
Evaluation of Entomopathogenic Nematodes against Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) on Cabbage under Laboratory and Glasshouse Conditions

Tolera, G.^{1*}, Hailu, T.¹, Dawd, M.¹, Negeri, M.² and Selvaraj, T.²

¹Ambo Plant Protection Research Center, P.O Box 37, Ambo, Ethiopia, East Africa;

²Department of Plant Sciences, College of Agriculture and Veterinary Sciences, Ambo; University, Ambo, P.O. Box 19, Ethiopia, East Africa.

Tolera, G., Hailu, T., Dawd, M., Negeri, M. and Selvaraj, T. (2016). Evaluation of entomopathogenic nematodes against diamond back moth, *Plutella xylostella* (Lepidoptera: Plutellidae) on cabbage under laboratory and glasshouse conditions. International Journal of Agricultural Technology 12(5):879-891.

Abstract Diamondback moth (DBM) (*Plutella xylostella* L., Lepidoptera: Plutellidae) is one of the most serious pest of cruciferous crops and causes huge economic losses. This study emphasized on determination of efficacy on EPN isolates against DBM larvae and also to evaluate the selected isolates of Entomopathogenic nematodes (EPN) for the control of DBM in cabbage plants under pot culture. Bioassay was conducted on three isolates of *Steinernema* (HI, AW3 and HH) and two isolates of *Heterorhabditis* spp. (AEH and Z9). All 5 Ethiopian EPN were tested in single dose efficacy experiment (400IJ/ml) under laboratory condition. The result indicates that among the EPN isolates, HI (33.33, 56.67 & 82.64) and AEH (26.67, 63.33 & 91.67) EPN isolates produce highest mortality in the 24, 48 and 72hrs after treatment application. The most effective or virulent EPN isolates were further tested for their potential on DBM on pot planted cabbage infested with 3rd instars larvae under glasshouse condition using HI and AEH were studied at 3 different concentration levels (300, 400 and 500 IJ/ml) including free control. The experimental result indicates that, HI (30, 54.81&72.6) and AEH (23.7, 44.44 & 71.52) at higher concentration levels (500 IJ/ml) produce higher mortality after 24, 48 and 72hrs treatment applications as compared to other concentration levels. The relative potency values indicated that *Steinernema* spp. (HI (1, 1)) was more effective than *Heterorhabditis* spp. (AEH (1.21, 1.77)) in potency against 3rd instar larvae of DBM at the LC50 and LC90 level, respectively. From the Current study, it can be revealed that, *Steinernema* spp. (HI) and *Heterorhabditis* spp. (AEH) was found promising isolate which is also recommended for further field application study to control DBM.

Keywords: DBM, Cabbage, *Steinernema* spp., *Heterorhabditis* spp.

Introduction

Cabbage (*Brassica oleracea* L. var *capitata*) is one of the most important cruciferous vegetable grown extensively in tropical and temperate regions of

* Corresponding author: Tolera, G.; Email: yonatentes@yahoo.com

the world. Between 1993 and 2009 the global area of brassica vegetable crops production increased by 39% and in 2009 an estimated 3.4 million hectares were covered by brassica worldwide (FAOSTAT, 2012). Both head and kale, is the second most important vegetable crop in Ethiopia both in area coverage as well as level of production next to red pepper, *Capsicum* spp. (MOA, 2002). It is among the major vegetables produced by private farmers and the state farms in Ethiopia (Lemma *et al.*, 1994).

However, Insect pests are among the major constraints of productions of crucifers including cabbage is often decline by a number of insect species including the diamondback moth (DBM), (Lepidoptera: Plutellidae), cabbage aphid *Brevicoryne brassicae* L. (Homoptera: Aphididae), mustard aphid *Lipaphis erysimi* Kalténbach (Homoptera: Aphididae), flea beetles *Phylloterta* spp. and cabbage leaf miner *Chromatomyia horticola* Goureau (Diptera: Agromyzidae), cabbage looper (*Trichoplusia ni* (Hubner) and cutworms (Agrotis species) (Abate and Ayalew, 1992) inflict damage on brassica crops in Ethiopia (Tsedeke and Gashawbeza, 1994). As in any of the tropical countries, the severity of damage inflicted by DBM on cabbage is higher than other insect pests (Talekar and Shelton, 1993). In Ethiopia, reported yield loss on cabbage from the pest ranges between 36.1% and 91.2% corresponding to 12 to 48.7 tons/ha respectively (Gashawbeza Ayalew, 2006).

