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Occurrence, isolation, and identification to genus level of entomopathogenic nematodes (epns) in Central Panay Island, Philippines

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Abstract Soil samples were isolated entomopathogenic nematodes (EPNs) from Central Panay Island, Philippines found to be positive for EPNs. However, no presence of EPNs was recorded in some sampling areas such as the Bingawan rice farm and fallow area; Tapaz, Capiz fallow area, and Lambunao, Iloilo residential area. The highest and lowest frequency occurrences were obtained from the municipalities of Calinog and Bingawan in Iloilo with a 100% and 50% recovery rate, respectively. There was a correlation between the vegetation types and the occurrence of EPNs. Regardless of vegetation cover, EPNs were often found in moderately acidic (pH 5.56) to neutral (pH 7.23) soils. Thus, the occurrence of EPNs was influenced by the vegetation type and soil pH value. The color of *Corcyra cephalonica* or rice moth cadavers infected by the EPN was gray and minute black spots. A compound microscope revealed that bursa is presented in males and first-generation adults are hermaphroditic. The collected EPN isolates belong to the genus *Heterorhabditis* of the family *Heterorhabtidae.*

Keywords: Central Panay, Entomopathogenic nematodes, Hermaphroditic, *Heterorhabditis,* Infective juveniles

Introduction

Nematodes are considered to be the most numerous Metazoa on earth (Abate *et al.,* 2017). Entomopathogenic nematodes (EPNs) are ubiquitous in distribution and occur naturally in different soils. Surveys have been conducted from almost all parts of the world in order to isolate indigenous EPN species. However, knowledge of the diversity of these beneficial nematodes is still limited, particularly in the Philippines despite being regarded as a megadiverse country.

EPNs belong to the families *Steinernematidae* and *Heterorhabditidae* (*Rhabditida*) and are symbiotically associated with entomopathogenic bacteria *Photorhabdus* and *Xenorhabdus*, respectively, which invariably results in the rapid death of parasitized insects (Chacon-

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Orozco *et al.,* 2019). EPNs are specific to insects, safe for non-target organisms including humans and other vertebrates, and do not pollute the environment. The infective juvenile (IJ) is the only free-living stage present and surviving in the soil until it locates and penetrates its hosts. IJs enter the host via natural body openings or by directly penetrating the cuticle until it reaches the haemocoel. Once inside, bacterial cells stored in the IJ gut are released that cause septicaemia killing the insects within 48–72 hours or may take a longer time for larger insects (Dillman *et al.,* 2012).

Aryal *et al.* (2021a) explained that the nematodes subsequently recover, start feeding on the bacteria-rich cadavers and develop into several generations until the food source is entirely exploited. Several species of entomopathogenic nematodes are being produced commercially and used as biological control agents against many soil insect pests and insects in cryptic habitats in many parts of the world (Hazer *et al.,* 2004). Caoili *et al.* (2018) stated that EPN can be integrated with other management strategies and reduce growers' dependence on chemical insecticides.

With very limited information about EPNs in different habitats, great potential can be seen in Central Panay Island, Philippines due to reports of high biodiversity in the area. Thus, the investigation of the occurrence and identification of indigenous EPNs is vital for EPN studies to progress in support of the integrated pest management strategy and organic farming for sustainable agriculture in the Philippines. Objectives of the study were to evaluate how indigenous entomopathogenic nematodes thrives in terms of its frequency in the municipalities situated in Central Panay Island, Philippines, to identify the type of associated vegetation in each sampling area that indigenous entomopathogenic nematodes, to identify the correlation of associated vegetation to the occurrence of indigenous entomopathogenic nematodes, to determine the soil pH level and correlation to different sampling sites and to determine the morphology of the identified indigenous entomopathogenic nematode isolation.

Materials and methods

The materials, tools, and equipment used in this study were spades, pail, sampling bag, meter stick, styrofoam box, pen, paper, protection gear, and weighing scale for soil sampling; bait insect (*Corcyra cephalonica)*, specimen cup, distilled water, test tube, beaker (150 ml), petri dish, masking tape, labeling stickers, filter paper (whatman), tissue paper, and spatula; compound microscope, dissecting microscope, fishing needles/loop, glass slides, syringe, cover slip, colorless nail polish, scalpel or scalpel blades, gloves, forceps, pipette dropper, and triethanolamine formalin (TAF) (Formalin 2%) fixative solution for identification.This study employed a quali-quanti type of research which involved a survey and laboratory analysis. Frequency and descriptive analysis were also used in the study. The study

was conducted in Central Panay Island, Philippines composed of the municipalities of Lambunao, Calinog, Bingawan, Iloilo and Tapaz, Capiz as shown in Figure 1 which is Geographical location of Central Panay Island.

