





Survey and Identification of Plant-Parasitic Nematodes Infecting Planting Materials of Garlic (*Allium sativum*) in Major Growing Areas of Ilocos Norte, Philippines

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Abstract

Ilocos region is the main producer of garlic in the Philippines. Bulbs from farmers' previous harvest constitute the primary source of planting materials. While fungi, viruses, and bacteria are monitored, the equally damaging nematodes are not being reported. Hence, this study was conducted to determine the occurrence of plant-parasitic nematodes (PPNs) in major garlic growing areas in Ilocos Norte, Philippines. A total of 198 planting materials were randomly collected from these municipalities: Pasuquin, Vintar, San Nicolas, Batac City, and Pinili. Two PPNs were isolated from plant parts such as roots, basal plates, and cloves. Ditylenchus dipsaci is abundant in the clove and basal plate, while Aphelenchoides besseyi is abundant in the clove only. The abundance index of PPN (1.56-2.07) is above the threshold level in all municipalities surveyed. Abundance indices of D. dipsaci, A. besseyi, and their combined population are recorded at 1.76, 2.05, and 2.23, respectively. The nematodes at these levels have the potential to become economically important parasites in the locality where they were found.

Introduction

Garlic (*Allium sativum* L.) is among the 19 major commodities produced in the Philippines and is mainly produced in Region 1. In 2015, Region 1 recorded the largest area harvested at 2.7 thousand hectares representing 75.6% of the total national area, followed by MIMAROPA at 11.2%. It contributed 69.7% of the country's 10.4 tons production, while MIMAROPA, Cagayan Valley, and Central Luzon contributed 18.6%, 6.2%, and 3.6% to the nation's garlic production, respectively. Nonetheless, the country's total production is insufficient; hence in the same

year, 90% of the domestic supply (74,000 metric tons) was imported (Philippine Statistics Authority [PSA], 2017). These figures indicate that the country is virtually import-dependent.

In Ilocos Norte of Region 1, garlic is among the five major commodities planted with a total area of 1,682 and 1,892.47 hectares in 2017 and 2018, respectively (Department of Agriculture-Regional Field Office 1 [DA-RFO1], 2020). Garlic is grown after rice in November to coincide with the cooler months, favorable for garlic crop production. But like any other crop, garlic production is faced with several problems that include plant diseases and the lack of quality seedlings. These problems severely reduced the quality and volume of production and triggered the entry of cheap imports and smuggled garlic and onion (De Vera-Ruiz, 2017). The common diseases of garlic are purple blotch (Alternaria porri), leaf blight (Stemphylium vesicarium), Cercospora spot (Cercospora duddiae) Fusarium bulb rot (Fusarium oxysporum f. sp. cepae (Hanzwa) Snyder and Jansen), pink rot (Phoma terrestris); and downy mildew (Peronospora destructor) (Bureau of Plant Industry [BPI], 2019). Additionally, PPNs associated with garlic such as Pratylenchus, Tylenchorhynchus, Helicotylenchus, Ditylenchus, Meloidogyne, and Rotylenchulus were observed in the Philippines (Baniqued, 1982; Vera Cruz & Alberto, 1988).

Ditylenchus dipsaci has a very low economic threshold level. Subbotin et al. (2005) recorded that 10 nematodes per 500 grams of soil can cause economic damage to crops, while Van Bezooijen (2006) observed even worse - at one nematode per kilogram of soil. Yield loss in fields heavily infested with D. dipsaci could reach 60-80% (Sturhan & Brzeski, 1991) and even complete crop loss (Testen et al., 2014). In Germany, 2-3 nematodes per 250cm³ soil indicate the risk of plants to infection (Center for Agriculture and Bioscience International [CABI], 2019). This characteristics explains why D. dipsaci is almost of worldwide quarantine concern (Jones et al., 2013), the fifth nematode of economic importance, and ranked 174 in the A2 list of European and Mediterranean Plant Protection Organization (EPPO) in 2017.

In the past decades, PPNs affecting garlic have not been given importance in the Philippines. However, in the last two years, garlic production in Region 1 was observed to have stunted, prematurely defoliated, and smaller bulb garlic that affected its quality and price. Symptoms of the disease were first associated with a virus; however, the researchers' initial diagnosis revealed the presence of PPNs in the samples collected from the field. This observation suggests that nematodes may be linked to the disease and accurate identification is key to effectively manage the parasite. The objectives of this study were to determine the occurrence of plant-parasitic nematodes and identify associated PPNs with the planting materials of garlic in the major growing areas of Ilocos Norte, Philippines.

