



Original article

Identification and prevalence of plant-parasitic nematodes associated with corn in Southern Cebu

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ABSTRACT

Plant-parasitic nematodes are reported to be associated with corn and are one of the limiting factors for yield reduction. A survey was conducted to identify genera of plant-parasitic nematode associated with corn and its prevalence in Southern Cebu, Philippines. Root and soil samples were collected from five corn growing municipalities of Cebu (Santander, Argao, Badian, Alegria and Barili, Cebu). Nematodes from roots and soil samples were extracted following standard methods and were identified to genus level based on morphology. A total of eight (8) plant-parasitic nematode genera (*Criconebella*, *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne*, *Paratylenchus*, *Pratylenchus*, *Tylenchorhynchus*, and *Tylenchus*) belonging to seven (7) different families were identified. Among these identified genera, *Pratylenchus* were the most abundant in corn roots and *Helicotylenchus* in soil rhizosphere. Both genera were found to have high frequency of occurrence and abundance in different corn fields surveyed. The other genera were less prevalent as manifested by their relatively low abundance and frequency of occurrence. This information may raise awareness of farmers on prevalent plant-parasitic nematodes in their area and guide in undertaking appropriate management actions.

KEYWORDS: *corn, Criconebella, Pratylenchus, Helicotylenchus, Meloidogyne, Hoplolaimus, Paratylenchus, Tylenchorhynchus, Tylenchus, morphological identification, nematode genera*

1 INTRODUCTION

Corn (*Zea mays* L.) is one of the most important cereal crops in the Philippines as it provides livelihood and food to millions of Filipinos. Corn is also a great source of animal feed and has a major role in the feed industry (Sailer, 2012; AMAD, 2011). Worldwide

production of corn reached 791.8M mt valued at US \$ 38.4 billion with a production area of around 158M hectares (FAOSTAT, 2009). In the Philippines, corn production attributed 7.38M mt in 2013 (BAS, 2014) and occupied 24% of the agricultural area consisting of about 1.8M farms. Majority of these farms are found in Mindanao and only 2.35% of the total production is from Central Visayas (BAS, 2014) which accounted for 53% of the regions' total output in 2013 (BAS, 2014). Corn is usually grown under an upland agro-ecosystem and prefers well-drained, fertile soils (Robberts, 2014).

Despite the advancement of corn production in the country, its yield has been hampered by several limiting factors that include the occurrence of plant-parasitic nematodes. These nematodes were reported as one of the greatest threats to crops throughout the world (Handoo, 1998). In corn, they caused considerable yield loss which was often undetected or attributed to other causes such as drought or nutrient deficiencies (Paul *et al.*, 2012). However, loss estimates of corn due to plant-parasitic nematodes reached up to 10% in the US (Tylka *et al.*, 2011). Nematode infestation in corn may result in thin stands, stunted plants, uneven plant height and tasseling, yellowing and small ears and kernels, root swelling, lack of fine roots and root branching and necrotic root lesions (Tylka, 2012).

About 120 species of plant-parasitic nematodes were reported to be associated with corn worldwide and more than 60 species were noted in North America (Tylka *et al.*, 2011) and in the Midwest (Tylka, 2012). Important genera reported included: *Xiphinema*, *Hoplolaimus*, *Longidorus*, *Paratylenchus*, *Mesocriconebella*, *Meloidogyne*, *Pratylenchus*, *Hemicycliophora*, *Helicotylenchus*, *Belonolaimus*, *Paratrichodorus*, *Tylenchorhynchus* and *Quinisulcius* (Tylka *et al.*, 2011). Lesion nematodes (*Pratylenchus* spp.) were considered the most important genus affecting corn production due to their prevalence (Niblack, 2003; Crow and Brammer, 2008). These nematodes were found to occur widely with population densities of 10,000 nematodes/cm³ of soil and noted in a wide range of soil

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types.

