Journal of Agriculture and Technology Management (JATM)
20(2): 8-13 (July-December 2017)

Original Article

Heterorhabditis taysearae and *Heterorhabditis bacteriophora:* Promising Indigenous Entomopathogenic Nematodes against Vegetable Insect Pest

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ABSTRACT

Commercial application of entomopathogenic nematodes (EPNs) against insect pest has been successfully applied in countries like USA, Australia and Europe. In biological control programs, however, indigenous control agents are often preferable than using exotic ones. Therefore, a study on the identification of promising indigenous EPNs as control to common insect pests attacking vegetables is deemed necessary. Four species of the genus *Heterorhabditis* were compared. Standard filter paper bioassay was followed to compare its effectivity at 0, 10, 25, 50, 100 and 200 IJs/larva in 0.5 ml distilled water against larvae of cutworm (*Spodoptera litura*), cabbage head moth (*Crocidolomia pavonana*) and diamond back moth (*Plutella xylostella).* All isolates caused mortality to diamond back moth, cabbage head moth and cutworm. However, percent mortality differed among EPN isolates/species, concentrations and insect pest. *H. taysearae* at 200 IJs/larva caused the highest mortality rate (65%) on diamond back moth larvae. Against cabbage head moth, generally the higher the concentrations the better effect. On cutworm larvae, concentrations of 50 to 200 IJs/larva were most effective. Moreover, *H. bacteriophora* together with *H. taysearae* and *H. marelatus* was the most effective isolates. The pupal stage of the insect life cycle seemed less susceptible to infection while its adult stage is more susceptible. Both stages (papae and adult) under normal conditions are spent away from the ground. From the different isolates considered, both *H. taysearae* and *H. bacteriophora* have the greatest potential for field trials considering their distribution.

KEYWORDS: *Cebu, effective concentration, indigenous entomopathogenic nematodes, insect pest larvae, pathogenicity, Philippines*

1 INTRODUCTION

One of the limiting factors affecting vegetable production is insect pest infestation. Currently, chemical control still plays a significant role in the management of insect pest. However, there is now a growing concern with the use of chemical pesticides on public safety, soil and water pollution, insecticide resistance, effects on non-target organisms, and enhanced biodegradation of pesticides.

Salad vegetables which are eaten fresh or with minimal preparation are supposedly healthy food; pose a great threat to human health because of chemical residues. Due to these increasing concerns, there is now a great need on finding alternatives to using chemical pesticides through organic agriculture.

Entomopathogenic nematodes (EPNs) belong to the families *Steinernematidae* and *Heterorhabditidae* (*Rhabditida*) and are symbiotically associated with entomopathogenic bacteria *Photorhabdus* (Boemare *et al.,* 1993) and *Xenorhabdus* (Thomas and Poinar, 1979), respectively. These nematodes kill the host insects by entering through natural body openings (i.e. anus, mouth and spiracles) or in some cases through the cuticle and release bacteria that kill the host within 48 hours (Poinar, 1990). They possess a unique combination of attributes such as broad host range, high virulence, long term efficacy, easy application, easy mass production, environmentally safe making it an ideal component of insect pest management system. Since they occur in nature and its pathogenecity are specific to insect, these microorganisms are safe to human.

In the Philippines, the use of EPNs is very limited. There is little or no information on the efficacy of indigenous EPNs. Thus, a study on the identification of promising indigenous EPN species for control of economically important insect pests attacking vegetables (e.g. cutworms, diamond back moth and cabbage head moth) is deemed necessary.

2 MATERIALS AND METHODS

Sampling and Collection of Indigenous EPNs

Soil samples were collected from 17 sampling sites based on the list of Organic Vegetable Farmers provided by the Department of Agriculture – Regional Field Unit VII across the province of Cebu. Soil samples representing each site was collected at 10-15

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cm deep using a shovel. At each site, ten soil samples were randomly collected, placed in a bucket and thoroughly mixed to come-up with a composite soil sample for each site. About one kilogram of soil was taken from the bucket and transferred to a plastic bag for processing. Samples were placed on coolers during transport and stored in refrigerator at 10^{o}C.