The result of this study can be used in crafting guidelines for monitoring PPNs not only in Region 1 but also in other garlic-producing areas in the Philippines. The guidelines could also be used in setting standards for policy formulation concerning the movement or transfer of garlic planting materials in and out of the region. Furthermore, the results can be used to develop management strategies against PPNs to mitigate potential economic loss.

Materials and Methods

Survey and Collection of Garlic Samples

Plant samples were collected in December 2018 to February 2019 from major growing areas of garlic in Ilocos Norte, Philippines, namely Pasuquin, Vintar, San Nicolas, Batac City, and Pinili. A total of 198 garlic samples were collected from 1.98ha planted with Ilocos White garlic variety. For every 1,000 m² area planted with garlic, a total of 10 garlic plants were randomly collected across the field in a W pattern. Samples obtained from each field were wrapped separately in paper, placed in a plastic container with a complete label, and stored in a cooler (Van Bezooijen, 2006).

Extraction of Nematodes

A composite sample of 10 sample plants per site were used. The soils attached to the roots of garlic samples were properly removed before extraction. Roots, basal plates, and cloves were finely chopped into \pm 1cm thin. These were placed in a sieve with tissue paper using the modified Baermann method. Water was added to cover the bottom of the plate lying beneath the plant tissue and was incubated for 48 hours. The suspension was collected and was placed in a labeled test tube. The suspension was allowed to settle for 2-3 hours, and excess water was siphoned, reducing it to 10ml.

Killing and Fixing Nematodes

This study adopted the procedure of Seinhorst (1962). The nematode suspension was settled for at least 1 hour, and the excess water was carefully siphoned, leaving approximately 5ml suspension. This suspension was transferred to a smaller tube with a cover and was settled again for an hour before siphoning as much water out from the



suspension. The suspension was then heated in a water bath for 4 minutes at 70°C, and an equal amount of heated (approximately 70°C) Formalin Acetic Acid combination was added to the suspension. It was mixed by gentle agitation before letting it stand for 24 hours or more for the fixative to act on the nematode tissue.

Morphological Characterization

Five nematodes were picked and transferred to a prepared slide with a paraffin ring. Before sealing, the position of the nematode was checked under a dissecting microscope and was carefully arranged to avoid overlapping. A coverslip was placed on top of the paraffin ring. Nematodes were observed under the microscope at 40x to 100x for characterization. Five males (if present) and females were picked for observation of morphological features and measurement of prominent body organs. Nematodes were identified using available taxonomic keys (Coyne et al., 2007; Luc et al., 2005). Major morphological characteristics of male and female PPNs were observed and recorded, such as the shape of head, stylet and stylet knob, median bulb, esophagus, the position of vulva, tail, and tail terminus, and spicule.

Population Density and Abundance of Nematodes

Nematodes were counted for each sample from every area surveyed in a counting dish in 10x magnification using the binocular compound microscope. The population density, frequency of occurrence, and abundance of PPNs were calculated following the formula by Fortuner and Merny (1973). Nematode occurrence and potential damage were estimated based on these calculated nematode indices.

The data gathered were population density and abundance index. Population density is the mean of observed nematodes per Barangay or collection site computed using the following formula:

Population Density = <u>Total Number of PPN observed</u> total samples

Abundance Index (AI) is the logarithm of the mean of observed nematode population density. Abundance (x) was plotted against Frequency (y). The population threshold of the nematode was set

at 20 individuals per g roots (AI $\frac{1}{4}$ 1.3), while the frequency was set at 30% (Pedroche et al., 2013).

Results and Discussion

A total of 198 garlic samples were collected from nine barangays in the five different major garlic growing municipalities of Ilocos Norte, namely: Pasuquin, Vintar, San Nicolas, Batac City, and Pinili. The total area surveyed was 1.98 hectares owned and managed by veteran garlic farmers with at least five years of farming experience. All the farmers interviewed had planted the same variety of garlic, i.e. Ilocos White. The majority (89%) of farmers obtained their planting materials from the Department of Agriculture, and 11% reported using their own produce as planting materials stored and taken from the previous harvest. Farmers in this region mainly practice rice-based cropping systems, and garlic is planted only after rice. Their cropping patterns depend on the availability of irrigation and planting materials and follow this sequence: rice-garlic/corn/tobacco-corn/vegetable/fallow.

In the areas surveyed, garlic plants manifested disease symptoms akin to nematode infection such as rotten roots and easily uprooted plants, stunted and uneven growth. According to some farmers, garlic production in Ilocos Norte has had the same disease problem the previous year, and the quality of their produce has been greatly affected (Salveda et al., 2020, personal communication). In Puyupuyan, Pasuquin, Ilocos Norte, stunted and yellowing garlic plants were observed in patches. In San Marcos, San Nicolas, Ilocos Norte, because of the state of their garlic plants, farmers no longer maintained their plant to avoid incurring additional expenses.

