Occurrence and Pathogenicity of Entomopathogenic Nematode Isolates in Maize Growing Regions of Ethiopia

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Abstract

The objective of this study was to find and test entomopathogenic nematodes (EPNs) native to regions of Ethiopia where maize is grown with the aim of developing a biocontrol program against fall armyworm (FAW) *Spodoptera frugiperda* (Smith) and other arthropod pests. Six hundred seventy-nine soil samples were collected from eight regional states of Ethiopia between August and October 2019. From these collections, 28 EPN isolates, 13 from Steinernema genera and 15 from Heterorhabditis genera have been identified. All the 28 isolates identified from the survey and nine Ambo Agricultural Research Center's isolates were tested using a single dose (500 infective juveniles ml^{-1}(IJ)/ml) under laboratory conditions using FAW larvae in completely randomized design. Isolates, such as Aso-Tes-287 from Steinernema genera and Am-Ger-Tes-74, Am-Adm-Tes-369, and Z9 from Heterorhabditis genera caused significant larval mortality within eight days. Moreover, the LT_{50} values i.e., 3.5 to 6.7 days showed that these isolates are more virulent. These most virulent isolates were further tested for their potential in a pot experiment under wire house conditions at three different concentration levels (250, 400, and 600 IJ/ml) in randomized complete block design. The isolates Aso-Tes-287 and Am-Ger-Tes-74 resulted in higher mortality of 74.7% and 78.3%, respectively at 600 IJ/ml. The EPN isolates Aso-Tes-287 and Am-Ger-Tes-74 which caused higher mortalities within shorter periods, were promising bio-agents for the management of FAW. A confirmatory study is suggested to use the two promising bioagents for the management of FAW and other arthropod pests.

Keywords: Bioassay, Entomopathogenic nematode, EPN isolates, Heterorhabditis, Steinernema

Introduction

Maize (*Zea mays* L.) is an annual crop mainly grown for food in tropical and subtropical regions worldwide, including several countries in sub-Saharan Africa and Ethiopia (Midega *et al*., 2015; Erenstein *et al*., 2022). In Sub-Saharan Africa (SSA) alone, maize is cultivated on more than 33 million hectares of land every year, and it is crucial for the economic and food security of over 208 million people in the region (Abate *et al*., 2015). In Ethiopia, maize is the major cereal crop, ranking second in yield and area coverage (CSA, 2020), and it occupies around 2 million hectares of land, with

smallholder farms accounting for more than 95% of the total area and production (CSA, 2020).

Maize productivity in SSA is low ranging from 2-ton ha^{-1} to 3-ton ha^{-1}, which is the world's lowest (Cairns *et al*., 2013; Abate *et al*., 2015; Assefa *et al*., 2020). This could be due to various abiotic and biotic factors, with insect pests causing the most significant damage (Emana *et al*., 2001; Kfir *et al*., 2002). Over 40 species of insects attck maize under field conditions (Abraham *et al*., 1993; Tolera *et al*., 2018). According to a survey by Emana *et al.* (2008), the most harmful insect pests of standing maize are the stem borers *Busseola fusca* (Fuller) and *Chilo partellu*s (Swinhoe). More recently, however, an invasive and polyphagous fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), has become a major insect pest causing substantial yield losses on maize (Birhanu *et al*., 2019; Teshome *et al*., 2019; De Groote *et al*., 2020). , The damage due to FAW on maize in Zimbabwe, Uganda, and Ethiopia is estimated to be 32%, 87.7% and 65%, respectively (Baudron *et al*., 2019; Sharon *et al*., 2020; Atnafu *et al*., 2021).

Chemical insecticides are the main means of control for FAW. However, the use of synthetic insecticides to control lepidopterous pests has caused environmental contamination and the emergence of resistance in a number of insect pests (Bloem and Carpenter, 2001). Hence, there is greater interest in exploring different ways to control using microbes such as viruses (Gómez-Valderrama *et al*., 2022), fungi (Birhanu *et al*., 2019; Kuzhuppillymyal-Prabhakarankutty *et al*., 2021), and genetically modified plants that contain *Bacillus thuringiensis* toxins (Horikoshi *et al*., 2016). Certain types of nematodes, specifically those in the Heterorhabditidae and Steinernematidae families, can also effectively control insect pests (Gozel and Gozel, 2016). These nematodes are referred to as entomopathogenic, and they function by penetrating the insect's hemocoel and releasing symbiotic bacteria (*Xenorhabdus* spp. in Steinernematidae and *Photorhabdus* spp. in Heterorhabditidae). As the bacteria multiply, they produce metabolites that eliminate the insect and provide a food source for the nematodes (Godjo *et al*., 2018; Danso *et al*., 2021; Wattanachaiyingcharoen *et al*., 2021). The susceptibility of *S. frugiperda* (Andaló *et al*., 2010; Acharya *et al*., 2020; Lalramnghaki *et al*., 2021) and *Mentaxya ignicollis* (Tesfaye *et al*., 2018) to EPNs have been reported. More information is needed regarding whether the local strains of EPNs (Heterorhabditidae and Steinernematidae) found in Ethiopia are capable of infecting FAW. Therefore, this study aimed to isolate and evaluate the potential of Ethiopia's EPN isolates to infect *S. frugiperda* larvae in both laboratory and wire-house settings.

Material and Methods

Soil sampling

Soil samples were randomly collected from Oromia, Amhara, Tigiray, Southern Nation Nationalities and peoples' (SNNP), Gambella, Benishangul-Gumuz, Somali and Afar maize growing areas during August to October 2019. A total of 679 soil samples were collected from 10–15 cm depth using auger at 5 to 10 kilometers intervals based on availability of maize farms. From each farm, 5 soil samples were randomly collected and composited (approximately 1 kg). The samples were transported to the Ambo Agricultural Research Center (AmARC) Entomology laboratory and kept at 12-15°C for later use (Abdel-Razek *et al*., 2018; Ashenafi *et al*., 2019; Yuksel and Canhilal, 2019). Additionally, dead FAW cadavers and other insects were collected and transported to the laboratory for further identification and isolation of EPNs.

Isolation of EPN

The insect-bait method was used to isolate EPN from the soil samples (Orozco *et al*., 2014). Briefly, the larvae of the greater wax moth *Galleria mellonella* was used as bait to isolate entomopathogenic nematodes from the soil samples. Ten third instar larvae of *G. mellonella* were placed into small glass jars of 500 ml. The soil samples were moistened and placed on top of the larvae until approximately two-thirds of the glass jars were filled. The glass jars were maintained in the dark at 22 to 25°C. The glass jars were inverted daily to enable the larvae move through the soil and were repeatedly exposed to infective juveniles in the soil. In the course of the experiment, data on mortality was recorded daily for ten days. The dead larvae were collected and submerged in 70% ethanol for one minute and washed in sterile distilled water for three minutes to remove saprophytes, the non-infective stage, and the host tissues (Orozco *et al*., 2014; Abdel-Razek *et al*., 2018).

The disinfected cadavers were placed in a modified white trap for recovery of nematode progeny and when there was the emergence of infective juveniles (IJ's), they were harvested and poured into the flask. The flask with nematode suspension was stored in an incubator between 10–20 °C for later use. The stored flasks were checked periodically for the availability of EPN.

Identification of EPN isolate

The EPN IJs outgrown from the cadaver were recultured on *G. mellonella* and pure cultures were obtained through a successive transfer for identification. For newly isolated EPNs, permanent slide was made using TAF (Triethanomine 2ml, Formalin (40% formaldehyde) 7ml, and 91 ml distilled water) (Gümüşsoy *et al*., 2022). Microscopic identification was made on live and slide-mounted specimens of IJs of EPNs using Olympus 33 camera-mounted compound microscope. Morphological identification was done based on growth stage and morphological characters of EPNs (Orozco *et al*., 2014; Ashenafi *et al*., 2019; Yuksel and Canhilal, 2019). The dead cadavers exhibited different colors due to the symbiotic bacteria associated with the

EPN species, i.e. cadavers with a brown or ochre coloration is a sign of parasitization by *Steinernematids*, whereas brick red to dark purple cadavers is parasitization by *Heterorhabditids*.

Preparation of EPN suspension

The suspension of each EPN isolate was prepared for the experiments. The EPN isolates were screened for their efficacy against FAW larvae at 500 IJs and for wirehouse experiment three concentrations (250, 400, and 600 IJs/ml) were prepared using the dilution method.The actual number of nematodes in the stock solution was calculated according to Abdel-Razek *et al*. (2018).

$$C = N \times (X + L) \times S$$

Where, C = Actual number of nematodes in the stock solution, N = Average number of nematodes per counted sample, S = Volume of original stock solution (ml), (X + L) = Total volume (ml) in the diluted sample.

