Plant-Parasitic Nematodes Associated with Pineapple (*Ananas comosus*) in Selected Provinces in Luzon, Philippines

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Abstract*: Plant parasitic nematodes are one of the major crop problems affecting various crop productions worldwide and pineapple is one of the most economically important crops in the Philippines which is also infected by these plant pathogens. Thus, this paper aimed to identify the species of plant parasitic nematodes associated with pineapple. In this study, the nematodes from soil and roots sample were extracted using Baermann tray method and were identified under the light microscope based on their morphological characteristics. Four species of plant parasitic nematodes were identified namely, Rotylenchus spp., Ditylenchus spp., Helicotylenchus spp., and Rotylenchulus spp. Wherein, Helicotylenchus spp. was the most frequently occurring species in the soil with a total of 75.8% whilst only 1% in the roots. The occurrence of these plant parasitic nematodes indicated that the soil environment in the area was favorable for these organisms.

Keywords:* *Ditylenchus spp., Helicotylenchus spp., pineapple, Rotylenchulus spp., Rotylenchus spp.,

1. INTRODUCTION

Pineapple is one of the most economically important crops. The country ranks second in the world, next to Thailand in terms of pineapple production with an estimated of 70,000 hectares planted with the crop. Most are exported thus, contributing to about 17% of the world supply [1]. Majority of the production is concentrated in Mindanao, with other provinces in Luzon close behind. However, 85% of the production is controlled by giant transnational corporations, led by Del Monte and Dole Philippines. However, even if there is a pedestal for the Philippine pineapple industry, there are still outlying concerns to be considered. These includes calamities, bug infestations, and infections of the crops by either fungi or plant parasitic nematodes. Plant parasitic nematodes are one of the major causes of plant infections and affect the production of crops worldwide [2]. Aside from pineapples, wheat, rice, maize, potatoes, sweet potatoes and many more are some of reported crops that have problem with crop losses associated with plant parasitic nematodes [3]. Nematodes are the most abundant group of multicellular creatures on earth in terms of numbers of individuals and about 10% of all nematode species are plant parasites. Plant parasitic nematodes are minute organisms that live underground [4]. Today, plant parasitic nematodes are apprehended as major rural pathogens and are known to assault plants and cause misfortunes over the world. They maintain the pedestal of one of the most harmful and hard to oversee agronomic irritations, and strain to decrease the utilization of exceptionally toxic nematicides [5]. In 2006, there were already 4,100 species of plant-parasitic nematode that have been identified (Jones *et al*., 2013) and yearly, they are causing an estimated annual crop loss of $78 billion worldwide with 10-15% average crop yield loss [6]. Even in this modern day, there are still a lot of producers, especially in developing countries that are ignorant of plant parasitic nematodes.

The feeding strategy of plant parasitic nematodes in pineapple is usually from the soil through the roots. Majority of the infections includes lesions of piths and vascular tissues necessary for water and food transport of the plant. When these tissues are disrupted, chlorosis will start to occur on the leaves. On the later stage of infection, leaves will facilitate necrosis, and the roots can be easily pulled from the infected soil. These infections can be contagious to its relative plants and causes permanent damage to the soil, leaving a permanent annual loss of production [2].

With the damage inflicted by PPN's on pineapples, this study aimed to determine the PPN's associated with the crop. Samples were taken from provinces of Cavite, Laguna and Batangas in Luzon, Philippines where the pineapple plantations are located. This study will help provide additional information on the occurrence of plant parasitic nematodes on pineapple crops in the Philippines and sends an awareness to pineapple farmers who are clueless on the effect of PPN's on their crops.

2. METHODOLOGY

A. Sampling site and sample collection

The soil and root samples of pineapples were collected from pineapple farms at Tagaytay City, Cavite, Laguna and Batangas. The surrounding soil of each pineapple plant as well as its roots was collected as the primary samples. Ensuing the protocol of Coyne *et al*. [7], the collection of both roots and soil were done randomly, following a zigzag pattern to ensure homogeneity. The surface litters at each sampling spot were removed first before drawing out the soil sample which was done by lashing the soil sample auger to a plough depth of 15 to 20 centimeters. On the other hand, the fine roots were gathered by digging the soil and cutting it from the main roots using a clean scissor. All the collected soil and root samples from the two different areas were separately packed in clean polyethylene bags and labeled. Then, all samples were put in an ice chest to maintain the moisture of the soil while being transported to the laboratory.

