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# Survey and identification of entomopathogenic nematodes in the province of Cotabato, Philippines, for biocontrol potential against the tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)

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## Abstract

**Background:** The tobacco cutworm, *Spodoptera litura* [Fab.] (Lepidoptera: Noctuidae), is a devastating insect pest of several crops. Entomopathogenic nematodes (EPNs) of the families Heterorhabditidae and Steinernematidae are used as an alternative control measure in lieu of the hazardous synthetic chemical applications.

**Results:** A survey of naturally occurring EPNs was conducted across the province of Cotabato, Philippines, covering a total of 5 municipalities with 25 villages. *Galleria*-baiting technique was employed to recover nematodes from peanut and grassland soils. Out of 50 soil samples collected, only 5 samples harbored nematodes, indicating a recovery of 10%. Preliminary morphological data identified only one EPN under the genera *Heterorhabditis* (1 isolate), whereas 4 were facultative necromenic nematodes from the genera *Metarhabditis* (2 isolates) and *Oscheius* (2 isolates). Analysis of D2D3 segments of the 28S rDNA confirmed high sequence similarity to *Heterorhabditis indica*, *Metarhabditis rainai*, *Oscheius insectivora*, and *Oscheius* sp. This is the first record of *H. indica* and *M. rainai* in the entire region, whereas the first record for *Oscheius* spp. in the Philippines. Furthermore, the biocontrol potential of the local *H. indica* infective juvenile (IJ) populations (PIGCD1) isolated from peanut was assessed against the tobacco cutworm, *S. litura*, under laboratory conditions. The mean percentage mortality caused by *H. indica* on *S. litura* at 7 different concentrations ranged from 0–100% at 24 h post inoculation. The lethal concentration (LC<sub>50</sub>) required to kill 50% of the *S. litura* larvae population with *H. indica* was 7.13±1 (IJs/larva).

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**Conclusions:** The use of *Galleria*-baiting method is a convenient approach to detect EPNs including other facultative necromenic nematodes from the soils. Obtained data indicated that the local *H. indica* isolate can be a promising alternative measure to suppress the economically important insect pest, *S. litura*, and this may provide significant outlook to establish the biocontrol program in the country.

**Keywords:** Entomopathogenic nematodes, New isolates, *Spodoptera litura*, Biocontrol, Virulence

## Background

The tobacco cutworm, *Spodoptera litura* [Fabricius] (Lepidoptera: Noctuidae), is a serious polyphagous insect pest, widely distributed throughout tropical and subtropical areas of Asia and Pacific Regions (Bragard et al. 2019). In the Philippines, it is one of the most economically important pests, reported infesting several crops. Control of *S. litura* is mainly dependent on synthetic insecticides, many of which have developed high insecticide resistance, leading to periodic out breaks and resurgence of pest and eventually, crop failure (Shad et al. 2012). The rampant application of harmful chemicals has caused perennial environmental pollution and deleterious effects on humans including other beneficial and non-target organisms (Jeyasankar et al. 2014). The increasing awareness of their several disadvantages have further revolutionized the exploration and utilization of alternative control measures like the biological control agents (BCAs), which are effective, eco-friendly, and safe (Grewal et al. 2005).

Entomopathogenic nematodes (EPNs) of the families Heterorhabdidae and Steinernematidae are known as most effective BCAs (Kaya and Gaugler 1993). The genera with the most successful stories are those of *Steinernema* and *Heterorhabditis* associated with their respective symbiont bacteria, *Xenorhabdus* and *Photorhabdus* (Ferreria and Malan 2014). The bacterial cells are released and rapidly kill the insect within 48–72 h (Boemare 2002).

The natural occurrence of EPNs has been documented in different soils, ranging from natural and managed ecosystems of all continents with the exception of Antarctica (Laznik and Trdan 2011). Numerous surveys in agricultural (managed) areas were conducted to collect and describe native EPNs using morphological and molecular diagnostic tools for the management of local target pests (Stock et al. 2008). These native EPN populations have mainly gained an increasing attention due to its beneficial attributes like better adaptation to local biotic and abiotic conditions (Stock et al. 2008 and Campos-Herrera et al. 2012). At present, about 100 *Steinernema* and 16 *Heterorhabditis* have been described worldwide (Bhat et al. 2020). These EPNs are broadly applied as biopesticides against insect pests (Lacey et al. 2015). For instance, *S. carpocapsae* induced a high

mortality against *S. litura* from tobacco and cotton (Abdel-Razek and Abdelgawad 2007). In the case of *H. indica*, it was reported to be highly virulent and effective for the control of *S. litura* larvae (Acharya et al. 2020) in corn, cotton, and vegetable crops (Caoili et al. 2018).

The province of Cotabato being located in the island of Mindanao, the Philippines is considered a predominantly agricultural area. This province is under Region 12 (SOCCSKSARGEN) which shared the highest number of farms with 126.7 thousand hectares, covering 275.5 thousand hectares of agricultural land (Philippine Statistics Authority 2004). The use of EPNs for integrated pest management (IPM) in the area has never been explored, which may offer valuable contributions in different farms. Collection of native EPNs in the area is a vital step toward the sustainable pest suppression in the agricultural areas.

This present study therefore aimed to survey EPNs from managed and unmanaged areas like the peanut and grassland fields within the province and to assess their biocontrol efficacy against the target local pest, *S. litura*. This study provided additional account to the EPNs and other insect-associated nematode diversity in the country. More importantly, this can serve as baseline information toward the selection of efficient native isolates for peanut pest control and other economically important crops.