Mass rearing of FAW

For mass rearing of FAW, larvae were collected from AmARC maize plantation fields and mass-reared in the entomology laboratory of AmARC on young seedlings of maize as a feed following the procedure of Mwamburi (2021). A total of 1140 third instar larvae of FAW of the second generation were used for the screening of the EPN isolates, while a total of 780 second to third instar larvae of FAW were used for the pot experiment.

Laboratory bioassay of EPN

All isolated EPN isolates were screened for their virulence on the 3rd instar FAW larvae in the entomology laboratory of AmARC. The isolates included the 37 native nematode isolates (28 new and 9 of AmARC's (Table 1)). Ten larvae per Petri dish (150 * 15 mm) were used with filter paper and young fresh chopped maize stems inside. For each isolate, an aqueous suspension containing 500 IJ/ml was prepared in distilled water and applied using a micro-pipette. Sterile distilled water was used as free control treatment. Treatments were incubated at 24 °C and 60% RH and maintained for 10 days in a growth chamber. The experiment was laid out in a completely randomized design (CRD) with three replications.

The mortality data were recorded daily for ten days starting from the first day. The dead larvae were collected and submerged for three seconds into 70% ethanol and 0.5% sodium hypochlorite for two minutes (Orozco *et al*., 2014) and washed in sterile distilled water for three minutes to remove saprophytes and all conidia found on the outer surface of cadavers. The disinfected cadavers were allowed to dry for ten minutes on Watman No.1 filter paper. Cadavers were held under high humidity on Petri dishes containing damp filter paper to provide sufficient humid conditions to promote EPN

outgrowth. A larva was considered dead by nematode when the cadavers burst and the nematodes were visible around it and those which showed pathogenic characteristics (i.e., the release of *Xenorhabdus* for *Steinernema* sp. and release of *Photorhabdus* for *Heterorhabditis* sp. of EPN isolates) of the entomopathogenic nematodes were recorded as infected.

Mortality data was corrected by the formula: $$CM(\%) = \frac{(T - C)}{(100 - C)} * 100$$

Where CM is corrected mortality, T is percent mortality in treated insects and C is percent mortality in untreated insects (Abbott, 1925).

The effect of native nematode isolates on larval mortality was analyzed using a one-way analysis of variance (SAS Institute, 2012). Moreover, the median Lethal Time (LT_{50}) value was also determined for all nematode agents using daily records of percent mortality data

Table 1. List of existing Ambo Agricultural Research Center 's (AmARC) EPN isolates tested against the fall armyworm in single dose experiment

No.	EPN isolate code	Habitat	Genus	Area of collection
1	HH	Soil	Steinernematidae	Harge Hirna
2	J-01	Soil	Steinernematidae	Jima
3	HI	Soil	Steinernematidae	Bule Hora
4	APPRC-p20692	Soil	Steinernematidae	Shambu
5	APPRC-p0508	Soil	Heterorhabditis	Jima Sokoru
6	AEH	Soil	Heterorhabditis	Ambo
7	HBWWM	Soil	*Hetrorabitidus bacteriophora*	South Africa
8	Z9	Soil	Heterorhabditis	Batu
9	APPRC PL 0697	Soil	Heterorhabditis	Fincha

Efficacy of selected *Steinernema* and *Heterorhabditis* species against FAW larvae under wire-house condition

The experiment was carried out at the AmARC wire-house during 2019/2020. For this experiment, the popular variety Jibat was used and five maize seeds were planted in each pot (21cm diameter and 19 cm height). The pots were initially filled with the composition of black soil, compost, and sand at a proportion of 2:1:1 and watered at three days intervals. Urea at the rate of 0.52 g per pot was applied one and a half months after planting. Treatments were applied after 50 days when the seedling attained a height of approximately 40 cm. Twenty third instar larvae of FAW were transferred into each pot. Four hours after infestation with the larvae, four potentially effective EPN isolates namely Z9, Am-Aso-Tes-287, Am-Ger-Tes-74 and Am-Adm-Tes-369 were applied at a concentration of 250 IJs ml^{-1}, 400 IJs ml^{-1} and 600 IJs ml^{-1} using the handheld sprayers (Arthurs *et al*., 2003). Sterilized distilled water was used as a control. Treatments were placed in the wirehouse in separate

cages. The experiment was laid out in a Randomized Complete Block Design (RCBD) in four replications. Data on larval mortality, and plant damage were recorded 12 days after artificial inoculation. Mortality was corrected using Abbot's formula (Abbott, 1925):

$$\%CM = \frac{(\%T - \%C)}{(100 - \%C)} * 100$$

Where CM is corrected mortality, T is mortality in treated insects, and C is mortality in untreated insects.

The percentage efficacy of the native isolate was determined using (Abbott, 1925) formula given as:

$$\%\ \text{Efficacy} = \frac{(Cd - Td)}{(Cd)} * 100$$

Where Cd is number of live individuals in the control plots after the treatment; Td is the number of live individuals in the treated plots after the treatment.

Plant damage was scored based on visual observation on a 0-9 scale (0=no damage, 1= only pinhole lesions on whorl leaves, 2 = pinhole and shoot-hole lesions on whorl leaves, 3 = A few small (0.5-1 cm) elongated lesions on leaves, 4= several leaves with mid-sized (1-3 cm) lesions, 5= several leaves with large elongated lesions or small portions eaten away, 6= several leaves with large elongated lesions and large portions eaten away, 7= many elongated lesions and large portions eaten from leaves, 8= many elongated lesions and many portions eaten from leaves, 9= many leaves destroyed (Davis *et al*., 1992; Navik *et al*., 2021).

The effect of native EPN isolates on larval mortality and plant damage was analyzed using a one-way analysis of variance (SAS Institute, 2012). Moreover, the LT_{50} value was also determined for all EPNs using daily records of percent mortality data. The data were arcsine transformed (Gomez and Gomez, 1984).

Results

Survey of Indigenous EPN in the maize-producing areas of Ethiopia

A total of 679 soil samples were collected from different regions, including Oromia, Amhara, Tigiray, SNNP, Gambela, Benishangul-Gumuz, Somali, and Afar. Out of these samples, 28 new EPN isolates were found. However, no nematode was recovered in the soil samples collected from Somali, Gambela, and SNNP regional states. The regions with the highest positive soil samples were Tigray and Oromia, with proportions of 13.09% and 5.34%, respectively. In contrast, only 3.48% of soil samples collected from the Amhara region were positive for EPN. The soil samples collected from Afar and Benishangul-Gumuz had a 2.89% positivity rate for EPN (Table 3 and Fig. 3).

Identification of EPN isolates

For detailed identification, morphological characterization of the isolated EPN was done under microscopy. Primarily,

the identification was made based on the cadavers colour. The cadavers parasitized by *Steinernematids* showed brown or ocher color, while cadavers with *Heterorhabditid* sp. are brick-red to dark purple. The morphometrics, such as observations on the reflex of the male spicule, total length, oesophagal length, body width, distance from the anterior or posterior end of vulva, and vulva to tail, considered for the identification of the newly isolated EPN and summarized in Table 2.

Am-Aso-Tes-287 isolate has a digitate, short, cone-shaped tail (Fig. 1A), whereas, Am-Adm-Tes-369 possesses cone shaped, short tail without digitation (Fig. 1B). Furthermore, the microscopic morphological description of the spicules, vulva, tail and oesophagus of the most effective isolates of Heterorhabditis (Am-Aso-Tes-287 and Am-Adm-Tes-369) and Steinernematidae (Am-Ger-Tes-74) were investigated (Fig. 1A-C and Fig. 2A-C). Accordingly, spicules of Am-Aso-Tes-287 and Am-Adm-Tes-369 were formed as slender, erected paired emerged from opposite sides (Fig. 1A & B), whereas, spicules of Am-Ger-Tes-74 were attached from one point and they were flaccid (Fig. 1C). Posterior part of the three male EPNs was cylindrical, narrow at the tip.

In Figure 1C, Steinernematidae (Am-Ger-Tes-74) is unique because it has a narrow and long tip tail. The vulva lips of Am-Aso-Tes-287 and Am-Adm-Tes-369 are swollen and protruded, as seen in Figures 2A and 2B. The female tail of Am-Aso-Tes-287 is conical and sharply pointed with a thin width in Figure 1A. The female tail of Am-Adm-Tes-369 is also conical and sharply elongated with a small width and a larger tail in Figure 1B. In contrast, the female tail of Am-Ger-Tes-74 is concave and longer with a larger width that is swallowed inside the vulva, as shown in Fig 1C.