B. Soil analysis

One kilogram of soil sample was collected from the sampling site and was sent to Bureau of Soils and Water Management (BSWM) in Quezon City, Philippines for the analysis of its nitrogen, phosphorus and potassium contents using a soil test kit.

C. Extraction of nematodes

Stones and debris were removed from the soil samples after thoroughly mixed. Five set-ups were prepared for the extraction of nematodes following the Baermann Tray technique [7, 8]. After the extraction period, soil samples were sieved wherein the water suspensions from the five trays were poured together onto three tilted layers of stainless steel sieve (45 μm, 105 μm, and 180 μm) with the smallest size at the bottom. The debris were condensed by gently but thoroughly rinsing 180 µm sieve with wash bottle mainly from the back to ensure that all debris and nematodes from the sieve surface were collected at the bottom point. All the debris from 180 µm sieve were disregarded while the debris from the remaining 105 µm and 45 µm were gently washed into a labeled glass bottle. As for the extraction of root nematodes, incubation technique with hydrogen peroxide (H_2O_2) was used. The root samples were gently washed in a running water removing all the associated soils, cut into 1-2 centimeters short and were mixed thoroughly. All the chopped root samples were weighed 15 grams for each of the two 30 milliliter amber bottles which were filled with 1% concentrated H_2O_2. The bottles were tightly covered and labeled with the crop name and date of preparation before incubating for 48 hours at room temperature. After incubation period, sieving method was done wherein all the contents of the two bottles were poured on to three tilted layers of stainless steel sieve (40 µm, 80 µm, and 200 µm) with the smallest size at the bottom. The debris were condensed by gently but thoroughly rinsing the 200 µm sieve with wash bottle mainly from the back to ensure that all debris and nematodes were collected from the mesh surface. The same process was done for the 80 µm and 40 µm sieve in where the nematode suspension began to be collected using a vial.

D. Killing and fixation of nematodes

The killing procedure for the nematodes from both soil and root samples were executed homogenously. Using an electric kettle, water was heated to nearly boiling point then, it was transferred to a flat container where the glass bottles and vials were immersed and shaken in a circular manner for about 40 seconds. The glass bottles were removed from the container then, a double strength (2x) formalin-glycerol fixative with an equal volume of the nematode suspension was poured into it. After fixation, the glass bottles were left in an undisturbed area for at least 24 hours to let the suspension settle down as well as to ensure the complete penetration of the fixative.

E. Preparation of microscope slides

Before picking and mounting the nematodes, proper layout of the microscope slides to be used were primed. By means of a ringer, each microscope slides were painted at the center with a clear nail polish in a circular manner. A microscope slide was clipped on the stage of a ringer with the tip of the brush immersed in nail polish slightly touching the portion of the slide while the stage was being spun [7].

F. Picking and mounting of nematodes

Based on the protocol of Coyne *et al.* [7], part of the nematode suspension was transferred to a small petri plate then, was observed on a dissecting microscope with under-stage lighting. The target nematode was located using the lowest magnification, verified at higher magnification and was picked out using a coconut splinter. Then, the picked out nematode was placed at the center of a glass slide with nail polish ring containing the single strength (1x) formalin-glycerol mounting medium. By means of a light microscope with under-stage lighting, the successful transfer of the nematode was confirmed as well as its possible position on the glass slide. Afterwards, a cover slip was placed on top of the specimen which was sealed by painting a clear nail polish on its four sides in which each microscope slides was labeled with pertinent information about the contained specimen.

G. Morphological Identification

The well mounted plant parasitic nematodes were observed under light microscopes in order to determine its genus identity. Morphological features such as body shape and stomatostylet structure were first identify to determine which was free living or PPN's. The initial identification on genus level was completed following the taxonomic keys of plant parasitic nematodes by Stirling *et al*. [9]. Furthermore, morphometric analysis of each nematodes were also executed using the ImageJ application following these sets of parameters from de Man‟s Indices [10]: *n* (number of specimens on which measurements were based), *L* (overall body length) *V* (length of vulva), *S* (stylet or spear length), and *s* = spicule length. Then, all the pre-identified nematodes were sent to the Department of Plant Pathology in University of the Philippines – Los Baños for further validation. Lastly, all the identified plant parasitic nematodes were photographed using the Image Focus 4 application. Also, appropriate labeling of the nematode body parts were completed which include the ventral (V) part, dorsal (D) part, stomatostylet, anterior and posterior ends of the body [9].