Rearing of Test Insects

Larvae of cutworm (*Spodoptera litura*), cabbage head moth (*Crocidolomia pavonana*) and diamond back moth (*Plutella xylostella)* as identified by the organic vegetable farmers as three major insect pests were used as test organisms. Rearing of test insect (Fig. 1) was necessary to achieve homogeneity of test materials and so that they were available throughout the conduct of bioassay.

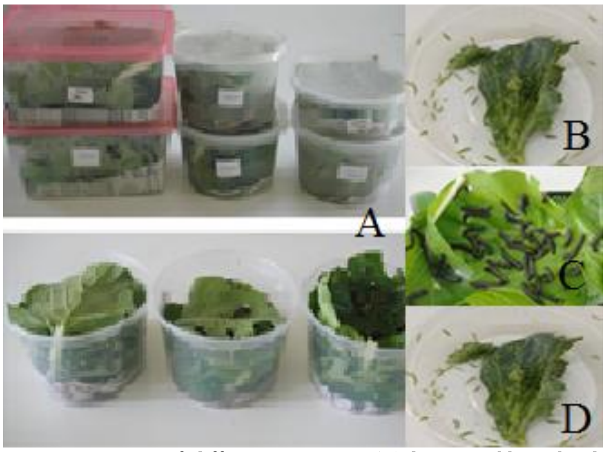

Figure 1. Rearing of different test insect (A) larvae: cabbage head moth (B), cutworm (C) and diamond back moth (D).

Nematode Isolation from Soil Samples

EPNs were recovered from soil samples using insect baiting methods described by Bedding and Akhurst (1975). Ten last instars of the identified test insect larvae were placed in 300 ml plastic container with moist soil sample (Fig. 2a). The covered containers were placed at room temperature for two weeks. Water was added to the soil samples to keep them moist at any point during baiting. The traps were checked every two days starting day five. Dead larvae from each container were placed in white traps to collect emerging effective juveniles (IJs) and infected to fresh larvae (Figs. 2b and 2c).

Maintenance of Insect Host Cultures

Lesser wax moth *(Achroia grisella)* larvae were used in culturing nematodes for mass production since maintenance of these larvae using artificial diet is well established. Insect cultures were maintained using artificial diet following the recommendation by van Zyl and Malan (2015), with modifications, containing 200 g rice powder, 100 ml honey, 100 ml glycerol and 50 g beer yeast in aerated plastic containers. Approximately 200-300 eggs were placed in artificial diet in plastic container and kept at room temperature. Eggs hatched after 3-4 days, after two weeks larvae were given fresh diet. After three weeks, late instars are ready for collection and those not immediately utilized were stored on paper shavings for 2-3 weeks at 10^{o}C.

Laboratory Screening

Standard filter paper bioassay (Woodring and Kaya, 1988) was conducted to compare infectivity of EPN isolates against test insects (Fig. 2d). Five petri plates were lined with filter paper for each nematode concentration and EPN isolates. Nematodes of different concentrations (0, 10, 25, 50, 100 and 200 IJs/larva in 0.5 ml of distilled water) were evenly distributed on a filter paper and was kept for 30 minutes. Three replicates with five sample larvae for each EPN species and concentration were laid-out in completely randomized design. Mortality was assessed daily over five days by gently probing with a soft brush. Percent mortality of test insect larvae was determined.