Based on the morphological characterization, the newly isolated EPN isolates belong to Heterorhabditis and Steinernematids genus. Of the 28 positive soil samples, 15 (53.57%) were Heterorhabditis while 13 (46.43%) were Steinernematids (Tables 3). The newly isolated EPNs were morphologically similar to *Steinernema ethiopiense*, *Steinernema yirgalemense*, *Heterorhabditis indica*, and *Heterorhabditis bacteriophora*. Six isolates (Am-Waz-Tes-68, Am-Tse-Tes-70, Am-Haw-Tes-71, Am-DeD-Tes-76 and Am-AnD-Tes-80) were morphologically similar to *S. ethiopiense* and five isolates (Am-Kor-Tes-7, Am-Gum-Tes-15, Am-UlM-Tes-20, Am-AsA-Tes-37 and Am-SeG-Tes-50) were *S. yirgalemense*. In addition, seven samples (Am-AdG-Tes-59, Am-Bel-Tes-60, Am-Wez-Tes-67, Am-Adm-Tes-369, Am-Aad-Tes-72, Am-Huj-Tes-73 and Am-She-Tes-244) showed the same character for *H. indica* and six isolates (Am-Kur-Tes-8, Am-KoD-Tes-19, Am-KuG-Tes-43, Am-Ben-Tes-292, Am-MeG-Tes-293 and Am-Tey-Tes-295) were identified as *H. bacteriophora*. The isolates Am-Amb-Tes-281 and Am-Aso-Tes-287 belonging to Heterorhabditis genus and Am-Adm-

Tes-69, Am-DiM-Tes-341 and Am-Ger-Tes-74 belonging to Steinernematids genus were found difficult to characterize them to the species level and thus require further study at molecular level.

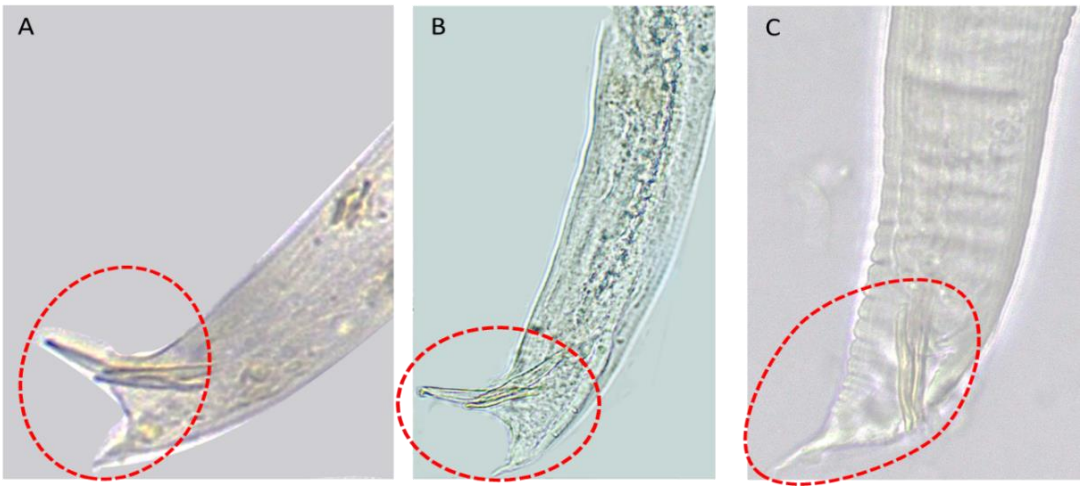

Figure 1. Morphological description of isolated male EPN spicules and tail. Heterorhabditis: A) Am-Aso-Tes-287 and B) Am-Adm-Tes-369 Steinernematidae: C) Am-Ger-Tes-74. Red circles indicate male spicules and tail

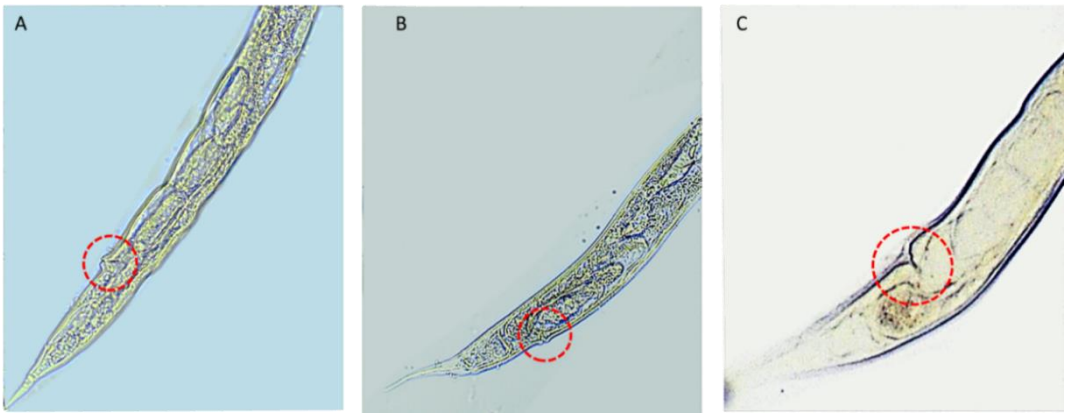

Figure 2. Morphological description of isolated female EPN vulva and tail. Heterorhabditis: A) Am-Aso-Tes-287 and B) Am-Adm-Tes-369 Steinernematidae: C) Am-Ger-Tes-74. Red circles indicate female vulva

Table 2. Morphometric measurements of infective juveniles (IJs) of the newly isolated entomopathogenic nematode (µm) (n = 5)

Character	Isolate Code									
	Am-Waz-Tes-68	**Am-Tse-Tes-70**	**Am-Haw-Tes-71**	**Am-DeD-Tes-76**	**Am-AnD-Tes-80**	**Am-Kor-Tes-7**	**Am-Gum-Tes-15**	**Am-UlM-Tes-20**	**Am-AsA-Tes-37**	**Am-SeG-Tes-50**
Body length Mean ± std										
Male	1222.2±0.6	1224.2±1.0	1225.9±0.4	1226.8±0.3	1223.1±1.2	1072.3±0.4	1072.2±0.2	1081.5±0.4	1098.1±0.5	1097.4±0.4
Female	1975.1±0.6	1970.8±0.2	1974.9±1.9	1975.3±0.1	1969.7±1.5	1374.9±1.0	1376.5±0.8	1388.5±0.4	1391.5±0.7	1390.4±0.1
Oesophagus Length (Mean ± std)										
Male	649.4±1.6	662.0±1.0	668.3±0.6	669.1±1.7	648.4±0.4	228.9±1.4	228.4±0.2	230.5±0.5	230.5±0.2	230.7±1.5
Female	205.3±0.4	201.9±0.5	206.2±0.7	206.7±1.3	202.0±0.9	412.6±0.3	413.6±0.5	422.5±0.4	422.7±0.1	422.4±0.2
Body width Mean ± std										
Male	38.8±0.9	39.5±1.4	40.1±0.6	40.1±0.4	38.4±0.7	84.3±0.7	81.5±0.6	82.6±0.2	82.1±0.1	83.4±0.4
Female	72.1±0.8	73.0±0.1	74.5±1.0	75.4±0.7	74.2±0.4	119.2±1.0	120.0±0.9	124.4±0.3	124.4±0.4	124.4±0.4
DP	1524.0±0.1	1521.7±0.6	1517.7±0.9	1517.8±1.2	1520.6±0.8	796.7±0.5	796.3±0.7	798.3±0.3	800.5±0.5	799.5±0.5
VTL	246.0±0.6	247.2±0.9	250.6±1.6	251.5±1.2	247.4±1.1	165.6±0.6	166.7±0.3	167.7±0.2	168.3±0.4	168.5±0.4
SL	99.2±0.4	98.5±0.3	114.6±0.3	114.8±0.2	101.1±0.9	148.4±0.2	148.5±0.2	150.0±0.0	150.2±0.4	150.0±0.3
ST	405.5±0.7	406.1±1.0	408.4±0.4	408.5±0.5	408.3±0.4	498.3±0.7	495.2±1.0	500.0±0.7	598.5±0.2	500.5±0.5
Tail length Mean ± std										
Male	148.3±1.1	148.1±0.4	151.4±0.6	152.7±0.4	148.2±0.2	55.7±0.1	56.0±0.0	62.4±0.2	62.8±0.1	62.4±0.2
Female	264.4±1.5	268.5±0.5	269.9±0.8	272.8±0.2	273.8±0.8	281.3±1.1	280.3±0.9	282.9±1.2	281.7±1.5	284.2±0.9

Table 2 (Continued)

