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EFFECTIVENESS OF TWO ENTOMOPATHOGENIC NEMATODES (NEMATODA: RHABDITIDA) POPULATIONS IN CONTROLLING FALL ARMYWORM [(*SPODOPTERA FRUGIPERDA*) LEPIDOPTERA: NOCTUIDAE] ON MAIZE

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ABSTRACT

The fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is a recent invasive pest significantly impacting maize production and food security in sub-Saharan Africa. While chemical insecticides have offered the most effective control in Ghana, their detrimental effects on human health and the environment necessitate the urgent exploration of alternative, environmentally friendly options. This study compared two entomopathogenic nematodes populations, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*, against a control group (no application) for their efficacy in managing fall armyworm on maize. In the second experiment, *S. carpocapsae* significantly outperformed both *H. bacteriophora* and the control in terms of seed weight, achieving a 12.9% increase over the former and a remarkable ninefold improvement over the latter. The first experiment, however, showed no significant difference in seed yield (59 g, 32 g, 2 g) between the two nematode treatments, *S. carpocapsae* demonstrated the highest yield within this group. Notably, the control group exhibited the highest degree of foliage damage (4.7; 5.0) on a 1-5 scale throughout the study period. Overall, this study demonstrates the superior efficacy of *S. carpocapsae* compared to *H. bacteriophora* in controlling fall armyworm on maize. These findings provide valuable understandings for advancing the practical application of entomopathogenic nematodes as a sustainable and eco-friendly biocontrol measure against this devastating pest, contributing to enhanced food security in sub-Saharan Africa.

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INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), has emerged as a major insect pest of maize (*Zea mays* L.) (Poaceae) in Ghana. In the southern and middle belt, where maize is planted in two seasons (major and minor), this continuous availability of feed resources facilitates unchecked multiplication of the destructive FAW larvae. Their voracious feeding on the cartridge and inflorescence of young maize plants inflicts substantial economic losses throughout the entire crop cycle, posing a significant threat to food security in sub-Saharan Africa (Day et al., 2017).

The primary approach to effectively combat FAW larvae infestations in maize fields in Ghana has been the application of synthetic insecticides, spearheaded by governmental initiatives since the pest's invasion in 2016. However, the excessive and indiscriminate use of these synthetic insecticides by farmers has resulted in severe environmental consequences, causing escalated chemical pollution (Malhat et al., 2015). Moreover, this misuse has led to the emergence of FAW populations that exhibit resistance to commonly employed insecticides (Neri et al., 2005). The persistent and excessive use of synthetic insecticides, encompassing carbamates, organophosphates, and pyrethroids, has given rise to significant challenges related to insecticide resistance across diverse food crops. Zhu et al. (2015) documented instances of multiple resistances, including resistance to organophosphates and *Bacillus thuringiensis* (Bt) (Bacillaceae), in FAW. Similarly, Huang et al. (2014) observed resistance to Cry1Fa proteins of Bt in FAW larvae from Puerto Rico and Florida (USA). Further evidence of resistance to Cry1Fa, pyrethroids, and organophosphates in FAW populations was reported by Santos-Amaya et al. (2016).

As an alternative approach, entomopathogenic nematodes (EPNs) and fungi have gained attention in the management of insect pests (Javed et al., 2019; Gulzar et al., 2020; Shehzad et al., 2021, 2022; Thakur et al., 2022) and offer potential integration into Integrated Pest Management (IPM) programs for FAW control in maize. Thakur et al. (2022) highlighted that the application of EPNs against insect pests carries a higher degree of safety for both humans and the environment compared to synthetic insecticides application. In the current study, the efficacy of FAW management on maize was assessed by employing two different EPN populations, namely *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*, under controlled plant house conditions. This research contributed to the exploration of sustainable and environmentally friendly alternatives for effective FAW control in agricultural settings.