## Methods

### Culture of insects

The greater wax moth, *Galleria mellonella* [L.] (Lepidoptera: Pyralidae), was used as bait to isolate EPN from soil sample and for subsequent identification of EPN. Rearing was carried out, using an artificial diet comprising of wheat bran (37.5%), honey (23.4%), bees wax (11.7%), glycerol (21.4%), nipagine (0.5%), and yeast (5.5%) at 37 °C with a 70% relative humidity (Kaya and Stock 1997). Egg masses of *S. litura* were collected from different peanut plantations in the province of Cotabato. After egg hatching, fresh vegetables, legume pods or seeds, and tomato fruits were provided for larval feeding until pupal stage under laboratory conditions, following the descriptions of Zhang et al. (2019) with modifications. Heat-treated soil was provided for pupation, after which the developed pupae were collected from the soil

and subsequently placed inside a cage for adult emergence. To increase the rate of fecundity, 10% honey solution mixed with a few drops of multi-vitamin was added for adult nourishment. Folded filter papers were provided for egg laying, then egg masses were recollected and allowed to hatch. This rearing process was repeated and only a single culture was maintained for virulence assays.

#### Soil sampling

Soil samples were randomly collected from peanut (managed) and grassland (unmanaged) areas in the province of Cotabato, Philippines, from December 2018 to February 2019. A total of 50 samples (2 from each site) were collected from different sampling sites, covering 5 towns with 25 barangays or villages. From each site, 5 subsamples were taken from a 20–25-cm deep, using a hand shovel and mixed together to obtain approximately 1 kg of composite samples (Orozco et al. 2014). The geographical coordinates and corresponding elevations were recorded along with some important edaphic parameters such as soil temperature, pH, moisture, and texture (Abate et al. 2017). The temperature, pH, and moisture of each soil sample were recorded in situ, using a 4-in-1 soil survey instrument (BGT-SM4, Beijing, China). Soil texture was characterized by ring method (Daddow and Warrington 1983). All soil samples were labeled, sealed, stored in a Styrofoam box, and was brought to the Department of Plant Pathology, College of Agriculture at the University of Southern Mindanao in Kabacan, Cotabato, for further processing.

#### Insect-baiting and nematode culture

Insect-baiting was carried out according to the original description of Bedding and Akhurst (1975). Ten last instar larvae of *G. mellonella* were added to each container with the soil samples and then incubated at 25±2 °C. The samples were regularly sprayed with water to avoid desiccation and monitored daily to check for successful insect infection. All collected cadavers were washed with distilled water and placed in a modified White trap (White 1927) until emergence of infective juveniles (IJs) was evident. IJs were then harvested, cleaned, and stored at 10–20 °C. To obtain pure culture of the nematodes recovered from the soil, re-inoculation was done 3 times to *G. mellonella* larvae (Hoy et al. 2008).

#### Morphological and molecular characterization

Infective juveniles, adult male and female nematodes, were heat-killed and processed for fixation according to De Gisse (1969). A series of transfer to anhydrous glycerin was done, using the following 3 solutions: solution 1 (containing a 50:50 ratio of 4% formalin and glycerin), solution 2 (containing a 50:50 ratio of 96% ethanol and

4% formalin), and solution 3 (containing pure glycerin). Morphological key characters of the genera were observed and further measurement of the body lengths was carried out using an Olympus CX22 compound microscope equipped with a digital camera. IJ and male measurements were undertaken, using the ImageJ software. The EPNs isolates were classified, using taxonomic keys from Nguyen and Hunt (2007), whereas the facultative necromenic nematode isolates were diagnosed based on Sudhaus (2011) until genus level only.

Molecular analysis was undertaken by amplification of the D2D3 expansion segments of the 28S rDNA for species identification. For this, DNA extraction was first performed, following the method described by Spiridonov et al. (2004) with modifications and using the DNA extraction kit (Dongsheng Biotech). Succinctly, 5 IJs were individually picked, placed on top of the glass slide with a drop of lysis buffer. IJs were cut into small pieces, using a sterile scalpel and transferred into a micro-centrifuge tube by adding the remaining 350 µl of the lysis solution. A stepwise process involving Proteinase K, different solutions, wash and TE buffers coupled with vortexing, and centrifuge process were done as indicated by the kit manufacturer. The extracted DNA was then stored at –20 °C in deep freezer for further processing. Products were sent for sequencing in Macrogen, Inc. (Seoul, South Korea). The universal primers D2A (5'-CAAGTACCGT GAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAA CCAGCTACT A-3') were used to amplify the D2D3 expansion segment of 28S rDNA (de Brida et al. 2017). PCR conditions were programmed for initial denaturation at 94 °C for 3 min, followed by 40 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 60 s, and a final extension at 72 °C for 15 min (Bhat et al. 2019).

#### Sequence alignment and phylogenetic analysis

Sequences obtained were first edited, using BioEdit 7.2. The D2D3 expansion segment of 28S rDNA sequences of all 5 isolates were aligned with their closest BLAST search matches in the National Center for Biotechnology Information (NCBI) GenBank (Altschul 1990). ClustalW multiple alignment was used for sequence alignment. A phylogenetic tree was constructed using the MEGA-7 (7.0) software (Kumar et al. 2016) by the maximum likelihood with a Tamura-Nei method (Tamura and Nei 1993). *Steinernema carpocapsae* (KY914572.1) and the model organism *Caenorhabditis elegans* (X03680.1) were used as outgroup taxa. All isolates were trimmed, annotated, and deposited in the NCBI GenBank with their accession numbers.