H. Frequency Count and Data Analysis of Plant Parasitic Nematodes

The occurrence of all the extracted plant parasitic nematodes from both soil and root samples were done by pouring the water suspension from a sample bottle into two petri plates that were divided into quadrants – this process was done for three trials. Separately, the petri plates containing water suspension were observed under a light microscope following a zigzag pattern to ensure homogeneity. This process was performed in order to confirm the infestation of certain plant parasitic nematode species in pineapple. Moreover, following the principle by Afolami and Daramola [11], the percentage frequency of occurrence of the PPN's was obtained.

3. RESULTS AND DISCUSSION

A. *Plant Parasitic Nematodes associated with Ananas comosus*

Four species were observed and recorded from the soil and root samples of pineapples. It includes *Rotylenchus* spp., *Ditylenchus* spp., *Helicotylenchus* spp., and *Rotylenchulus* spp., respectively. The *Helicotylenchus* spp., was noticeably in higher number in soil samples but lesser in the root samples. The results obtained coincides with the study of Sipes and Wang [12] and Afolami and Daramola [11] where all the four PPN's obtained in the samples were also the PPN's they have observed in pineapple.

Helicotylenchus spp. belongs to the order *Tylenchida* and family *Hoplolaimidae*. It is the most rampant species observed in this study and known for its spiral body shape with body length that ranges from 438 to 506 μm. However, the shape of the stylet knobs varies from one individual to another. As shown in Figure 1B, the representative female species of *Helicotylenchus* that was picked from the soil samples has rounded knob, conversely, anteriorly flattened stylet knob was present in the female species found in the root samples (Figure 2A). Likewise, the stylet length of the extracted species from both soil and root samples ranged from 12 to 16μm long with 4 μm vulva length, however, the vulva of the female species from the soil is not prominent. The identification of these organisms under this genera were based from the dichotomous key of Stirling *et al.*[9] which were further supported by a study of Palomares-Rius *et al.* [13] proving that *Helicotylenchus* spp. is characterized by forming a spiral shape when killed and a robust stylet with knobs flattened and indented anteriorly. Moreover, an extremely inconstant reproduction is extant amongst species of *Helicotylenchus* [14]. Some species under this genus have males and females which sexually reproduced. Others are hermaphrodites that self-fertilize their own eggs without mating while some species only have females which reproduced through parthenogenesis since males are either absent or extremely rare [15]. Additionally, spiral nematodes are generally considered as ectoparasite but, species such as *Helicotylenchus pseudorobustus* have an occasional semi-endoparasitic feeding strategy. They infest wide range of crops such as pineapples, soybean, cotton, corn and banana as well as in turf grasses like Bermuda grass and *Paspalum vaginatum* [14].

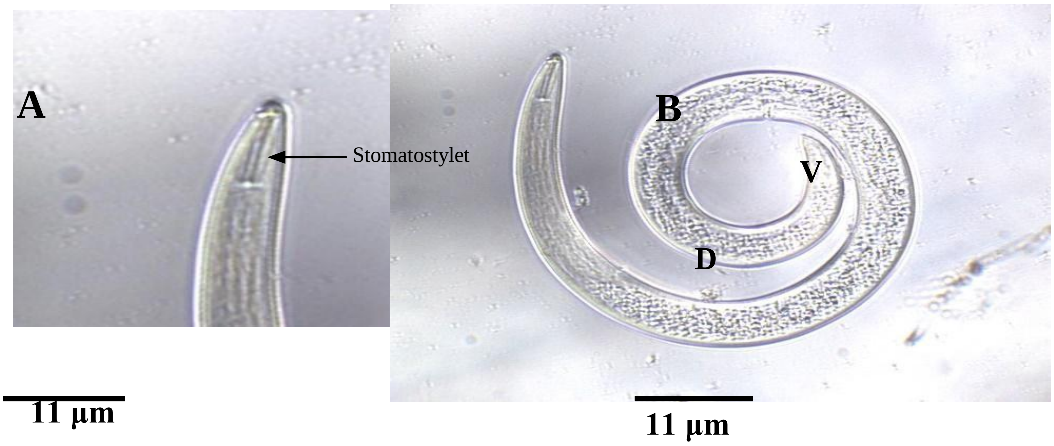

Fig 1 Micrographs of female *Helicotylenchus* spp. picked from the soil samples. **(A)** Anterior portion of the PPN body displaying the stomatostylet; **(B)** General morphology of the parasitic nematode.