Character	Isolate Code								
	Am-AdG-Tes-59	**Am-Bel-Tes-60**	**Am-Wez-Tes-67**	**Am-Adm-Tes-369**	**Am-Aad-Tes-72**	**Am-Huj-Tes-73**	**Am-She-Tes-244**	**Am-Kur-Tes-8**	**Am-KoD-Tes-19**
Body length									
Male	1101.9±0.9	1101.6±0.4	1115.5±0.5	1120.5±0.5	1115.7±0.7	1104.5±1.9	1135.6±0.6	1083.7±0.9	1082.9±1.2
Female	1498.6±0.6	1498.2±0.1	1502.8±0.8	1518.2±0.5	1510.9±0.8	1499.2±0.7	1515.8±0.8	1232.5±1.4	1232.5±0.5
Oesophagus length									
Male	477.6±1.0	471.1±0.9	483.5±1.6	485.7±0.3	485.0±1.2	481.2±0.2	485.3±0.7	341.3±0.6	349.0±0.6
Female	307.9±0.2	308.0±0.1	310.5±0.4	311.4±0.5	310.3±0.6	308.4±0.4	311.4±0.4	204.3±0.6	204.3±0.6
Body width									
Male	56.0±0.6	55.4±0.9	52.6±0.2	55.5±0.4	54.7±1.1	51.3±0.9	55.4±0.3	41.1±0.5	41.2±1.1
Female	176.0±1.2	179.3±0.5	175.2±0.5	179.4±1.0	175.6±1.5	163.1±1.4	125.1±1.1	87.5±1.1	91.3±1.1
DP	518.2±0.4	517.7±0.1	518.2±1.0	525.3±0.4	521.6±0.4	517.8±0.9	523.7±0.4	355.6±0.9	355.4±0.4
VTL	672.5±0.5	672.5±0.1	674.1±0.2	681.4±0.3	679.0±0.7	672.1±0.8	680.8±1.0	672.6±0.3	672.7±0.4
SL	93.8±1.7	91.3±0.6	94.0±0.9	96.3±1.4	95.3±0.3	91.4±1.0	96.6±0.3	40.4±0.4	40.7±2.0
ST	373.8±0.7	373.5±1.1	373.3±0.9	381.0±1.0	380.2±0.7	373.6±1.5	381.7±0.2	373.3±1.0	372.7±0.2
Tail length									
Male	118.4±1.5	119.6±1.4	121.5±0.2	125.6±0.9	125.5±1.4	118.4±1.5	127.6±1.4	28.7±0.8	29.6±0.8
Female	369.7±0.8	370.8±0.7	371.6±1.2	384.8±0.7	389.4±0.7	369.4±0.7	395.9±0.8	148.5±1.0	149.1±1.2

Table 2 (Conitinued)

Character	Isolate Code								
	Am-KuG-Tes-43	**Am-Ben-Tes-292**	**Am-MeG-Tes-293**	**Am-Tey-Tes-295**	**Am-Amb-Tes-281**	**Am-Aso-Tes-287**	**Am-Adm-Tes-69**	**Am-DiM-Tes-341**	**Am-Ger-Tes-74**
Body length									
Male	1081.4±1.1	1084.7±1.8	1085.8±1.6	1071.2±2.0	1335.4±0.9	1363.0±1.2	1103.4±1.1	1121.4±1.2	1148.2±0.2
Female	1231.0±1.5	1248.7±1.3	1249.43±0.8	1245.4±1.2	2193.2±0.9	2262.0±0.2	1468.3±0.0	1506.8±0.2	1617.0±1.0
Oesophagus length									
Male	349.8±1.3	362.3±2.0	361.1±0.5	363.6±0.9	844.5±2.0	856.0±1.0	420.6±0.9	428.5±0.2	429.1±0.8
Female	204.6±0.7	212.6±0.3	212.2±0.2	212.5±0.4	728.5±0.5	740.7±0.3	289.6±0.4	291.0±0.9	392.3±0.5
Body width									
Male	41.6±1.0	45.5±1.4	43.6±0.4	43.6±0.3	165.7±0.7	172.5±1.4	86.1±0.4	88.3±0.6	89.4±1.0
Female	94.6±1.2	97.4±0.9	92.3±1.0	91.7±1.3	197.8±1.2	198.6±0.6	149.3±0.9	154.5±0.6	126.8±1.5
DP	354.1±0.5	355.2±0.6	355.3±0.4	354.5±0.4	564.3±0.1	568.4±0.2	476.2±0.4	476.4±0.7	478.2±0.7
VTL	672.2±1.0	680.9±1.0	682.0±0.6	678.4±1.5	900.4±0.6	952.9±0.2	702.4±0.8	739.3±0.4	746.6±1.8
SL	40.7±0.3	42.9±1.2	42.4±0.5	42.8±0.5	376.7±0.7	381.3±0.3	410.6±1.7	114.2±0.8	416.4±1.8
ST	372.2±2.0	379.9±9.5	382.3±0.7	387.2±0.9	309.3±1.1	357.1±0.9	404.2±0.9	439.6±0.6	444.3±1.6
Tail length									
Male	29.6±0.5	34.4±0.6	34.2±1.5	34.2±0.3	218.5±1.8	236.7±0.4	163.8±1.4	172.4±1.7	174.2±0.4
Female	138.7±1.0	154.9±1.7	152.2±1.0	152.8±1.3	397.1±1.6	416.6±1.6	324.7±1.7	371.0±1.5	378.4±0.2

DP= Distance from the anterior or posterior end of vulva (µm), VTL= Vulva to Tail length, SL= Spicule length, ST= Spicule to tail

Table 3. Summary of positive and negative samples for native entomopathogenic nematodes from the maize producing areas.

Site	No. of fields surveyed	Total No. of positive samples	EPN isolates obtained
Oromia	187	10	4 Steinernematidae & 6 Heterorhabditis
Amhara	86	3	2 Steinernematidae & 1 Heterorhabditis
SNNP	60	0	No
Tigray	84	11	4 Steinernematidae & 7 Heterorhabditis
B.Gumuz	86	2	2 Steinernematidae
Somali	54	0	No
Afar	69	2	1 Steinernematidae & 1 Heterorhabditis
Gambela	53	0	No

NB: B.Gumuz = Benishangul-Gumuz, SNNP = Southern Nation, Nationalities, and Peoples'

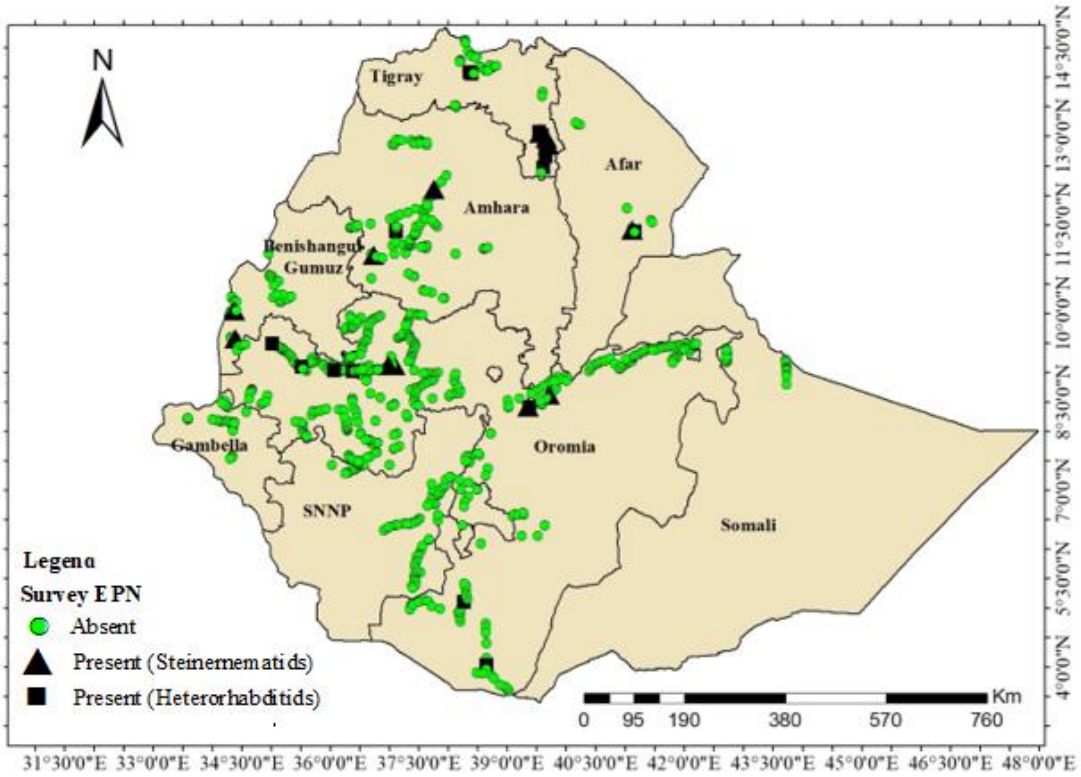

Figure 3. Soil collection sites for assessing indigenous EPN from eight maize growing regions of Ethiopia