#### Virulence tests for *H. indica*

Virulence test was measured on insect mortality of the 3<sup>rd</sup> instar larvae of *S. litura*. Following a modified

version of the methods used by Godjo et al. (2018), approximately 50 g of heat-treated sand with 20% moisture content was placed in Petri dishes (60×15 mm). The following nematode concentrations or dosages were prepared and added to each plate: 10, 50, 100, 150, 200, 250, and 300 IJs/ml with 4 replications. After an hour, 10 3<sup>rd</sup> larval instars of *S. litura* were subsequently added to the set-ups where they burrowed into the sand naturally. The control set-up only received sterile distilled water (SDW). After 24 h, insects were retrieved from the Petri dishes, recording the total number of dead larvae. Insect cadavers were dissected to confirm EPN infection (Sumaya et al. 2018).

#### Data analyses

The distribution of nematode species in the province was determined and shown in a map using QGIS version 3.10.1 A Coruna. The concentrations required to kill 50% of *S. litura* insect population ( $LC_{50}$ ) for each replicate was determined, using Probit analysis (Finney 1952). The mortality data showing a non-normal distribution was analyzed using a Kruskal-Wallis test and a post hoc Dunn's test for multiple comparisons was used. Data analyses were performed in R version 4.0.2 (R Core Team 2020).

## Results

### Nematode survey and recovery

Soil samples were randomly collected from peanut and grassland areas of Cotabato province in the Philippines. From a total of 50 soil samples collected from 5 towns covering 25 villages or barangays, only 5 soils harbored nematodes indicating a recovery rate of 10%. Out of 5 nematode-positive areas, 2 isolates were recovered from peanut fields in Kabacan (Osias) and Pigcawayan (North Manuangan), and 3 isolates from grassland areas in Libungan (Ulamian), Aleosan (Dualing), and Midsayap (Bual Norte). The elevation ranged between 12.6 and 61.9 masl with the following soil parameter values: soil pH (6.5-7), soil temperature (33-34 °C), and soil texture (clay, clay loam, silty clay loam, and sandy clay loam). All soil samples were dry during the sampling period (Table 1).

### Morphological and molecular characterization

Morphological key characters for the genera were examined and measurement of the body lengths (e.g., IJs and males) was carried out, using an Olympus CX22 compound microscope equipped with a digital camera. The EPN isolate was classified, using taxonomic keys of Nguyen and Hunt (2007) until genus level only. For PIGCD1 isolate, the body length range of IJs was 530-622 µm and males were from 759-808 µm, belonging under the genus *Heterorhabditis*.

Moreover, the facultative necromenic nematodes, *Metarhabditis* and *Oscheius* (Rhabditida: Rhabditidae), were morphologically diagnosed based on Sudhaus (2011) until genus level only. For isolates LIBCD1 and MIDCD4, the body length range for IJs and males were 328-407 µm and 745-981 µm, respectively. For isolates KABCD2 and ALCD3, the body size of IJ ranged from 419-539 µm and males from 840-990 µm. The isolates LIBCD1 and MIDCD4 were identified as *Metarhabditis* whereas KABCD2 and ALCD3 were under *Oscheius*.

Analysis of D2D3 expansion segments of 28S rDNA region confirmed the nematode species and distribution of *Heterorhabditis indica*, *Metarhabditis rainai*, *Oscheius insectivora*, and *Oscheius* sp. in the soil samples collected from different areas in the province of Cotabato (Fig. 1). Sequences for all nematode isolates were deposited in GenBank. The isolate PIGCD1 from peanut fields in Pigcawayan yielded 606 bp fragments, which were ≥99% identical to *H. indica* from isolates in Switzerland, India, and Pakistan (MK421435.1, MF801427.1, MH316165.1, and JQ838180.1). The 2 isolates (LIBCD1 and MIDCD4) from Libungan and Midsayap grasslands had a total of 604 and 700 bp fragments, respectively with ≥99% sequences similarity to the *Metarhabditis* (formerly identified as *Rhabditis*) *rainai* (EU195966.1 and JN572919.1). The isolate KABCD2 from peanut field in Kabacan yielded 730 bp which showed homology to *O. insectivora* (EU195968.1). Finally, isolate ALCD3 from grassland in Aleosan yielded 710 bp and was found identical to *Oscheius* sp. (MF441252.1 and MK932087.1).

### Sequence alignment and phylogenetic analyses

The phylogenetic tree using maximum likelihood (Fig. 2) obtained from 25 aligned sequences of the D2D3 expansion segments of the 28S rDNA genes in nematodes revealed three distinct groups of Strongyloidea (Heterorhabditidae): *H. indica* (PIGCD1) Rhabditodea (Rhabditidae): *M. rainai* (LIBCD1, and MIDCD4), *O. insectivora* (KABCD2), and *Oscheius* sp. (ALCD3). They are well-separated from the outgroup taxa, *Steinernema* (KY914572.1) (Strongyloidea: Steinematidae) and the model nematode, *C. elegans* (X03680.1).