In the Philippines, studies on the major plant-parasitic nematodes associated with corn are very limited. Only *Pratylenchus zaei* was reported as the most important nematode associated with corn in the country (Davide, 1988). Knowledge on the identification and prevalence of plant-parasitic nematodes associated with corn are important in understanding their contribution to corn yield reduction and in undertaking appropriate management actions. This study was carried out to identify plant-parasitic nematode genera associated with corn and its prevalence in Southern Cebu.

2 MATERIALS AND METHODS

Sampling

Plant and soil samples were collected from five corn growing municipalities in Southern Cebu (Alegria, Argao, Badian, Barili and Santander) last January, 2015. In each municipality, three corn fields were randomly sampled and soil characteristics (texture, pH, temperature, moisture), corn varieties grown and management practices employed were recorded. In each field, five sample plants with symptoms of nematode infection or damage were collected by carefully uprooting the plant. Approximately 200 g of soil samples were also collected from the soil rhizosphere of up to 15 cm depth using hand trowel or bolo. Soil samples collected in each field were mixed to make a composite soil sample of about 1 kg. Plant and soil samples were placed in plastic bags and were stored in a cooler to avoid drying which were then brought to the laboratory for extraction and identification.

Nematode extraction from plant roots and soil

Nematode extraction was done at the Crop Protection laboratory of the Department of Crop Science, CTU- Barili Campus. Plant root samples were washed with running water and were chopped into small pieces approximately up to 1 cm using a pair of scissors. Then, 5g of chopped roots were macerated for about six seconds at low speed in a kitchen blender (Osterizer). Macerated roots were placed into an improvised net sieve (~0.6 mm) lined with tissue paper and placed over a bowl following a modified Baermann tray method. Water (~200 ml) was added to the tray just enough to touch the bottom part of the mesh to get a clear suspension. Then, the set-up was incubated at room temperature for 48 hours to obtain young and mobile adults. Application of water mists were done whenever necessary to avoid drying up of set-ups.

Nematodes were extracted from 300 ml soil taken from a composite sample using a combination of decanting and sieving (using a 212 μm and 53 μm aperture size) methods followed by incubation in a modified Baermann tray method. Soil sample was placed on an improvised net sieve (~0.6 mm) lined with tissue

paper, placed into a dish with water (~200 ml) and left at room temperature for 48 hours.

Nematode preservation

After the specified extraction time, nematode suspensions were collected into test tubes and were allowed to settle for three hours. The supernatant was sucked using a pipette to reduce the volume of suspension. Nematodes, then, were killed and fixed by adding hot (70°C) formaldehyde (4%) into the nematode suspensions. Preserved nematodes were placed in the refrigerator at 4 °C until identification and assessment of population density were done.

Identification of plant-parasitic nematode genera

Extracted nematodes from soil and root samples were manually picked using a picking needle from the clear suspension under the stereomicroscope (Olympus, Germany) and were mounted to a glass slide with a drop of water covered with cover slip and sealed with colorless nail polish to prevent drying up of mounts. Plant-parasitic nematodes were identified to genus level following keys and references of Siddiqi (2006) and Mekete *et al.* (2012).

Assessment of nematode population densities and its prevalence

The number of extracted plant-parasitic nematode genera from root and soil samples per plant per farm was counted in three 1 ml aliquots in a counting dish under a stereomicroscope (Olympus, Germany). Nematode counts were expressed as nematodes per 1L soil and one-gram roots. The prevalence of plant-parasitic nematode genera in roots and soil was determined using the relationship between abundance index and frequency of occurrence. Abundance index (AI) was calculated as the total number of nematodes per liter of soil or per gram of fresh roots for all samples containing that genus divided by the number of positive samples for that genus and expressed as a decimal logarithm. Frequency of occurrence (F) was computed through division of the number of farms or crops where the nematode was observed on the total number of farms or crops and expressed as a percentage. Based on the limits established by Fortuner and Merny (1973), a nematode was regarded as abundant if abundance ≥ 1.3 (20 individuals/g of roots) ≥ 2.3 (200 individuals/L of soil) in roots and soil, respectively. A nematode was regarded as frequent in the soil or roots when observed in at least 30% of the samples.