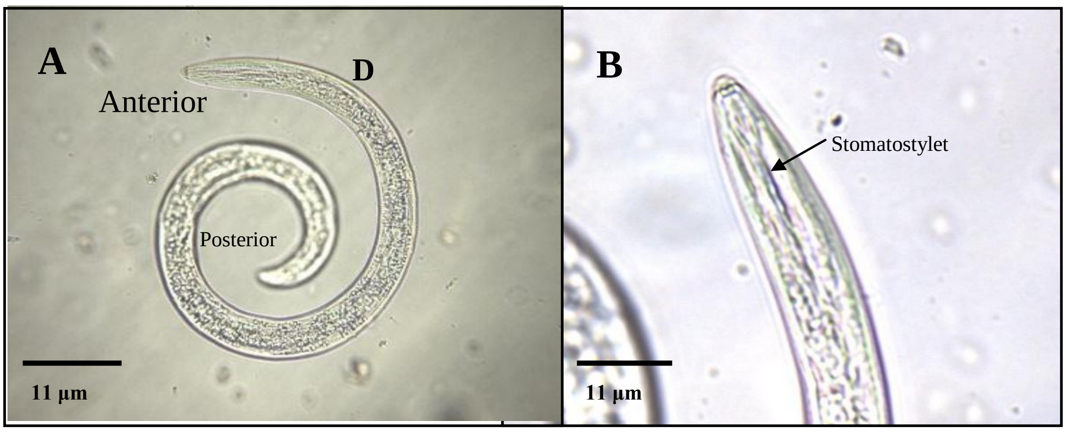

Fig 2 Micrographs of female *Helicotylenchus* spp. picked from the root samples. General morphology of the species; **(B)** Anterior part of the body showing the flattened stylet knob

Another PPN observed in pineapple's soil and root samples was *Rotylenchulus* [16] belonging to the order Tylenchida and family Hoploloamidae. Further, the extracted *Rotylenchulus* spp. from both soil and root samples showed a curved or C-shaped body situation (Figures 3A and 4B) with immature females having a body length that ranged from 266 to 282 µm long stylet that has rounded basal knobs, not prominent vulva and slightly tapering to a narrow and rounded terminal tail. On the other hand, males have approximately 299 µm body length, barely notable or weak stylet that was about 15 µm and at least 14 µm spicule with its curved posterior part of the body. The morphometric results of this study coincide with the study of several researchers [13, 17].

Based on the research of [17],,*Rotylenchulus* spp. have both female and male species. However, only the female species are infective since the males remain outside of the host's roots. The immature female imbeds her head intro the root tissue while the posterior portion protrudes from the root surface and swells during maturation. The infective stage of its female species stretched from one to two weeks after hatching and matures after one or two weeks once they penetrated into the roots of the host plant. Subsequently, species under this genus are pests of wide range of plants which includes pineapple, cotton, soybean and cowpea to name a few. Among these crops, pineapple is one of the severely affected by the species *Rotylenchulus* spp. The amount and type of damage frequently relies upon the host species or cultivar as well as the population of nematodes, which causes a reduced root system, leaf chlorosis, overall stunting of host plants, and reduced yields and plant longevity [18].