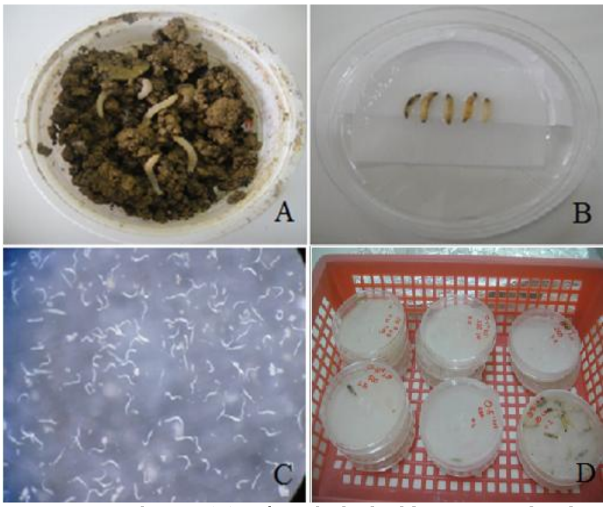

Figure 2. Insect baiting (A). After which, dead larvae were placed in white traps (B) and emerging infective juviniles were viewed under microscope (C). These IJs were screened using standard filter paper bioassay (D).

3 RESULTS AND DISCUSSION

EPNs of the family *Heterorhabditidae* (Poinar, 1976) are obligate insect parasites which can kill a wide range of host (Kaya and Gaugler, 1993). *Heterorhabditis* species are hermaphroditic and only one infective juvenile (IJ) in the host is sufficient for successful reproduction. In the second generation, the species is amphimictic, requiring both male and female for reproduction (Poinar, 1990).

All isolates caused mortality to diamond back moth, cabbage head moth and cutworm as shown in Figure 3. All control treatments, no EPN IJ, resulted to zero mortality.

However, percent mortality differed among EPN isolates/species, concentrations and insect pest as shown in Tables 1-3. On the dose-mortality response of diamond back moth (*P.* x*ylostella*), results revealed that the concentration of EPNs (IJ) and the species of nematode used has a significant interaction. The application of *H. taysearae*, at 200 IJs, resulted to 65% mortality. On the study on citrus root nematode, the

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population density *T. semipenetrans* was drastically reduced with the application of *H. taysaerae* (Abd-Elgawad *et al.,* 2013). The same species of nematode, *H. taysaerae*, was also found to be most effective against cabbage head moth (*C. pavonana*) resulting to mortality of more than half of its population (51%). Furthermore, 200 IJ also proved to be the best concentration of nematodes, regardless of isolate, resulting to 46% mortality of cabbage head moth larvae. As reported by Monteiro *et al.*, (2014), all treatments with EPNs of the genus *Heterorhabditis* caused significant reduction ($p < 0.05$) in the egg mass weight and hatching percentage of *Rhipicephalus microplus* larvae. Also on the dose-mortality against cabbage head moth (Table 2), dosage (200IJ) proved to be the more significant factor. For the species, *H. marelatus, H. indica,* and *H. bacteriophora* resulted to 57, 54 and 52% mortality, respectively. However, it is noteworthy that both *H. indica and H. bacteriophora* were isolated from most of the organic farms visited in Cebu, Philippines while only one area was positive of *H. marelatus*. Based on LC_{50} value on a study on sawfly (*A. lugensproxima*) larvae, *H. indica* was the most pathogenic species (Yakay and Lalramliana, 2012).

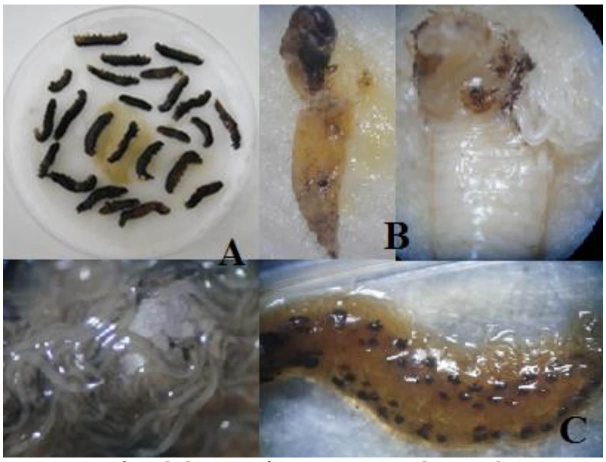

Figure 3. Infected larvae of cutworm (Spodoptera litura) (A), diamond back moth (Plutella xylostella) (B) and cabbage head moth (Crocidolomia pavonana) (C).