Moreover, garlic bulbs collected from different sites were notably small and exhibited symptoms of the disease. Usually, a healthy garlic bulb retains its whitish to tan color when cut along the basal plate. Roots are strongly attached to the basal plate and do not fall off or easily detached when pulled (Figure 1A). Diseased garlic bulbs showed light brown to dark brown discoloration of the basal plate. The basal plate and roots are easily separated, and the middle of the basal plate and the bulb's base become disintegrated (Figure 1B). In other instances, no discoloration was observed; however, the loss of roots in undeveloped bulbs leads to premature defoliation and death of the plant (Figure 1C). In advance infections, discoloration and cracking of the base of the clove (Figure 1D) are commonly observed, and invasion of the weakened bulb by secondary pathogens is inevitable, leading to rotting of bulb and death of the entire plant. As a result, some farmers harvest their crops and sell them as "green garlic" making them dependent on traders or other farmers for the source of seedling for the next cropping season.

Morphological Characterization of Plant-Parasitic Nematodes

Two PPNs were observed from the garlic samples collected from different municipalities. Moreover, both nematodes were present in all sampling areas and all the parts of garlic, namely roots, basal plates, and cloves. Characterization of the two predominant nematodes revealed the diagnostic features for important PPNs of garlic.

The first predominant nematode observed is

slender and has a straight to ventrally arcuate body shape (Figure 2A). The head or cephalic region is hemispherical and barely set off from the body. The stylet is delicate with a very small distinct knob which sometimes disappears when nematodes are fixed in reagents (Figure 2B). The median bulb is oblong to pear-shaped, and the valve is not prominent and sometimes missing (Figure 2C). No overlapping was observed in the esophagus-intestine region (Figure 2F). The vulva is very distinct, sclerotized, large, slit-like, and observed at 60-70% from the anterior (Figure 2E). The tail is tapered with a single mucron (Figure 2D). For this sample, no male was observed.

Comparison and analysis of the morphofeatures with available reports show that the first PPN is identified under genus *Ditylenchus*, similar to the observations of various authors (French et al., 2017; Indarti et al., 2018; CABI, 2019; EPPO, 2017). Compared to other *Ditylenchus* species, distinct characteristics such as the tail and tail terminus conform to *Ditylenchus dipsaci* (Kuhn, 1857) *Filipjev 1936* (International Plant Protection Convention [IPPC], 2015).

Figure 1

Healthy (A) and Diseased Samples of Garlic Bulbs Used as Planting Materials in Ilocos Norte Showing Various Stages of Symptom Development: Necrotic Lesion (B); Presence of Secondary Fungal Infection (C); and Necrosis with Cracking (D)



Morphological Traits of Ditylenchus dipsaci at 100x Magnification (A - Whole Body Image; B - Head Region and Stylet (Arrow); C - Nematode Median Bulb; D - Nematode Tail and Tail Terminus with 1 Mucron (Arrow); E - Vulva of Female D. dipsaci (Arrow); F – Esophagus Showing a Slight Overlapping with the Intestine)



The second predominant nematode observed is also slender and vermiform in shape. Female is almost straight to ventrally arcuate when relaxed, while the juvenile stage resembles the female but lacks genital structures and the male is almost straight but bent ventrally at the posterior part (Figure 3A). The head/cephalic region is hemispherical to flat and is set off from the body by a constriction. The stylet knob is small, resembling the shape of an immature onion or garlic (Figure 3B). The median bulb is prominent, roundish to oblong, valvate, and large, almost filling the cavity (Figure 3C). The esophagus slightly overlaps the intestine dorsally (Figure 3E). Female vulva, observed at 60-70% distance from the head, is also sclerotized, slit-like, and slightly raised (Figure 3D). Post vulva sac extends more than halfway towards the anus. The tail terminus is conoid bearing mucron. In females, stellate mucron, appearing to have three mucrons, was observed (Figure 3F1), while only one mucron was observed in males and juveniles. Males are common, possess large, rose thorn-shaped and smooth spicule with short and simple gubernaculums (Figure 3F2). All the developmental stages of the nematode, except the egg, were observed.

The morpho-features of the second PPN are of genus *Aphelenchoides* and are consistent with the observations of Kohl (2011), CABI (2019), and EPPO (2017). Its identification down to species level proved difficult since some of the morphological features observed were similar to different species reported. However, the majority of the morphological characteristics observed conform to the description of *A. besseyi* Christie, 1942 (IPPC, 2016).