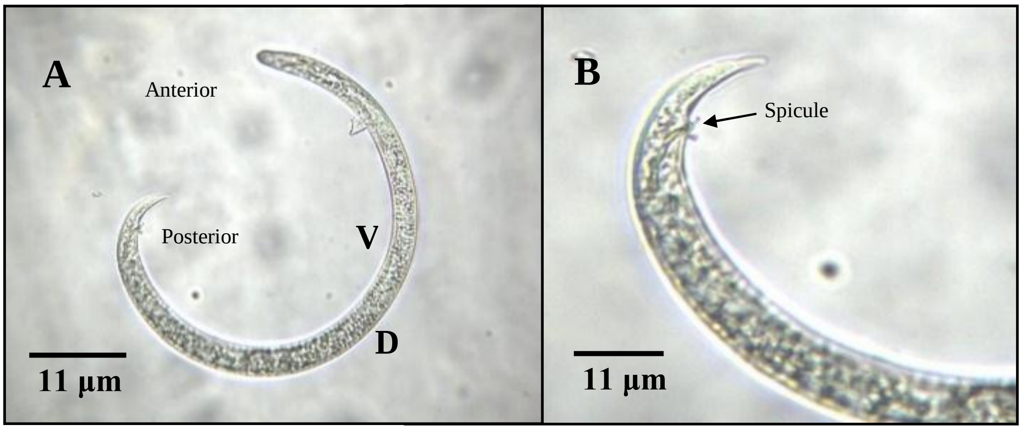

Fig 3 Micrographs of male *Rotylenchulus* spp. picked from the soil samples. **(A)** General morphology showing the spicule at the posterior end; **(B)** posterior end with the spicule (14 µm).

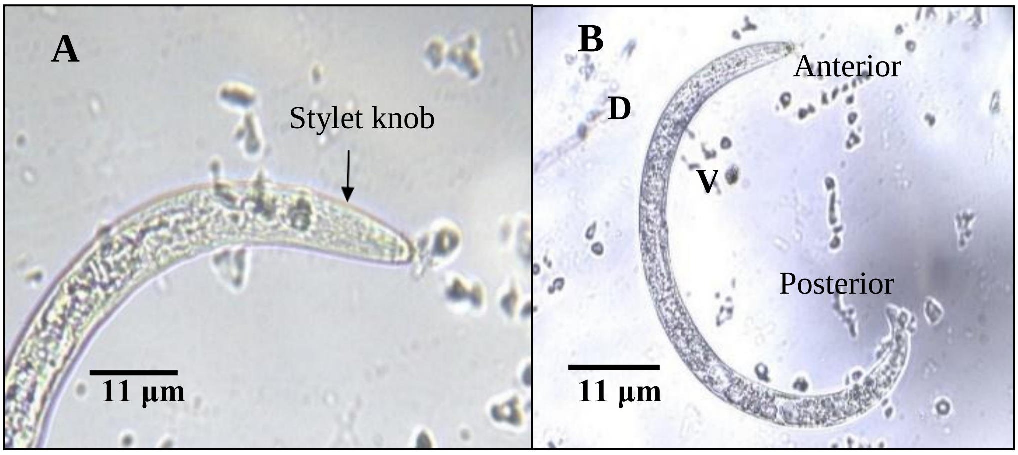

Fig 4 Micrographs of immature female *Rotylenchulus* spp. picked from the root samples. **(A)** Buccal cavity of the species; **(B)** general morphology of the nematode showing the habitus mortis.

Ditylenchus spp. [19] belongs to order Tylenchida and family Anguinidae. The *Ditylenchus* spp. (Figure 8) collected from the soil samples was observed having a slightly curved to straight body shape with a body length of 1100 µm long, a stylet of 21µm long and slightly pointed anterior and posterior ends. The identification of this species using its morphological features was done following a dichotomous key from Stirling *et al.*, (2014) and was further supported by the study of Esmaeili *et al.*[20] which also described the tapering at both body ends of *Ditylenchus* spp., short stylet and its rounded knobs.

Based on some studies, *Ditylenchus* spp. is well-known plant parasitic nematodes of garlics, onions and rice that are that are present on the stem and bulb of the crops thereby being commonly known as stem nematodes. Species under this genera are considered as migratory endoparasites of plants but they can also be mycophagous or fungivorous that feeds on fungi [21], which causes stunted growth, discoloration, swellings and distortion of aerial plant parts (stems, leaves, flowers) and necrosis or rotting of stem bases, bulbs, tubers and rhizomes. The juveniles of *Ditylenchus* spp. can survive in a dry condition for several years and may persist in clay soils. Cool and moist conditions are favorable for the invasion these plant parasitic nematodes [22].

