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Application of Entomopathogenic Nematodes in Management of Fall Armyworm Infesting Maize in Ghana: A Greenhouse Study

Danso, Yaw; U.S. Issa; J. Adomako; I. Adama; K. Osei; E. Agyei Obeng; M. Opoku; W.B. Amoabeng and B. Abugri

CSIR-Crops Research Institute, P.O. Box 3785, Kumasi, Ghana.

Corresponding author email: abiacea@yahoo.co.uk

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ABSTRACT

Fall armyworm infestation poses a serious threat to the food security and livelihoods of smallholder maize farmers in Ghana. The most sustainable management approach is Integrated Pest Management. Entomopathogenic nematodes have the potential for inclusion in IPM to manage crop insect pests. The study aimed at finding a sustainable option to manage fall armyworms in maize. Maize plant rhizosphere soils were sampled from maize farms in 2019 for entomopathogenic nematodes. On an acre maize farm, 10 core soil samples were collected using soil augur at $\leq$20 cm soil depth and composited weighing 0.5 kg. Each composite soil sample was supplied with five 5th instar stage fall armyworm larvae and incubated (25℃; 85% RH) in a dark room. After 4 days, the fall armyworm larvae cadavers were removed from the soil for culturing and collection of infective entomopathogenic nematodes using modified White traps. A partitioned plant house accommodated each of three treatments: *(T_1)* Supa ataka (Emamectin benzoate), *(T_2)* Entomopathogenic nematodes, and *(T_3)* No application – Control. The third instar fall armyworm larvae were added to the maize seedlings 14 days after emergence. The data collected were subjected to Multivariate Analysis of Variance (MANOVA) in IBM SPSS Statistics 21. About 99.9 and 99.4% of the variability in the dependent variables in a canonical MANOVA derived estimate is accounted for by the treatment effect in experiments one and two respectively. Entomopathogenic nematode application against fall armyworm is promising for incorporation into IPM strategies against the pest. This will minimize over-reliance on synthetic insecticides in maize production.

Keywords: Insecticidal nematodes, Environmental safety, *Spodoptera frugiperda*, *Zea mays*.

INTRODUCTION

Maize (*Zea mays* L.) is the most important cereal staple crop in Ghana. It accounts for more than 50% of total cereal production (MoFA, 2012). It is commonly cultivated and serves as food and cash income to many households (Tachie-Obeng et al., 2010; GSS, 2008). It ranks first in farmland area cultivated (MoFA, 2013). On this account, maize was selected as the number one crop in the government of Ghana's flagship program '*Planting for food and jobs* in 2018. Despite the numerous benefits of maize to the Ghanaian economy, the average economic yield is one of the lowest in the world hovering between 1.2 and 1.8 MT/ha in the face of the 6.0 MT/ha potential (MoFA, 2010). Recent invasion by fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is posing threat to the livelihoods of smallholder maize farmers (GhanaWeb, 2020). Ghana lost US$64 million through the FAW infestation on maize in 2017 (GNA, 2018). The absence of sustainable and cheaper management options is causing huge losses in maize yields seasonally in the country. Maize farmers in Ghana have used synthetic insecticides to control FAW since its invasion in 2017 mostly through central government support. Over-reliance on synthetic pesticides has human health, environmental threat, and global insecticidal resistance concerns. The most preferred approach to deal with FAW infestation in maize is Integrated Pest Management (IPM) (FAO, 2018). This combines a variety of control options into a sustainable package to lower infestation levels. Entomopathogenic nematodes (EPNs) have the potential for inclusion in IPM as biocontrol agents to manage crop insect pests. These insecticidal nematodes are more specific and have a higher degree of human and environmental safety (Georgis et al., 2006). The objective of this study was to assess the efficacy of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) against FAW infestation in maize.