The morphological characteristics provided important information to reveal the identity of the isolated nematodes from the diseased samples. *Ditylenchus dipsaci* and *A. besseyi* were easily differentiated from each other based on the morpho-features previously mentioned. Both PPN females have the same body shape when relaxed, they possess a stylet with a delicate knob, and their vulvas were positioned at almost the same distance from the anterior body region. However, the position of the esophagus, shape of the median

Morphological Traits of Aphelenchoides besseyi at 100x Magnification (A - Whole Body Image Showing Adult Female (A1), Juvenile Stage (A2) and Male (A3); B - Head Region and Stylet (Arrow); C - Nematode Median Bulb; D1 - Vulva of A. besseyi Female; D2 - Spicule of Male; E - Nematode Esophagus Showing No Overlapping with the Intestine; F – Nematode Tail and Tail Terminus with A. besseyi Female with 3 Mucrons (F1) and Male with 1 Mucron (F2))



bulb, characteristics of the vulva, shape of the tail, and the number of mucrons have set them apart. The cephalic region was also slightly different. In both cases, molecular identification is essential to confirm the identity of the two PPNs.

The occurrence and identification of two of the most important plant parasites of garlic in this study, D. dipsaci and A. besseyi, would likely pose a threat as they are known to cause significant damage to crops. D. dipsaci has been reported to cause damage to garlic and other economically important crops worldwide (Douda, 2005; Saeed, 2015; Indarti et al., 2018; French et al., 2017 & Testen et al., 2014). Likewise, A. besseyi, the causal organism of white tip disease of Oryza sativa has been found to infest vegetables in Allium family (IPPC, 2016). Other species of Aphelenchoides, A. saprophilus Franklin, 1957, has been reported parasitizing garlic, for which it could be an important pest (Balasubramanian et al., 2002). It was also observed in the flowers, stalk,

leaf, scales and bulb of tuberose in India (Khan & Ghosh, 2011). In a similar study, *A. subtenuis* was observed to cause infection on the root and bulb of Narcissus in Israel. The symptoms in the plant include premature yellowing of the leaves, root rot, and discolored and sunken basal plates (Mor & Spiegel, 1993).

Population Density of Plant-Parasitic Nematodes

Table 1 summarizes the mean population density of nematodes per sampling site. The data showed that both PPNs were observed in all collection sites but varied in population. This result indicates that PPNs were widely distributed in the major garlic growing areas in Ilocos Norte, Philippines, and probably other areas in the province. The very high frequency of occurrence could possibly be attributed to the introduction of infected planting materials from one place to another since this nematode can persist in the

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bulb for a long time. In the Philippines, 40% of garlic farmers were reported to use their own seeds as planting materials, 24% obtained it from the Department of Agriculture, 8% from traders, and 7.56% from input dealers (PSA, 2014).

Mean population densities of D. dipsaci range from 6-15 nematodes with the highest mean population density observed in samples obtained from Dariwdiw, Batac City, Ilocos Norte, and the lowest mean population density observed in samples obtained from Sta. Asuncion, San Nicolas, Ilocos Norte. On the other hand, A. besseyi has a mean population density ranging from 16-29 nematodes. The highest mean population density was observed from Puritak, Pinili, Ilocos Norte samples, while the lowest was observed from Cabisuculan, Vintar, and Ilocos Norte samples. The combined mean population densities of both D. dipsaci and A. besseyi per sampling site range from 24-40 nematodes. The highest combined PPN population was noted in Puritak, Pinili, Ilocos Norte samples while samples from Cabisuculan, Vintar, and Sta. Asuncion, San Nicolas have the lowest combined PPN population. Further analysis revealed that *D. dipsaci* has an average population of 2 nematodes per plant while A. besseyi has an average population of 5 nematodes per plant. Moreover, it was observed that in all collection sites, A. besseyi is the predominant PPN.

This study has shown and confirmed the presence of PPNs in garlic in various barangays

and municipalities in Ilocos Norte. Their number varied from high (>20 individuals/g root) to low (≤20 individuals/g root). However, the mere presence of these nematodes in garlic bulb planting materials is alarming as they could already be damaging in low numbers. Similar studies have reported these nematodes in various countries (Indati et al., 2018; Subbotin & Chitambar, 2018; Balasubramanian et al., 2002). In Indati et al. (2018) study, D. dipsaci was observed in garlic with an average of 2.67 nematodes per 100g of soil and 2.67-189.33 nematodes per garlic bulb. On the other hand, Subbotin and Chitambar (2018) reported a population density of 3,609 nematode/gram of garlic tissue. Sundaram et al. (1990) observed the presence of A. saprophilus from soil and garlic bulb samples in India, and it has been reported as a parasite of garlic that may be considered an important pest (Balasubramanian et al., 2002).