Figure 1. Geographical location of Central Panay Island

Soil sampling

Soil samples were collected from the different municipalities of Central Panay Island namely: Lambunao, Calinog, Bingawan in Iloilo and Tapaz in Capiz. In each municipality, sampling was done in a certain barangay with Vegetable Farms, Rice Farms, Fallow, and Residential areas. In each municipality, four soil samples were collected. In each site, 10 sub-samples were collected at a 10-cm deep, using a hand shovel and mixed together to obtain approximately 1kg soil composite soil. The collected soil samples were sealed in a plastic bag, labeled with date and place of collection, placed in a styrofoam box (Aryal *et al.,* 2021b) and brought to the laboratory center for isolation, baiting, extraction, and identification. The vegetation was recorded. Soil pH in every site was noted and was brought to the Regional Organic Soils Laboratory for analysis.

The step-by-step procedure of soil sampling was adapted from the Department of Agriculture Bureau of Soils and Water Management. Ten soil samples were taken randomly at equally distant points throughout the lot in a zigzag direction. Before digging the pit, the soil surface was cleared of litter and vegetation. Soil samples were collected at 10 inches deep using a spade because preliminary studies showed a greater abundance of nematodes and roots in this zone. From one vertical side of the pit, a slice was taken with two to three centimeters thick with a single downward thrust of the spade. Using a knife or a trowel, the sliced soil was trimmed on both sides to a bar measuring 3-4 centimeters in width. This bar of soil was then placed in a pail or any suitable clean container. After collecting all the spot soil samples in a

particular area, they were pulverized and mixed thoroughly. Stones and other objects were removed from the soil. The samples were divided into quarters to get the composite samples per area.

Nematode extraction and isolation

Entomopathogenic nematodes were isolated from the collected soil samples from different municipalities of Central Panay Island by insect baiting method (Akhurst, 1984). Rice moths (*Corcyra cephalonica*) were used instead of the conventional host bait greater wax moth (*Galleria melonella*) due to its similar phylogenetic relationship. Five of the third and last instar larvae were placed in the plastic cup (100 ml) filled with moistened soil samples for each sampling site. The traps were stored at room temperature in a dark place and were checked after 2 days. Dead larvae from each container with signs of EPNs infection, such as fost, flaccid, odorless larvae with a change in color, were recovered and examined under the dissecting microscope for the presence of EPNs.

The collected infected cadavers were rinsed with sterile distilled water (Benseddik *et al.,* 2020) to remove soil particles and were transferred to modified white traps (Kaya and Stock, 1988) which consisted of a petri dish filled with distilled water to a depth of 0.5 cm. The bottom of an inverted petri dish was placed in the bigger petri dish. A sheet of filter paper was placed on the smaller petri dish allowing the edge of the paper to come in contact with the distilled water. The dead larvae positive for EPNs were then placed on the filter paper. Five of the third and last instar living *C. cephalonica* larvae were added to obtain the first generation of EPNs and incubated at room temperature until all the nematode progeny had moved down into the water of the bigger petri dish.

The emerging infected juveniles (IJ's) were then collected by the suspension method. The solution was set aside for suspension. After 10 minutes, the excess liquid was siphoned and the remaining suspension of IJs was then transferred to modified white trap following the same process to collect the second generation of EPNs. The first and second-generation adults were obtained by dissecting infected cadavers after 2 and 5 days, respectively. Re-inoculation was done 3 times to *C. cephalonica* to obtain a pure IJs population (Kaya and Stock, 1988). Collected IJs and adults were used for identification.

Identification of Entomopathogenic nematodes

The isolated entomopathogenic nematodes were subjected to morphological analysis. Preliminary morphological identification to genera was performed using the following criteria: (1) observation of the color of *C. cephalonica* cadavers infected by the EPN. The color of the cadaver killed by

Heterorhabditis is grey (Hunt *et al.*, 2007) light grey, minute black spots, red, brick-red, or orange, and yellow-brown or black in *Steinernema* (Emelianoff *et al.,* 2008); (2) the presence of bursa in male *Heterorhabditis* and bursa absence in *Steinernema* (Kaya and Stock, 1988; Nguyen and Smart, 1996). (3) In *Heterorhabditis* first generation adults are Hermaphroditic, and second-generation adults are amphimictic while in *Steinernema* first- and second-generation adults are amphimictic (Hazer *et al*., 2004; Hunt and Nguyen, 2007).