Table 1. Dose-mortality response (%) of diamond back moth (*P. xylostella*) larvae at different concentrations of EPS isolates.

Species	Concentration (IJs)					Mean
	C1 (10)	C2 (25)	C3 (50)	C4(100)	C5 (200)	
H. indica	20.00gh	26.67fgh	28.33fg	38.33def	45.00cde	31.67c
H. bacteriophora	34.67ef	38.67def	37.33def	44.00def	62.67ab	43.47b
H. taysearae	39.05def	55.24abc	45.71cde	50.47bcd	64.76a	51.05a
H. marelatus	13.33h	20.00gh	26.67fgh	13.33h	13.33h	17.33d
Mean	26.76c	35.14b	34.51b	36.53b	46.44a	

C.V. (%) = 21.69
Means within a column followed by a common letter is not significantly different from each other at P ≤ 0.05 determined by Student Newman Keul's (SNK) Test.

Table 2. Dose-mortality (%) response of cabbage head moth (*C. pavonana*) larvae at different concentrations of EPS isolates.

Species	Concentration (IJs)					Mean
	C1 (10)	C2 (25)	C3 (50)	C4(100)	C5 (200)	
H. indica	25.00	30.00	26.67	60.00	73.33	43.00b
H. bacteriophora	21.33	37.33	52.00	62.67	85.33	51.73ab
H. taysearae	41.90	39.05	49.53	63.81	75.24	53.91a
H. marelatus	40.00	46.67	46.67	73.33	80.00	57.33a
Mean	32.06d	38.26cd	43.72c	64.95b	78.48a	

C.V. (%) = 22.87
Means within a column followed by a common letter is not significantly different from each other at P ≤ 0.05 determined by Student Newman Keul's (SNK) Test.

On the dose-mortality (%) response of cutworm (*S. litura*) to application of different EPN isolates at varying concentration, results revealed high mortality at concentrations ranging from 50 IJ to 200IJ using any of the four species of nematodes: *H. bacteriophora, H. indica, H. taysearae* and *H. marelatus*. Among the four-species identified, *H. bacteriophora* consistently registered the highest mortality on cutworms at 95%. According to Gómez and Sáenz-Aponte (2015), *H. bacteriophora* has been effective in controlling *Strategusaloeus* L (Coleoptera: Scarabaeidae) known as "Little bull" or oil palm "chiza", a limiting pest in palm plantation in Cesar Colombia. The dosage applied ranged from 160, 290, 420, 550, 680, 810 IJs/cm ^{2}. Furthermore, Monteiro *et al.* (2014) also reported that along with *H. indica*, *H. bacteriophora* is effective in reducing the egg mass weight and hatching percentage of *Rhipicephalus microplus* larvae. In this study, the effectiveness of *H. bacteriophora* was similar to that of *H. taysearae* and *H. marelatus*.

This result shows the selectivity of EPN species against insect host were *H. taysearae w*as the most effective against larvae of cabbage head moth and diamond back moth, while *H. bacteriophora* was most effective against cutworm. This also shows the potential of co-application and compatibility trails of the two species against wide range of insect pest. In the field, EPNs attack a significantly narrower host range than in

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the laboratory (Akhurst, 1990; Bathon, 1996; Georgis, 1991; Peters, 1996), adding to their safety as biological control agents. The isolation of new nematode strains/species is usually done using larvae of the lesser wax moth, *A. grisella*, and therefore, the host range of known nematode species tends to be biased towards generalists or species adapted to lepidopterous insects. However, some nematode species that have been isolated from host cadavers in the field have a restricted host range with *S. kushidai* (Mamiya, 1989) and *S. scarabaei* (Stock and Koppenhfer, 2003) being adapted to scarab larvae. *S. scapterisci* appears to be adapted to mole crickets and poorly infects other insects (Grewal *et al.*, 1993; Parkman and Smart, 1996), but Bonifassi *et al.* (1999) demonstrated that a combination of *Xenorhabdus* strain UY61 and *S. scapterisci* readily infects the wax worm, *A. grisella*.

