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# Morphological identification of nematodes found in *Hylocereus* spp. (Dragon fruit)

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**Key words:** Dragon fruit, Free-living, Plant-parasitic nematodes, Morphological identification

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**Abstract**

As a recently introduced crop in the Philippine neighbouring Asian countries, the Dragon fruit or Pitaya (*Hylocereus* spp.) had gained popularity to farmers and was widely cultivated for its nutritive and medicinal benefits. As a non-native and newly introduced plant species, the researchers determined if plant parasitic nematodes also feed, parasitize, and damage these plants. Investigations included the physicochemical factors present in the soil samples in the collected in the farm. All specimens were identified based on their morphological features.

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## Introduction

Infections produced by parasitic nematodes in plants are now threats to different agricultural crops in tropical and subtropical countries. Crop protection approaches differ widely among organic growers both globally and regionally, yet organic farming faces the same plant-parasitic nematode (PPN) issues as conventional farming (Briar*, et al.,* 2016). A crop yield loss due to these tiny unseen pests in various countries is enormous. They caused projected yield loss of 12.3% ($157 billion dollars) worldwide (Singh, *et al.,* 2015). In the Philippines, past research during the last twenty (20) years revealed that nematodes are considered major pests on most economic crops particularly on banana, pineapple, citrus, tomato, ramie and sugarcane. Damage may range from 10 to 50%, and in severe cases crops have become unproductive and had to be changed (Davide, 1988).

In 2017, a taxonomic survey on nematodes found in the soil samples collected from organic and conventional farms in Laguna Province and isolated seven plant-parasitic nematode generas namely, *Aphelenchoides* sp., *Aphelenchus* sp*., Helicotylenchus* sp., *Meloidogyne sp., Pratylenchus* sp., *Rotylenchulus* sp. and *Tylenchus* species. *Meloidogyne* sp. and *Rotylenchulus* sp. were the most prevalent and abundant among the taxa (Pascual, *et al.,* 2017).

Dragon fruit (*Hylocereus* spp.) is a popular primary agricultural product in the Philippines for its commercial value, gaining popularity to farmers and was widely cultivated for its nutritive and health benefits. As a newly introduced species, no studies had been made yet regarding its association with plant-parasitic nematodes and its impact to the plant. No previous study was conducted yet on dragon fruit and its symbiotic relationship with the nematodes. The researchers conducted this local pilot study and determined the plant parasitic nematodes that fed, parasitized, and damaged these plants. The investigation included the prevailing physicochemical conditions of the soil samples while the nematode specimens collected were identified based on their morphological features. To effectively fight nematode pests, it is necessary to define the problem to correctly search for lasting management strategies *(Hassan, et al.,* 2013).

## Material and methods

*Sampling and Sampling Area*

Soil Samples were taken from Silan's Dragon Fruit Agrifarm in Cavite and Mendoza's Backyard Farm in Tagaytay last February-March 2019. 10 samples were dug out using auger or hand trowel 12 cm. deep following a zigzag pattern and placed inside a plastic bag (Fig. 1), then labelled for processing. Some portions were submitted to BSWM for soil analysis (e.g., soil texture, pH, Nitrogen, Phosphorus and Potassium content).

*Nematode Extraction using Modified Baermann Method*

To extract the nematodes, the researchers employed the modified *Baermann* Method. 100 cc. of soil sample were spread evenly over a 1-ply tissue paper placed on top of the wire screens that were wrapped around a 3-cm PVC pipe before placing on top of a bowl. The bowl was then filled with water to a level enough to reach the edge of the PVC pipe. The set-up was left for 3 -7 days depending on the extraction of the target nematode. The water from the bowl was then filtered 3 times using a 38$\mu m$ mesh sieve (Bezooijen, 2006). Assuming that the nematodes were trapped in the sieve, the sieve will be sprayed gently with water to release them from the mesh and the water drip was transferred on a petri dish for examination under the microscope. For the plant roots, it was cut into pieces and placed in a bottle with 100 ml of 1% of $H_2O_2$ and left for 48 hours at room temperature.

*Killing and Fixation*

All contents were poured on 38um mesh sieves and the filtrates were placed on vials for killing and fixation (Tarjan, 1967). Water filtrate was then heated to near boiling point using a water bath. Vials containing the nematodes were immersed in hot water for 40 seconds and then removed before adding the fixative solution and left for 24 hours (Orajay, 2005).