Control measures of DBM, including cultural, chemical and microbial methods (Gashawbeza Ayalew, 2014, Liu *et al.*, 1996; Perez and Shelton, 1997; Wright *et al.*, 1997) have been attempted. However, they were not adequate to decrease the harmfulness of DBM and the yield loss due to the pest. Use of synthetic pesticides causes some unfortunate consequences, such as environmental pollution, pest resistance and toxicity to other non-target organisms. The problem of insecticide resistance as well as environmental and consumer health hazards associated with insecticide residue in plant materials have been changed the attention of scientists towards looking for alternative methods for the control of DBM.

Entomopathogenic nematodes have a wide host range, can effectively suppress their hosts and are naturally available under nearly in all environmental conditions, increasing their potential as successful biological control agents (Uğur GÖZEL 2013, Gaugler and Kaya, 1990; Koppenhöfer, 2000; Nguyen *et al.*, 2004a). Now a day, entomopathogenic nematodes are applied with success against many target pests in biological control (Uğur GÖZEL, 2013, Kaya and Gaugler, 1993; Fenton *et al.*, 2000; Koppenhöfer, 2000).

Thus, the aims of this study were to determine the efficacy of EPN isolates against DBM larvae and also to evaluate the selected isolates of EPN for the control of DBM in cabbage plants under pot culture.

Materials and methods

Study Area

The experiment for studying Evaluation of Entomopathogenic nematodes was carried out in the Ethiopian Institute of Agricultural Research (EIAR), Plant Protection Research Center Ambo (PPRC).

Growing of cabbage Plants

Cabbage: Copenhagen market, *Brassica oleraceae* (*Capitata Group*) was planted on earthen 50cm height and 50cm diameter wide pot filled with soil. The soil was composed of sand, compost and loam soil at the ratio of 1:2:1. The seedlings were transplanted to the pot (18 cm height and 20 cm diameter) after one month. This cabbage seedlings were then used for DBM rearing and final experiment.

DBM Rearing

The DBM larvae were collected from Guder and brought to the laboratory of APPRC for rearing. Larvae were released on six week old cabbage seedling planted on pot in the rearing cage. The cabbage seedlings were watered daily and cages kept clean. Cabbage was planted step by step for the continuous rearing of DBM. Emerged adults (male and female moths) of DBM were transferred to other cage to allow mating by providing 20 % honey solution with cabbage leaves. The female laid Eggs either singly or in small clusters on the upper and lower side of leaves. The cabbage seedlings were replaced weekly and transfer to other cages. The hatched larvae were reared on fresh cabbage leaves and third instars larvae were taken and used for pathogenicity test of EPNs under laboratory and glasshouse condition. The experiment was conducted at the temperature of $(26 \pm 2^{\circ}\text{C}$ and 65+5% RH).

Culture and Preparation of EPN

Both EPN isolates (*Steinernema* and *Heterohabditis* spp.) were obtained from APPRC, entomology bio-control laboratory, collected from different parts of the country (Table 1). The suspension of each isolate was then diluted 100

times to determine the nematodes infective juvenile (IJ) concentrations of the stock solution with sterilized distilled water. Counting slide under dissected microscope was used to count the infective juvenile(IJ) and suspension of 300, 400 and 500 IJ ml⁻¹ respectively for laboratory and glasshouse were prepared for the experiment and counted by dilution technique. The actual number of nematodes in the stock solution was calculated by using the formula as suggested by Woodring and Kaya, (1988).

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

Where, C is actual number of nematodes in the stock solution, N is average number of nematodes per counted sample, S is volume of original stock solution (ml) and (X + L) is total volume (ml) in the diluted sample

To prepare dilution with a given number of nematodes per ml, from the counted suspension, the formula of Navon and Ascher (2000), $V_a = V \times [(i/c) - 1]$ where, I is initial concentration, c is final concentration, V is volume of the suspension (ml), V_a is the amount of water (ml) to be added (if positive) or to be removed (if negative) from the suspension was used.

Source of entomopathogenic nematod isolates

Five different isolates of entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis* spp. were used for virulence test. They were supplied by the Ethiopian Institute of Agricultural Research (EIAR), Plant Protection Research Center Ambo (PPRC), and (Table 1). The nematodes were isolated from different agro-ecological zones of Ethiopia at different times and properly isolated in PPRC laboratory.

Table 1. Entomopathogenic nematodes used for this study.

