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OCCURRENCE OF ENTOMOPATHOGENIC NEMATODES (STEINERNEMATIDAE, HETERORHABDITIDAE) AS POTENTIAL BIOCONTROL AGENTS AGAINST *Spodoptera frugiperda* INFESTING *Zea mays*

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ABSTRACT

Synthetic insecticides are used worldwide to manage invasive fall armyworm (*Spodoptera frugiperda*) in cereals, in spite of the chemical's prohibitive cost and threats to environmental health. In Ghana, little attention has been given to entomopathogenic nematodes as insect pest biocontrol agents, due to the existence of dismal expertise in the field. This study evaluated maize (*Zea mays* L.) farms for endemic entomopathogenic nematodes as potential biological control agents against fall armyworms infesting maize in Ghana. Entomopathogenic nematode juveniles were extracted using an insect baiting technique, White traps. These Rhabditid nematodes (*Heterorhabditis*; *Steinernema*) were found in 75% of 200 soil samples; and 92.5% of 40 districts targeted by this study. The Greater Accra region recorded the greatest population density (1,820 juveniles per 5 fall armyworm larvae cadavers); while; the Central region recorded the lowest density (81 juveniles per 5 fall armyworm larvae cadavers). There was high presence of entomopathogenic nematodes across the study areas. Therefore, entomopathogenic nematodes offer a promising alternative strategy for managing fall armyworm infestation of maize in Ghana to minimise over-reliance on synthetic insecticides.

Key Words: Bio-insecticidal nematodes, *Heterorhabditis*, *Steinernema*

RÉSUMÉ

Les insecticides chimiques de synthèse sont utilisés dans le monde entier pour lutter contre les chenilles légionnaires d'automne (*Spodoptera frugiperda*) envahissants le maïs (*Zea mays* L.), malgré le coût élevé de ces produits et les menaces qu'ils représentent pour l'environnement. Au Ghana, peu d'attention a été accordée aux nématodes entomopathogènes comme agents de lutte biologique contre les insectes ravageurs en raison du peu d'expertise dans le domaine. Cette étude a permis de prospecter des champs de maïs à la recherche de nématodes entomopathogènes endémiques comme agents potentiels de lutte biologique contre les chenilles légionnaires d'automne infestant le maïs. Des juveniles de nématodes entomopathogènes ont été extraits à l'aide de la technique d'appât pour insectes et du White trap. Ces nématodes Rhabditid (*Heterorhabditis*; *Steinernema*) ont été trouvés dans 75% des 200 échantillons de sol; et 92,5% des 40 Communes ciblées. La région du Grand Accra a enregistré la plus grande densité de population (1820 juvéniles/ 5 cadavres de larves de la chenille légionnaire d'automne) tandis que la région centrale a enregistré la plus faible (81 juvéniles/ 5 cadavres de larves de la chenille légionnaires d'automne). Il y avait une forte présence de nématodes entomopathogènes enregistrés en nombres importants dans toutes les zones d'étude. Par conséquent, les nématodes entomopathogènes offrent une stratégie alternative prometteuse pour gérer l'infestation par les chenilles légionnaires d'automne au Ghana. S'ils étaient davantage exploités, ils permettraient de réduire la dépendance excessive à l'égard des insecticides de synthèse dans la production de maïs.

Mots Cles: Nematodes entomopathogènes, *Heterorhabditis*, *Steinernema*

INTRODUCTION

Maize (*Zea mays* L.) serves as food and cash income crop for many households in sub-Saharan Africa (Tachie-Obeng *et al.*, 2010) and offers direct and indirect employment to many (GSS, 2018). As such, maize was chosen as the 'star' crop in the Ghana government's flagship programme "*Planting for food and jobs*" in 2018. However, maize production still lags behind demand, owing to proliferation of the fall armyworm (FAW), *Spodoptera frugiperda* (Noctuidae), which is endangering sustainable maize production in the country (MoFA, 2020). GhanaWeb (2020) also opined that the pest was endemic and, was thus posing a threat to livelihoods in the country. In fact, in 2018 Ghana lost maize worth US$ 64 million in revenue due to the FAW infestation of maize fields (GNA, 2018).