MATERIALS AND METHODS

Soil sampling for entomopathogenic nematodes

Soil samples were collected from 39 maize farms in Ashanti, Bono East, Bono, Ahafo, and Western North regions using soil auger and hand trowel. The farms were located in four different agroecologies: Forest Savanah Transition, Moist-semi-deciduous Forest/ Guinea Savanah Woodland, Forest, and Forest Savanah Transition. These agroecologies have a bimodal rainfall pattern. The GPS coordinates of the farms were recorded with a GARMIN GPSMAP64s device. The altitude of the farms ranged from 147 m to 430 m above sea level. For each farm, 10 core soil samples were taken from an acre (0.4 ha) plot and mixed thoroughly into a composite sample weighing 500 g for EPN extraction. The soil samples were stored in plastic containers with a capacity of 635 cm^3. The lids of the containers had small holes to allow air circulation. The holes were small enough to prevent the escape of the greater wax moth larvae, *Galleria mellonella* (Lepidoptera: Pyralidae), that were used as bait insects. Each container had five last instar *G. mellonella* larvae (Bedding and Akhurst, 1975). The containers were sealed and inverted to ensure even distribution of the larvae in the soil. The containers were then incubated in a controlled chamber at 25 ±2°C, 60% relative humidity, and 12-hour photoperiod (Woodring and Kaya, 1988; Parra, 1998).

Multiplication and application of fall armyworm larvae

Last instar fall armyworm (FAW) larvae were obtained from the Entomology Section, CSIR-Crops Research Institute, Kumasi, in controlled cages (25 ± 2°C, 70 ± 10% RH, 12 h photoperiod). The FAW larvae were applied to the maize seedlings 14 days after emergence at one FAW larva per maize plant.

Culturing and extraction of *Steinernema* and *Heterorhabditis* populations from parasitized *G. mellonella* cadavers

The EPNs were grown and multiplied on last instar *G. mellonella* larvae. The populations of *S. carpocapsae* and *H. bacteriophora* were determined based on peculiar cuticular color differences the *G. mellonella* larvae cadavers assumed upon infection by the EPNs. *Heterorhabditis bacteriophora*-infected cadavers turned red or yellowish, while *S. carpocapsae*-infected cadavers turned brown, tan, or black (Sayed et al., 2022). The *G. mellonella* cadavers assuming those peculiar color changes were grouped for EPNs population extraction using modified White traps (Woodring and Kaya, 1988). The modification involved the use of kitchen tissue paper (Papyrus, Delta Paper Mill Ltd, Ghana) wrapped around an 8 cm diameter Petri dish. The wrapped Petri dish was inverted and placed in 14 cm diameter Petri dish. The cadavers were dipped in double-distilled water (ddH_2O) to remove adhering soil particles and disinfected with 0.1% sodium hypochlorite (NaOCl) as an antimicrobial agent.

Five cadavers were placed on each White trap setup. The placement of the cadavers was carried out carefully to ensure they did not touch each other. A 100 ml quantity of double-distilled water (ddH_2O) was poured into the 14 cm diameter Petri dish. The setup was covered and placed in an incubation chamber (25 ± 2°C) to allow the infective juveniles of EPNs to migrate into the ddH_2O for collection. After 14 days, the EPNs suspension was collected and concentrated to 20 ml each for assessment using a stereomicroscope (100× magnification) with a tally counter to facilitate the counting process.

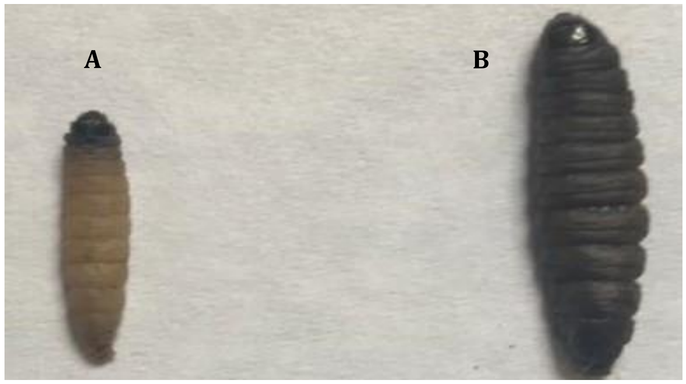

Figure 1. Entomopathogenic nematode infected *G. mellonella* cadavers. Cadaver colour change was observed few days after death. A (*H. bacteriophora*) infected cadavers turned red or yellowish); B (*S. carpocapsae* infected cadavers turned brown, tan, or black) (Sayed et al. 2022).