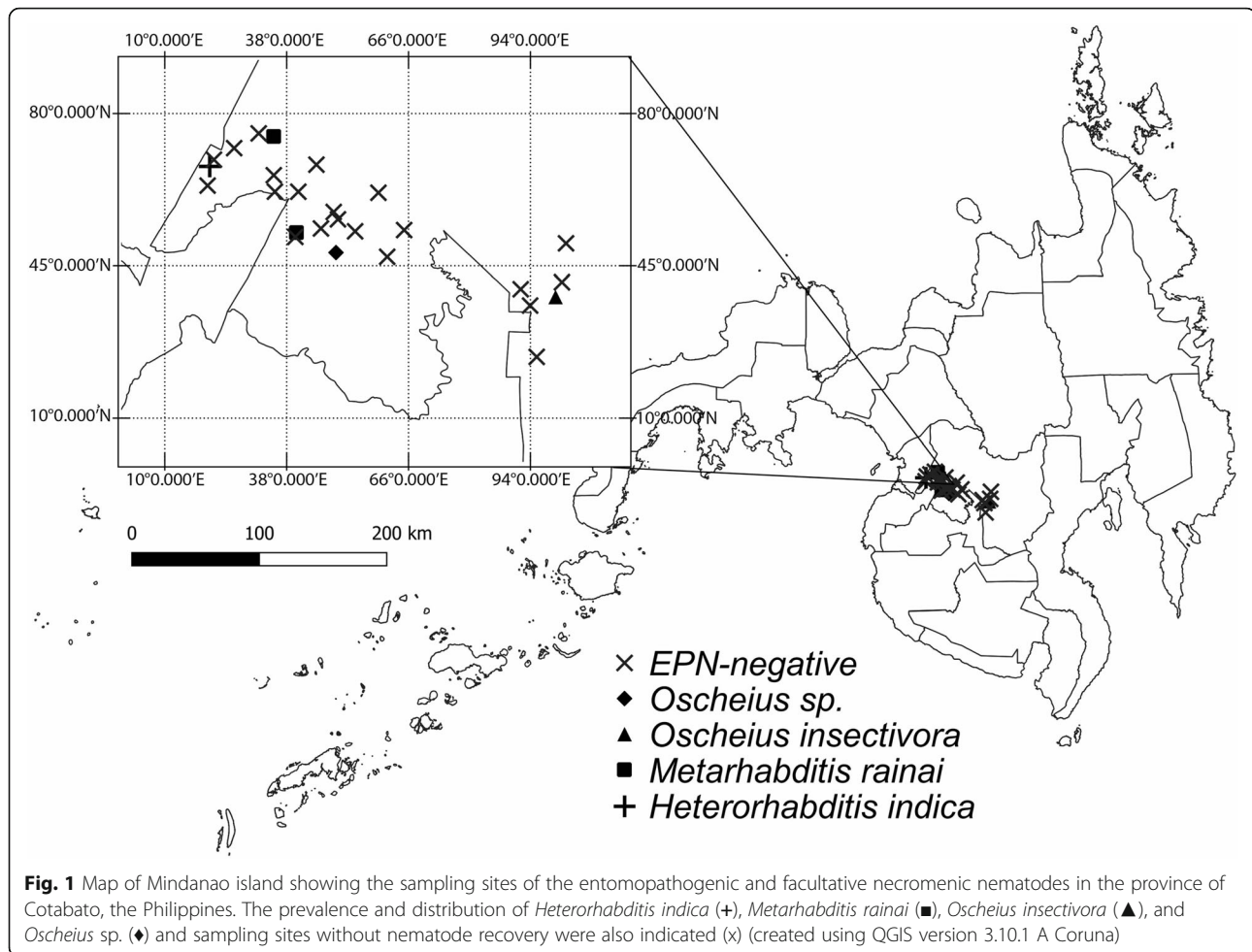
### Virulence of *H. indica* against *S. litura*

Virulence of the lone *H. indica* PIGCD1 isolate from peanut field was evaluated based on the mean mortality (%) to the 3<sup>rd</sup> instar larvae of *S. litura* 24 h post inoculation (Fig. 3). The larvae were exposed to 7 different concentrations, namely, 10, 50, 100, 150, 200, 250, 300 IJs/larva, each with 4 replications. Significant differences among treatments ( $\chi^2 = 26.85$ ,  $df = 6$ ,  $p = 0.0001$ ) were observed for the mean mortality (%) at different

**Table 1** Occurrence of entomopathogenic and facultative necromenic nematodes in the province of Cotabato, Philippines, with different soil parameters

Location	Sampling site (villages/ barangays)	Coordinates	Elevation (masl)	Habitat	Soil texture	Soil temperature (°C)	Soil pH	Nematode
<b>Pigcawayan</b>	North Manuangan	7.2745° N, 124.4060° E	12.6	Peanut	Silty clay loam	33	7	+
	South Manuangan	7.2832° N, 124.4115° E	9.1	Peanut	Silty clay loam	34	7	–
	Malagakit	7.2496° N, 124.4032° E	5	Peanut	Clay loam	33	7	–
	New Igbaras	7.2981° N, 124.4378° E	23	Peanut	Silty clay loam	33	6.5	–
	Kimarayang	7.3171° N, 124.4697° E	225.9	Peanut	Silty clay loam	34	7	–
<b>Libungan</b>	Montay	7.2763° N, 124.5445° E	69.8	Grassland	Clay loam	34	6.5	–
	Baguer	7.2413° N, 124.5209° E	15.7	Grassland	Clay loam	33	7	–
	Ulamian	7.3131° N, 124.4891° E	61.9	Grassland	Silty clay loam	34	7	+
	Sinawingan	7.2628° N, 124.4891° E	12.1	Grassland	Clay loam	33	7	–
	Gumaga	7.2415° N, 124.4905° E	17.3	Grassland	Silty clay loam	34	7	–
<b>Midsayap</b>	Bual Norte	7.1883° N, 124.5182° E	13.8	Grassland	Clay	33	7	+
	Bual Sur	7.1827° N, 124.5168° E	19.5	Grassland	Clay loam	34	7	–
	Kimagango	7.2149° N, 124.5667° E	46.7	Grassland	Clay	33	7	–
	Milaya	7.2053° N, 124.5722° E	62.5	Grassland	Clay loam	33	6.5	–
	Sadaan	7.1935° N, 124.5501° E	32.2	Grassland	Silty clay loam	34	7	–
<b>Aleosan</b>	Dualing	7.1622° N, 124.5695° E	61.4	Grassland	Sandy clay loam	34	6.5	+
	Tomado	7.1565° N, 124.6359° E	62	Grassland	Clay loam	33	6.5	–
	Santa Cruz	7.1897° N, 124.5944° E	59.2	Grassland	Clay loam	33	7	–
	New Leon	7.1911° N, 124.6580° E	76.8	Grassland	Silty clay loam	34	7	–
	Pentil	7.2396° N, 124.6248° E	195.5	Grassland	Clay loam	34	7	–
<b>Kabacan</b>	Kayaga	7.1141° N, 124.8089° E	24.3	Peanut	Clay loam	34	6.6	–
	Kilagasan	7.0929° N, 124.8210° E	15	Peanut	Clay loam	33	7	–
	Cuyapon	7.0263° N, 124.8293° E	12.5	Peanut	Clay loam	33	6.5	–
	Osias	7.1037° N, 124.8541° E	21	Peanut	Clay loam	34	6.5	+
	Pisan	7.1731° N, 124.8679° E	34.6	Peanut	Silty clay loam	34	6.5	–