Virulence screening of EPN isolates

All thirty-seven isolates of *Steinernematidae* sp. and *Heterorhabditis* sp. were pathogenic to FAW and caused varying levels of larval mortality within 4 to 10 days after treatment (DAT) (Table 4). The isolates had significantly different virulence ($P<0.01$) at 4, 6, 8 and 10 DAT (Table 4). At 4 DAT, Z9 (48.9%), Am-Ger-Tes-74 (46.9%), Am-Adm-Tes-369 (43.1%), Am-Aad-Tes-72 (43.0%), caused significantly higher cumulative mortality followed by the isolates Am-Aso-Tes-287 (26.48%), Am-Kur-Tes-8 (22.26%), Am-SeG-Tes-50 (18.04%), APPRC-P20692 (18.04%), and Am-MeG-Tes-293 (13.7%). The rest 22 EPN isolates did not cause larval mortality similar to the control (Table 4). Besides, the percent mortalities of isolates Am-Waz-Tes-68, Am-Tse-Tes-70, Am-Amb-Tes-281, Am-AsA-Tes-37, Am-Gum-Tes-15 and Am-Waz-Tes-68 were not significantly different from each other (Table 4).

Out of thirty-seven isolates of EPNs, the highest cumulative mortality was observed in Am-Aso-Tes-287 (89.0%) isolate followed by Am-AdG-Tes-59 (68.9%), Am-Adm-Tes-369 (66.1%), Am-Ger-Tes-74 (66.1%), Am-SeG-Tes-50 (63.9%), Z9 (63.9%), Am-DiM-Tes-341 (61.2%), APPRC-p0508 (63.4%), Am-KoD-Tes-19 (59.0%), Am-Ben-Tes-292 (55.9%), Am-Huj-Tes-73 (55.4%), Am-Tey-Tes-295 (55.0%), Am-Tse-Tes-70, Am-Gum-Tes-15, Am-Kur-Tes-8 (51.1%), HH and Am-Bel-Tes-60 (50.9%), respectively, at 6 DAT. The remaining EPN isolates of *Steinernematidae* and *Heterorhabditis sp*. caused the lowest mortality on FAW larvae (Table 4).

In most of the isolates, the mortality of larvae was significantly higher in 6, 8 &10 DAT when compared to the mortality in 4 DAT (Table 4). Most of the tested isolates (22 isolates) caused 50 to 75 % FAW larval mortality within 8 DAT. Isolates Am-Aso-Tes-287, Am-Adm-Tes-369, Am-Ger-Tes-74, Z9, Am-SeG-Tes-50, Am-Gum-Tes-15, Am-AdG-Tes-59, APPRC PL 0697 and AEH caused significantly higher larval mortality (71– 89%) at 8 DAT, whereas isolates HBWWM, APPRC-PL0697 caused significantly lower mortality (Table 4).

Mortality level ranged between 26.1 and 89.0 at 10 DAT. FAW larval mortality on this day was more or less similar with the 8 DAT. Am-Aso-Tes-287, Am-Adm-Tes-369, Am-Ger-Tes-74, and Z9 isolates resulted in higher mortality than the other isolates. Differences between isolates for lethal time (LT_{50}) against FAW larva at 500 IJs/ml was highly significant ($P<0.01$) (Table 4). Isolate Am-Aso-Tes-287 resulted in significantly lower LT_{50} (3.5) than the rest of the treatments. Am-BuT-Tes-369 and Am-Ger-Tes-74 with LT_{50} of 6.7, and the isolate Z9 with LT_{50} of 6.6 resulted in significantly lower LT_{50} than the rest of the treatments other than Am-Aso-Tes-287. On the other hand, the isolates HBWWM and APPRC PL 0697 with LT_{50} of 16.7 gave significantly higher

LT_{50} value than the rest of the treatments (Table 4).

Wirehouse pot experiment

Effect on larval mortality and leaf damage

The percent larval mortality of FAW increased with the increase in the concentration of the Infective juveniles (IJs) of the EPNs. The lowest larval mortality of 36.5, 48.9, 46.7 and 42.4%, respectively were recorded on the 12 DAT for Z9, Am-Aso-Tes-287, Am-Ger-Tes-74, and Am-Adm-Tes-369, at the lowest concentration of 250 IJs/ml, which increased on medium concentration (400 IJs/ml) to 51.2%, 68.0%, 65.3%, 57.8% for Z9, Am-Aso-Tes-287, Am-Ger-Tes-74, and Am-Adm-Tes-369, respectively (Table 5). The larval mortality increased to (53.2, 74.6, 74.3, and 63.4%) at the concentration of 600 IJs/ml on the same isolates. On other hand, the EPN concentration of 600 IJs/ml caused the highest larval mortality (74.6 and 74.3%) on isolates Am-Aso-Tes-287, and Am-Ger-Tes-74.

Moreover, the percentage of damage decreased to isolate Z9 (50.8%, 42.1%, 35.3%), Am-Aso-Tes-287 (46.0%, 38.2%, 30.9%), Am-Ger-Tes-74 (45.0%, 37.2%, 28.9%) and Am-Adm-Tes-369 (49.9%, 41.2%, 40.0%) at the tested concentrations of 250, 400 and 600 IJs/ml, respectively. The percentage damage by FAW decreased with increasing the concentration of *Steinernema* sp. (Am-Aso-Tes-287) and *Heterorhabditis* sp. (Z9, Am-Adm-Tes-369 and Am-Ger-Tes-74) (Table 5). At the lowest concentration of the isolates (250 IJs/ml), the highest damage and the lowest mortality of FAW larva were recorded on 4 isolates (Table 5).

Discussion

Results of this study revealed the occurrence of EPNs with varying virulence in various maize-producing areas of Ethiopia including Tigray, Oromia, Amhara, Afar, and Benishangul-Gumuz regions. However, no EPNs were discovered in Somali and SNNP regions. The occurrence and distribution of EPNs is dependent on the soil physical and chemical properties (Kandji *et al*. 2001). The absence of EPNs in Somali and SNNP could be related to the soil properties in those areas. EPNs were absent in clay, silty clay, silty loam, silty clay loam, and clay loam soil (Kour *et al*., 2020). Additionally, Yohannes *et al*. (2020) reported that the majority of the soils in the Somali region are clay loamy.

Based on morphological descriptions, 54% of the isolated EPNs belonged to *Heterorhabditis* and the remaing 46% belonged to *Steinernematidae*. Moreover, cadavors parasitize by Steinernematidae showed brown or ochre coloration whereas the cadavers parasitized by Heterorhabditids exhibited brick reds to dark purple colors (Dolinski *et al*., 2012; Lalramnghaki, 2018).

Table 4. Percentage mortality and LT_{50} of FAW 10 days after treatment with isolates of *Steinernematidae* and *Heterorhabditis* sp. at the rate of 500 IJs/ml