Species	Isolate code	Location Collected	Origin
<i>Steinernema</i> spp.	HI	Hagermariyam	Soil
	AW3	Awaro (Ambo)	Soil
	HH	Hagermariyam to Hagereselam	Soil
<i>Heterorhabditis</i> spp.	AEH	Ambo Ethiopia Hottel	Soil
	Z9	Zeway	Soil

Source: Ambo Plant Protection Research Center

Efficacy of *Stienernema* and *Heterorhabdtis* spp. against Larvae of DBM under Laboratory conditions

Bioassay was conducted on three isolates of *Stienernema* (HI, AW3 and HH) and two isolates of *Heterorhabdtis* spp. (AEH and Z9). Infective juveniles

(400 individual nematodes/ml) of each entomopathogenic nematode species were evaluated against 3rd instar DBM larvae under laboratory conditions with untreated larvae as control. (Nyasani *et al.*, 2008, Mahnaz Hassani-Kakhki, 2013). Total of 240 3rd instar DBM larvae were placed in 24 Petridishes (9cm diameter) for the experiment. Cabbage leaves were washed with distilled water and cut into 9 cm leaf discs and allowed to air-dry for 45 min before being used as the nutrient medium in the Petri dishes. Ten 3rd instar of DBM larvae were placed in 9cm diameter filter paper-padded-Petri-dishes there after 2ml of nematode suspension of each isolate was applied onto the filter papers as described by Rosa *at al.* (2002). Individual treatments were sprayed using micro pipette. The Petridishes were incubated at room temperature for 72hrs and weperiment was arranged by completely randomized design with 6 treatemnts and repeated four times for each nematode isolates. Larval mortality was recorded at 24hrs, 48hrs and 72hrs after treatment application.

Efficacy of selected *Stienernema* and *Heterorhabditis* species against Larvae of DBM under Glasshouse Conditions

The experiment was conducted at APPRC, Entomology Glasshouse and arranged according to randomized completely block design containing four treatments that were replicated four times.

Two potential EPN isolates, namely AEH (*Heterorhabditis* spp.) and HI (*Steinernema*) were used for this experiment. The nematode isolates were selected based on the observed potential in the screening experiment conducted in laboratory. Suspension of the isolates were prepared, diluted and counted using nematodes counting chamber. Three different doses of each nematode isolate at 300, 400 and 500 Ij/ml including untreated check were used.

The 3rd instar larvae of DBM were placed into each cabbage planted per pot. The total number of larvae used for this study was 160. The doses were chosen from a preliminary trial carried out by Somvanshi *et al.* (2006). Individual treatments were sprayed using hand sprayer. Larval mortality was assessed every 24 hour after application of treatments for consecutive three days.

Data Analysis

Larval mortality under each treatment in both laboratory and glasshouse conditions were corrected using Abbott (1925), and the data subjected to Arcsine transformed when necessary to meet assumptions of normality and homogeneity of variances. In the laboratory bioassays, probit analysis on the

larval mortality data was carried out to assess the two EPN at three concentration and LC₅₀ and LT₅₀ for each nematode strain. The data of larval mortality was subjected to one-way analysis of variance (ANOVA) to calculate the nematode efficacy, and were followed by Duncan Multiple Range Test (DMRT) to compare larval mortality. Statistical analysis software (SAS version 9.1) was used for all statistical analyses.

$$\text{Corrected per cent mortality} = \text{CM} (\%) = \frac{\text{T}-\text{C}}{100-\text{C}} * 100$$

Where CM is Corrected mortality, T is Percent mortality in treated larvae of DBM and C is Percent mortality in untreated larvae of DBM

Results and Discussion

Secreanig of Stienernema and Heterorhabdtis spp. against Larvae of DBM under Laboratory conditions

Five nematodes isolates obtained from APPRC, Ambo, Ethiopia were capable of infecting and killing the larval stages of DBM under laboratory conditions.

Mortality caused by the nematode isolates was confirmed based on the visual observation of nematode growth on surface of cadavers and all the dead DBM supported mycosis of the respective isolates. All the five isolates tested were capable of infecting and killing the DBM larve. Mortality was observed 24 hours after treatment. However, variations were seeing among the tested nematode isolates in the extent of mortality and the time taken to kill DBM larve.