Experimental setup

The study was conducted at the CSIR-Crops Research Institute, Kumasi (N06°43.079' W001°31.979'; Altitude 295 m), Ghana. A partitioned plant house accommodated each of the three treatments. The treatments were;

T1 = *Steinernema carpocapsae*

T2 = *Heterorhabditis bacteriophora* and

T3 = untreated control.

Steam-sterilized topsoil weighing 8.5 kg per vessel (dimensions: 31 × 28 × 20 cm, top × height × base) was used (sterilized at 120°C for 30 min). The vessels were arranged in Completely Randomized Design (CRD) fashion with four replications. *Zea mays* L. cv. Abontem was sown and thinned to two seedlings per vessel seven days after emergence.

Application of treatments

Third instar FAW larvae, multiplied in controlled cages (25 ± 2℃, 70 ± 10% RH, 12 h photoperiod) according to the method by Greene et al. (1976), were applied to the maize plants at a rate of one FAW larva per plant, totaling 24 FAW larvae per cage 14 days after seedling emergence. The treatments were administered 14 days after introduction of FAW larvae. A 200 ml EPNs suspension was prepared for each pure population at a density of 2000 infective juveniles per ml and stored in tissue culture flasks at 15°C. EPN stocks, less than 2 weeks old as recommended by Laznik and Trdan (2013), were applied using a standard pesticide hand sprayer after assessing nematode viability. Only EPN stocks with over 90% survival were used. Re-infestation was carried out with FAW larvae, and the same treatments were re-applied 30 days after the initial at same rates.

An NPK 15:15:15 fertilizer was applied through band placement seven days after emergence at rate of one matchbox per pot. Urea (46% N) was applied 21 days after the NPK using the same rate and application method. Data collected included the number of infected cobs, severity of cob damage on a 1-5 scale, foliage damage on 1-5 scale (dos Santos et al., 2020), plant height (cm), stalk dry matter weight (g), maize cobs weight (g), and maize seeds weight (g). The foliage damage rating scale (1-5) was defined as follows: (1) no damage; (2) little damage – 10 to 25% leaf damage; (3) medium damage – 25 to 50% leaf damage; (4) heavy damage – 50 to 75% leaf damage, with most of the plant showing damage symptoms; and (5) very heavy or total damage – the plant is almost dying. The study was concluded 12 weeks after emergence and was repeated under same conditions.

Data analysis

The collected data were analyzed using multivariate analysis of variance (MANOVA) in RStudio version 4.1.3. Suppose y was a response vector of pathological or yield parameters consisting of q components and $y_{ki} = \alpha + \beta_k + \varepsilon_{ki}$; where: k = 1, 2, …, c ; i = 1, 2, …, d. [α denotes an overall level, β_k denotes the fixed effect of treatment application] (Johnson and Wichern, 2007). The treatment means were separated with Tukey's HSD at 5% significance level.

RESULTS

The MANOVA results for the impact of treatments on the response variables in experiments 1 and 2 were significant, with Pillai Test Statistics of 2.4 (App. F= 2.5, p=0.05) for experiment 1 and 2.5 (App. F= 2.9, p=0.03) for experiment 2. Approximately 82-84% of the variability in the dependent variables in the canonical MANOVA estimate was explained by the treatment effect in experiments 1 and 2, respectively.

There were no significant differences observed between *H. bacteriophora* and *S. carpocapsae* in terms of maize cob infection severity, plant height, and stalk dry matter weight. The control group exhibited the highest foliage damage, scoring 4.7 on 1-5 scale over the study period (Figure 2). This damage was 57% and 66% higher than that observed in *H. bacteriophora* and 57% and 60% higher than in *S. carpocapsae*, respectively.