EPNs were manually picked using a picking needle from the clear suspension under dissecting microscope. Then, the specimens were mounted to a glass slide in a drop of triethanolamine formalin (TAF) (Formalin 2%: 0.05ml) fixative (Kaya and Stock, 1988) and covered with cover slip sealed with colorless nail polish. Observations were conducted using a compound microscope under 40x magnification.

Data gathered

The data gathered included the occurrence of EPNs in selected areas. It also covered the frequency of indigenous EPNs in each municipality. Moreover, morphological identification up to the genus level was also determined. To get the frequency, the number of occurrences per area in every municipality was counted and divided by the total number of areas sampled multiplied by 100. Specific standing crop per area was also determined, along with the soil pH which interpretation was based on the United States Department of Agricultural National Resources Conservation Service.

The interpretation of soil pH value are as follows:Less Than 3.5 = Ultra Acidic, 3.5–4.4 = Extremely Acidic, 4.5–5.0 = Very Strongly Acid, 5.1–5.5 = Strongly Acidic, 5.6–6.0 = Moderately Acidic, 6.1–6.5 = Slightly Acidic, 6.6–7.3 = Neutral, 7.4–7.8 = Slightly Alkaline, 7.9–8.4 = Moderately Alkaline, 8.5–9.0 = Strongly Alkaline, and Greater Than 9.0 = Very Strongly Alkaline.

Results

All municipalities surveyed in Central Panay namely, Lambunao, Calinog, and Bingawan in Iloilo and Tapaz in Capiz were positive of EPNs as shown in Table 1 and Figure 2. These results show that EPNs had a wide distribution and clearly agree with the literature reviews that Central Panay has high biodiversity due to the presence of a wide spectrum of flora and fauna which may be related to the survival of nematodes.

Table 1. Occurrence of Indigenous Entomopathogenic Nematodes in Central Panay

Municipality	Occurrence
Lambunao	+
Calinog	+
Bingawan	+
Tapaz	+

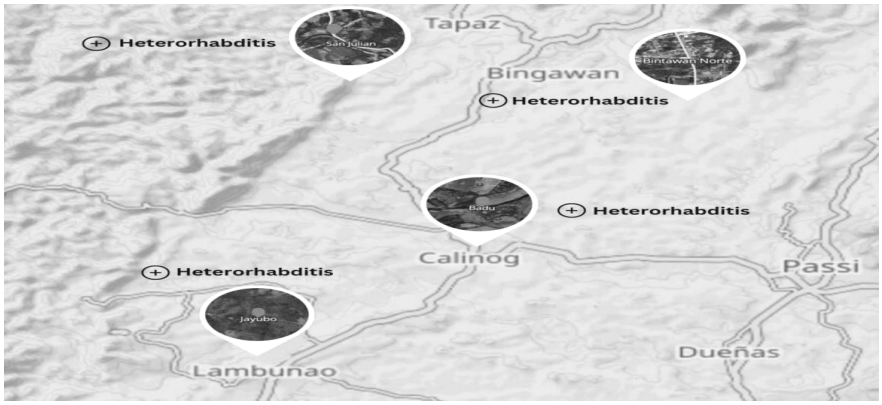

Figure 2. Map of Central, Panay showing the sampling sites of the entomopathogenic nematodes. The occurrence of *Heterorhabditis* was indicated (+)

Sampling sites were grouped into four areas: vegetable farms, rice farms, fallow areas, and residential areas in certain barangay from the municipalities of Central Panay. It was noted that EPNs were detected in all soil samples collected from vegetable farms as shown in Table 2. Meanwhile, only soil samples from Lambunao and Calinog, Iloilo and Tapaz, Capiz rice farms were positive for the occurrence of EPNs. In the fallow area, the occurrence of EPNs was reported in soil samples collected from Lambunao and Calinog. While, Calinog and Bingawan, Iloilo and Tapaz, Capiz residential areas were EPNs-positive. The rest of the results are delineated below. Based on the result of the study, two out of four municipalities in fallow areas were positively recorded of EPNs occurrence. Moreover, the apparent absence of EPNs from fallow areas where spruce budworm larvae and pupae were present on trees may be significant.