The ANOVA indicated that mortality of 3rd instar larvae of DBM at 24, 48 and 72hrs after treatment application were significantly different from each other. The bioassay result showed that there were significant variations in the percentage mortality among the different EPN isolates tested. EPN isolates, HI (33.33, 56.67 & 82.64) and AEH (26.67, 63.33 & 91.67) incurred highest mortality in the 24, 48 and 72hrs after treatment application. EPN isolates AW3 (16.67, 33.33 & 46.67), HH (10, 20 & 40) and Z9 (10, 40 & 46.67) caused less mortality as compared to HI and AEH, but highest mortality when compared to the control treatment. On other hand, there was no larval mortality recoreded from the control (Table 2).

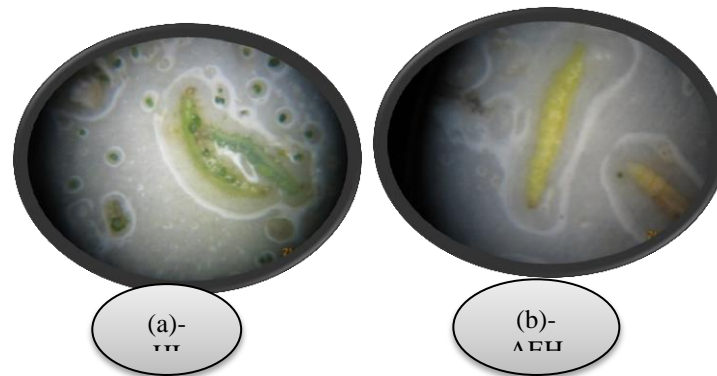


Figure 1. Treated Larvae of DBM with (a) *Steinernema* spp. (HI) and (b) *Heterorhabditis* spp. (AEH) under Laboratory Bioassay

Table 2. Mean Percent Mortality of 3rd Instar Larvae of DBM when Treated with *Steinernema* spp. (HI) and *Heterorhabditis* spp. (AEH) Isolates under Laboratory Conditions.

Treatment	Doses(IJ/ml)	Mean 3 rd Instar larvae of DBM Mortality \pm SD		
		24hrs	48hrs	72hrs
HH	400	10 \pm 0 ^d	20 \pm 0 ^d	40 \pm 0 ^c
AW3	400	16.67 \pm 2.71 ^c	33.33 \pm 5.77 ^c	46.6 \pm 5.77 ^b
HI	400	33.33 \pm 5.77 ^a	56.67 \pm 5.77 ^a	82.64 \pm 5.77 ^a
AEH	400	26.67 \pm 2.21 ^b	63.33 \pm 5.77 ^a	91.67 \pm 5.77 ^a
Z9	400	10 \pm 0 ^d	40 \pm 0 ^b	46.67 \pm 5.77 ^b
Control		0 \pm 0 ^e	0 \pm 0 ^e	0 \pm 0 ^d

Note: In the column, means followed by the same letters are not significantly different by the DMRT.

Under Glasshouse Conditions

The study was conducted using the two more virulent result showing on laboratory experiment namely, *Steinernema* spp. (HI) and *Heterorhabditis* spp. (AEH) at three different concentrations 300, 400 and 500 IJ/ml including untreated check indicates that there was significant difference in mortality among EPN isolates concentration levels at 24 48 and 72 hrs after treatment applications, but the is no significant difference among EPN isolates tested in glasshouse.

However, the individual nematode isolates at the different levels of IJ concentration showed variability in their effect against the insect (Table 3). The statistical analysis of the percentage mortality at 24hrs after application showed that *Steinernema* spp. (HI) 10% and *Heterorhabditis* spp. (AEH) 10% at the amount of 300IJ/ml and at 48hrs for HI (24.07) and AEH (20.74) and 72hrs for

HI (37.77) and AEH (34.44). Moreover, on 400IJ/ml concentration level for both entomopathogenic nematodes isolates at 24hrs, 48hrs and 72hrs after application showed that *Steinernema* spp. (HI) 13.33, 34.44 and 44.81 and *Heterorhabditis* spp. (AEH) 10, 23.7, 44.81, respectively.

The EPN isolate HI (30, 54.81 and 72.6) and AEH (23.7, 44.44 and 71.52) produce significantly superior mortality at the higher (500IJ/ml) concentration level in 24, 48 and 72 hrs after treatment application, respectively, as compared to other concentration levels of entomopathogenic nematodes isolates. Similarly, all entomopathogenic nematodes isolates at the lowest conidial concentration produced significantly lower larval mortality than the other treatments except the untreated check. All of the EPN isolates showed increased efficacy as the isolates concentration increased.