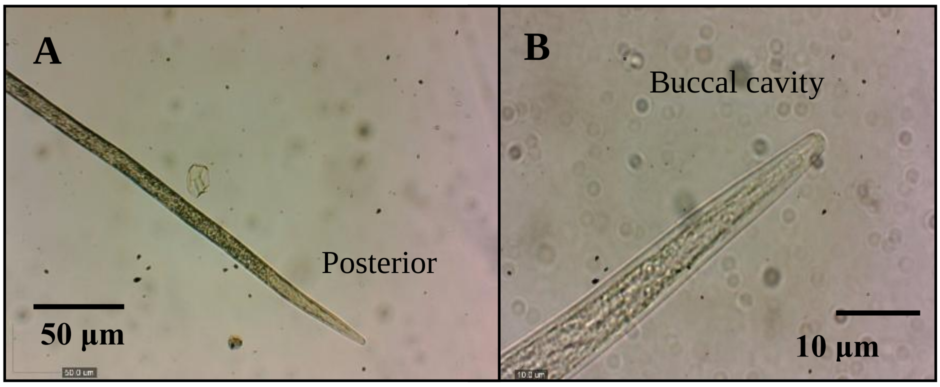

Fig 8 Micrographs of female *Ditylenchus* spp. picked from the soil samples. **(A)** Posterior morphology of the species through high power objective; **(B)** buccal cavity through oil immersion objective.

Likewise, the identification of *Rotylenchus* spp., (Figure 5) extracted from the soil samples was distinctive with their semi-spiral body shape of 518 µm long, a stylet length that was 13µm, flattened knobs, 9µm vulva and a hemispherical tail which were supported by a dichotomous key [9]. Its identification was further supported on the study of Palomares-Rius *et al.* [13] in where morphometrics of female *Rotylenchus* spp. were elaborated with having close C to spiral habitus that varies depending on species, body length of 500–1856 µm long, and 20 to 51 µm stylet with rounded to hemispherical tail.

Plant parasitic nematode species of genus *Rotylenchus* spp. are generally ectoparasitic, rarely endoparasite that feed on roots of wide range of plants such as strawberry, sunflowers, carrots, pineapples and boxwood among others [11, 23], which causes stunting andyellowing of the plant host that have been associated with large numbers of *Rotylenchus* spp. [24]. *Rotylenchus* belongs to the family of Tylenchida and order Hoplolaimidae.

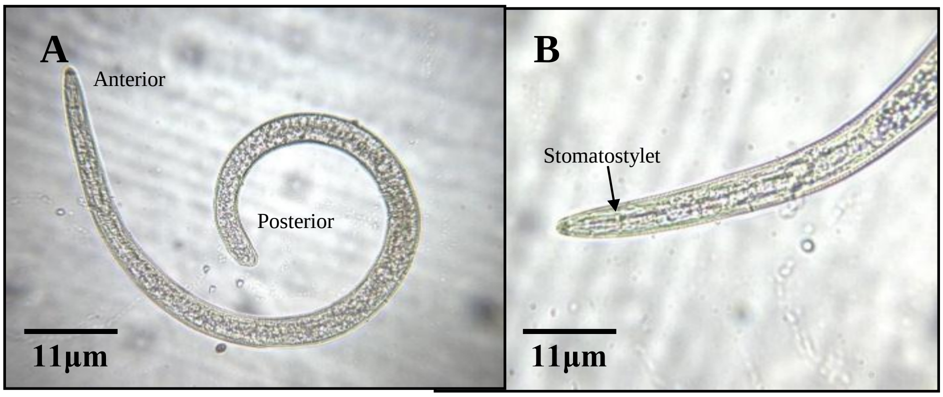

Fig 5 Micrograph of female *Rotylenchus* spp. picked from the soil samples. **(A)** General morphology of the species; **(B)** anterior part of the body.

All the extracted plant parasitic nematodes in this study are root-feeders and are considered as herbivores since they feed directly on plants and its tissues thereby being included at the first tropic level of the soil food web excluding *Ditylenchus* spp. since species of this genus have life styles that ranges from being plant parasites to fungal feeders. Thus, its species belong to different trophic level depending on its life cycle; mycophagous and plant parasite species belong to the second and first trophic level, respectively [25].

Identifying plant parasitic nematodes using only the morphological technique could really be difficult as some species bear resemblance to others in terms of body shape. Nevertheless, despite the innovating usage of molecular method, conventional taxonomy using reliable morphological characters is still a vital tool for the identification of nematodes primarily because it allows a clear link between function and morphological aspects of the specimen analyzed, it is suitable for quantitative evaluations, it is also used for population surveys of plant parasitic nematodes to name a few but more importantly, it is cheap [18].