3 RESULTS AND DISCUSSION

Plant-Parasitic Nematode Genera Associated with Corn

Different genera of plant-parasitic nematodes were recovered from corn fields in Southern Cebu, Philippines (Table 1, Fig. 1). A total of eight (8) genera of plant-parasitic nematodes belonging to seven (7) different families were noted in the surveyed areas. The genera of plant-parasitic nematodes found associated with corn included: *Criconemella* (Criconematidae), *Helicotylenchus* (Hoplolaimidae), *Hoplolaimus* (Hoplolaimidae), *Meloidogyne* (Meloidogynidae), *Paratylenchus* (Tylenchulidae), *Pratylenchus* (Pratylenchidae), *Tylenchorhynchus* (Dolichodoridae), and *Tylenchus* (Tylenchidae). All of the nematode genera were found in the soil but only three (3) genera were recovered in the roots of corn plants. Distribution and prevalence of these genera varied in different sites.

The identified nematode genera of the present study were also reported from previous works (Niblack, 2003; Windham, 1988; and Tylka, 2007). This indicated that they are common nematodes associated with corn. Windham (1988) reported that *Meloidogyne*, *Pratylenchus*, *Belonolaimus*, *Longidorus*, *Paratrichodorus*, *Dolichodorus*, *Heterodera*, *Xiphinema*, *Hoplolaimus*, *Criconemella*, *Helicotylenchus*, and *Tylenchorhynchus* were some of the nematodes that caused yield loss of corn in the US.

Table 1. Plant-parasitic nematodes associated with roots and rhizosphere of corn grown in Southern Cebu.

Roots		Rhizosphere	
Family	Genus	Family	Genus
Hoplolaimidae	<i>Helicotylenchus</i>	Criconematidae	<i>Criconemella</i>
Meloidogynidae	<i>Meloidogyne</i>	Meloidogynidae	<i>Meloidogyne</i>
Pratylenchidae	<i>Pratylenchus</i>	Tylenchulidae	<i>Paratylenchus</i>
		Pratylenchidae	<i>Pratylenchus</i>
		Dolichodoridae	<i>Tylenchorhynchus</i>
		Tylenchidae	<i>Tylenchus</i>
		Hoplolaimidae	<i>Helicotylenchus</i>
			<i>Hoplolaimus</i>



Figure 1. Some of the plant-parasitic nematode genera under light microscope. *Criconemella* (A); *Helicotylenchus* (B); *Meloidogyne* (C); and *Pratylenchus* (D). A-D, 10x magnification.

Prevalence of plant-parasitic nematodes associated with corn

Population densities of plant-parasitic nematodes associated in corn roots (Figure 2), and in the rhizosphere soil (Figure 3) differed. Of the three (3) genera found in corn root samples, *Pratylenchus* had

the highest population density of 14/300 g soil followed by *Meloidogyne* (8/300 g soil) and *Helicotylenchus* (6/300 g soil). In soil, *Helicotylenchus* had the highest population density of 100/300 g soil among the nematode genera present. *Pratylenchus* was found to occur at higher population density (55/300 g soil) along with *Hoplolaimus* (36/300 g soil), *Meloidogyne* (30/300 g soil), and *Tylenchus* (27/300 g soil). The rest of the nematode genera (*Paratylenchus*, *Tylenchorhynchus* and *Criconemella*) were found to occur at lower densities that ranged only from 15 to 20/300 g soil. Results of the present study confirmed that *Pratylenchus* is an important nematode associated with corn as reported by Davide (1988). He identified *Pratylenchus zaeae* as the most important plant-parasitic nematode of corn. The high population densities of *Pratylenchus* in corn roots can be accounted to the fact that they are migratory endoparasites. As such, they are usually found inside the root tissues of corn. While *Helicotylenchus* are ectoparasitic (Tylka, 2009) thus found mostly on soil resulting in high recovery in the soil samples.