Table 3. Mean Percent Mortality of 3rd Instar Larvae of DBM when Treated with *Steinernema* spp. (HI) and *Heterorhabditis* spp. (AEH) Isolates under Glasshouse Conditions .

Treatment	Doses(IJ/ml)	Mean 3 rd Instars larvae of DBM Mortality \pm SD		
		24hrs	48hrs	72hrs
HI	300	10 \pm 0 ^b	24.07 \pm 5.25 ^b	37.77 \pm 3.85 ^c
	400	13.33 \pm 5.77 ^b	34.44 \pm 5.1 ^b	44.81 \pm 5 ^b
	500	30 \pm 0 ^a	54.81 \pm 8.98 ^a	72.6 \pm 4.5 ^a
AEH	300	10 \pm 0 ^b	20.74 \pm 1.28 ^c	34.44 \pm 5.1 ^c
	400	10 \pm 0 ^b	23.7 \pm 10.91 ^b	44.81 \pm 5.01 ^b
	500	23.7 \pm 0 ^a	44.44 \pm 9.62 ^a	71.52 \pm 2.57 ^a
Control		0 \pm 0 ^c	3.33 \pm 5.77 ^d	3.33 \pm 5.77 ^d
CV		9.15	18.96	8.86

Note: Values followed by the same letter in the same column do not differ significantly

In line with these results, a number of laboratory bioassays on various insects have been conducted by various researchers and DBM was found as a good host for *S. thermophile* which showed 100% mortality of DBM larvae within 48hrs after infection (Ganguly and Gavas, 2004). *Heterorhabditis indica*, *Steinernema kariii*, *Steinernema wesieri*, *Steinernema* spp. and *Heterorhabditis* spp. caused 96.0%, 93.3%, 92.0%, 88.0% and 86.7% at the concentration of 400 IJs/ml in the DBM larvae mortality within 72 hrs (Nyasani *et al.*, 2008). *Steinernema* species is more effective than *Heterorhabditis* species against DBM (Ratansinghe and Hague, 1995, 1998). *Steinernema siamkayai* and *Heterorhabditis bacteriophora* caused 62.5 and 60% diamondback moth larvae mortality at dosages of 400 IJs at 60hrs whereas; *S. carpocapsae* caused 92.5% larval mortality (Sasnarukkit, 2003). The effectiveness of *Steinernema* spp. is also similarly confirmed by the present finding. Shinde and Singh (2000) tested eight nematode species/strains against

DBM and found that all of them were pathogenic but that *Heterorhabditis bacteriophora* Poinar exhibited the greatest pathogenic potential due to its lowest LD₅₀ (9.16 IJs/larvae), LT₅₀ (43.26 hrs) and LT50 (3.24 hrs), and its greatest propagation potential (average of 271.4 IJs/mg host body weight).

Furthermore, the result of this study agrees with the study done by Ratansinghe and Hague, 1995, 1998, who reported that *Steinernema* spp. to be more effective than *Heterorhabditis* spp. against DBM. The present findings are also in the agreement with authors who reported the efficacy of the different *Steinernema* spp. and *Heterorhabditis* spp. against DBM (Belair *et al.*, 2003; Maher *et al.*, 2004; Somvanshi *et al.*, 2006). The *S.thermophilum* caused mortality of 46%, at 400 IJs/ml (Somvanshi *et al.*, 2006). A linear increase in the percent mortality of DBM with increased in concentration was observed. The result of the study presented here demonstrated that both *Heterorhabditis* spp. (AEH) and *Steinernema* spp. (HI) are virulent on DBM. However, *Heterorhabditis* spp. (AEH) was found less virulent than *Steinernema* spp. (HI) under laboratory and glasshouse conditions. In order to obtain better control of DBM through the use of EPNs, the selected nematodes should be sprayed onto the foliage at a suitable time of the day when UV light levels are low, relative humidity is high, and temperature is optimal for their survival. Generally, early morning or late evenings after sunset are the best times to spray. As DBM feeds nocturnally on leaf surfaces, late evening foliar applications are better for DBM infection on the leaf than the day. Therefore, the appropriate conditions were also conducted for the current experiment for the application of all selected Nematodes.