MATERIALS AND METHODS

Soil and fall armyworm larvae sampling

Maize plant rhizosphere soils were sampled from FAW-infested maize farms in EPN hotspot areas of Brong-Ahafo, Volta, and Greater Accra regions of Ghana. All the sampling areas fall under Forest Savannah Transition and Coastal Savannah agro-ecologies and experience bi-modal rainfall patterns. On an acre maize farm size, 10 core soil samples were collected systematically in an N-shaped fashion. Soil sampling was done using soil augur at ≤20 cm soil depth and composited weighing 0.5 kg. Fifth instar FAW larvae were sampled from infested maize plants on the same farms. The harvested FAW larvae were applied to the soil for infestation by indigenous EPN (Steinernematidae, Heterorhabditidae) (Molina-Ochoa, 2003). The dry soil samples were moistened with water sprays to facilitate the movement of nematodes in the soil.

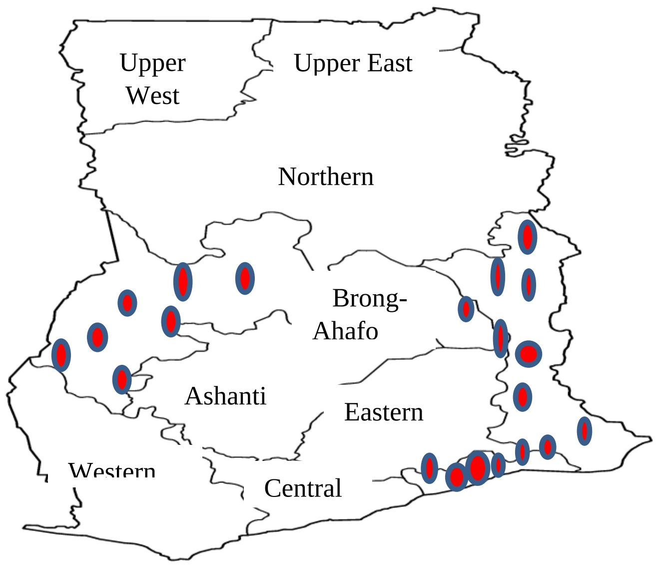

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Figure 1. Sampling point locations in three regions of Ghana in the major planting season, 2019.

Isolation of entomopathogenic nematodes from fall armyworm cadavers

Each composite soil sample was supplied with five live FAW larvae in a lid-perforated plastic container (5.5 x 11 x 10.5 cm) (Molina-Ochoa, 2003). The covered plastic container and its content were turned upside down 5x to ensure uniform mixing of the FAW larvae and the soil. The samples were stored in insulated cool boxes to reduce dehydration and transported to the lab for incubation in a dark room (25℃; 85% RH) (Bedding and Akhurst, 1975). After 4 days, the FAW larvae cadavers were removed from the soil for culturing, emergence, and collection of EPN infective juveniles using modified White traps (Woodring and Kaya, 1988). The EPN suspensions were harvested and concentrated to 20 ml each after 14 days. Quantification was done using a stereomicroscope (100x magnification) and a tally counter.

Greenhouse experiment set-up

A partitioned greenhouse accommodated each of three treatments. Treatments used were *(T_1)* Supa ataka (Emamectin benzoate), *(T_2)* Entomopathogenic nematodes (Steinernematidae, Heterorhabditidae), and *(T_3)* untreated (Control). The study was stationed at CSIR-Crops Research Institute, Kumasi (N06°43.079’ W001°31.979’; Altitude 295 m), Ghana. Steam pasteurized (120℃ for 30 mins) topsoil weighing 8.5kg per pot [(31 x 28 x 20 cm) (top x height x base)] was used. The plastic pots were arranged in a Completely Randomized Design with four replications. Abontem maize cultivar was sowed on 15 October 2019 and thinned to two seedlings per pot seven days after emergence. Third instar FAW larvae multiplied in controlled cages (25 ±2℃, 70 ± 10% RH, 12 h photoperiod) following Greene et al. (1976) were applied to the maize seedlings 14 days after emergence at one larva per plant. The treatments were applied 14 days after the FAW larvae application. The emamectin benzoate was applied (30 mL/ 16 L knapsack sprayer) (Fig. 2a). A 200 mL EPN suspension was prepared (1,028 infective juveniles / mL) and sprayed using a standard pesticide sprayer (Fig. 2b). Re-infestation was done 30 days after the first and the same treatments were re-applied at the same rates. An NPK 15: 15: 15 fertilizer was applied by band placement seven days after emergence. Urea fertilizer was applied 28 days after emergence using the same rate and mode of application.