Isolates		Mortality ±SD*				LT_{50}
		4DAT	6DAT	8DAT	10DAT	
Am-Aso-Tes-287*	Steinernematidae Sp	26.48±12.1cd	89.0±0.0a	89.0±0.0a	89.0±0.0a	3.5±0.3l
Am-Waz-Tes-68		9.56±4.8efg	26.6±0.0ghi	49.8±11.4defghi	49.8±11.4defgh	13.5±0.3c
Am-Tse-Tes-70		9.56±4.8efg	51.1±12.01cdef	62.4±0.9bcdef	62.4±0.9bcdef	11.6±0.3defg
Am-MeG-Tes-293		13.7±3.6cdef	53.1±9.42bcdef	53.5±10.4defgh	53.5±10.4cdefg	12.25±0.3de
Am-Kor-Tes-7		0.99±0.0g	38.9±12.1efgh	44.0±21.0fghij	53.1±4.8cdefg	12.5±0.3d
HH		9.52±4.8efg	50.8±0.0cdef	53.6±7.3defgh	53.6±7.1cdefg	12.5±0.3d
Am-SeG-Tes-50		18.04±7.8defg	63.9±24.9bc	71.4±15.2ab	71.4±15.2abc	11.3±0.3ghi
Am-Haw-Tes-71		0.99±0.0g	30.8±7.3fgh	31.3±10.5jk	31.3±10.5hi	15.3±0.2b
Am-AnD-Tes-80		0.99±0.0g	55.0±7.3bcde	62.4±0.9bcdef	71.4±15.2abc	11.08±0.4ghij
J-01		0.99±0.0g	39.0±12.1efgh	54.3±13.1cdefgh	54.3±13.1cde	12.25±0.4de
Am-DeD-Tes-76		0.99±0.0g	37.0±9.4fgh	55.5±7.6cdefgh	55.5±7.6cdefg	12.08±0.4def
Am-Gum-Tes-15		0.99±0.0g	51.1±12.1cdef	71.4±15.2abc	71.4±15.2abc	11.17±0.3ghij
HI		0.99±0.0g	9.5±4.7ij	45.5±17.8efghij	45.5±17.8efghi	13.73±0.8c
Am-UIM-Tes-20		0.99±0.0g	39.2±0.0efgh	43.1±4.6hijk	43.1±4.6fghi	14.07±0.2c
Am-Amb-Tes-281		9.56±4.8efg	9.5±4.7ij	45.2±5.2fghij	45.2±5.2efghi	13.64±0.4c
Am-AsA-Tes-37		9.56±4.9efg	55.4±13.9bcde	62.4±0.9bcdef	71.4±15.2abc	11.07±0.2ghij
APPRC-p20692		18.04±7.8cdef	41.2±3.3efgh	51.1±5.0	64.7±21.5bcde	11.6±0.1efg
Am-Kur-Tes-8	Heterorhabditis Sps	22.26±9.5cde	51.1±12.1cdef	61.1±9.9bcdefg	70.1±19.1abcd	11.4±0.2fgh
Am-Bel-Tes-60		0.99±0.0g	50.9±10.1cdef	63.1±6.9bcde	63.1±6.9bcdef	11.65±0.4efg
Am-AdG-Tes-59		0.99±0.0g	68.9±4.7b	74.3±13.2ab	74.3±13.2abc	10.82±0.1hij
Am-KoD-Tes-19		0.99±0.0g	59.0±3.8bcd	62.4±0.9bcdef	62.4±0.9bcdef	11.82±0.1defg
APPRC-p0508		0.99±0.0g	63.4±0.0bc	74.3±13.2ab	74.3±13.2abc	10.68±0.2ij

Table 4 (Continued)

Isolates		Mortality ±SD*				LT_{50}
		4DAT	6DAT	8DAT	10DAT	
Am-KuG-Tes-43	Heterorhabditis Sp.	0.99±0.0g	43.1±6.6defg	45.4±14.4efghij	45.5±14.4efghi	13.7±0.1c
AEH		0.99±0.0g	38.9±16.2efgh	74.3±13.2ab	74.3±13.2abc	10.7±0.2ij
Am-She-Tes-244		9.6±4.9efg	49.6±22.5cdef	57.2±16.2bcefgh	57.2±16.2cdef	12.1±0.3def
Am-BuT-Tes-369*		43.1±3.3ab	66.1±4.6bc	89.0±0.0a	89.0±0.0a	6.7±0.1k
Am-Huj-Tes-73		30.8±7.3bc	55.4±13.9bcde	61.1±9.9bcdefg	61.1±9.9bcdef	12.07±0.2def
Am-Wez-Tes-67		0.99±0.0g	36.1±16.3fgh	38.6±14.7ijk	38.6±14.7ghi	15±0.3b
HBWWM		0.99±0.0g	23.9±4.7hi	26.1±11.4k	26.1±11.4i	16.7±0.2a
Am-Ger-Tes-74*		46.9±3.3a	66.1±4.7bc	89.0±0.0a	89.0±0.0a	6.7±0.1k
Am-DiM-Tes-341		6.8±10.1gf	61.2±3.8bc	71.4±15.2ab	71.4±15.2abc	11.1±0.2ghij
Am-Adm-Tes-69		0.99±0.0g	32.3±12.0gh	31.3±10.5jk	31.3±10.5hi	15.6±0.6b
Z9*		48.9±6.8a	63.9±7.4bc	89.0±0.0a	89.0±0.0a	6.6±0.3k
APPRC PL 0697		0.99±0.0g	28.8±3.8gh	28.9±9.9ij	28.9±9.9hi	16.7±0.2a
Am-Tey-Tes-295		0.99±0.0g	55.0±7.3bcde	60.0±4.6bcdefg	60.0±4.6bcdef	11.6±0.2defg
Am-Aad-Tes-72		43.0±3.3ab	54.8±3.5bcde	58.2±6.4bcdefgh	67.2±19.7bcd	11.5±0.3efgh
Am-Ben-Tes-292		0.99±0.0f	55.9±7.3bcde	66.9±20.6bcd	80.5±14.8ab	10.5±0.3j
Control		0.99±0.0g	0.99±0.0j	0.99±0.0l	0.99±0.0j	
CV		9.84	22.89	19.05	22.2	
F Value		7.19	8.52	9.26	6.8	

* Indicate isolates selected for further screening. Means in the same column followed by similar letters are not significantly different according to the LSD test at 0.05.

Table 5. Percentage larval mortality and leaf damage due to FAW after 12 days inoculation of the entomopathogenic nematodes ***Steinernematidae*** sp. (Am-Aso-Tes-287) and ***Heterorhabditis*** sp. (Z9, Am-BuT-Tes-369 and Am-Ger-Tes-74), in Wirehouse conditions.

Treatment	First Experiment		Second Experiment	
	Mean Mortality (%)	Mean % Damage	Mortality (%)	Damage (%)
Z9 250	36.5±15.8e	50.8±0.0b	28.9±1.8f	46.9±1.7bc
Z9 400	51.2±3.3cde	42.1±±2.9bcd	38.1±4.4def	39.2±2.9cd
Z9 600	53.2±4.5bcd	35.3±1.8def	50.2±4.9bc	35.2±3.5de
Am-Aso-Tes-287 250	48.9±11.9cde	46.0±4.4bc	44.3±10.7bcd	45.9±1.7bc
Am-Aso-Tes-287 400	68.0±5.3ab	38.2±1.7cdef	49.9±1.5bc	39.2±5.0cd
Am-Aso-Tes-287 600	74.6±6.2a	30.9±4.9ef	74.7±13.1a	28.9±1.9e
Am-Ger-Tes-74 250	46.7±10.8de	45.0±5.8bc	42.1±7.9cde	46.9±4.4bc
Am-Ger-Tes-74 400	65.3±4.6abc	37.2±4.5cdef	52.2±9.8bc	37.1±1.7cde
Am-Ger-Tes-74 600	74.3±13.2a	28.9±2.0f	78.3±18.6a	33.0±6.3de
Am-BuT-Tes-369 250	42.4±12.4de	49.9±6.1b	31.7±4.5ef	51.8±1.7b
Am-BuT-Tes-369 400	57.8±8.4bcd	41.2±3.3bcd	43.9±6.1bcd	41.1±1.7cd
Am-BuT-Tes-369 600	63.4±4.9abc	40.0±4.6cde	54.5±6.4b	40.9±7.1cd
Control	0.99±0.0f	66.1±4.7a	0.99±0.0g	65.2±7.6a
P Value	<.0001	<.0001	<.0001	<.0001
F Value	12.13	8.48	25.23	7
CV	18.47	13.57	14.89	14.33

Means in the same column followed by similar letters are not significantly different according to the LSD test at 0.05.

The *Steinernematids* and *Heterorhabditids* sp. were the most commonly collected species in Ethiopia similar to reports from different countries including Mexico, Turkey, India, and Egypt (Mekete *et al*., 2005; Girón-Pablo *et al*., 2012; Tewodros *et al*., 2012; Gonfa *et al*., 2016; Devi *et al*., 2017; Abdel-Razek *et al*., 2018; Ashenafi *et al*., 2019; Yuksel and Canhilal, 2019). The isolates Am-Aso-Tes-287, Am-BuT-Tes-369, Am-Ger-Tes-74, and Z9 caused the highest mortality within 8-days. This indicates that these isolates have the potential to be used for the management of the FAW. similarly, *Steinernematids* and *Heterorhabditids* sp. demonstrated the highest mortality against different insect pests, for instance, *Tuta absoluta*, *Phyllophaga vetula* (Batalla-Carrera *et al*. 2010; Girón-Pablo *et al*., 2012), *Spodoptera litura* (Adithya and Shivaprakash, 2021), and storage insect (Qader *et al*., 2021). In addition, Abbas, (2010) and Shahina *et al*. (2009) reported that *Steinernematids* and *Heterorhabditids* sp. caused 100%

mortality on red palm weevil in the third and fifth instars at a concentration of 400 IJs /ml. Andaló *et al*. (2010) also reported 97.6 and 100 %mortality of *S. frugiperda* larvae by *Steinernema* sp. and *Heterorhabditis* in laboratory and greenhouse.