Pratylenchus, *Helicotylenchus* and *Meloidogyne* occurred in most roots and soil rhizosphere of corn plants among the plant-parasitic nematode genera identified (Figure 3). *Pratylenchus* and *Helicotylenchus* were present in all soil samples while *Pratylenchus* and *Meloidogyne* occurred in 83% and 73% of the root samples, respectively. *Helicotylenchus* were found in 47% of the root samples and the rest of the genera occurred at relatively low frequency. In soil samples, *Hoplolaimus* and *Meloidogyne* had a frequency of occurrence of 67% followed by *Tylenchorhynchus* (F, 60%) and *Paratylenchus* (F, 60%) while the other genera had less than 50% frequency of occurrence.

Prevalence of plant-parasitic nematodes in terms of frequency and abundance in roots and rhizospheres varied (Figure 4). In roots, the prevalent nematode genus in terms of frequency of occurrence was *Pratylenchus* (F, 87%) followed by *Meloidogyne* (F, 73%) and *Helicotylenchus* (F, 47%) (Figure 4A). However, their abundance index ranged only from 0.4 to 1.1 which was below the abundance limit (AI, 1.3) given by Fortuner and Merny (1973). In soil, *Helicotylenchus* and *Pratylenchus* were the most prevalent in terms of frequency of occurrence as they were found to occur in all corn fields sampled (Figure 4B). However, their abundance index (AI, 2.2 and 1.7, respectively) was below the threshold limit (AI, 2.3). *Hoplolaimus*, *Meloidogyne*, *Tylenchorhynchus* and *Paratylenchus* were also considered frequent nematodes of corn as their frequencies ranged from 48% to 68% which were above the frequency limit (30%). *Tylenchus* was the only genus that had abundance (AI, 2.6) above the threshold limit but they were noted only in 20% of the fields. The remaining nematode genera found in soil were less prevalent and occurred below the frequency and abundance limits.

Among the genera identified in this study, *Pratylenchus*, *Meloidogyne* and *Helicotylenchus* were considered as omnipresent pathogens (Fortuner and Merny, 1973) in roots due to their high frequency of occurrence but not considered as potential pathogens since their abundance were low. In the soil, the same classification for these three genera along with *Hoplolaimus*, *Tylenchorhynchus* and *Paratylenchus* can be observed. The rest of the nematodes (*Criconebella* and *Tylenchus*) were considered as fortuitous parasites or don't have the ability to cause economic loss due to both low frequency and abundance as described by Fortuner and Merny (1973).

The low population density of plant-parasitic nematodes observed in this study can be attributed to several factors such as the management practices employed by the farmers. In fact, Norton (1978) stated that management practices such as tillage, crop rotation, and planting time can alter nematode populations. In relation, all sites practiced integrated farming (mixed farming-both organic and inorganic practices) which might account for the low population densities of nematodes. Rodriguez (1986) reported that organic amendments such as chicken litter, composts, and green manure released ammonia in the soil which can affect nematode population due to their low carbon: nitrogen ratio.

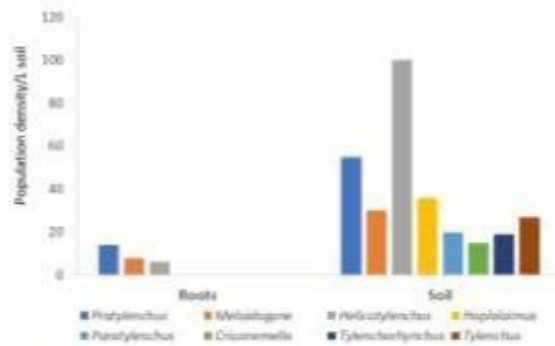


Figure 2. Population density of plant-parasitic nematode genera associated with roots and rhizosphere of corn grown in Southern Cebu.

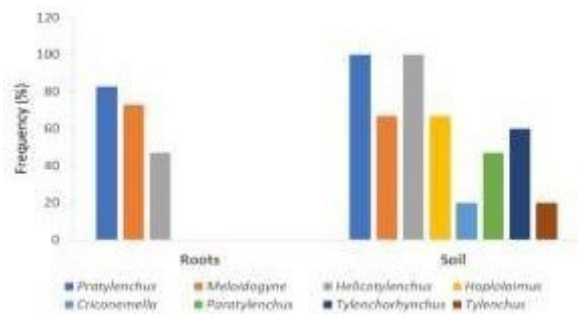


Figure 3. Frequency of occurrence (F) of plant-parasitic nematode genera associated with roots and rhizosphere of corn grown in Southern Cebu.