B. *Soil Characteristics of the Sampling Site*

Soil nutrient content such as nitrogen, phosphorus and potassium and soil characteristics such as pH and texture could influence the proliferation of plant parasitic nematodes on the sampling site. Among the different PPNs, *Helicotylenchus spp.* has been said to thrive in all soil types [26].

Changes in the soil chemical factors could affect the nematode's ability to locate plant roots and reproduction. Presence and distribution of plant parasitic nematodes vary depends on abiotic characteristics of the soil [26]. As shown on the result of soil analysis (Table 2), the soil sample collected from the study area have a medium or clay loams to sandy clay loams texture with a pH level of 4.9. This result concurred with an article by the Bureau of Plant Industry which indicated that pineapples optimally thrive in sandy loam to clay loam soils and has a pH of 4.5 to 5.5. Soil texture plays a significant role in nematode distribution for it has a direct impact to the population density of plant parasitic nematodes which supports the conclusion of this research [27]; the soil type requirement for the growth of pineapple is a well-drained sandy loam and has an optimum range of pH level of 4.5 to 5.5, which is also ideal for nematode reproduction and infectivity [28, 29]. Additionally, it was indicated that *Rotylenchulus* spp. has an optimal pH level of approximately 4.8 to 5.2 thereby [16], supporting the extraction of *Rotylenchulus* spp. from the soil samples of this study.

Moreover, the result of physicochemical soil analysis of the soil samples shows that it has low nitrogen level, medium content of phosphorus and sufficient amount of potassium. Nitrogen (N) significantly influences the aboveground ecosystem productivity and below-ground pools. It plays a vital role in biochemical and physiological functions of plant since nitrogen is a major component of chlorophyll that is used by plants for its food production as well as amino acids which is the building blocks of protein necessary for proper development of plants [30]. However, in relation to other nutrients, too much nitrogen could also induce lateness in flowering or fruit formation, unproductive growth, and prone to disease [31].

Table 2. Physicochemical analysis of the soil sample from the sampling site

Description	**Test Result**			
	pH	**N**	**P**	**K**
Soil Sample	4.9	Low	Medium	Sufficient

On the other hand, phosphorus (P) is an essential macro-element used by plants during photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in plants. Phosphorous was also taken by plants in relatively small quantities and shortage of this macro-element results in blue coloration while excess amount of phosphorous on the plant was extremely difficult to define [31]. Whereas, potassium (K) in soil is known for its role in regulation of plant metabolic activities as well as its help in minimizing plant stress. It is present in soils in larger quantities than either nitrogen or phosphorus [31] which supported the result. It is required in large amount particularly by those flowering plants or fruit producing plants and shortage of this macro-element causes marginal leaf scorch while excess amount results in stunted dark blue tinted growth and sometimes burning of the tips of the leaf. Additionally, there is a close relationship between the effects of nitrogen and those of potassium, and successful cultivation of most plants depends on the correct ratio of nitrogen to potassium, while phosphorus playing little part provided that it is still present in sufficient quantity[31].

In the greenhouse study which evaluated the influence of soil nutrients in the presence of reniform nematodes, low level of P can suppress plant development and increase the number of the *Rotylenchulus* spp. whereas, increase in nitrogen level can suppress plant parasitic nematodes which means that if the soil has a low amount of N [32]. Then, there will be an increase in the population of plant parasitic nematodes [7]. On the other hand, unlike the two minerals, potassium shows no effect on the reproduction of plant parasitic nematodes [32]. Nonetheless, several studies have proved that the combination of these three minerals with high amount of availability have a strong correlation with the population decline of plant parasitic nematodes [32, 33]. Hence, high amount or deficiency of these vital soil macro-nutrients as well as high population of plant parasitic nematodes can both greatly affect the development of a plant since low amount of nitrogen in the soil may result to stunted growth with discoloration on the leaves since, these symptoms are indications of protein deficiency in the plant and nitrogen deficiency in the soil. However, stunted growth and chlorosis are also above ground symptoms of disease [26] caused by plant parasitic nematodes such as *Ditylenchus* spp. and *Rotylenchus* spp. [24].