*Preparation of Nail Polish Ring for mounting*

An improvised well glass slide was made to mount the nematodes before observation. To do this, the slides were fixed on a clip to fix it in place of a free-revolving platform. Using a nail polish, the enamel was placed evenly using the tip of the newly opened nail polish while the revolving platform is rotating to form a circle, then left to dry (Orajay, 2005).

*Mounting and Morphological Identification*

Using a dissecting microscope, Plant parasitic nematodes were picked out using the tip of a camel hairbrush. A drop of the fixed nematode suspension was placed on a glass slide to free the specimen from the brush and was examined under a light microscope in OIO objective. In fixing the specimen gently, a cover slip was placed on top and the slides were sealed using nail polish. Slides were labelled with the following information: the plant, place of collection, date of mounting, and number of mounted specimens (Seinhorst, 1959). Identification of nematodes was based on the morphological features using the oil immersion objective (OIO) of the light microscope (EuroMex) and manuals as reference. Distinct characteristics of each nematode were used to identify them accordingly to their genera level as possible using the following features: presence and type of stylet, shape of the cephalic and caudal region, body shape, body size, presence of metacorpus, and the position of vulva or spicule (Eisenback, 1991).

**Result and discussion**

Soil sample results showed that Silan's Agrifarm and Mendoza backyard farm are both acidic (pH value of 5.5 and 4.4), respectively. Soil analysis records from Silan's farm showed high nitrogen content, moderate phosphorus, and a moderate level in potassium, while the samples from Tagaytay showed low Nitrogen content, moderate Phosphorus, and moderate Potassium level. Adding nutrients like fertilizers, or manures were applied to optimize crop yields. Addition of organic matter influences the important and diverse biological activities of soils. A new energy source is provided by the amalgamation of organic materials that results in the increased activities of soil microbes and its diversity. A recent study conducted by Widmer and Abawi (2000) showed that the incorporation of sundan grass increased populations of total fungi, bacteria, and nematodes. Many of these organisms or their produced metabolites are advantageous for plant growth or, oppositely, hostile toward plant pathogens, including nematodes (Widmer, Mitkowski, & Abawi, 2002).

Of all plant nutrients, Nitrogen is vital for yield and growth. High levels of nitrogen results in the formation of new saps and tissues and can prolong the vegetative state and builds up the number of feeding sites in the roots, which attracts nematode attack. Nevertheless, a plant that is lacking in nitrogen can become incapacitated, suffer decelerated growth, and become more vulnerable.

Phosphorus is important to plant growth and can also influence diseases caused by nematodes. Plants with high amounts of phosphorus secrete fewer root exudates and are therefore less attractive to nematodes and lead to lessening the incidence of the diseases. Moreover, plants become more resistant when supplied with appropriate measures of phosphorus, resulted in an increase in cell activity, protein synthesis, and production of ammonia, polyphenols, and peroxidase.

Potassium plays an important role in enzymatic activation (over 60 enzymes), potassium also participates in neutralization of anions, membrane crossover, and retaining membrane potential (Santana-Gomes, *et al.*, 2013). Adequate amount of potassium helps lessen the incidence of disease due to improved resistance to the dissemination and development of pathogens, is considered that has the greatest influence on diseases (Santana-Gomes *et al.,* 2013).

It is recognized that soil pH can affect the development of nematode indirect to host reactions and by changing the chemical composition of soil or the present antagonistic organisms. Nematodes are mostly tolerant to a wide range of pH levels, but acidic and alkaline soil pH, caused by soil alterations (generation of ammonia and its nitrification or organic acids), may contribute to nematode suppression with no connection to pH-dependent nematicidal compounds (i.e., ammonia, nitrous acid, and volatile fatty acids). It was also observed that only non-ionized forms of the organic acids can pass though the nematodes' cell membrane and affect them. Decreasing soil pH may increase the percentage of non-ionized organic acids, thus enhancing nematicidal activity, in addition to the direct adverse effect of low pH on nematodes (Hosseinikhah Choshali, Seraji, Rezaee, & Shirinfekr, 2013).

A total of 27 individual nematodes belonging to 1 family and 6 genera, both plant-parasitic and free living, were isolated from the samples. Based on their morphologies, they were identified under the *Family Rhabditidae* (free living nematode) and *Genera Aphelencoides* sp., *Helicotylenchus* sp., *Rotylenchus* sp., *Rotylenchulus* sp., and *Meloidogyne* sp. (plant-parasitic nematodes) (Fig. 1-4).