Photo Credit: Bismark Abugri

Figure 2A: Emamectin benzoate application; **2B:** Entomopathogenic nematodes application using a standard pesticide sprayer.

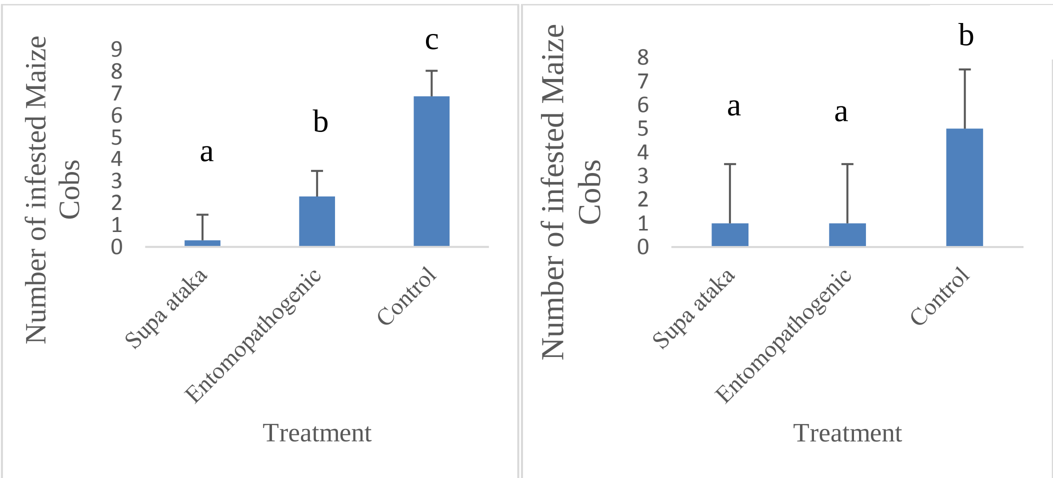
Data collected included; the number of dead FAW larvae 24 hours after treatments application, number of FAW larvae at harvest, number of maize cobs, the weight of maize cobs (kg), the weight of maize seeds (kg), frass incidence, and severity of maize cobs damage (scale of 1-5). The study was terminated 12 weeks after sowing. It was repeated under the same conditions to consolidate the results.

Data analysis

The experiment was laid out as a Completely Randomized Design (CRD) with four replicates. All essential data were taken at the appropriate time and growth stage of the crop. Collected data were analyzed using the Multivariate Analysis of Variance (MANOVA) design. Suppose Y is a response vector of pathological or yield parameters consisting of p components and $Y_{hj} = \propto + \beta_h + \varepsilon_{hj}$; where $h = 1, 2, \ldots, b$; $j = 1, 2, \ldots, a$. α denotes an overall level, β_h denotes the fixed effect of treatment application (Johnson and Wicherin, 2007). The data were analyzed with IBM SPSS Statistics 21 and the means were separated with Tukey's HSD at a 5% significance level.

RESULTS AND DISCUSSION

The MANOVA output of the effect of treatment on the response variables for experiment 1 (F=233.86, p=0.004) and experiment 2 (F=26.97, p=0.036) was significant. About 99.9 and 99.4% of the variability in the dependent variables in a canonical MANOVA derived estimate is accounted for by the treatments for experiments one and two, respectively.