Determination of Medium Lethal Concentration of Steinernema spp. (HI) and Heterorhabditis spp. (AEH) Isolates against 3rd Instar Larvae of DBM under Glass house Conditions

The comparative virulence of nematodes *Steinernema* spp. (HI) and *Heterorhabditis* spp. (AEH) on the 3rd instar larvae of DBM was determined under laboratory condition. The estimated LC₅₀ and LC₉₀ values based on the mortality trends across dosage and relative potency are presented in Table 4. The results obtained in the concentration -mortality relationship studies further indicated that there were differences among the two tested isolates. *Steinernema* spp. (HI) showed the highest virulence with the lowest LC₅₀ 191.55 IJ/ml and LC₉₀ 605.46IJ/ml, whereas, *Heterorhabditis* spp. (AEH) showed the lowest virulence with the highest LC₅₀ 231.81 IJ/ml and LC₉₀ 1072IJ/ml.

The relative potency values indicated that nematode isolate of *Steinernema spp.* was the most effective than *Heterorhabditis spp.* with 1.21 times the highest potency at the LC₅₀ and 1.77 times great highest potency at the LC₉₀ level, respectively.

Table 4. LC₅₀ and LC₉₀ of *Steinernema spp.* (HI) and *Heterorhabditis spp.* (AEH) Isolates (IJ/ml) to 3rd Instar Larvae of DBM at 72hrs after application.

Treatments	LC ₅₀ (95% CI) ^a	LC ₉₀ (95% CI)	Relative potency ^b	
			LC ₅₀	LC ₉₀
HI	191.55(125.89-246.37)	605.46(387.03-4224)	1	1
AEH	231.81(0.25-604)	1072(477.16-1.58)	1.21	1.77

^a LC₅₀ or LC₉₀ and 95% fiducial limits (CLs) are given in IJ/ml

^b Relative potency is calculated as LC₅₀ or LC₉₀ of the tested EPN isolates/LC₅₀ or LC₉₀ of the most effective isolates.

Shapiro (2001) reported that the virulence of *H. bacteriophora* to be less than the virulence of *Steinernema spp.* on *G. mellonella*. In this study *steinernema spp.* (HI) was found to be the most virulent species against DBM 3rd instar larvae. The result of this study agrees with the study of virulence of EPN done by Shapiro (2001) where *steinernema spp.* was found to cause greater mortality than *Heterorhabditis spp.* the present finding is also supported by previous studies which reported *Steinernema species* to be more effective than *Heterorhabditis species* against DBM (Ratansinghe and Hague, 1995, 1998). This difference may be attributed to differences in infection strategies.

However, Shinde and Singh (2000) tested eight nematode species/strains against DBM and found that all of them were pathogenic but that *Heterorhabditis bacteriophora* Poinar exhibited the greatest pathogenic potential due to its lowest LD₅₀ (9.16 IJs/larvae) and LT₅₀ (43.26 hrs) and its greatest propagation potential (average of 271.4 IJs/mg host body weight) Comparative studies on the efficacy of different EPNs have produced contradictory results. This discrepancy may have been caused by differing experimental conditions. In those studies, the nematodes were produced and formulated under different conditions. Therefore, differences in virulence might involve nematode production and formulation as well as species differences. The result of the present study demonstrated that *steinernema spp.* (HI) is more virulent than isolates of *Heterorhabditis spp.* (AEH).

Based on the present study, *steinernema spp.* (HI) can be recommended as primary candidate for further research in order to develop an insecticidal nematode for DBM management in Ethiopia. However, *Heterorhabditis spp.* (AEH) could be considered as an alternative. Taking into account further aspects, such as, mass production, formulation, storage, spectrum of activity to

the pest and safety to non-target organisms will help focus on EPN isolates for product development.

Time-mortality Response of Steinernema spp. (HI) and Heterorhabditis spp. (AEH) Isolates against 3rd Instar of DBM Larvae

The median lethal time values of the EPN tested on the 3rd larval instar of DBM are shown in Table 5. It is indicated that the time required for 50% death decreased with increasing concentrations in both tested isolates on 3rd larval instar. At lower concentration (300IJ/ml), nematode isolate *Steinernema* spp. (HI) 8.11 days to kill 50% of the DBM larve and *Heterorhabditis* spp. (AEH) 8.76 days to kill 50% of the DBM larve and at 400IJ/ml concentration level, nematode isolate *Steinernema* spp. (HI) 6.0 days to kill 50% of the DBM larve and *Heterorhabditis* spp. (AEH) 6.98 days to kill 50% of the DBM larve. And at 400IJ/ml concentration level, nematode isolate HI 4.6 days to kill 50% of the DBM larve and AEH 5.1 days to kill 50% of the DBM larve.