In terms of the infected parts of garlic, *D. dipsaci* was less abundant in roots and more abundant in basal plates and bulbs. On the other hand, *A. besseyi* was less abundant in basal plates and more abundant in bulbs (Table 2). From this result, it could be inferred that the movement of *D. dipsaci* is restricted in stem and bulb while *A. besseyi* is more of a foliar nematode. This result also shows that *D. dipsaci* was able to establish feeding in both the basal plate and bulb while *A. besseyi* was found to infect all plant parts. This result is consistent with Charchar et al. (1980) that noted high population densities of

Municipality	Barangay	Ν	Mean Population Density per Barangay		
			Ditylenchus dipsaci	Aphelenchoides besseyi	Total PPNs
Pasuquin	Nagsanga	30	11	20	31
	Puyupuyan	30	9	19	28
Vintar	Cabisuculan	25	8	16	24
San Nicolas	San Marcos	30	11	21	32
	Sta Asuncion	8	6	18	24
Batac City	Tabug	32	10	17	27
	Dariwdiw	30	15	23	38
Pinili	Puritak	7	11	29	40
	Capangdanan	6	9	24	33

N= Total number of samples observed; high (>20 individuals/g root) and low (\leq 20 individuals/g root)

Table 1

Summary of Population Density of Plant-Parasitic Nematodes (PPN) per Barangay in Ilocos Norte, Philippines

Table 1

Summary of Population Density of Plant-Parasitic Nematodes (PPN) Extracted from Different Garlic Plant Parts in Ilocos Norte, Philippines

Plant Part	Ν	Mean Population	Mean Population of Plant Parasitic Nematodes		
		Ditylenchus dipsaci	Aphelenchoides besseyi		
Root	10	8	21		
Basal Plate	10	11	20		
Bulb	10	11	26		

N= Total no. of samples observed

D. dipsaci in both the bulb and stem of garlic. Indarti et al. (2018), on the other hand, reported that *D. dipsaci* also infects the root, tuber, and stem of the host. The nematode is also found in seed stocks of broad beans, red beets, carrots, shallot, leeks, and onions (Green & Sime, 1979).

On the other hand, earlier reports showed the association of a species of *Aphelenchoides*, *A. saprophilus*, in garlic (Sundaram et al., 1990), and its parasitism has been confirmed (Balasubramanian et al., 2002). Another species of *Aphelenchoides*; *A. besseyi*, was observed to be present in the flowers, stalk, leaf, scales, and bulb of tuberose in India (Khan & Ghosh, 2011). Similarly, *Aphelenchoides* sp. is a migratory ectoparasite and endoparasite affecting leaves, buds, stems, and sometimes corms (IPPC, 2016).

The infection of nematodes in various parts of the garlic plant can also be attributed to the movement within the plants as it may be influenced by environmental factors such as food availability, low and high temperatures, and host response. If they are exposed to extreme conditions, both nematodes have the capacity to produce survival structures and may aggregate in one area, thus showing levels of population in various plant parts. Aphelenchoides sp. may undergo anhydrobiosis, and adult and fourth-stage juveniles have been shown to overwinter in dried leaves and dormant buds but not in plant roots (Kohl, 2011). Ditylenchus sp. may also undergo cryptobiosis and form "nematode wool" on seeds and plant debris when plants begin to dry (IPPC, 2016). The observation of infection in various plant parts is essential, particularly in conducting post-harvest and pre-planting activities in garlic production to minimize inoculum levels in garlic planting materials. It may also lead to

the use of practical control methods such as roguing or removal of infected parts only prior to planting.

Abundance of Plant-Parasitic Nematodes

Figure 4 shows the abundance and potential of nematodes as plant parasites in the five major growing municipalities of Ilocos Norte. The abundance index of D. dipsaci as sole agent ranges from 0.92 to 1.41. In Pasuquin, San Nicolas, and Vintar, Ilocos Norte, its population is below the threshold level with values of 1.29, 1.24, and 0.92, respectively. But in Batac City and Pinili, Ilocos Norte, D. dipsaci has an abundance index value of 1.41 and 1.39, which are above the population threshold level. On the other hand, the abundance index of A. besseyi as sole agent ranges from 1.20 to 1.73. Its population threshold is low in Vintar, Ilocos Norte with a value of 1.20, while high in Pasuquin, San Nicolas, Batac City, and Pinili, Ilocos Norte with values of 1.58, 1.60, 1.60, and 1.73, respectively.