Subsequently, similar symptoms of abnormal discoloration showed when phosphorus is deficient. In pineapple, aside from chlorosis, phosphorus deficiency also results to the reduction of plant height, length and number of the leaves which is due to the reduction in the cell division [29]. Conversely, potassium is not organically bound which means that when the plant dies and decomposes, it is immediately released back on the soil. Yet, if some crops have no adequate amounts of potassium they exhibit symptoms such as yellow or white spots on the margins of the leaflets with symptoms first appearing on the older plant tissue and reduction in plant growth, root development, as well as fruit development [27]. Consequently, plant parasitic nematodes that feed on the roots such as *Helicotylenchus* spp., *Rotylenchus* and

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Rotylenchulus spp. also caused above ground symptoms that mostly resulted into many root injuries such as root necrosis (Figure 10) resulting in severe root pruning [34]. Also, *Rotylenchulus* spp. attacked the fibrous roots reducing the absorption ability and other physiological functions of the plant.

Fig 10 Pineapple plants in the sampling site showing symptoms of plant parasitic nematode infection. **(A)** Rotten and impaired root system; **(B)** stunted growth and discoloration on the leaf.

Nevertheless, plant diseases are also caused by unfavorable environmental conditions. Temperature, moisture, light and the properties of the soil are environmental factors which could affect the plant growth. There is an optimum range for each environmental factors within which plants grow best, however, growth abnormalities might exhibit if the level of environmental factor varies greatly from its optimum, or severely impacts on another factor (Ogle, n.d.). Therefore, biotic and abiotic factors not only greatly affect the growth of plants but are also considered as factors of plant diseases, which were observed in the sampling area.

C. *Nematode Population*

Of the ten set-ups each with 100 g of soil samples, *Helicotylenchus spp*. was the most prevalent plant parasitic nematode species encountered. The population density of *Helicotylenchus spp.*, observed from the soil samples is shown in Table 3.

Table 3. Population Density of *Helicotylenchus spp*. Associated with Soil and Root Samples of *A. comosus*

Collection Schedule	**Nematode Population (%)**			
	Soil Sample	**Frequency Percentage**	**Root Sample**	**Frequency Percentage**
1st collection	39.54%	51.8%	25%	0.6%
2nd collection	37.18%	99.8%	30.43%	1.4%
Total Percentage	37.95%	75.8%	28.57%	1%

The result showed that *Helicotylenchus* spp. covered 39.54% of the nematode population with 51.8% frequency rating for the first collection. Subsequently, comparatively 37.18% of *Helicotylenchus* spp. manifested on the second sample size with 99.8% of frequency rating. It is noticeable that the frequency rating of *Helicotylenchus* spp. on the second collection was relatively higher than the first collection nevertheless, the attained combined frequency rating data still suggest that *Helicotylenchus* spp., favors the clay loams type of soil; this result corresponds with those of other researchers who have conducted similar studies [18, 19].

On the other hand, Table 4 presented the low occurrence of Helicotylenchus spp., in the collected root samples with 0.6% frequency rating from the first collection and a fair increase to 1.4% in the second collection thereby, proposing that not all plant parasitic nematodes that infest the soil can also be abundant in roots. Nematode occurrence is highly dependent on the presence of host plants. However, its presence is likewise influenced, more or less, by most of the biological, chemical and physical components of the soil environment [24]. Having been observed, the trophic level of the recorded nematode species shows its dependence on the presence of their host plant. Furthermore, chlorosis, necrosis, and stunted growth on the pineapples can be inferred that, that is due the presence of the identified plant parasitic nematodes.

4. CONCLUSIONS AND RECOMMENDATIONS

Plant parasitic nematodes identified in pineapple fields in Cavite, Batangas and Laguna were *Rotylenchus* spp., *Ditylenchus* spp., *Helicotylenchus* spp., and *Rotylenchulus* spp. The most prevalent nematode identified was *Helicotylenchus spp* that might have affected the crops manifesting symptoms such as chlorosis, necrosis and stunting. Studies on plant parasitic nematodes in the Philippines are still limited and so does the knowledge of farmers regarding these parasites, thus, further studies with wide sampling sites on the occurrence of plant parasitic nematodes in pineapples is recommended. To further identify the PPN into species level, molecular identification is recommended.

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