*Helicotylenchus* sp. is the only species found in roots collected from Mendoza's backyard farm, together with both the male and immature female *Rotylenchulus* sp. from the soil samples.

One nematode isolated from the soil samples obtained from Silan's Agrifarm was identified as *Meloidoygne* spp. As described by Eisenback (2002), the body is short and thin, approximately 207 *µm* long. Obviously, this nematode is a male because it exhibits a lightly sclerotinized cephalic framework (Nino-Castaneda & Vezina, 2016). Stylet is short and weak with indistinct knobs. Esophagus overlaps the intestine ventrally and the tail is pointed and narrow having clear terminus (Eisenback, 2002), as shown in Fig. 3*D* and *C*, respectively.

The species under Family Rhabditidae a free-living nematode (Fig. 5), and Genera *Aphelenchoides* sp., *Meloidogyne* sp. and *Rotylenchus* sp. were isolated from the soils of Silan's Agrifarm in Indang. Unlike in Mendoza's Backyard farm (Tagaytay), Dragon fruit cultivars in Silan's Agrifarm (Indang, Cavite) used fertilizers, particularly, humus, in their soil.

Free-living nematodes inhabit the interstitial spaces in soil as well. Unlike the plant-feeders, free-living nematodes are rarely sedentary, continually moving to feed on a diverse range of food, including algae, bacteria, protozoa, fungi, small invertebrates, and other nematodes. They use similar set of sensilla to locate the source of their food. Nevertheless, their feeding structures are quite modified to suit their meal. Bacterial feeders graze using a comparatively simple tubular mouthpart, though the cuticle surrounding the oral opening may be modified sumptuously to direct food toward the stoma (Neher & Powers, 2005).

The maintenance and addition of high levels of organic matter, particularly the active fraction, will greatly improve the chemical, physical, and biological properties of soil, thus increasing productivity. High levels of soil organic matter contents were correlated with the lower abundance of plant-parasitic nematodes, signifying that addition of organic compounds is an alternative way for suppressing these nematodes (*de Barros, Pedrosa, Cardoso, & Rolim, 2017).*

**Table 1.** The soil analysis results.

| pH, Nitrogen, Phosphorus and Potassium level | | | | |
|---|---|---|---|---|
| | pH | Nitrogen (N) | Phosphorus (P) | Potassium (K) |
| Silang's Agrifarm in Indang, Cavite | 5.5 | High | Moderate | Moderate |
| Mendoza Backyard farm in Tagaytay, Cavite | 4.4 | Low | Moderate | Moderate |

Table 1 showed the physicochemical properties of the soil collected

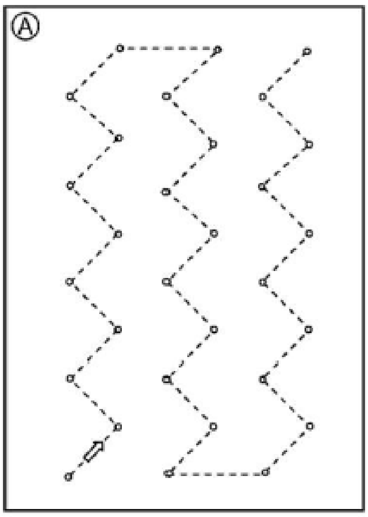
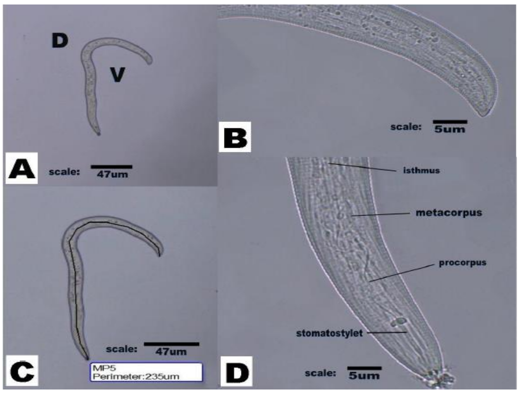
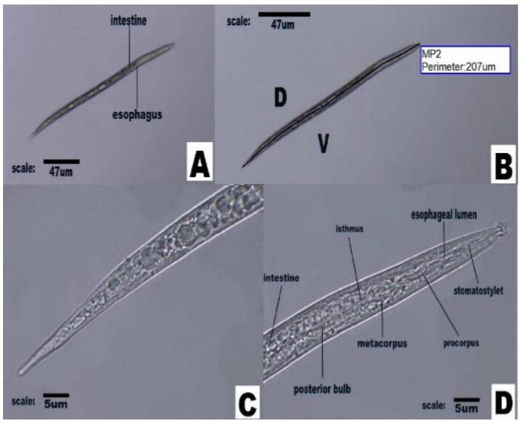