To check the efficacy of EPN on other stages of the insect pest life cycle, pupal stage of diamond back moth and adult stage of cutworm were also tested. Table 4 reveals that the insect pest was less susceptible to EPN infection at pupal stage. It is clear, however, that *H. taysearae* (42%) remains the most promising among EPN isolates with 200 IJs (46%) being the best dosage with the best combination of using *H. taysearae* at 200IJ. The adult stage of cutworm, on the other hand, shows high susceptibility to EPN for both *H. taysearae* and *H. taysearae* at 100% mortality at approximately four-day infection rate. Figure 4 also shows that mass reproduction can be better achieved in the adult stage of cutworm. At adult stage, only 200 IJ was tested (data not presented). Pupae were less susceptible since they were cocooned and thus less exposed to infection.

Table 3. Dose-mortality (%) response of cutworm (S. *litura*) at different concentrations of EPS isolates.

Species	Concentration (IJs)					Mean
	C1 (10)	C2 (25)	C3 (50)	C4(100)	C5 (200)	
H. indica	56.67	76.67	93.33	96.67	96.67	84.00b
H. bacteriophora	86.67	98.67	96.00	97.33	94.67	94.67a
H. taysearae	74.28	88.57	93.33	95.33	99.05	90.11a
H. marelatus	80.00	93.33	100.00	100.00	100.00	94.67a
Mean	74.40c	89.31b	95.67a	97.33a	97.59a	

C.V. (%) = 8.45
Means within a column followed by a common letter is not significantly different from each other at P ≤ 0.05 determined by Student Newman Keul's (SNK) Test.

Table 4. Dose-mortality response (%) of diamond back moth (*P.xylostella*) pupae at different concentrations of EPS isolates.

Species	Concentration (IJs)					Mean
	C1 (10)	C2 (25)	C3 (50)	C4(100)	C5 (200)	
H. indica	11.67fg	36.67de	36.67de	33.33de	50.00abc	33.67b
H. bacteriophora	28.00de	26.67de	25.33e	37.33cde	40.00bcd	31.47b
H. taysearae	29.52de	32.38de	38.10cde	51.43ab	59.05a	42.10a
H. marelatus	0.00g	13.33f	6.67fg	26.67de	33.33de	16.00c
Mean	17.30d	27.26c	26.69c	37.19b	45.60a	

C.V. (%) = 23.30
Means within a column followed by a common letter is not significantly different from each other at P ≤ 0.05 determined by Student Newman Keul's (SNK) Test.

Figure 4. Adult cutworm infected with EPNs

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4 CONCLUSION

All EPN isolates caused mortality on larvae of diamond back moth, cabbage head moth and cutworm, pupae of diamond back moth and adult cutworm. Their effectiveness varied with dosage and species. *H. taysearae* was most effective against larvae of cabbage head moth, *H. marelatus* against diamond back moth, while *H. bacteriophora* was most effective against cutworm. The highest dosage, 200IJ, generally proved to be most effective for all EPN isolates against the selected insect pest. Effectivity of EPNs seems to reduce at pupal stage and increase at adult stage and under field condition, these stages maybe less susceptible to EPN infection. In laboratory condition, adult stage of insect maybe a good material for mass production of EPN as substitute to lesser wax moth larvae.

ACKNOWLEDGMENT

The authors wish to acknowledge the Department of Agriculture – Bureau of Agricultural Research through the Department of Agriculture- Organic Agriculture Fund for providing the necessary funds for the conduct of the project. They would like to acknowledge further the support extended by Cebu Technology University.

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