Isolate Am-Aso-Tes-287, Am-BuT-Tes-369 and Am-Ger-Tes-74, Z9 caused 50% mortality within 3.5 and 6.6 days, respectively, which is faster than the LT_{50} reported by Adithya and Shivaprakash (2021) on *Spodoptera litura* using the most active symbiotic bacterial of EPN and *Steinernematids.* Similarly, Bhairavi *et al*. (2021) reported significant variations in lethal time to 50% mortality using *Heterorhabditis bacteriophora* for the control of *Odontotermes obesus* and *Agrotis ipsilon*. In the current study, the intermediately virulent isolates Am-Aad-Tes-72, Am-AdG-Tes-59, Am-KuG-Tes-43, and AEH, had LT_{50} of 10.5, 10.82, 10.68, and 10.7 days, respectively, compared to those categorized as weakly virulent. Further evaluation of the four highly virulent EPN isolates on pot planted maize showed that increase on the concentration levels of the EPN increased mortality in *S. frugiperda* larvae. Similar results have been reported by Shahina *et al*. (2009) and Gonfa *et al*. (2016) using *Heterorhabditis* and *Steinernema* sp. isolates that caused the highest mortality against red palm weevil and Diamondback moth under laboratory and greenhouse conditions. Data on plant damage showed that there was less plant damage when the isolate concentration was higher.

Conclusion and Recommendation

Native strains of the Entomophatogenic nematodes, Steinernematids and Heterorhabditids species were isolated from soils of maize-producing areas of Ethiopia with the objective to find out efficient strains to manage FAW. Among the newly isolated EPN strains Am-Aso-Tes-287, Am-BuT-Tes-369 and Am-Ger-Tes-74, and Z9 were the most pathogenic to FAW in laboratory and wire-house pot experiments. The isolates Am-Aso-Tes-287 and Am-Ger-Tes-74 performed better than the other isolates at all concentrations, exposure times, and in cumulative mortality of the insects. Therefore, these isolates have the potential for the development of microbial insecticide against FAW to be used as a component of an integrated management strategy of the pest. However, future studies are needed on collection of EPNs from different agroecology, molecular characterization of entomopathogenic nematode isolates, field evaluations using appropriate formulation under high insect population conditions, and more researches on mass production, and shelf-life of the isolates.

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Reference

Abate Tsedeke, Shiferaw Bekele, Menkir Abebe, Wegary Dagne, Kebede Yilma, Tesfaye Kindie, Kassie Menale, Bogale Gezahegn, Tadesse Berhanu, Keno Tolera, 2015. Factors that transformed maize productivity in Ethiopia. Food security, 7: 965-981.

Abbas M.S.T. 2010. IPM of the red palm weevil, *Rhynchophorus ferrugineus*, . In: Ciancio A, Mukerji K.G. (eds.), Integrated management of arthropod pests and insect borne diseases, Integrated Management of Plant Pests and Diseases 5, pp. 209-233.

Abbott W.S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.

Abdel-Razek A.S, Hussein M, Shehata I. 2018. Isolation and identification of indigenous entomopathogenic nematode (EPN) isolate from Egyptian fauna. Archives of Phytopathology Plant Protection, 51: 197-206.

Abraham Tadese, Ferdu Azerefegne, Asefa Gebreamlak, Adhanom Negasi, 1993. Research highlights on maize insect pests and their management in Ethiopia. Proceedings of the National Maize Workshop. May 5-7 1992, Institute of Agricultural Research, Addis Ababa, Ethiopia.

Acharya R, Hwang H.-S, Mostafiz M.M, Yu Y.S, Lee K.Y. 2020. Susceptibility of various developmental stages of the fall armyworm, *Spodoptera frugiperda*, to entomopathogenic nematodes. Insects, 11: 868.

Adithya S, Shivaprakash M. 2021. Evaluation of Pathogenicity of Symbiotic Bacteria of Entomopathogenic Nematodes against the Larvae of Tobacco Caterpillar, *Spodoptera litura* F.(Lepidoptera; Noctuidae). International Journal of Plant & Soil Science, 33: 1-9.

Andaló V, Santos V, Moreira G.F, Moreira C.C, Moino Junior A. 2010. Evaluation of entomopathogenic nematodes under laboratory and greenhouses conditions for the control of *Spodoptera frugiperda*. Ciência Rural, 40:1860-1866.

Arthurs, S, Heinz, K.M, Thompson S, Krauter, P.C. 2003. Effect of temperature on infection, development and reproduction of the parasitic nematode *Thripinema nicklewoodi* in Frankliniella occidentalis. Biological Control, *48*: 417-429.

Ashenafi Kassaye, Emana Getu, Awol Seid, Mulatu Wakgari, Muluken Goftishu, Solomon Yilma, Temesgen Addis, 2019. Occurrence and Distribution of Entomopathogenic Nematodes in Termite Prone Areas of Eastern and Western Ethiopia. Pest Management Journal of Ethiopia, 22: 15-28.

Assefa Banchayehu, Chamberlin J, Reidsma P, Silva J.V, van Ittersum M.K. 2020. Unravelling the variability and causes of smallholder maize yield gaps in Ethiopia. Food Security, 12: 83-103.

Atnafu Wondimu, Beyene Petros, Zemede Asfaw, Yitbarek Woldehawariat, Sciences A. 2021. Prevalence and impacts of fall army worms (*Spodoptera frugiperda*) on maize (*Zea mays*) production and productivity in Ethiopia. Journal of Food, 10: 21-27.

Batalla-Carrera L, Morton A, García-del-Pino F. 2010. Efficacy of entomopathogenic nematodes against the tomato leaf miner, (*Tuta absoluta*) in laboratory and greenhouse conditions. Biological Control, 55: 523-530.

Baudron F, Zaman-Allah M.A, Chaipa I, Chari N, Chinwada P. 2019. Understanding the factors influencing fall armyworm (*Spodoptera frugiperda* JE Smith) damage in African smallholder maize fields and quantifying its impact on yield. A case study in Eastern

Zimbabwe. Journal of Crop protection, 120: 141-150.

Bhairavi K.S, Bhattacharyya B, Devi G, Bhagawati S, Das P.P.G, Devi E.B, Manpoong N.S. 2021. Evaluation of two native entomopathogenic nematodes against *Odontotermes obesus* (Rambur)(Isoptera: Termitidae) and *Agrotis ipsilon* (Hufnagel)(Lepidoptera: Noctuidae). Egyptian Journal of Biological Pest Control, 31: 1-8.

Birhanu Sisay, Simiyu J, Esayas Mendesil, Likhayo P, Gashawbeza Ayalew, Mohamed S, Subramanian S, Tadele Tefera. 2019. Fall armyworm, (*Spodoptera frugiperda*) infestations in East Africa: Assessment of damage and parasitism. Insect, 10: 195.

Bloem S, Carpenter J.E. 2001. Evaluation of population suppression by irradiated Lepidoptera and their progeny. The Florida Entomologist, 84: 165-171.

Cairns J.E, Hellin J, Sonder K, Araus J.L, MacRobert J.F, Thierfelder C, Prasanna, B.M. 2013. Adapting maize production to climate change in sub-Saharan Africa. Food Security, 5: 345-360.

CSA (Central Statistics Agency). 2020. Agricultural sample survey 2020/2021 (2013 E.C): Report on farm management practices (private peasant holdings, Meher season). The Federal Democratic Republic of Ethiopia Central Statistical Agency, Addis Ababa.

Danso Y, Issa U, Adama I, Osei K, Agyei Obeng E. Opoku M, Amoabeng W, Abugri B, Adomako J. 2021. Application of Entomopathogenic Nematodes in Management of Fall Armyworm Infesting Maize in Ghana: A Greenhouse Study. Egyptian Journal of Agronematology, 20: 159-166.

Davis F.M, Ng S, Williams W. 1992. Visual rating scales for screening whorl-stage corn for resistance to fall armyworm. Technical bulletin, Mississippi Agricultural and Forestry Experiment Station, 186:1-9.

De Groote H, Kimenju S.C, Munyua B, Palmas S, Kassie M, Bruce A.J.A. 2020. Spread and impact of fall armyworm (*Spodoptera frugiperda* JE Smith) in maize production areas of Kenya. Agriculture, Ecosystems and Environment, 292: 106804.

Devi G, Sarma A, Thakuria R, Nath D, Bhuyan P, Somvanshi V.S, Sultana R, Kumar P, Das P, Bhagawati B. 2017. Isolation and Identification of Entomopathogenic Nematodes from Assam, India. Indian Journal of Nematology, 47: 65-74.

Dolinski C, Choo H, Duncan L. 2012. Grower acceptance of entomopathogenic nematodes: case studies on three continents. Journal of Nematology, 44: 226.

Emana Getu, Overholt W, Kairu E. 2001. Distribution and species composition of stemborers and their natural enemies in maize and sorghum in Ethiopia. International Journal of Tropical Insect Science, 21: 353-359.