Table 2. Occurrence of indigenous entomopathogenic nematodes from the soil in each sampling area

Location	Sampling Area			
	Rice Farms	Vegetable Farms	Fallow Area	Residential Area
Lambunao	+	+	+	-
Calinog	+	+	+	+
Bingawan	-	+	-	+
Tapaz	+	+	-	+

The frequency distribution of indigenous entomopathogenic nematodes was delineated in Table 3. To get the frequency, the number of occurrences per area in every municipality was counted and divided by the total number of areas sampled multiplied by 100. The highest frequency was noted in Calinog, Iloilo where from a total of 4 soil samples collected from four municipalities covering four sampling sites, all soils harbored indigenous EPNs indicating a recovery of 100%. It was followed by Lambunao, Iloilo, and Tapaz, Capiz with a 75% frequency occurrence of indigenous EPNs. The lowest frequency of occurrence was observed in Bingawan, Iloilo with 50%.

Table 3. Frequency of indigenous entomopathogenic nematode recovered from the municipalities of Central Panay

Municipality	Frequency				%
	Rice Farms	Vegetable Farms	Fallow Area	Residential Area	
Lambunao	/	/	/	X	75
Calinog	/	/	/	/	100
Bingawan	X	/	X	/	50
Tapaz	/	/	X	/	75

The standing crop grown in each sampling site were noted as shown in Table 4. The survey was conducted to check different vegetations for EPN's presence in the selected sampling area of the municipality of Central Panay. In Lambunao, Iloilo the vegetation covers such as rice, string beans, pechay, okra, and banana was reported positive EPNs occurrence in the research area. In Calinog, Iloilo all sampling areas were reported positive for EPNs occurrence, and vegetation types in the area include rice, papaya, bell pepper, taro, and mango tree. In Bingawan, Iloilo, vegetation types such as cassava, bamboo, banana, mango, and cacao report a positive occurrence of EPNs. Lastly, in Tapaz, Capiz, the associated vegetation covers rice, pechay, peanut, and pomelo was reported EPNs-positive.

Table 4. Associated vegetation in each sampling area

Location	Sampling Site	Associated Vegetation
Lambunao	Rice Farms	rice
	Vegetable Farms	string beans, pechay, okra
	Fallow Areas	banana
	Residential Areas	mango, papaya, eggplant
Calinog	Rice Farms	rice
	Vegetable Farms	papaya, bell pepper
	Fallow Areas	taro
	Residential Areas	mango
Bingawan	Rice Farms	rice
	Vegetable Farms	cassava
	Fallow Areas	mahogany, bamboo, coconut
	Residential Areas	bamboo, banana, mango, cacao
Tapaz	Rice Farms	rice
	Vegetable Farms	pechay, peanut
	Fallow Areas	bamboo
	Residential Areas	pomelo

In this study, it was observed that there was a correlation between the vegetation types and occurrence of EPNs. In this study, the collected samples from fallow areas having vegetation types such as bamboo, mahogany, and coconut had no presence of EPNs.

The pH levels in each sampling area were noted as shown in Table 5 which shows that in Lambunao, Iloilo, rice farm area generated a pH value of 6.98; fallow area generated a pH value of 7.23, which were both tagged as neutral. It was followed by vegetable farm that generated a pH value of 6.56, tagged as slightly acidic. While the residential area generated a pH value of 6.05, tagged as moderately acidic. In Calinog, Iloilo, the rice farm generated a pH value of 5.80; the vegetable farm generated a pH value of 5.79; the fallow area generated a pH value of 5.93; residential area generated a pH value of 5.78, which were all tagged as moderately acidic. In Bingawan, Iloilo, rice farm generated a pH value of 6.70, tagged as neutral. The vegetable farm generated a pH value of 5.56; fallow area generated a pH value of 5.70 which were both tagged as moderately acidic. The residential area generated a pH value of 5.46, tagged as strongly acidic. In Tapaz, Capiz, rice farm generated a pH value of 6.56, which is tagged as slightly acidic, vegetable farm generated a pH value of 7.05, tagged as neutral, fallow area generated a pH value of 5.54, tagged as strongly acidic and lastly the residential area of the said municipality generated a pH value of 6.07, tagged as moderately acidic.