Maize farmers in Ghana almost exclusively use synthetic insecticides to manage FAW infestations, in spite of their prohibitive costs and threat to environmental health. FAO (2018) recommended integrated pest management (IPM) as the most appropriate approach to managing FAW infestations. Integrated pest management is a combination of a variety of control tactics into one sustainable package to lower infestation levels. Entomopathogenic nematodes (EPN), also known as insect parasites, have great potential against FAW as biocontrol agents and should, therefore, be included in the IPM package recommended for its control. These insecticidal nematodes are more specific and have a higher degree of human and environmental safety (Georges *et al.*, 2006). In Ghana, however, limited attention has been given to EPN in insect pests' biocontrol due to dismal expertise available in the field. The objective of this study was to bio-prospect maize farms across Ghana for entomopathogenic nematodes in an effort to integrate them into IPM strategies for management of FAW in maize.

MATERIALS AND METHODS

This study was conducted in 40 districts in Ghana, namely Mampong, Ejura, Ejisu, Asante Akyem Central, Pru East, Nkoranza North, Sunyani South, Dormaa Central, Bia East, Prestea Huni-Valley, Ahanta West, Shama, Cape Coast, Abura Asebu Kwamankese, Assin South, Assin North, Nkawkaw, Kwahu West, Akuapem North, Yilo Krobo, Tema, Ningo Prampram, Ga West, Amasaman, South Tongu, Ketu South, Hohoe, Nkwanta South, Garu, Bolgatanga, Kasena Nankana, Builsa North, Sisala East, Lawra, Nadowli Kaleo, Wa, West Gonja, Tamale, Mamprusi West and Saboba.

In each district, five FAW-infested maize farms were soil sampled; making a total of 200. GARMIN GPSMAP64s handset was used to record district sampling locations (Table 1). The sampling areas fell under diverse natural vegetation agro-ecologies (Table 2). On a 0.4-hectare maize farm, 20 core soil samples were collected. Four sub-samples were hand mixed thoroughly into a composite sample weighing 500 g, and used for laboratory analysis. The soil augur was cleaned thoroughly with one-ply disposable table napkins (three-

TABLE 1. District sampling locations for entomopathogenic nematodes in maize fields in Ghana

Region	District	Sampling district coordinates
Ashanti	Mampong	N07°11'.637' W01°24.806'
	Ejura	N07°22'.958' W01°23.015'
	Ejisu	N06°41'.234' W01°23.674'
	Asante Akyem Central	N06°36'.881' W01°15.768'
Brong Ahafo	Pru East	N08°12'.966' W00°40.753'
	Nkoranza North	N07°40'.340' W01°44.181'
	Sunyani South	N07°22'.691' W02°12.274'
	Dormaa Central	N07°15'.940' W02°46.571'
Western	Bia East	N06°42'.508' W03°04.086'
	Prestea Huni-Valley	N05°24'.730' W01°59.796'
	Ahanta West	N04°55'.995' W01°58.648'
	Shama	N05°02'.505' W01°39.545'
Central	Cape Coast	N05°07'.369' W01°15.698'
	Abura Asebu Kwamankese	N05°14'.008' W01°11.999'
	Assin South	N05°23'.412' W01°08.965'
	Assin North	N05°50'.126' W01°19.116'
Eastern	Nkawkaw	N06°36'.049' W01°52.608'
	Kwahu West	N06°28'.065' W01°38.418'
	Akuapem North	N06°01'.215' W00°05.087'
	Yilo Krobo	N06°03'.786' W00°01.311'
Greater Accra	Tema	N05°38'.501' W00°07.555'
	Ningo Prampram	N05°46'.104' E00°03.978'
	Ga West	N05°41'.949' W00°17.113'
	Amasaman	N05°46'.475' W00°19.672'
Volta	South Tongu	N06°00'.438' E00°37.028'
	Ketu South	N06°07'.222' E01°08.755'
	Hohoe	N07°11'.467' E00°28.560'
	Nkwanta South	N08°16'.230' E00°31.486'
Upper East	Garu	N10°50'.822' W00°10.861'
	Bolgatanga	N10°48'.403' W00°50.273'
	Kasena Nankana	N10°50'.241' W01°00.056'
	Builsa North	N10°44'.767' W01°16.304'
Upper West	Sisala East	N10°50'.820' W01°59.739'
	Lawra	N10°39'.470' W02°53.925'
	Nadowli Kaleo	N10°23'.054' W02°40.796'
	Wa	N10°05'.101' W02°28.999'
Northern	West Gonja	N09°04'.842' W01°46.446'
	Tamale	N09°39'.112' W00°50.532'
	Mamprusi West	N10°24'.246' W00°47.193'
	Saboba	N09°44'.525' E00°05.234'