+ presence; – absence



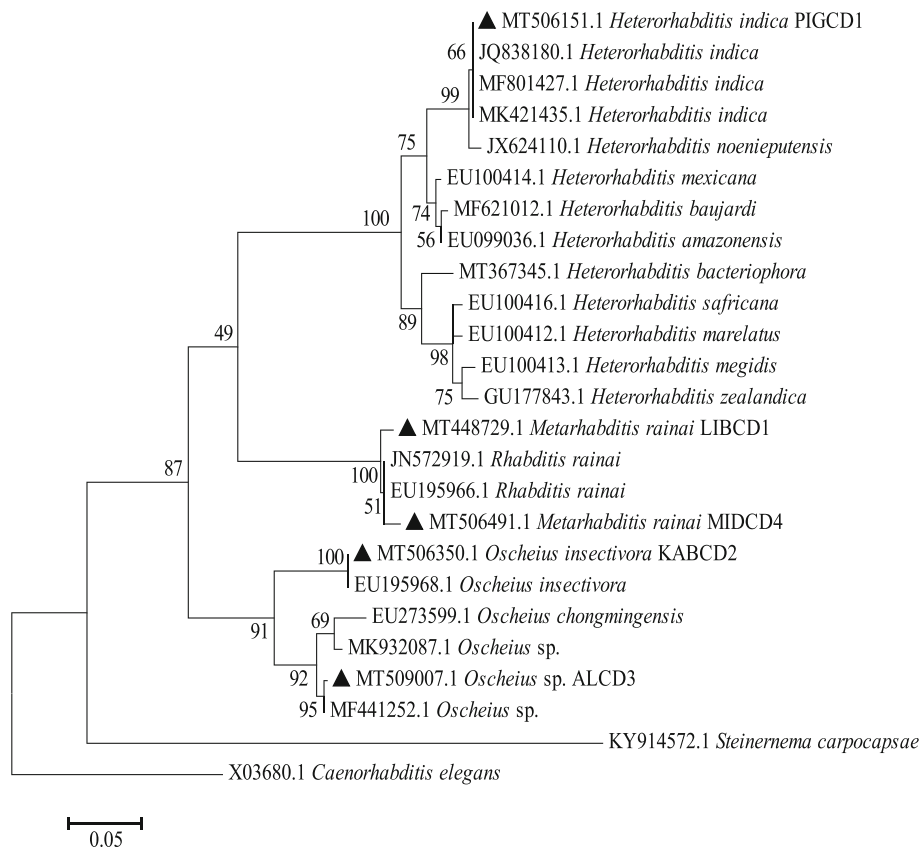
concentrations with mean mortality ranging from 0–100%. While the highest mean mortality was obtained starting from the concentrations of 200–300 IJs/larva, no mortality was recorded in the concentration of 10 IJs/larva and the control set-up. Further on, the lethal concentration required to kill 50% of the insect population ( $LC_{50}$ ) was estimated to be  $7.13 \pm 1$  (IJs/larva), implying the high virulence and biocontrol potential of the local *H. indica* isolate.

## Discussion

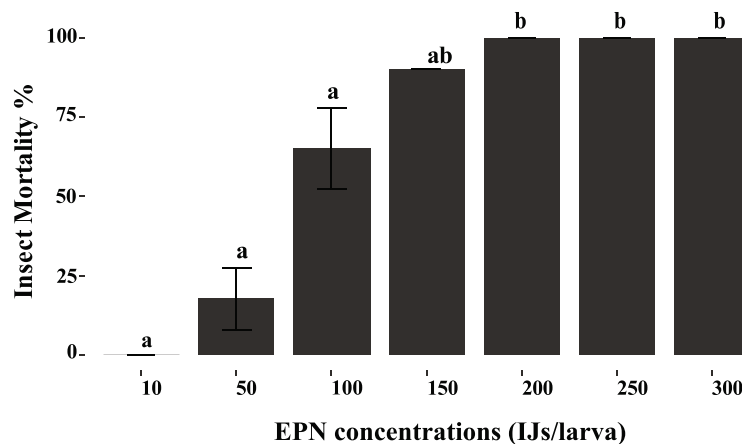
Entomopathogenic nematodes are used as biocontrol agents with several success stories in suppressing insect pests of different crops (Kaya et al. 2006). In this study, occurrence and distribution of EPNs in different areas of Cotabato province, Philippines, was conducted in order to assess their biocontrol potential to economically important insect pest like the tobacco cutworm, *S. litura*. From a total of 50 soil samples collected from peanut and grassland areas, only 5 harbored nematodes indicating a recovery rate of 10%. This low recovery rate is congruent with several previous studies on EPNs recovery

in different surveys worldwide (Caoili et al. 2018 and Kour et al. 2020). Several abiotic and biotic factors may likely influence the recovery of nematodes in the soil such as soil texture, moisture, pH, and vegetation (Abate et al. 2018 and Campos-Herrera et al. 2019). Although response of EPNs to these abiotic and biotic factors may vary within each species, their prevalence and abundance in sampling areas have nevertheless optimal levels, preference, and tolerance.