It also showed that increasing the concentration and exposure time helped to increase the effective searching and infection chances of nematode. This indicates that *Steinernema* spp. (HI) requires less exposure time (4.6 days) to kill 50% of the DBM larvae. Whereas *Heterorhabditis* spp. (AEH) requires the higher exposure time to kill 50% of DBM larvae in all concentration.

Table 5. LT₅₀ *Steinernema* spp. (HI) and *Heterorhabditis* spp. (AEH) Isolates to 3rd Instar Larvae of DBM under Laboratory Conditions.

Treatments	LT ₅₀ (95% fiducial limit) ^a		
	300IJ/ml	400IJ/ml	500IJ/ml
HI	8.11	6.0	4.64
AEH	8.76	6.98	5.1

^a LT₅₀ and 95% fiducial limits (FLs) are given in hrs.

Conclusion

From the study it can be concluded that, *Steinernema* spp. (HI) and *Heterorhabditis* spp. (AEH) is a promising isolate (at the dose of 500IJ/ml) which is recommended for further study to control DBM. These agents can be considered as effective and environmentally friendly if used with appropriate manner.

Therefore it suggested further field trials that should provide evidence on its utility before recommending these bio-control agents to farmers. More work is also needed to study the application technology and environmental factors that influence the effectiveness of the EPNs during field applications.

References

- Abate, G. and Ayalew, G. (1992). Progresses on vegetables pest management research in: Proceedings of the 2nd international horticulture workshop. FAO, Addis Ababa, Ethiopia. pp. 187-193.
- Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18:265-267.
- Belair, G., Fournier, Y. and Dauphinais, N. (2003). Efficacy of steinernematid nematodes against three insect pests of crucifers in Quebec. *Journal of Nematology* 35:259-265.
- FAOSTAT. (2012). Production statistics. Rome: FAO. Retrieved from <http://faostat.fao.org/site/567/default.aspx# ancor>.
- Fenton, A., Norman, R., Fairbairn, J. P. and Hudson, J. P. (2000). Modelling the efficacy of entomopathogenic nematodes in the regulation of invertebrate pests in glasshouse. *Journal of Applied Ecology* 37:309-320.
- Ganguly, S. and Gavas, R. (2004). Host range of entomopathogenic nematode, *Steinernema thermophilum* Ganguly and Singh (Steinernematidae: Rhabditida). *International Journal of Nematology* 14:221-228.
- Gaugler, R. and Kaya, H. K. (1990). Entomopathogenic nematodes in biological control. Boca Raton, Florida: CRC Press. pp. 365.
- Gashawbeza, A. (2006). Comparison of yield loss on cabbage from diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) using two insecticides. *Crop Protection* 25:915-919.
- Gashawbeza, A. (2014). Citrus woolly whitefly and major insect and mites pests of tomato and cabbage: Recommendation and Management. Ethiopian Institute of Agricultural Research, Integrated Pest Management Manual. pp. 19-34.
- Kaya, H. K. and Gaugler, R. (1993). Entomopathogenic nematodes. *Annual Review of Entomology* 38:181-206.
- Koppenhöfer, A. M. (2000). Nematodes, 283-301. In *Field Manual of Techniques in Invertebrate Pathology*. Dordrecht, The Netherlands: Kluwer. 886 pp.
- Lemma, D., Hearth, E., Belehu Temesgen, B. Lemaga and Seyfu, G. (1994). Horticultural Research - past, present and future trends. In Hearth, E. and Dessalegne, L. (Eds.). *Proceedings of the Second National Horticultural Workshop*, Addis Ababa, Ethiopia, pp. 19-28.
- Liu, Y.B., Tabashnik, B. E. and Pustzai-Carey, M. (1996). Field-evolved resistance to *Bacillus thuringiensis* toxin Cry1C in diamondback moth (Lepidoptera: Plutellidae) *Journal of Economic Entomology* 89:798-804.
- Maher, A. N., Munir, M. and Elawad, S. (2004). Microbial control of diamondback moth, *plutella xylostella* L. (Lepidopter: Yponomeutidae) using bacteria (*Xenorhabdus nematophila*) and its metabolites from the entomopathogenic nematode *Steinernema carpocapsae*. *Journal of Zhejiang University SCIENCE B* 5:1183-1190.
- Mahnaz, H. K., Javad, K. and Mojtaba, H. (2013): Efficacy of Entomopathogenic nematodes against potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae) under laboratory conditions. *Biocontrol Science and Technology* 23:146-159.
- Navon, A. and Ascher, K. R. S. (2000). Bioassays of entomopathogenic microbes and nematodes. UK: CABI Publishing, pp. 229-234.
- Nguyen, K. B., Tesfamariam, M., Gozel, U., Gaugler, R. and Adams, B. J. (2004a). *Steinernema yirgalemense* n. sp. (Rhabditida: Steinernematidae) from Ethiopia. *Nematology* 6:839-856.
- Nyasani, J. O., Kimenju, J. W., Olubayo, F. M. and Wilson, M. J. (2008). Laboratory and field investigations using indigenous entomopathogenic nematodes for biological control of *Plutella xylostella* in Kenya. *International Journal of Pest Management* 54:355-361.