When both PPNs are combined, all areas have abundance indices exceeding the population threshold. This result is because both PPNs are present in the same plant. This result may indicate that individually, and in some areas, the nematode is not yet considered damaging but may pose a serious threat when the two species are infecting at once.

In Batac City and Pinili, Ilocos Norte, the abundance index of each PPN species exceeded the economic threshold level indicating potential economic yield loss as sole pathogen of garlic. Their combined population may even cause greater problems in the crop. The abundance index of combined PPN was highest in Pinili,

Abundance Index of D. dipsaci and A. besseyi in 5 Garlic Major Growing Areas in Ilocos Norte, Philippines, Namely: Pasuquin, Vintar, San Nicolas, Batac City, and Pinili.



Note: Nematode is Frequent if Found in More Than 30% of the Survey Area and Abundant if $AI \ge 1.3$

Ilocos Norte, with a value of 2.07, while lowest in Vintar, Ilocos Norte, with a value of 1.56. Taken overall, the cumulative abundance index value of *D. dipsaci* and *A. besseyi* in the province is already above the threshold level at 1.76 and 2.05, while the combined nematode abundance index value was even higher at 2.23 (Figure 5). This result means that as individual parasites, each PPN species can cause economic yield loss in garlic but even more so when both are present in the plant.

Plant-parasitic nematodes have varying economic threshold levels as affected by different factors like the crop, soil type, and other environmental factors. The economic threshold of *D. dipsaci* is mostly very low (Subbotin et al., 2005). A population density of 10 nematodes per 0.5kg of soil may lead to significant crop losses in onion, sugar beet, carrot, rye, and other crops; hence, planting nematode-free seeds is important in controlling the nematode. In the subtropic and tropical countries, 10 nematodes/400ml dry soil threshold was suggested by Sikora et al. (2018) in onion. A lower *D. dipsaci* damage threshold level of 1-5/500cc soil in onion was reported by Reddy (2019). In Germany, they are able to establish the risk of nematode transmission to seed with the tolerance level of five nematodes/ 300 seeds of faba beans, while a tolerance level of 2-3 nematodes/250ml soils is used to indicate

Frequency of Occurrence (%) and Abundance of Plant-Parasitic Nematodes in Ilocos Norte, Philippines.



Note: Nematode is Frequent if Found in More Than 30% of the Survey Area and Abundant if $AI \ge 1.3$

potential plant infection (CABI, 2019). On the other hand, the economic damage threshold level of *A. besseyi* in rice is 2.48 AI or 300 live nematodes/100 seeds (CABI, 2019; Feng et al., 2014).

Conclusions

Two plant-parasitic nematodes were found associated with the planting materials of garlic in the major growing areas of Ilocos Norte, Philippines. Based on their morphology, the nematodes were identified as Ditylenchus dipsaci (Kuhn, 1857) Filipjev, 1936 and Aphelenchoides besseyi Christie, 1939. They are widely distributed in garlic farms in Ilocos Norte, occurring in all the samples collected. The nematode D. dipsaci is highly abundant in farm areas in Batac City and Pinili, Ilocos Norte, while A. besseyi is highly abundant in Pasuquin, San Nicolas, Batac City, and Pinili, Ilocos Norte. Both nematodes are considered to have the potential to become economically important parasites in the succeeding cropping seasons of garlic and in the local farming areas where they occur without management interventions.

Recommendations

As the population of nematodes in garlic were found to be abundant in the areas surveyed, the extent of distribution of *D. Dipsaci* and *A. besseyi* must be determined to properly assess yield losses and determine sources of healthy planting materials. The PPNs must be subjected to further molecular analysis to verify their identity, including pathogenicity, alternate hosts, and management.

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References

- Balasubramanian, P., Devrajan, K., & Natrajan, S. (2002). Susceptibility of garlic accessions to white tip nematode, Aphelenchoides saprophilus Franklin, 1957. *Indian Journal of Nematology*, (32) 2: 208-209.
- Baniqued, C.A. (1982). A preliminary survey of the plant-parasitic nematodes on garlic (Allium sativum), cassava (Manihot esculenta), corn (Zea mays) and cotton (Gossypium spp) in Ilocos and the Visayas regions (Philippines). In: 13th Anniversary and Annual Convention of the Pest Control Council of the Philippines, Baguio City (Philippines). http://agris.fao.org/agris-search/ search.do?recordID=XB8540214.
- Bureau of Plant Industry. (2019). *Garlic Production Guide*. http://bpi.da.gov.ph/bpi/images/Produc tion_guide/pdf/Garlic.docx.
- Centre for Agriculture and Bioscience International. (2019). Ditylenchus dipsaci (Stem and Bulb Nematode). In: Invasive Species Compendium. Wallingford, UK: CAB International. https:// www.cabi.org/isc/datasheet/19287.