Interestingly, by using the morphological and molecular taxonomic data, not only EPNs (one heterorhabditid) but also facultative necromenic nematodes (4 rhabditids) in the *G. mellonella* infected cadavers were detected. Generally, the *H. indica* isolate had similar IJ and male body size range and morphology as the type strain from India (Poinar et al. 1992), Vietnam (Phan et al. 2003) and the Philippines (Pascual et al. 2017). Analysis of D2D3 expansion segments of 28S rDNA region confirmed the following nematode species: *H. indica*, *M. rainai*, *O. insectivora*, and *Oscheius sp.* Obtained lone *H. indica* (PIGCD1 isolate) recovered from peanut fields in Pigcawayan had a  $\geq 99\%$  similarity to isolates from



**Fig. 2** Phylogenetic tree showing the relationship of *Heterorhabditis indica*, *Metarhabditis rainai*, *Oscheius insectivora*, and *Oscheius* sp. (▲) with other related species using the maximum likelihood method (with 1000 bootstrap values). *Caenorhabditis elegans* and *Steinernema carpocapsae* were used as outgroup taxa. Numbers before each species indicate the GenBank Accession numbers



**Fig. 3** Mean percentage mortality of *Spodoptera litura* third instar larvae after exposure to different dosages (IJs/larva) of the entomopathogenic nematode *Heterorhabditis indica* (PIGCD isolate) 24 h post inoculation at 25±2 °C. Error bars indicate standard deviation of four replicates. Different letters next to the error bars indicate significant differences with Dunn's test for multiple comparisons

Switzerland, India, and Pakistan. The detection of both EPN and facultative necromenic nematodes agree with the study of Campos-Herrera et al. (2015) who reported that all cadavers recovered from baited samples of Swiss agricultural soils produced free-living nematodes (FLNs). In their study, about 80% contained a mixture of EPN, *Acrobeloides*-group and from the genus *Oscheius* was reported to be in competition with EPN and characterized further as scavengers. Campos-Herrera et al. (2019) also reported the presence of FLNs in Algarve, Southern Portugal, and De Brida et al. (2017) detected *Metarhabditis rainai* and *Oscheius tipulae* in different agricultural crops of Brazil using D2D3 segments of 28S rRNA. Notably, these rhabditid nematodes were earlier found to have a biocontrol potential (Dillman et al. 2012 and Torrini et al. 2015). Other entomophilic nematodes also known as facultative necromenic nematodes from the genera *Metarhabditis* and *Oscheius* have demonstrated their biocontrol potential against vegetable cruciferous pests (Park et al. 2012 and Torrini et al. 2015). In this study, however, biocontrol potential of these 4 facultative necromenic isolates which were one of our research outlooks was not assessed.

Several previous studies have demonstrated the efficacy of *H. indica* against a variety of insect pests including *S. litura* (Acharya et al. 2020). Similar result, *H. indica*, was reported to be very effective against *S. litura* under laboratory and field conditions (Gokte-Narkhedkar et al. 2019). Caoili et al. (2018) likewise reported that *H. indica* (PBCB), an isolate from Luzon island, Philippines, was highly virulent against *S. litura*, with percentage mean mortality of 88.67%. However, they had a higher LC<sub>50</sub> value (8.89 IJs/larva) at 48 h post inoculation. Moreover, in Egypt, *Heterorhabditis* sp. ELG, *H. indica*, and *Heterorhabditis* sp. ELB were found to have the highest in activity, obtaining a 100% mortality to *S. littoralis* larvae in a Petri dish assay 24 h post exposure (Abdel-Razek and Abdelgawad 2007), which is in agreement with obtained present data.

## Conclusions

Nematode surveys in different areas of Cotabato province, using *G. mellonella* baiting method, were a convenient approach to detect EPNs including other facultative necromenic nematodes from the soils. One local EPN, *H. indica* isolate (PIGCD1) from peanut fields was documented in the town of Pigcawayan and 4 facultative necromenic rhabditid nematodes (*M. rainai*, *O. insectivora*, and *Oscheius* sp.), providing additional account of EPNs and other nematode species in the country and extending their habitats' range and geographic distribution. This is the first record of *H. indica* and *M. rainai* in Region 12 or SOCKSARGEN, whereas *Oscheius* spp. was the first report in the

Philippines. The virulence of the local *H. indica* isolate on the 3<sup>rd</sup> instar larvae of *S. litura* was recorded to be very high. Therefore, this lone isolate can be further used as biocontrol agent of economically important insect pests.

## Abbreviations

EPN: Entomopathogenic nematodes; BCA: Biological control agent; IPM: Integrated pest management; NCBI: National Center of Biotechnology Information; *S. litura*: *Spodoptera litura*; *H. indica*: *Heterorhabditis indica*; *M. rainai*: *Metarhabditis rainai*; *O. insectivora*: *Oscheius insectivora*

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## Authors' contributions

All authors contributed significantly to this research study from conceptualization to data analyses including the preparation, writing, and review of the manuscript. CAD carried out the main experiments and RRR assisted in the field sampling and the virulence experiments. SA was involved in molecular/data analysis and writing. NPDS was involved in the design of the study, analysis, supervision to CAD and RRR during the field sampling, the virulence experiments and writing. NHS was the in-charge of resources, supervision during the morphological and molecular works, data analysis, and writing. All authors have read and approved the manuscript.

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## Availability of data and materials

The data and materials of this manuscript are available upon reasonable request.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

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## References

- Abate BA, Slippers B, Wingfield MJ, Malan AP, Hurley BP (2018) Diversity of entomopathogenic nematodes and their symbiotic bacteria in South African plantations and indigenous forests. *Nematol* 20(4):355-371. <https://doi.org/10.1163/15685411-00003144>.
- Abate BA, Wingfield MJ, Slippers B, Hurley BP (2017) Commercialisation of entomopathogenic nematodes: should import regulations be revised?.