Table 5. Corresponding pH level value of soil samples taken from different sampling sites in the different municipalities of Central Panay

Location	Sampling Site	Soil pH
Lambunao	Rice Farms	6.98
	Vegetable Farms	6.56
	Fallow Areas	7.23
	Residential Areas	6.05
Calinog	Rice Farms	5.80
	Vegetable Farms	5.79
	Fallow Areas	5.93
	Residential Areas	5.78
Bingawan	Rice Farms	6.70
	Vegetable Farms	5.56
	Fallow Areas	5.70
	Residential Areas	5.46
Tapaz	Rice Farms	6.56
	Vegetable Farms	7.05
	Fallow Areas	5.54
	Residential Areas	6.07

Entomopathogenic nematodes were recovered from soil samples taken from different sampling areas in the municipalities of Central Panay. In the present study, EPN isolates were identified to genus level by morphological analysis. Genus identification of adult EPNs revealed that the isolates belong to genera *Heterorhabditis* as reflected in Table 6 and showing its comparative illustration in Figures 3 and 4.

Table 6. Distinguishing features of Heterorhabditis and Steinernema

Phenotypic trait	*Heterorhabditis*	*Steinernema*
First generation adults	Hermaphroditic	Males and females
Second generation males	With bursa	Without bursa

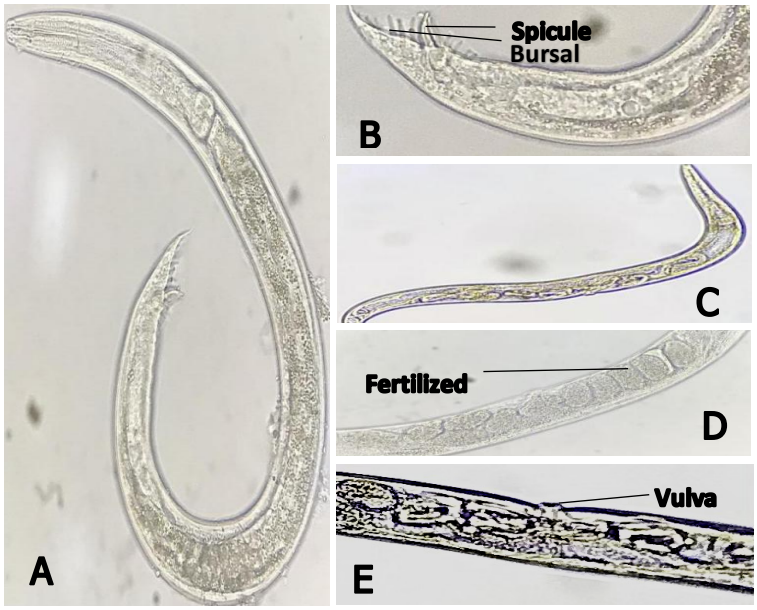

Figure 3. Features of *Heterorhabditis* and *Steinernemay: B, Bursa region; C, hermaphroditic; D-E: female, D, fertilized eggs; E, vulva*

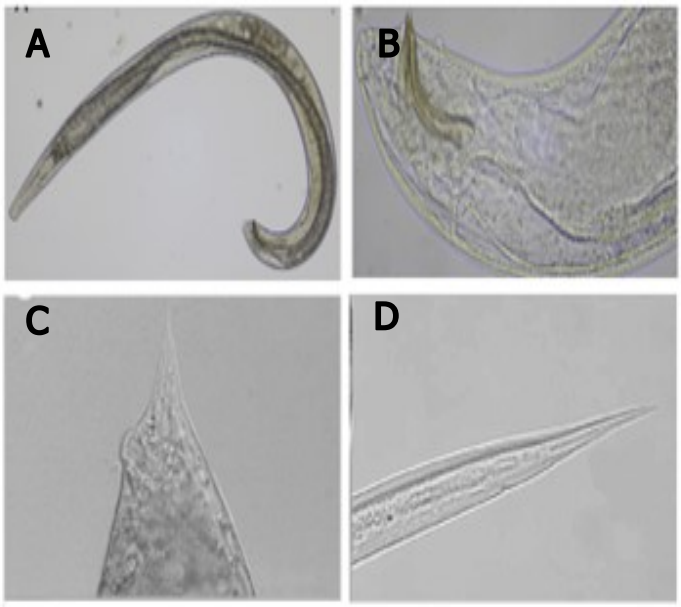

Figure 4. Steinernema (40X). A-B: male, A, lateral view entire body: B, posterior region; C-D: female adult, C, close up photo of tail region of adult female steinernema: D, entire tail region of female steinernema