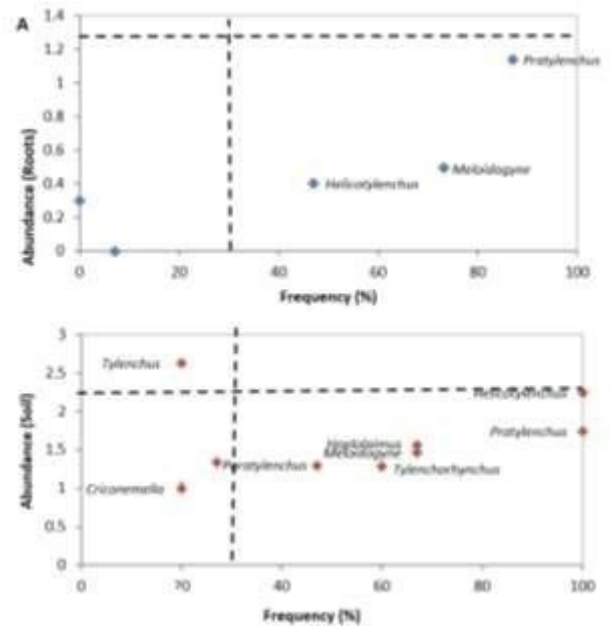


Figure 4. Frequency and abundance of plant-parasitic nematode genera associated with roots (A) and rhizosphere (B) of corn. Dotted vertical lines represent nematode frequency limit (F , 30%) and dotted horizontal lines represent the abundance threshold in roots (AI, 1.3) and in soil (AI, 2.3) according to Fortuner and Merny (1973).

Occurrence and abundance of plant-parasitic nematodes in the localities

Nematode genera from different municipalities in Southern Cebu varied in their frequency of occurrence and abundance (Table 2). *Helicotylenchus* were found to occur frequently and abundantly in all areas where survey was done. *Pratylenchus* were also prevalent in all locations except in Alegria, while *Meloidogyne* was prevalent in Argao and Badian. The rest of the nematodes were frequent but had a very low abundance in various areas visited. Based on the groupings by Fortuner and Merny (1973), *Pratylenchus*, *Helicotylenchus* and *Meloidogyne* are potential pathogens of corn in the area where they were found to occur frequently and abundantly while the other genera were identified as omnipresent nematodes because of their high frequency of occurrence but not potential nematodes due to their very low abundance.

4 CONCLUSIONS

Various plant-parasitic nematodes that differ in densities and prevalence are found associated with corn. *Pratylenchus* and *Helicotylenchus* are the most prevalent nematodes in roots and in soil, respectively, which pose problems in corn production if not managed. This information is relevant in raising farmer's awareness on

plant-parasitic nematodes prevalent in their area. Further efforts are necessary to study dynamics of plant-parasitic nematodes in other areas at different seasons and to identify prevalent nematodes to its species level for a thorough knowledge on the identity of important nematodes associated with corn to employ appropriate management strategies.

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Table 2. Frequency of plant-parasitic nematodes associated with corn in different localities.

Genus	Alegria	Argao	Badian	Barili	Santander
<i>Criconemella</i>	+		+		+
<i>Helicotylenchus</i>	+++	+++	+++	+++	+++
<i>Hoplolaimus</i>		+	+	+	+
<i>Meloidogyne</i>		+++	+++	+	+
<i>Paratylenchus</i>	+	+	+	+	
<i>Pratylenchus</i>	+	+++	+++	+++	+++
<i>Tylenchorhynchus</i>	+	+	+	+	
<i>Tylenchus</i>		+++			

Notes: + = frequent (present in more than 30% of the fields surveyed); ++ = abundant (AI ≥ 2.3/L soil); and +++ = frequent and abundant (F ≥ 30% and AI ≥ 2.3/L soil).

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