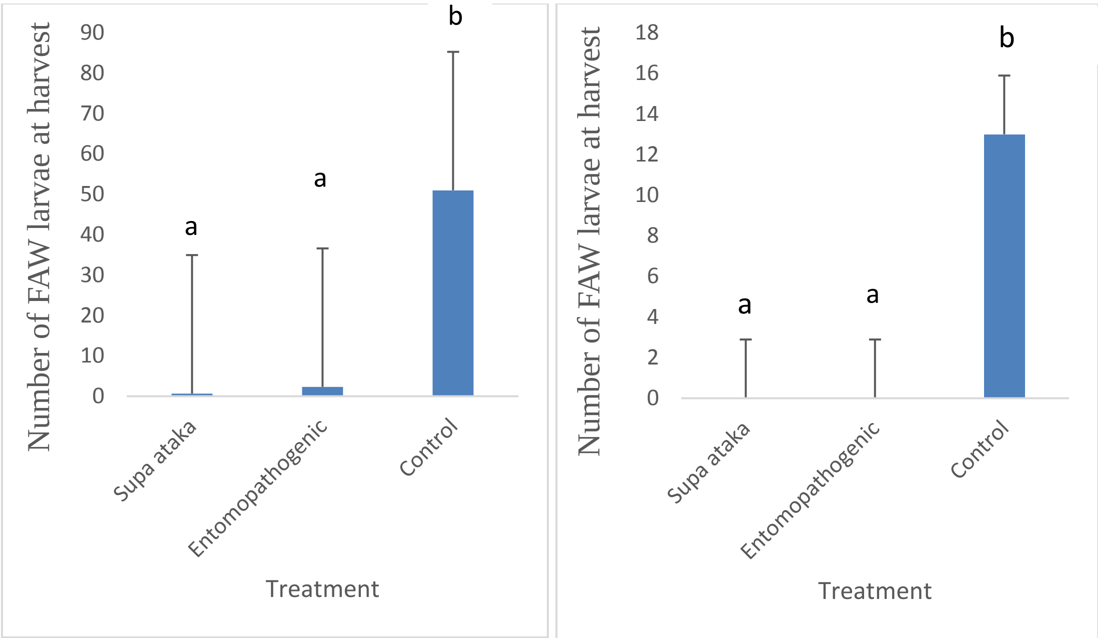


Experiment 1 Experiment 2

Figure 3: Number of fall armyworm infested maize cobs at harvest.

Entomopathogenic = Steinernematidae and Heterorhabditidae entomopathogenic nematodes.

The number of FAW-infested maize cobs at harvest variable for experiment 1 (F=4.42, p=0.001) and experiment 2 (F=1.39, p=0.001) were significant. In experiment 1, emamectin benzoate had significantly the lowest FAW infestation, followed by entomopathogenic nematodes, which was also significantly lower than that of the control (Fig. 3). In experiment 2, emamectin benzoate and entomopathogenic nematodes recorded similar FAW infestations and were significantly lower than infestations recorded by the control (Fig.3). Thus, emamectin benzoate and entomopathogenic nematodes were effective in reducing the number of FAW infestations on maize cobs.



Experiment 1 Experiment 2

Figure 4: Number of fall armyworm larvae at harvest.

The number of FAW larvae at harvest for experiment 1 (F=13.09, p=0.001) and experiment 2 (F=126.75, p=0.001) were significant over the controls. The emamectin benzoate and entomopathogenic nematodes recorded a similar number of FAW larvae and were significantly lower than that of the control for the two experiments (Fig. 4).

Table 1: Treatments effect on maize crop under greenhouse conditions.

Treatment	Frass incidence		Cob weight (kg)		Seed weight (kg)	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Supa ataka (Imamectin benzoate)	0.000	0.000	0.867	1.033	0.500	0.600
Entomopathogenic nematodes	3.000	1.667	0.567	0.733	0.267	0.367
Control	6.667	7.667	0.367	0.300	0.087	0.067
HSD (5%)	1.670	1.867	0.145	0.313	0.089	0.187

Exp. = experiment; HSD = honestly significant difference.