TABLE 2. Climatic and vegetation features of the study areas in Ghana

Region	District	Natural vegetation	Rainfall regime	Rainfall (mm $year^{-1}$)	Monthly temperature (°C)
Ashanti	Mampong	Savanna Transitional Forest	Bimodal	1,270	27
	Ejura Sekyedumase	Derived Deciduous Forest	Uni/bimodal	1,350	28.2
	Ejisu	Semi-deciduous Forest	Bimodal	968	29
	Asante Akyem Central	Semi-deciduous Forest	Bimodal	1,177	26.1
Brong Ahafo	Pru East	Forest Savanna Transition	Unimodal	1,600	26
	Nkoranza North	Forest Savanna Transition	Bimodal	1,002	26
	Kintampo South	Woodland Savannah	Bimodal	1,600	29.1
	Techiman North	Guinea Savanna Woodland	Bimodal	1,460	25
Western	Bia East	Semi-deciduous Forest	Bimodal	1,625	28.3
	Prestea Huni-Valley	Tropical Rain Forest	Bimodal	1,870	28.2
	Ahanta West	Deciduous Forest	Bimodal	1,700	27
	Shama	Deciduous Forest	Bimodal	1,380	25
Central	Cape Coast	Coastal Savanna	Bimodal	875	26
	Mfantseman	Coastal Savanna	Bimodal	1,175	24
	Assin South	Moist Evergreen Forest	Bimodal	1,625	23
	Adanse South	Semi-deciduous Forest	Bimodal	1,500	28.1
Eastern	Nkawkaw	Semi-deciduous Forest	Bimodal	1,257	24.3
	Akuapem North	Semi-broken Forest	Bimodal	1,260	23.4
	Yilo Krobo	Transitional Forest	Bimodal	1,175	27
	Kwahu West	Semi-deciduous Forest	Bimodal	1,226	28.3
Greater	Tema	Coastal Savannah	Bimodal	1,011	32.3

TABLE 2. Contd.

Region	District	Natural vegetation	Rainfall regime	Rainfall (mm $year^{-1}$)	Monthly temperature (^{O}C)
Accra	Ningo-Prampram	Coastal Savannah	Bimodal	991	31
	Ga West	Coastal Savannah	Bimodal	1,027	27.2
	Amasaman	Coastal Savannah	Bimodal	1,030	29
Volta	South Tongu	Coastal Savannah	Bimodal	975	26
	Ketu South	Dry Coastal Savannah	Bimodal	1,000	27.2
	Jasikan	Moist Deciduous Forest	Bimodal	1,500	28.4
	Nkwanta South	Savannah Woodland	Bimodal	1,398	32
Upper East	Garu	Sudan Savannah	Unimodal	800	27.6
	Bawku West	Sudan Savannah	Unimodal	956	34
	Kasena Nankana	Sahel Savannah	Unimodal	950	27.8
	Builsa North	Savannah Woodland	Unimodal	802	28.1
Upper West	Sisala East	Guinea savannah	Unimodal	1,211	27.9
	Lambussie	Savannah	Unimodal	1,000	30.2
	Lawra	Guinea savannah	Unimodal	1,117	32
	Wa	Guinea Savannah Grassland	Unimodal	1,120	31.4
Northern	Gonja West	Guinea Savannah	Bimodal	1,144	27.9
	Mamprusi West	Guinea Savanna Woodland	Unimodal	1,075	27.6
	Saboba	Guinea Savannah	Bimodal	1,050	26.8
	Nanumba North	Guinea Savannah	Unimodal	1,268	35.2

Source: District weather information, Ghana Meteorological Service (2020) star brand, Accra, Ghana), in between sampling sites.

Fifth and 6th instar FAW larvae were collected from infested maize plants on the same farms, and supplied to the soil as bait insects to be infected by soil endemic entomopathogenic nematodes in plastic containers (5.5 cm x 11 cm x 10.5 cm). The soil samples were kept in plastic containers (635 cm^3 capacity) with perforated covers to ensure aeration. Care was taken to ensure that the perforations made in the cover were not large enough to allow the applied FAW larvae to exit. The containers were covered tightly and their contents turned upside down to ensure uniform mixing of the FAW larvae with the soil. The samples were stored in insulated cool boxes to reduce dehydration. They were incubated in a dark laboratory room at 25 ^oC and 85% relative humidity.

After 4 days, the FAW larvae cadavers were removed from the soil for culturing and entomopathogenic nematode isolation using modified White traps (Woodring and Kaya, 1988). The modification involved the use of kitchen tissue (Fresh, 3-star paper works, Ghana) to wrap 9 cm Petri dishes instead of filter papers (Fig. 1). The entomopathogenic nematode-water suspension was poured after 14 days and concentrated to 20 ml in a 50 ml flat bottom beaker. Soil samples that showed negative nematode recovery results were rebaited to confirm whether truly those samples contained no EPN. The nematode population was quantified using a stereomicroscope (40X magnification) and a tally counter to aid counting.