- Perez, C. P. and Shelton, A. M. (1997). Resistance of *Plutella xylostella* to *Bacillus thuringiensis* Berliner in Central America. *Journal of Economic Entomology* 90:87-93.
- Ratansinghe, G. and Hague, N. G. M. (1995). The susceptibility of diamond back moth *Plutella xylostella* (Lepidoptera: Yponomeutidae) to entomopathogenic nematodes. *Afro-Asian Journal of Nematology* 5:20-23.
- Ratansinghe, G. and Hague, N. G. M. (1998). The invasion, development, and reproduction of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) in the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Nematropica* 28:1-6.
- Rosa, J. S., Cabral, C. and Simoes, N. (2002). Differences between the pathogenic processes induced by *Steinernema* and *Heterorhabditis* (Nemata: Rhabditida) in *Peudaletia nipuncta* (Insecta: Lepdoptera). *Journal of Invertebray Pathology* 80:46-54.
- Sasnarukkit, A. (2003). Efficacy of an Entomopathogenic Nematode, *Steinernema siamkayai* stock, somsook and reid on controlling diamondback moth, *Plutella xylostella* (Linnaeus). (Ph.D. thesis), Kasetsart University.
- Shapiro-Ilan, D. I., Lewis, E. E., Behle, R. W. and McGuire, M. R. (2001). Formulation of Entomopathogenic nematode-infected cadavers. *Journal of Invertebrate Pathology* 78:17-23.
- Shinde, S. and Singh, N. P. (2000). Susceptibility of diamondback moth, *Plutella xylostella* L. to entomopathogenic nematodes. *Indian Journal of Experimental Biology* 38:956-959.
- Sithole, R. (2005). Life history parameters of *Diadegma mollipla* (Holmgren), competition with *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) and spatial and temporal distribution of the host, *Plutella xylostella* (L.) and its indigenous parasitoids in Zimbabwe. (Ph.D. thesis). University of Harare, Zimbabwe.
- Somvanshi, V. S., Ganguly, S. and Paul, A. V. N. (2006). Field efficacy of the entomopathogenic nematode *Steinernema thermophilum* Ganguly and Singh (Rhabditida: Steinernematidae) against diamondback moth (*Plutella xylostella* L.) infesting cabbage. *Biological Control* 37:9-15.
- Talekar, N. S. and Shelton, A. M. (1993). Biology, ecology, and management of the diamondback moth. *Annual Review Entomology* 38:275-301.
- Tsedeke, A. and Ayalew, G. (1994). Progress in vegetable management research: 1985-1992. *Proceedings of the Second National Horticultural Workshop*. Addis Ababa, Ethiopia, pp. 187-193.
- Uğur, G. (2013). Effect of entomopathogenic nematode species on the corn stalk borer (*Sesamia cretica* Led. Lepidoptera: Noctuidae) at different temperatures. *Türky. Entomology Dermatology* 37:65-72.
- Woodring, J. L. and Kaya, H. K. (1988), *Steinernematid and Heterorhabditid nematodes: A handbook of biology and techniques*. South cooperative Sericulture Arkansas. Agricultural Experimental Station, Fayetteville. 331 pp.
- Wright, D., Iqbal, M., Granero, F. and Ferre, J. (1997). A change in the single receptor in diamondback moth (*Plutella xylostella*) is only in part responsible for field resistance to *Bacillus thuringiensis* sbs. Kurstaki and *B. thuringiensis* sbs. aizawi. *Applied and Environmental Microbiology* 63:1814-1819.

(Received: 19 April 2016, accepted: 5 September 2016)