**Fig. 1.** The zigzag method when selecting sample site in a big farm with plants arranged in a pattern.



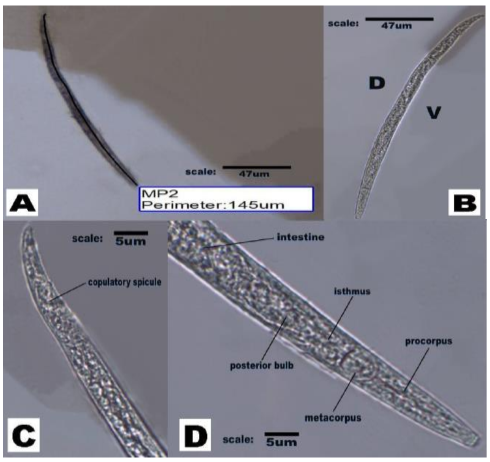

(A) Under (4.5X); (B) Caudal region under (45X); (C) Measurement with approximately 235 µm; (D) Cephalic region under (45X)

**Fig. 2.** The image of *Rotylenchus* sp.



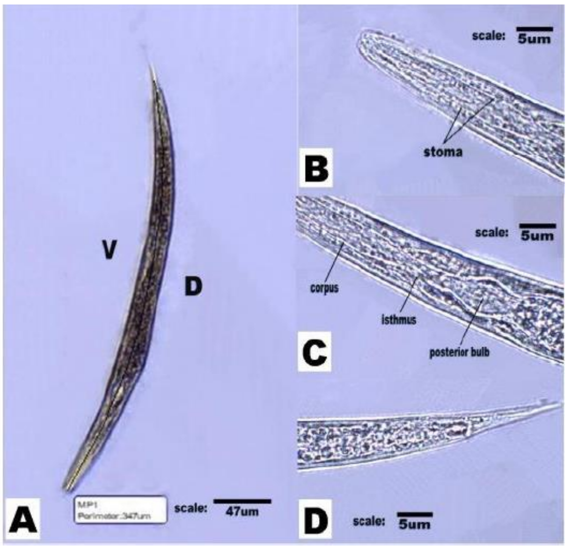

(A) Under LPO (4.5X); (B) Measurement with approximately 207 µm; (C) Caudal region under OIO (45X); (D) Cephalic region under OIO (45X)

**Fig. 3.** The image of juvenile *Meloidogyne* sp.




(A) Measurement with approximately 145 µm; (B) under LPO (4.5X); (C) Caudal region under OIO (45X); (D) Cephalic region under OIO (45X)

**Fig. 4.** The image of *Aphelenchoides* sp.




(A) Measurement with approximately 347µm; (B) Cephalic region under OIO (45X); (C) Upper midbody under OIO(45X); (D)Caudal region under OIO(45X);

**Fig. 5.** Shows the image of *Rhabditida* sp.

## Conclusion

About 6 nematode species were identified from the two sampling sites: one species under the *Family Rhabditidae* (a free-living nematode) and 6 plant-parasitic nematodes species: *Aphelenchoides* sp., *Helicotylenchus* sp., *Rotylenchus* sp., *Rotylenchulus* sp., and *Meloidogyne* species. The correlation between the high numbers of free-living nematodes found in Silan's Agrifarm was directly proportional to the high amounts of organic matter and was inversely proportional on the number of plant-parasitic nematodes. Notably, Plant damages may not be directly caused by the nematodes. According to research, the severity of infection may depend on the interaction of agronomic factors, e.g., soil texture, weather conditions and the association of some other organisms and other physicochemical factors possibly affecting the plant pathogens within the rhizosphere

## Recommendations

For future researchers, it is recommended to use other forms of extraction to process not only the active, locomotive nematode species but also the passive (sedentary) ones as well. Additionally, exploitation of molecular analysis to ensure the proper identification of species of nematodes is highly suggested. High quality laboratory equipment is also

needed for distinguishing the morphological characteristics of nematodes. For other researchers who aspire to continue this study, we suggest including abundance and frequency of the nematodes in their objective to know the distribution of a particular species. The authors thank the Commission of Higher Education's (CHED) Dareto Grant who funded the research, and Prof. Romnick Latina of University of the Philippines-Los Baños for sharing his expertise on nematode identification.

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