Data on entomopathogenic nematode population counts were standardised as ln (x+1) to improve the homogeneity of variance before analysis and back-transformed for clarity. Statistical analysis was performed with analysis of variance using GenStat (12); significant means were separated using Tukey's test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Entomopathogenic nematodes were recorded in 92.5% of the districts sampled, representing 75% of 200 maize farms (Table 3). Only three districts (Cape Coast, Mfantseman, and Sisala East) recorded no such beneficial nematodes. The highest population density (1,820 per 5 FAW larvae cadavers) was recorded in the Greater Accra region (Table 4). This was significantly ($P<0.05$) greater than those of other regions, except the Volta and Western regions. The central region recorded the lowest density (81per 5 FAW larvae cadavers). Our results corroborate several other studies, where entomopathogenic nematode surveys in tropical and sub-tropical regions recorded 6.5% in Nigeria (Akyazi *et al.*, 2012), 6.9% in Ethiopia (Makete *et al.*, 2005), 7.0% in Chile and South Africa (Edghinton *et al*, 2010).

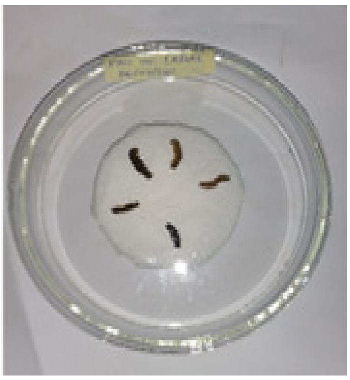


Figure 1. Modified White traps for culturing and isolation of entomopathogenic nematodes from fall armyworm larvae cadavers.

TABLE 3. Frequency of entomopathogenic nematodes in fourty districts of Ghana

Region	District	Presence/ absence (+/ 0)
Ashanti	Mampong	+
	Ejura Sekyedumase	+
	Ejisu	+
	Asante Akyem Central	+
Brong	Pru East	+
Ahafo	Nkoranza North	+
	Kintampo South	+
	Techiman North	+
Western	Bia East	+
	Prestea Huni-Valley	+
	Ahanta West	+
	Shama	+
Central	Cape Coast	0
	Mfantsiman	0
	Assin South	+
	Adanse South	+
Eastern	Nkawkaw	+
	Akuapem North	+
	Yilo Krobo	+
	Kwahu West	+
Greater	Tema	+
Accra	Ningo-Prampram	+
	Ga West	+
	Amasaman	+
Volta	South Tongu	+
	Ketu South	+
	Jasikan	+
	Nkwanta South	+
Upper	Garu	+
East	Bawku West	+
	Kasena Nankana	+
	Builsa North	+
Upper	Sisala East	0
West	Lambussie	+
	Lawra	+
	Wa	+
Northern	Gonja West	+
	Mamprusi West	+
	Saboba	+
	Nanumba North	+

TABLE 4. Entomopathogenic nematode populations per five fall armyworm larvae cadavers in Ghana

Region	Entomopathogenic nematode populations
Ashanti	277 (13.6) b
Brong Ahafo	401 (11.1) b
Central	81 (5.7) b
Eastern	313 (14.6) b
Greater Accra	1,820 (41.4) a
Northern	300 (15.8) b
Upper East	151 (11.3) b
Upper West	104 (8.8) b
Volta	898 (28.2) a b
Western	469 (18.2) a b
LSD [ln (x+1)]	(14.14)

Values are means of 12 replications. Values in parentheses are results of transformed values used for mean separation. Values followed by the same letters are not significantly different at a 95% confidence interval

Griffin *et al*. (1994) also sampled 270 soils from 30 different habitats in 10 geographic regions in Ghana; 26.3% tested positive for entomopathogenic nematodes. The occurrence and abundance of such beneficial nematodes in tropical and sub-tropical soils imply that EPN can be integrated successfully into the management of FAW on maize farms in Ghana.

CONCLUSION

Entomopathogenic nematodes exist in maize farms across the study areas in Ghana. As such, they can be integrated in IPM as a potential biocontrol agents against invasive fall armyworms for maize production across Ghana. The richness of the distribution reflects the diversity of entomopathogenic nematode soil habitats across the country. To advance the use of entomopathogenic nematodes as biological control agents, locally-adapted species from undisturbed soil habitats need to be explored. Entomopathogenic nematode application against insect pests does not pose negative effects on human health and the environment.

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