Cob and seed yield were generally higher in experiment 2 (Table 2). Specifically, *S. carpocapsae* recorded the highest cob weight (113.7 g; 1397.0 g) compared to *H. bacteriophora* (71.7 g; 1296.0 g) and the control (4.7 g; 160.7 g) in experiments 1 and 2, respectively.

Table 1. Treatment effect on fall armyworm infestation and maize yield in experiment one.

Treatment	Infected cobs number	Infected cobs severity (1-5)	Foliage damage (1-5)	Plant height (cm)	Stalk dry matter weight (g)	Cobs weight (g)	Seeds weight (g)
Heterorhabditis bacteriophora	2.3a	3.0ab	2.0b	174.0a	82.9a	71.7a	32.0a
Steinernema carpocapsae	4.0a	2.7ab	2.0b	130.7a	47.3a	113.7a	59.0ab
Control	5.7a	5.0a	4.7a	119.0a	18.7a	4.7b	2.0b
HSD (5%)	2.7	2.2	1.3	82.9	37.4	73.0	45.1

The values are means of four replicates.

Table 2. Treatments effect on fall armyworm infestation and maize yield in experiment two.

Treatment	Infected cobs number	Infected cobs severity (1-5)	Foliage damage (1-5)	Plant height (cm)	Stalk dry matter weight (g)	Cobs weight (g)	Seeds weight (g)
Heterorhabditis bacteriophora	1.3cb	3.0ab	1.7bc	173.3a	58.3a	1296.0b	796.0a
Steinernema carpocapsae	3.3b	2.0ab	2.0b	130.3a	51.3ab	1397.0b	899.7b
Control	10.3a	5.0a	5.0a	119.0a	17.3b	160.7c	86.3d
HSD (5%)	2.9	3.8	0.8	99.0	36.2	132.8	82.7

The values are means of four replicates

The differences between *H. bacteriophora* and *S. carpocapsae* in maize cob infection severity, plant height, and stalk dry matter weight were not significant. The control recorded the greatest foliage damage (4.7; 5.0) on a 1-5 scale over the study period (Figure 2). This was (57; 66%) and (57; 60%) higher than *H. bacteriophora* and *S. carpocapsae*, respectively. Cob and seed yield were generally higher in experiment 2 (Table 2). *S. carpocapsae* recorded the greatest (113.7 g; 1397.0 g) compared to *H. bacteriophora* (71.7 g; 1296.0 g), and the control (4.7 g; 160.7 g) in cob weight in experiments 1 and 2, respectively.

DISCUSSION

Nematodes belonging to the Steinernematidae and Heterorhabditidae families form associations with pathogenic bacteria to eliminate insect hosts, typically accomplishing this within 48 hours of infection (Chaston and Goodriech-Blair, 2010). Functioning as biocontrol agents, both *H. indica* and *S. carpocapsae* exhibited 100% mortality against FAW larvae within 24 hours post-exposure, utilizing nematode concentrations ranging from 150 to 2400 infective juveniles (IJs) (Mohamed and Shaira, 2023) in an entomopathogenicity study.