- Centre for Agriculture and Bioscience International. (2019). *Aphelenchoides besseyi (Rice Leaf nematode)*. In: *Invasive Species Compendium*. Wallingford, UK: CAB International. https://www.cabi.org/isc/ datasheet/6378.
- Charchar, J.M., Huang, C.S., Menezes-Sobrinho, J.A., & Lopes, C.A. (1980). Plant-parasitic nematodes identified from garlic plants (*Allium sativum* L. and *A. ampeloprasum* L.) collected from the main growing areas in Brazil. https:// worldveg.tind.io/record/12594/.
- Coyne, D.L., Nicol, J.M., & Caludius-Cole, B. (2007). Practical plant Nematology: A field and laboratory guide. SP-IPM Secretariat, International Institute of Tropical Agriculture (IITA), Cotonou, Benin. Pp. 72-74
- Department of Agriculture-Regional Field Office 1. (2020). *High Value Crop Program and Development Database on Garlic production area in Region I.* Unpublished.
- De Vera-Ruiz, E. (2017). DAR Cites Decline in Garlic, Onion Production. *Manila Bulletin*. https://news. mb.com.ph/2017/11/16/dar-cites-decline-ingarlic-onion-production/
- Douda, O. (2005). Host Range and Growth of Stem and Bulb Nematode (*Ditylenchus dipsaci*) Populations Isolated from Garlic and Chicory. *Plant Protect. Sci.*, 41(3): 104–108. https:// www.agriculturejournals.cz/publicFiles/16844. pdf. Accessed on March 6, 2020.
- European and Mediterranean Plant Protection Organization. (2017). *Aphelenchoides besseyi*. Bulletin *OEPP/EPPO Bulletin* 47(3): 384–40.
- European and Mediterranean Plant Protection Organization). (2017). *Ditylenchus dipsaci*. Bulletin *OEPP/EPPO Bulletin*.
- Feng, H., Shao, Y., Wei, L., GAo, C., & Zhou, Y. (2014). The white-tip nematode, *Aphelenchoides besseyi*, exhibits an auxin-orientated behaviour affecting its migration and propagation. *Nematology*, 16(7): 837-845. https://doi.org/10.1163/15685 411-00002812
- French, J.M., Beacham, J., Garcia, A., Goldberg, N.A., Thomas, S.H., & Hanson, S.F. (2017). First report of Stem and Bulb Nematode of Garlic in New

Mexico. http://dx.doi.org/10.1094/PHP-12-16-0069-BR.

- Fortuner, R., & Merny, G. (1973). Les ne´matodes parasites des racinesassocie´ s au rizen Basse Casamance (Senegal) et en Gambie, Cahiers de l'Orstom. Se´rie. *Biologique*. 21: 3–20.
- Green, C.D., & Sime, S. (1979). The dispersal of Ditylenchus dipsaci with vegetable seeds. Annals of Applied Biology. Wiley Online Library. https:// onlinelibrary.wiley.com/doiabs/10.1111/j.1744-7348.1979.tb03872.x.
- Indarti, S., Subandiyah, S., Wibowo, A., & Ajri, M. (2018). First Record: A stem and Bulb Plant-Parasitic Nematode at Garlic Area Centre Temanggung, Central Java, Indonesia with Species Reference to Ditylenchus dipsaci. https:// www.researchgate.net/publication/330099755_ First_Record_A_Stem_and_Bulb_Plant_ Parasitic_Nematode_at_Garlic_Area_Centre_ Temanggung_Central_Java_Indonesia_with_ Species_Reference_to_Ditylenchus_dipsaci.
- International Plant Protection Convention. (2015). Diagnostic Protocol for Regulated Pest *Ditylenchus dipsaci* and *Ditylenchus destructor*. DP_08_2015_ En_2015-12-22_Reformatted.pdf.
- International Plant Protection Convention. (2016). Diagnostic Protocol for Regulated Pest. DP 17: Aphelenchoides besseyi, A. fragaria and A. ritzemabosi. DP_17_2016_En_2016-11-01_iaK6 His.pdf.
- Jones, J.T., Haegeman, A., Danchin, E.G.J., Gaur, H.S., Helder, J., Jones, M.J.K., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J.E., Wesemael, W.M.L., & Perry, R.N. (2013). Top 10 plantparasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*, 14(9): 946-961. https://doi.org/10.1111/mpp.12057
- Khan, M.R., & Ghosh, S. (2011). Survival and Population Dynamics of Foliar Nematode, Aphelenchoides besseyi Infecting Tuberose in West Bengal, India. Indian Journal of Nematology. https://www.researchgate.net/publication/ 265519872_Survival_and_population_dynamics _of_foliar_nematode_Aphelenchoides_besseyi_ infecting_tuberose_in_West_Bengal_India.