Discussion

Sampling areas in Central Panay Island, Philippines includes Lambunao, Calinog, Bingawan in Iloilo and Tapaz in Capiz were positive of Entomopathogenic nematodes. These were grouped into four different ecosystems such as vegetable farms, rice farms, fallow areas, and residential areas. It was noted that EPNs were detected in all soil samples collected from vegetable farms. Meanwhile, only soil samples from Lambunao and Calinog, Iloilo and Tapaz, Capiz rice farms were positive for the occurrence of EPNs. In the fallow area, the occurrence of EPNs was reported in soil samples collected from Lambunao and Calinog. While Calinog and Bingawan, Iloilo and Tapaz, Capiz residential areas were EPNs-positive.

The results of the current study were in agreement with the study of Eivazian *et al*. (2009) in the northwest mountainous area of Iran which indicated that the highest recovery rate of EPNs was recorded in cropland and vegetable areas. Similar accounts were reported by Alburo *et al.* (2017) that the highest natural occurrence of Steinernematidae and Heterorhabditidae was obtained in vegetables area also reported that EPNs were found to occur in both lowland and highland vegetable organic farms in Cebu, Philippines.

It was observed in the current study that frequent human-powered tillage in vegetable farms significantly affects the natural occurrence of EPNs. The findings of various studies support the idea that EPNs are often more frequently detected in reduced tillage regimes (Akhurst and Brooks, 1984; Mracek and Webster, 1993) found that the greater complexity of the soil environment associated with relatively high levels of crop residue in conservation tillage regimes might influence the abundance of EPNs through provision of a greater number and diversity of hosts. Cochrane *et al.* (2021b) reported that surface residues could benefit nematode persistence through protection from desiccation or ultraviolet light, and increased insect pest suppression by EPNs or enhanced nematode movement.

Moreover, it is possible that the prevalence of EPNs occurrence in vegetable farms is caused by the presence of a diversity of insect host due to monocropping practice, particularly in Bingawan, Iloilo. This is in agreement with the study by Mracek and Webster (1993) which states greatest proportion of nematode-positive sample sites occurred in areas where there are outbreaks of insect pests with visible damage to foliage associated with intensive crop monocultures. They also suggested that the sufficient numbers of insect host in an area are the most important factor influencing the occurrence of these EPNs. Also, this is in line with the report of Campos-Herrera *et al.* (2017) who found out that EPNs are frequently detected in reduced tillage but less in conventional tillage regimes since conventional tillage regime disrupt the physico-chemical state of the soil and its biological properties.

In rice farms and residential areas, 3 out 4 municipalities surveyed were positive of EPNs. The mechanical disturbance such as conventional

tillage is practiced in the study area. This observation contradicts the work of Arellano *et al.* (2002) who reported that none of the soil samples screened for EPNs in the disturbed agroecosystem such as farmland were positive for EPN which may be due to a myriad of anthropogenic activities bordering on land use or land cover activities. The results agreed with the literature review conducted by Akhurst and Brooks (1984) which showed that EPNs occurred at sites where human impact on nature has been substantial such as agricultural land, roadsides or moderate such as forest logged areas or regions adjacent to those with severe impact. No nematode-positive soil samples were recorded at sites where the impact of humans has been slighted such as alpine or steppe meadows, unlogged mountain forests, natural parks, and virgin rain forest.

Empirical studies supported the claim that entomopathogenic nematodes appear to be more prevalent in agricultural fields than in natural habitats which is consistent with the findings of other studies (Akhurst and Brooks, 1984; Briscoe and Hominick, 1990; Griffin *et al.,* 1991; Mracek and Webster, 1993; Garcia del Pino and Palomo, 1996; Campos-Herrera *et al*., 2007; Benseddik *et al*., 2020). The effects of tillage on EPNs are variable and depend on the EPN species present and their associated foraging strategies (Campbell and Gaugler, 1997). This result explains the work of Campbell and Gaugler (1997) why both H. bacteriophora and S. riobrave were favored by tillage whereas S. carpocapsae was favored by the conservation tillage regime.