Emana Getu, Abrham Tadesse, Mulugeta Negeri, Tadele Tefera, Hadush Tsaheye and Asmare Dejene, 2008. Review of Entomological Research on Maize, Sorghum and Millet. Abraham Tadesse (ed.) Proceedings of the 14th Annual Conference of the Plant Protection Society of Ethiopia (PPSE), 19-22 December 2006, Addis Ababa, Ethiopia. PP 167-244.

Erenstein O, Jaleta M, Sonder K, Mottaleb K, Prasanna B. 2022. Global maize production, consumption and trade: trends and R&D implications. Food Security, 14: 1295-1319.

Girón-Pablo S, Ruiz-Vega J, Pérez-Pacheco R, Sánchez-García J, Aquino-Bolaños T. 2012. Isolation of entomopathogenic nematodes and control of *Phyllophaga vetula* Horn in Oaxaca, Mexico. African Journal of Biotechnology, 11: 16525-16531.

Godjo A, Zadji L, Decraemer W., Willems A, Afouda L. 2018. Pathogenicity of indigenous entomopathogenic nematodes from Benin against mango fruit fly (*Bactrocera dorsalis*) under laboratory conditions. Biological Control, 117: 68-77.

Gómez-Valderrama J, Cuartas-Otálora P, Espinel-Correal C, Barrera-Cubillos G, Villamizar-Rivero L. 2022. Fungal and viral entomopathogens as a combined strategy for the biological control of fall armyworm larvae in maize. *CABI Agriculture and Bioscience*, 3(1): 24.

Gonfa Tolera, Tesfaye Hailu, Mohammed Dawd, Mulugeta Negeri, Selvaraj, T. 2016. Evaluation of entomopathogenic nematodes against Diamondback moth, *Plutella xylostella* (L.)(Lepidoptera: Plutellidae) on cabbage under laboratory and glasshouse conditions. International Journal of Agricultural Technology, 12: 879-891.

Gomez, K.A. and Gomez, A.A. 1984. Statistical Procedures for Agricultural Research. 2nd ed. New York, An International Rice Research Institute Book. Pp. 680.

Gozel U, and Gozel C. 2016. Entomopathogenic nematodes in pest management. In: Gill H. G, and Goyal G. (eds), Integrated pest management: Environmentally sound pest management, Pp55-69.

Gümüşsoy A, Yüksel E, Özer G, İmren M, Canhilal R, Amer M, Dababat A.A. 2022. Identification and biocontrol potential of entomopathogenic nematodes and their endosymbiotic bacteria in apple orchards against the Codling moth (*Cydia pomonella*) (L.) (Lepidoptera: Tortricidae). Insects, 13: 1085.

Horikoshi R.J, Bernardi D, Bernardi O, Malaquias J.B, Okuma D.M, Miraldo L.L, Amaral F.S.d.A, Omoto C. 2016. Effective dominance of resistance of *Spodoptera frugiperda* to Bt maize and cotton varieties: implications for resistance management, Scientific reports. 6: 34864. doi: 10.1038/srep34864.

Kandji S.T, Ogol C.K, Albrecht A. 2001. Diversity of plant-parasitic nematodes and their relationships with some soil physico-chemical characteristics in improved fallows in western Kenya. Applied Soil Ecology, 18: 143-157.

Kfir R, Overholt W, Khan Z, Polaszek A., 2002. Biology and management of economically important lepidopteran cereal stem borers in Africa. Annual Review of Entomology, 47: 701-731.

Kour S, Khurma U, Brodie G, Hazir S. 2020. Natural occurrence and distribution of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) in Viti Levu, Fiji Islands. Journal of Nematology, 52: 1-17.

Kuzhuppillymyal-Prabhakarankutty L, Ferrara-Rivero F.H, Tamez-Guerra P, Gomez-Flores R, Rodríguez-Padilla M.C, Ek-Ramos M.J. 2021. Effect of *Beauveria bassiana*-seed treatment on *Zea mays* L. response against *Spodoptera frugiperda*. Applied Sciences, 11: 2887.

Lalramnghaki H.C. 2018. Morphological and molecular characterization of entomopathogenic nematode, *Heterorhabditis baujardi* (Rhabditida, Heterorhabditidae) from Mizoram, northeastern India. Journal of Parasitic Diseases, 42: 341-349.

Lalramnghaki H.C, Lalremsanga H.T, Lalramchuani M. 2021. Susceptibility of the fall armyworm (*Spodoptera frugiperda*) (JE Smith, 1797) (Lepidoptera: Noctuidae), to four species of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from Mizoram, North-Eastern India. Egyptian Journal of Biological Pest Control, 31: 1-11.

Mekete Tesfemariyam, Gaugler, R., Nguyen, K., Wondirad Mandefro Wondirad Mandefro, Tessera Mesifin, 2005. Biogeography of entomopathogenic nematodes in Ethiopia. Nematropica, 31-36.

Midega C.A, Bruce T.J, Pickett J.A, Khan Z.R. 2015. Ecological management of cereal stemborers in African smallholder agriculture through behavioral manipulation. Ecological Entomology, 40: 70-81.

Mwamburi L.A. 2021. Endophytic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, confer control of the fall

armyworm (*Spodoptera frugiperda*) (JE Smith)(Lepidoptera: Noctuidae), in two tomato varieties. Egyptian Journal of Biological Pest Control, 31: 1-6.

Navik O, Shylesha A, Patil J, Venkatesan T, Lalitha Y, Ashika T, 2021. Damage, distribution and natural enemies of invasive fall armyworm (*Spodoptera frugiperda*) (JE smith) under rainfed maize in Karnataka, India. Crop Protection, 143: 105536.

Orozco R.A, Lee M.M, Stock S.P. 2014. Soil sampling and isolation of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae). Journal of Visualized Experiments, 89: 52083.

Qader F, Mohammed B, Ameen H. 2021. Efficacy of Entomopathogenic Nematodes Against Three Species of Stored Product Insects. Earth and Environmental Science, 910(1): 012047.

SAS (Analytics Software & Solutions) Institute. 2012. *SAS/OR 9.4 User's Guide: Mathematical Programming Examples*. SAS institute.

Shahina F, Gulsher M, Javed S, Khanum T.A, Bhatti M.I. 2009. Susceptibility of different life stages of red palm weevil, (*Rhynchophorus ferrugineus*) to entomopathogenic nematodes. International Journal of Nematology, 19: 232-240.

Sharon B, Michael M, Bwayo M.F. 2020. Severity and prevalence of the destructive fall armyworm on maize in Uganda: A case of Bulambuli District. African Journal of Agricultural Research, 16: 777-784.

Tesfaye Hailu, Belay Habtegebriel, Teshal Daba, 2018. In-vitro and In-vivo Evaluation of Entomopathogenic nematodes, *Steinernema yirgalemense* and *Heterorhabditis bacteriophora* for control of Red Teff Worm (*Mentaxya ignicollis*). Journal of Plant Sciences and Agricultural Research, 3: 1-17.

Teshome Kumela, Josephine S, Birhanu Sisay, Likhayo P, Mendesil E, Gohole L, Tadele Tefera. 2019. Farmers' knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. International Journal of Pest Management, 65: 1-9.

Tewodros Tamiru, Waeyenberge L, Tesfaye Hailu, Ehlers, R.-U., Půža, V., Mráček, Z., 2012. *Steinernema ethiopiense* sp. n.(Rhabditida: Steinernematidae), a new entomopathogenic nematode from Ethiopia. Nematology 14, 741-757.

Tolera Keno, Girum Azmach, Dagne Wegary, Worku M, Berhanu Tadesse, Legese Wolde, Temesegen Deressa, Belay Abebe, Temesegen C, Mahabaleswara, S. 2018. Major biotic maize production stresses in Ethiopia and their management through host resistance. African Journal of Agricultural Research, 13(21): 1042-1052, DOI: 10.5897/AJAR2018.13163

Wattanachaiyingcharoen W, Lepcha O, Vitta A, Wattanachaiyingcharoen D. 2021. Efficacy of Thai indigenous entomopathogenic nematodes for controlling fall armyworm (*Spodoptera frugiperda*)(JE Smith) (Lepidoptera; Noctuidae). Egyptian Journal of Biological Pest Control, 31: 1-7.

Yohannes Gerezihier, Bajigo Aklilu, Yohannes Mekiso, Tsegay weldu. 2020. Characterization of Soil Physicochemical properties and its implications to the Water quality of Elbahi Dam, Somali Region, Ethiopia. Journal of Ecology and Natural Resources, 4(4): 000204.

Yuksel E, Canhilal R. 2019. Isolation, identification, and pathogenicity of entomopathogenic nematodes occurring in Cappadocia Region, Central Turkey. Egyptian Journal of Biological Pest Control, 29: 1-7.