant, and pod yield/plant), as compared to untreated control (T1). The maximum number of branches per plant were in T3 (EPN bioagent-enriched powder formulation) with a nominal value of 12.88 ± 1.54. The number of pods per plant showed the pattern of T3 (62.13 ± 1.86) > T2 (61.72 ± 5.10) > T1 (52.87 ± 2.72). The pod yield per plant in the case of all treatments ranged between 14.57 ± 1.07 (T1)-19.33 ± 1.42 g (T3).

Grain yield of wheat and chickpea

The effects of different treatments on three years' mean grain yield and test weight of wheat and chickpea are given in Table 3. Among the test treatments, the powder treatment revealed the highest grain yield and test weight in both wheat and chickpea. In wheat, the grain yield in EPN bioagent-enriched powder formulation was 27.46 ± 2.89 q/ha, as compared to 14.62 ± 5.26 q/ha in untreated control. A similar pattern was observed in chickpeas. The grain yield of 13.16 ± 1.38 q/ha was recorded in EPN bioagent-enriched powder formulation, which was superior to the untreated control (6.64 ± 1.33 q/ha). The test weight (1000-seed weight) of wheat ranged from 28.42 ± 2.21 g (T1) - 35.41 ± 1.50 g (T3). In chickpea, the test weight (100-seed weight) ranged in between 16.53 ± 0.21 g (T1) - 17.00 ± 0.35 g (T3).

Table 3. Three years' mean grain yield and test weight of wheat and chickpea

Treatments	Wheat		Chickpea	
	Grain yield (q/ha)	**Test weight (g/1000 seeds)**	**Grain yield (q/ha)**	**Test weight (g/100 seeds)**
T1	14.62 ± 5.26^{b}	28.42 ± 2.21^{b}	6.64 ± 1.33^{b}	16.53 ± 0.21^{a}
T2	26.39 ± 2.81^{a}	34.84 ± 1.43^{a}	8.70 ± 2.14^{b}	16.86 ± 0.23^{a}
T3	27.46 ± 2.89^{a}	35.41 ± 1.50^{a}	13.16 ± 1.38^{a}	17.00 ± 0.35^{a}
LSD (P=0.05)	7.65	3.50	3.32	NS

(T1: Untreated control, T2: Chlorpyriphos 20 % EC, T3: EPN-enriched Powder, applied during sowing; The results are shown as the mean ± standard deviation of mean over three years; Means with dissimilar superscript letters within a column are significantly different as per Fisher's LSD at P=0.05; NS; non-significant)

These results are consistent with the earlier reports. Although chemical pesticides are used to manage termites (2), several EPN species and bio formulations comprising EPNs have the ability to control termites (5). Under laboratory condition, Khanum and Javed (17) evaluated the termite (*Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae)) control efficacy of four *Steinernema* species, and reported 95 % and 100 % virulence effects of *S. pakistanense* NNRC-AS.04 and *S. bifurcatum* NNRC-As.65, respectively at a dosage of 150 IJs/ml. Javed *et al*. (13) in another study reported 100 % mortality of *Microtermes obesi* (Holmgren) (Blattodea: Termitidae) 48 h after application of *S. bifurcatum* N-KA.93 and *S. pakistanense* N-KA.04. Regarding the field studies to manage the termite damage, Gupta *et al*. (12) investigated the impact of chemical pesticide seed treatment on termite infestation patterns in wheat. They found that application of fipronil 40 % + imidacloprid 40 % WG was the best of the seven insecticides tested in terms of increasing yield and reducing termite damage. Among the bioformulations tested, Visalakshi *et al*. (32) reported that the two-time application of the entomopathogenic nematode *Heterorhabditis indica* (NBAII-H38) and entomopathogenic fungi *Metarhizium anisopliae* (NBAIR Ma-4) was successful in sugarcane by reducing the bud damage and seedling mortality caused by termites. In another study, a single treatment of Pusa NemaGel containing *S. thermophilum* IJs in a hydrogel matrix decreased the termite incidence in wheat crops by 48-78 % than controls (26). Due to the drop in termite occurrence, wheat yield increased from 22.2 to 43.3 % in this study.

Effects of field soil samples on *Galleria* mortality (*in-vitro*)

The impact of soil samples taken from various wheat and chickpea treatment plots on *Galleria mellonella* mortality (*in-vitro*) showed that the three years' mean mortality of the test insect in untreated control and Chlorpyriphos-treated plots were 0 % in both cases, whereas it was 66.67 ± 5.77 % and 70 ± 10 % in wheat and chickpea fields, respectively. Figure 3 displays representative illustrations of this bio-efficacy technique in wheat field soils. This finding implies that the EPN IJs applied to the fields, remain infective at 30 days of their application holding their bioinsecticidal potential.

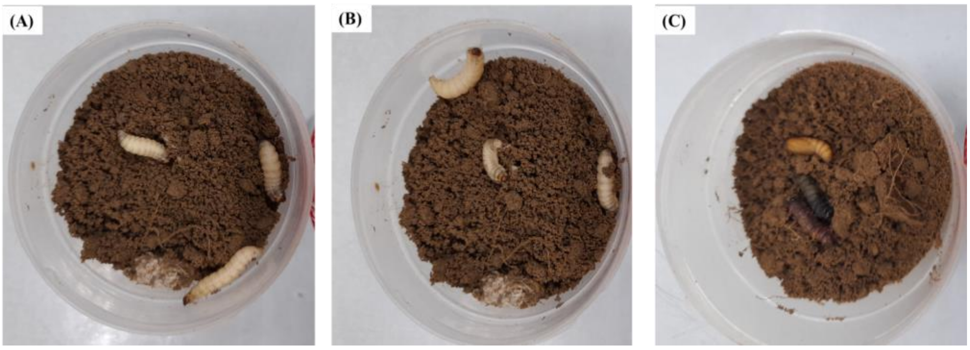

Figure 3. Virulence of untreated control (A), Chlorpyriphos 20 % EC-treated (B), and EPN powder-treated (C) field soil (wheat) against *Galleria mellonella* (4th instar larvae)

CONCLUSIONS

We evaluated the effects of *Steinernema abbasi* enriched bioinsecticidal powder formulation on termite management in wheat and chickpea under field conditions from 2020-2023. The mean termite damage expressed as infested tillers (%) in wheat, and percent infested plants in chickpea were lowest at harvest in the treatment comprising powder formulation, superior to the untreated control, but statistically at par to the synthetic insecticide (Chlorpyriphos 20 % EC). Moreover, the agronomic parameters namely (plant height, number of spikelets per spike, spike weight) in wheat, number of branches per plant, number of pods per plant, and pod yield per plant in chickpea were increased by the powder formulations. The nematode IJs applied through the powder formulation were virulent for 30-days of their application. Further studies needs to be done on shelf life experiments and the application of this biocontrol formulation for integrated pest management programs.

AUTHORS' CONTRIBUTIONS

AM and SC conducted the experiments. AS, VSS, and SMN provided resources and supervised the experiments. AS conceptualized the study. AS, VSS, SB, and AM prepared the original draft. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The Authors declare no conflict of Interest.

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