In addition, the application of fertilizer in vegetable areas and rice farms indirectly influence the occurrence of EPNs. Similar findings were observed to the work of Campos-Herrera *et al.* (2007) that nutrient disturbance have profound direct and indirect effects on the abundance and community composition of soil biota. Blanco-Pérez *et al.* (2017) indicated that inorganic fertilizers affect EPN occurrence by reducing their infectivity and virulence in the soil.

The results of the study clearly agreed with the literature review which states that maximum number of EPNs was recovered from okra, chilies fields, root base of brinjal, cabbage, rice, cassava, and mango (Caoili *et al*., 2018). Heterorhabditis isolates were also reported from peanut fields in Pigcawayan which provides additional account of EPNs and other nematode species in the country and extending their habitat's range and geographic distribution.

The results of the study contradicted with the study of Campos-Herrera *et al.* (2017) who reported that EPNs activity are poor in annual crops because of the management that reduces the numbers of suitable hosts for EPN. They hypothesized that the high frequency of EPNs occurrence where annual crops are cultivated is due to the monocropping system and reduced tillage operations in the study area. Furthermore, the results of the study opposed the work of Campos-Herrera *et al.* (2007) which stated that natural and perennial systems have been shown to provide more consistent conditions

for the settlement and persistence of these nematodes by supporting a stable rhizosphere community since zero EPNs were reported with this vegetation.

In this study, the soil pH level of the EPN-positive and EPN-negative did not differ significantly. EPNs are often found in moderately acidic (pH 5.56) to neutral (pH 7.23) soils. Regardless of vegetation cover, the occurrence of EPN populations were more evident in these soil acidity ranges, which is consistent with other local researches such as the work of Alburo *et al.* (2017) in Cebu, Philippines, in which EPNs were found from pH level ranging from slightly acidic to slightly basic (6-8.0) soil and in the study of Aryal *et al.* (2021b) in Cotabato, Philippines, in which EPNs-positive soil samples were found in pH level ranging from 6.5 -7 soils.

Similar findings were reported by Briscoe and Hominick (1990) where Heterorhabditis and Steinernema species were found in soils with pH <6, whereas representatives of Heterorhabditis commonly detected in soils with pH >6. Khatri-Chhetri *et al.* (2010) found 90% of EPN in acidic soils and six isolates occurring in soils with less than pH <4. Benseddik *et al.* (2020) reported EPNs occurrence in slightly alkaline (pH7.5-8) to neutral (pH 7-7.5) soils and reported EPNs were often found in pH ranging from 7.2 to 8.6. Amorim *et al*. (2018) found that low acidity directly decreases population of the nematodes due to the effect on activity of enzymes, the increased levels of metabolism of body, as well as intensification of proteolytic enzymes. Comparison of adult EPNs with the key to two major genera of entomopathogenic nematodes by Kaya and Stock (1988); Nguyen and Smart (1996); Hazer *et al*. (2004); Hunt and Nguyen (2007) strongly agree with the description of the genus. A compound microscope revealed that bursa is present in males and first-generation adults of *Heterorhabditis* are hermaphroditic.

All municipalities surveyed in Central Panay Island, Philippines were positive of EPNs. The highest and lowest frequency occurrence were obtained from the municipality of Calinog, and Bingawan, Iloilo, respectively. The collected samples from fallow areas having vegetation types such as bamboo, mahogany, and coconut have no presence of EPNs. These crops are identified as perennial crops. Moreover, annual crops were spotted in the research area and there were reports of EPNs occurrence. It was observed that the lifespan of crops cultivated and present in the study area may influence the EPNs occurrence and persistence in the soil. It was observed that there is a correlation between the vegetation types and occurrence of EPNs that is also observed in various studies conducted. The occurrence of EPNs was observed in all municipalities with pH values ranging from moderately acidic (pH 5.56) to neutral (pH 7.23) soils. It was observed that the soil pH level of the EPN-positive and EPN-negative did not differ significantly. Regardless of vegetation cover, the occurrence of EPNs populations was more evident in these soil acidity ranges. Lastly, genus identification of adult EPNs revealed that the isolates belong to the genera *Heterorhabditis.* For future studies, the researchers present recommends that this may be replicated and conducted in

other parts of Western Visayas Region or other region of the Philippines with wider coverage and increase the growing literature about entomopathogenic nematodes. Conducting another survey involving abiotic parameters such as but not limited to natural physical or chemical factors (soil texture, GSIS mapping, weather) as well as those resulting from human activities (physical or chemical disturbance). Morphometrics study of nematodes is likewise suggested.

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