- Biocontrol Sci Techn 27(2):149–168. <https://doi.org/10.1080/09583157.2016.1278200>
- Abdel-Razek AS, Abd-Elgawad MM (2007) Investigations on the efficacy of entomopathogenic nematodes against *Spodoptera littoralis* (Biosd.) and *Galleria mellonella* (L.). Arch Phytopath Plant Protect 40:414–422
- Acharya R, Yu YS, Shim JK, Lee KY (2020) Virulence of four entomopathogenic nematodes against the tobacco cutworm *Spodoptera litura* Fabricius. Biol Control 150:104348. <https://doi.org/10.1016/j.biocontrol.2020.104348>
- Altschul SF, Gish W, Miller W, Myers EW, Lipman D J (1990) Basic local alignment search tool. J Mol Biol 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Bedding RA, Akhurst RJ (1975) A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica 21:109–110
- Bhat AH, Chaubey AK, Askary TH (2020) Global distribution of entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*. Egypt J Biol Pest Co 30:1–5
- Bhat AH, Chaubey AK, Shokoohi E, Mashela PW (2019) Study of *Steinernema hermaphroditum* (Nematoda, Rhabditida), from the West Uttar Pradesh, India. Acta Parasitol 64(4):720–737. <https://doi.org/10.2478/s11686-019-00061-9>
- Boemare N (2002) Biology, taxonomy and systematics of *Photorhabdus* and *Xenorhabdus*. In: Gaugler R (ed), Entomopathogenic nematology. CABI publishing, Wallingford, pp. 35–56.
- Bragard C, Dehnen-Schmutz K, Di Serio F, Gonther P, Jacques MA, Miret JAJ, Justesen AF, Milonas P, Navas-Cortes JA, Parnell S (2019) Pest categorisation of *Spodoptera litura*. J EFSA 17(7)
- Campos-Herrera R, Barbercheck M, Hoy CW, Stock SP (2012) Entomopathogenic nematodes as a model system for advancing the frontiers of ecology. J Nematol 44:162–176
- Campos-Herrera R, Blanco-Pérez R, Bueno-Pallero FÁ, Duarte A, Nolasco G, Sommer RJ, Martín JAR (2019) Vegetation drives assemblages of entomopathogenic nematodes and other soil organisms: evidence from the Algarve, Portugal. Soil Biol Biochem 128:150–163
- Campos-Herrera R, Půža V, Jaffuel G, Blanco-Pérez R, Čepulytė-Rakauskienė R, Turlings TC (2015) Unraveling the intraguild competition between *Oscyeius* spp. nematodes and entomopathogenic nematodes: implications for their natural distribution in Swiss agricultural soils. J Invertebr Pathol 132:216–27.
- Caoili BL, Latina RA, Sandoval RF, Orjay JI (2018) Molecular identification of entomopathogenic nematode isolates from the Philippines and their biological control potential against lepidopteran pests of corn. J Nematol 50(2):99
- Daddow RL, Warrington GE (1983) Growth-limiting soil bulk densities as influenced by soil texture. USDA Forest Service Watershed Systems Development Group, Fort Collins, Colorado. General Technical Report No. WSDG-TN-00005.
- De Brida AL, Rosa JM, De Oliveira CM, BM EC, Serrão JE, Zanon JC, Leite LG, Wilcken SR (2017) Entomopathogenic nematodes in agricultural areas in Brazil. Sci Rep 7:45254
- De Grisse AT (1969) Redescription of modifications de quelques techniques utilisées es dans l'étude des nématodes phytoparasitaires. Mededelingen Rijksfakulteit Land-bouwwetenschappente Gent. 34:351–369
- Dillman AR, Chaston JM, Adams BJ, Ciche TA, Goodrich-Blair H, Stock SP, Sternberg PW (2012) An entomopathogenic nematode by any other name. PLoS Pathog 8: e1002527. <https://doi.org/10.1371/journal.ppat.1002527>
- Ferreira T, van Reenen CA, Endo A, Tailliez P, Pages S, Spröer C, Malan AP, Dicks LM (2014) *Photorhabdus heterorhabditis* sp. nov., a symbiont of the entomopathogenic nematode *Heterorhabditis zealandica*. Int J Syst Evol Microbiol 64(5):1540–1545
- Finney DJ (1952) Probit analysis: a statistical treatment of the sigmoid response curve. Cambridge university press, Cambridge
- 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. Biol Control 117:68–77
- Gokte-Narkhedkar N, Bhanare K, Nawkarkar P, Chilliveri P, Fand BB, Kranthi S (2019) Parasitic potential of entomopathogenic nematode *Heterorhabditis indica* against two Lepidopteran insect pests of cotton, *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fabricius). Phytoparasitica 47(1):31–41
- Grewal PS, Ehlers R-U, Shapiro-Ilan DI (2005) Critical issues and research needs for expanding the use of nematodes in biocontrol. In: Grewal PS, Ehlers R-U, Shapiro-Ilan DI (eds). Nematodes as biocontrol agents. CABI publishing, Wallingford, pp. 479–489.
- Hoy CW, Grewal PS, Lawrence JL, Jagdale G, Acosta N (2008) Canonical correspondence analysis demonstrates unique soil conditions for entomopathogenic nematode species compared with other free-living nematode species. Biol Control 46:371–379
- Jeyasankar A, Chinnamani T, Chennaiyan V, Ramar G (2014) Antifeedant activity of *Barleria buxifolia* (Linn.) (Acanthaceae) against *Spodoptera litura* Fabricius and *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). Int J Nat Sci Res 2:78–84