- Kohl, L.M. (2011). Foliar nematodes: A summary of biology and control with a compilation of host range. The American Phytopathological Society (APS). https://apsjournals.apsnet.org/doi/abs/ 10.1094/PHP-2011-1129-01-RV.
- Luc, M., Sikora, R.A., & Bridge, J. (2005). Plantparasitic nematodes in subtropical and tropical agriculture. 2nd ed. CABI Wallingford. Pp 11-85, Pp 319-392.
- Mor, M., & Spiegel, Y. (1993). Infection of Narcissus Roots by Aphelenchoides subtenuis. Journal of Nematology, 25(3):476-479. The Society of Nematologists 1993.
- Pedroche, N.B., Villaneuva, L.M., & De Waele, D. (2013). Plant-parasitic nematodes associated with semi-temperate vegetables in the highlands of Benguet Province, Philippines. Archives of phytopathology and plant protection, 46(3): 278-294
- Philippine Statistics Authority. (2014). *Selected Statistics on Agriculture*. https://psa.gov.ph/
- Philippine Statistics Authority. (2017). *Selected Statistics on Agriculture*. https://psa.gov.ph/
- Reddy, P.P. (2019). Introductory Plant Nematology. E-book. https://books.google.com.ph/books?id= U82MDwAAQBAJ&dq=population+threshold+ of+Ditylenchus+in+onion&source=gbs_nav links_s
- Saeed, M.R.M. (2015). Plant-Parasitic Nematodes Associated with Garlic in Yemen. *Egypt. J. Agronematol.*, 14(1): 37-44.
- Seinhorst, J.W. (1962). On the killing, fixation, and transferring to glycerin of nematodes. *Nematologica*, 8: 29-32. https://brill.com/view/ journals/nema/8/1/article-p29_7.xml?language =en&ebody=previewpdf-49929
- Sikora, R.A., Claudius-Cole, B., & Sikora, E.J. (2018). Nematode parasites of food legumes, in Plant-Parasitic Nematodes in Subtropical and Tropical Agriculture, 3rd Ed, eds R. Sikora, D. Coyne, J. Hallmann, and P. Timper (Boston, MA: CABI), 290–345. doi: 10.1079/9781786391247.0290
- Sturhan, D., & Brzeski, M.W. (1991). Stem and bulb nematodes, Ditylenchus spp. In: Nickle WR, editor.

Manual of Agricultural Nematology. New York, NY, USA

- Subbotin, S.A., & Chitambar, J.J. (2018). Plant-Parasitic Nematodes in Sustainable Agriculture of North America. Springer Nature Switzerland Vol. 2
- Subbotin, S.A., Madani, M., Krall, E., Sturhan, D., & Moens, M. (2005). Molecular Diagnosis, Taxonomy, and Phylogeny of the Stem and Bulb Nematode Ditylenchus dipsaci Species Complex Based on the Sequences of the Internal Transcribed Spacer-rDNA. Phytopathology, 95(11): 1308-15. DOI: 10.1094/PHYTO-95-1303.
- Sundaram, R., Rajendran, G., Lakshmanan, P.L., & Nanjan, K. (1990). Nematodes associated with garlic and vegetables in Nilgris district. J. South Indian Horticulture, 38(6): 353.
- Testen, A.L., Walsh, E.K., Taylor, C.G., Miller, S.A., & Lopez-Nicora, H.D. (2014). First Report of Bloat Nematode (Ditylenchus dipsaci) Infecting Garlic in Ohio. Plant Disease, 98(6): 859. https/:apsjournals.apsnet.org/doi/10.1094/ PDIIS-11-13-1121-PDN.
- Van Bezooijen, J. (2006). Methods and Techniques for Nematology. Wageningen University, the Netherlands. p. 20
- Vera Cruz, M.O., & Alberto, R.T. (1988). Survey and identification of plant-parasitic nematodes associated with garlic (Allium sativum) in San Jose City, Nueva Ecija, Philippines. Central Luzon State University, Munoz, Nueva Ecija. AGRIS, 9(2): 1-8. https://agris.fao.org/agrissearch/search.do?recordID=PH9010375