In experiment 1, the control recorded significantly (F=6.7; p=0.001) the most frass incidence; followed by entomopathogenic nematodes which was also significantly (3.0; p=0.0) more than emamectin benzoate (Table 1). In experiment 2, the difference between entomopathogenic nematodes and emamectin benzoate in frass incidence was not significant (Table 1).

Also, cob weight (kg) for experiment 1 (F=57.00, p=0.001) and experiment 2 (F=26.21, p=0.001) were significant. In experiment 1, emamectin benzoate recorded significantly (F=0.87; p=001) the highest cobs weight (Plate 1). Entomopathogenic nematodes out yielded the control in the same experiment. However, in experiment 2, emamectin benzoate and entomopathogenic nematodes recorded similar cobs weight which was significantly higher than that of the control (Table 1).

The seed weight (kg) for experiment 1 (F=99.97, p=0.001) and experiment 2 (F=38.60, p=0.001) were significant. Emamectin benzoate still recorded significantly heaviest seed weight, followed by entomopathogenic nematodes. The control recorded the least in both experiments (Plate 2).

Plate 1: Treatments effects on maize cobs (1a) Emamectin benzoate; (1b) Control; (1c) EPN

Plate 2: Treatments effects on maize seed yield, (2a) Emamectin benzoate; (2b) Control; (2c) EPN

Entomopathogenic nematodes performed comparably to the emamectin benzoate in reducing FAW infestations and protecting maize cobs against FAW. In a related study, Molina-Ochoa et al. (1999) demonstrated that entomopathogenic nematodes increased FAW larval and prepupal stages mortality. The current study corroborated that of Molina-Ochoa et al. (1996) where all EPN strains of *Steinernema carpocapsae* and *S. riobravis* were pathogenic against 7-day-old FAW larvae, prepupae, and pupae stages. It was demonstrated that the FAW prepupal stage was the most susceptible. Molina-Ochoa et al. (1999) showed that smaller prepupae entering the soil from FAW larvae resulted in increased mortality by entomopathogenic nematodes and other biocontrol agents. This prevented adults from emerging to migrate to start infesting other host crops. Castro et al. (1986) also found up to 71% natural parasitism of FAW larvae by EPN in southern Honduras. Cabanillas et al. (1994) also worked on the EPN, *S. riobravis* on soils sampled from maize farms in Texas, USA. Prepupae and pupae FAW were observed to be naturally infected by EPN, *Steinernema* spp. in 24% of the maize farms sampled. Other successful results have been obtained with *Steinernema* spp. for controlling other insect pests such as root weevil, *Diaprepes abbreviates* (Schroeder, 1994), and pink bollworm, *Pectinophora gossypiella* (Henneberry et al., 1995). Molina-Ochoa et al. (2003) demonstrated the natural occurrence of Steinernematidae and Heterorhabditidae EPN in soil samples collected in 64 localities (corresponding to 10.9%) from six Mexican states.

It was detected that entomopathogens were associated with FAW populations in maize, sorghum, and pastures. They showed huge potential to control FAW in such agroecosystems.

Steinernematidae and heterorhabditidae EPN reduced FAW infestations and increased maize yield in our study. Fall armyworm has naturally occurring soil pathogenic microbes including EPN. These are biological control agents that feed on insect host pests. They are effective and have no existent mammalian and environmental toxicity. Interest in the usage of EPN as bio-insecticides to complement the biocontrol 'fight' against FAW is high. Application of Steinernematidae and Heterorhabditidae EPN at 1,028 infective juveniles per ml reduced FAW infestations in maize. Further testing will be done especially under natural field conditions to consolidate EPN efficacy against FAW for increased maize crop productivity in Ghana and beyond.

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