- Kaya HK, Aguilera MM, Alumai A, Choo HY, De la Torre M, Fodor A, Ganguly S, Hazir S, Lakatos T, Pye A, Wilson M (2006) Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. Biol Control 38(1):134–155
- Kaya HK, Gaugler R (1993) Entomopathogenic nematodes. Annu Rev Entomol 38: 181–206
- Kaya HK, Stock SP (1997) Techniques in insect nematology. In: Lacey LA (ed) Manual of Techniques in Insect Pathology. Academic Press, San Diego, pp 281–324
- Kour S, Khurma U, Brodie G, Hazir S (2020) Natural occurrence and distribution of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) in Viti Levu, Fiji Islands. J Nematol 52:1–17. <https://doi.org/10.21307/jofnem-2020-017>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874
- Lacey LA, Grzywacz D, Shapiro-Ilan DI, Frutos R, Brownbridge M, Goettel MS (2015) Insect pathogens as biological control agents: back to the future. J Invertebr Pathol 132:1–41. <https://doi.org/10.1016/j.jip.2015.07.009>
- Laznik Ž, Trdan S (2011) Entomopathogenic nematodes (Nematoda: Rhabditida) in Slovenia: from tabula rasa to implementation into crop production systems. In: Perveen F (ed) Insecticides -advances in integrated pest management. InTech, Rijeka, pp 627–656
- Nguyen KB, Hunt DJ (2007) Entomopathogenic nematodes: systematics, phylogeny and bacterial symbionts. In: Hunt DJ, Perry RN (series eds). Nematology monographs and perspectives, Brill NV, The Netherlands.
- Orozco RA, Lee MM, Stock SP (2014) Soil sampling and isolation of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae). J Vis Exp 89:e52083 <https://doi.org/10.3791/52083>
- Park HW, Kim HH, Youn SH, Shin TS, Bilgrami AL, Cho MR, Shin CS (2012) Biological control potentials of insect-parasitic nematode *Rhabditis blumi* (Nematoda: Rhabditida) for major cruciferous vegetable insect pests. Appl Entomol Zool 47:389–397
- Pascual PR, Pascual ML, Albuero HM (2017) First report of Entomopathogenic nematode Heterorhabditidae (Rhabditida) in organic vegetable farms in Cebu, Philippines. J Agric Manag 20:1–9
- Phan KL, Subbotin S, Nguyen NC, Moens M (2003) *Heterorhabditis baujardi* sp. n. (Rhabditida: Heterorhabditidae) from Vietnam and morphometric data for *H. indica* populations. Nematology 5:367–382. <https://doi.org/10.1163/156854103769224368>
- Philippine Statistics Authority (2004) Review of the agricultural sector in SOCCSKSARGEN. Retrieved from <https://psa.gov.ph/content/review-a-gricultural-sector-soccsksargen>
- Poinar GO Jr, Karunakar GK, David H (1992) *Heterorhabditis indicus* n. sp. (Rhabditida: Nematoda) from India: separation of *Heterorhabditis* spp. by infective juveniles. Fundam Appl Nematol 15:467–472
- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. In: <https://www.R-project.org/>.
- Shad SA, Sayyed AH, Fazal S, Saleem MA, Zaka SM, Ali M (2012) Field evolved resistance to carbamates, organophosphates, pyrethroids, and new chemistry insecticides in *Spodoptera litura* Fab.(Lepidoptera: Noctuidae). J Pest Sci 85: 153–162
- Spiridonov S, Reid AP, Kasia P, Subbotin S, Moens M (2004) Phylogenetic relationships within the genus *Steinernema* (Nematoda: Rhabditida) as inferred from analyses of sequences of the ITS1-5.8S-ITS2 region of rDNA and morphological features. Nematology 6:547–566
- Stock SP, Al Banna L, Darwish R, Katbeh A (2008) Diversity and distribution of entomopathogenic nematodes (Nematoda: Steinernematidae, Heterorhabditidae) and their bacterial symbionts ( $\gamma$ -Proteobacteria: Enterobacteriaceae) in Jordan. J Invertebr Pathol 98:228–234. <https://doi.org/10.1016/j.jip.2008.01.003>
- Sudhaus W (2011) Phylogenetic systematisation and catalogue of paraphyletic "Rhabditidae" (Secernentea, Nematoda). J Nematode Morphol System 14:113–178
- Sumaya NH, Gohil R, Okolo C, Addis T, Doerfler V, Ehlers R-U, Molina C (2018) Applying inbreeding, hybridization and mutagenesis to improve oxidative stress tolerance and longevity of the entomopathogenic nematode

*Heterorhabditis bacteriophora*. J Invertebr Pathol 151:50–58. <https://doi.org/10.1016/j.jip.2017.11.001>

- Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526
- Torrini G, Mazza G, Carletti B, Benvenuti C, Roversi PF, Fanelli E, De Luca F, Troccoli A, Tarasco E (2015) *Oscheius onirici* sp. n. (Nematoda: Rhabditidae): a new entomopathogenic nematode from an Italian cave. Zootaxa 3937:533–548
- White GF (1927) Scientific apparatus and laboratory methods: a method for obtaining infective nematode larvae from cultures. Science 66:302–303
- Zhang M, Demeshko Y, Dumbur R, Iven T, Feussner I, Lebedov G, Ghanim M, Barg R, Ben-Hayyim G (2019) Elevated  $\alpha$ -linolenic acid content in extraplastidial membranes of tomato accelerates wound-induced jasmonate generation and improves tolerance to the herbivorous insects *Heliothis peltigera* and *Spodoptera littoralis*. J Plant Growth Regul 38:723–738

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