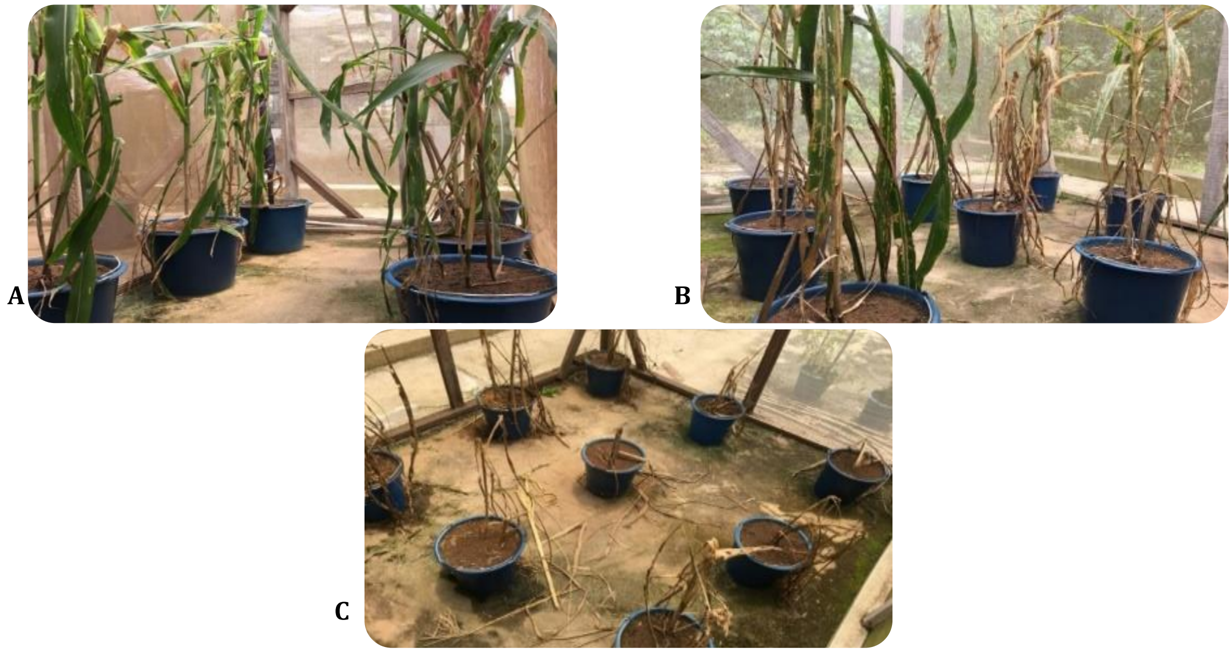

Figure 2. A: Maize plants under *H. bacteriophora* infestation; B: Maize plants under *S. carpocapsae* infestation; C: Untreated control treatment.

In the current investigation, *S. carpocapsae* demonstrated a significant increase in seed weight compared to *H. bacteriophora* and the control, showing a 12.9% and approximately 9-fold improvement, respectively, in experiment 2. Consistent with our findings, Mohamed and Shaira (2023) reported that *S. carpocapsae* displayed greater virulence against various stages of FAW larvae compared to *H. indica*. Numerous studies have substantiated the variability in the efficacy of different entomopathogenic nematode species on FAW larvae and other insect pests in terms of mortality and damage caused (Rahoo et al., 2011, 2018a; Fallet et al., 2022). *Steinernema* nematodes exhibited a high potential in controlling FAW larvae (Acharya et al., 2020). Conversely, FAW larvae were found to be susceptible to *H. indica* infection (Shinde et al., 2022).

The superiority of *S. carpocapsae* over *H. bacteriophora* in reducing infections and subsequently increasing maize seed yield in this study is supported by other research. *Steinernema* nematodes were highly effective against *Spodoptera* spp. (Fallet et al., 2022). According to Elbrense et al. (2021), differences in virulence or infectivity between *Steinernema* and *Heterorhabditis* nematodes may be attributed to host insect specificity, morphological features of the nematode species, and their tolerance to the host's immune response (Rahoo et al., 2017, 2018b, 2019a, b).

CONCLUSION

Steinernema carpocapsae has demonstrated superiority over *H. bacteriophora* nematodes in effectively reducing fall armyworm infections in maize, resulting in a higher seed yield and an improvement in farmers' income and livelihoods. This study highlights the variability in entomopathogenic nematode populations as potential biocontrol agents for managing fall armyworm infections, ultimately contributing to an increase in maize yield and farmers' income.

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AUTHORS' CONTRIBUTION

YD, JA and BWA designed the study and did experimental works; BA collected data, nematode extractions and quantification; EAO and KFA contributed in statistical analysis and paper writing; MO and MBM did monitoring, evaluation, and edited the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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