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DIVERSITY AND ABUNDANCE OF NEMATODES IN GUAVA (*Psidium guajava* L.) CULTIVATION IN LAMPUNG

Nabilah[1], I Gede Swibawa[2], Radix Suharjo[2], & Yuyun Fitriana[2]

[1] Department of Agrotechnology, Faculty of Agriculture, Universitas Lampung, Indonesia
Jl. Prof. Sumantri Brojonegoro 1 Lampung 35145
[2] Department of Plant Protection, Faculty of Agriculture, Universitas Lampung, Indonesia
Jl. Prof. Sumantri Brojonegoro 1 Lampung 35145
E-mail: igede.swibawa@fp.unila.ac.id

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ABSTRACT

Diversity and abundance of nematodes in guava (*Psidium guajava *L.) cultivation in Lampung. Crystal guava agroecosystem is inhabited by many species of plant parasitic nematodes. However, information regarding this topic was still limited. This study aimed to understand the species dominancy of nematodes in crystal guava cultivation in Lampung. Sampling was carried out in three locations of guava crystal plantations: Lampung Timur, Lampung Tengah, and Tanggamus. The laboratory analysis was done at the Plant Pest Science Laboratory and Agricultural Biotechnology Laboratory, Universitas Lampung. The study was conducted in December 2019 – July 2020. Nematodes were identified to the level of the genus. The Prominance value (PV) was used to assess the nematodes genus dominancy. The results showed that the nematodes inhabiting the crystal guava agro-ecosystem in Lampung was both plant parasitic and free-living nematodes. The plant parasitic nematodes were identified as *Meloidogyne*, *Aphelenchus*, *Hemicriconemoides*, *Tylenchus*, *Aphelenchoides*, and *Xiphinema*, while free-living nematodes was *Rhabditis*, Dorylaimine, *Dorylaimus*, and *Mononchus*. The dominant plant parasitic nematode was *Meloidogyne* and the dominant free-living nematode was *Rhabditis*. The abundance of *Meloidogyne* /300 mL of soil was 351.47 individuals in Lampung Timur, 124.27 individuals in Lampung Tengah, and 82.18 individuals in Tanggamus. The dominant free-living nematode in the three locations was *Rhabditis.*

Key words: *Meloidogyne,* prominance value*, Rhabditis*

INTRODUCTION

The diversity of nematodes in the tropics is higher than in temperate regions. Luc *et al.* (2005) mentioned that, the diversity of nematodes in subtropical and tropical areas was high due to higher diversity of cultivated plants compared to temperate regions. Yanto & Swibawa (2016) reported 13 genera of plant parasitic nematodes associated with banana cultivation in East Lampung. In Crystal guava cultivation, there are many species of plant parasitic nematodes that inhabit the agroecosystem. El-Borai & Duncan (2005) reported that, more than three genera of plant parasitic nematodes inhabited the guava crop agroecosystem including, *Meloidogyne*, *Helicotylenchus*, and *Tylenchorhynchus*.

Plant parasitic nematodes are serious problem in the cultivation of certain types of plants due to its ability in causing serious damage to crop. These nematodes were attacking and damaging the roots so that their function as a transport of nutrients and water is not optimal. Root systems that do not function optimally could caused disorder of growth and physiological processes in plant. As a result, plants could easily wilt in dry season, stunted, chlorosis, and die (Agrios, 1996). To overcome this problem, an effective control of plant parasitic nematode population is needed.

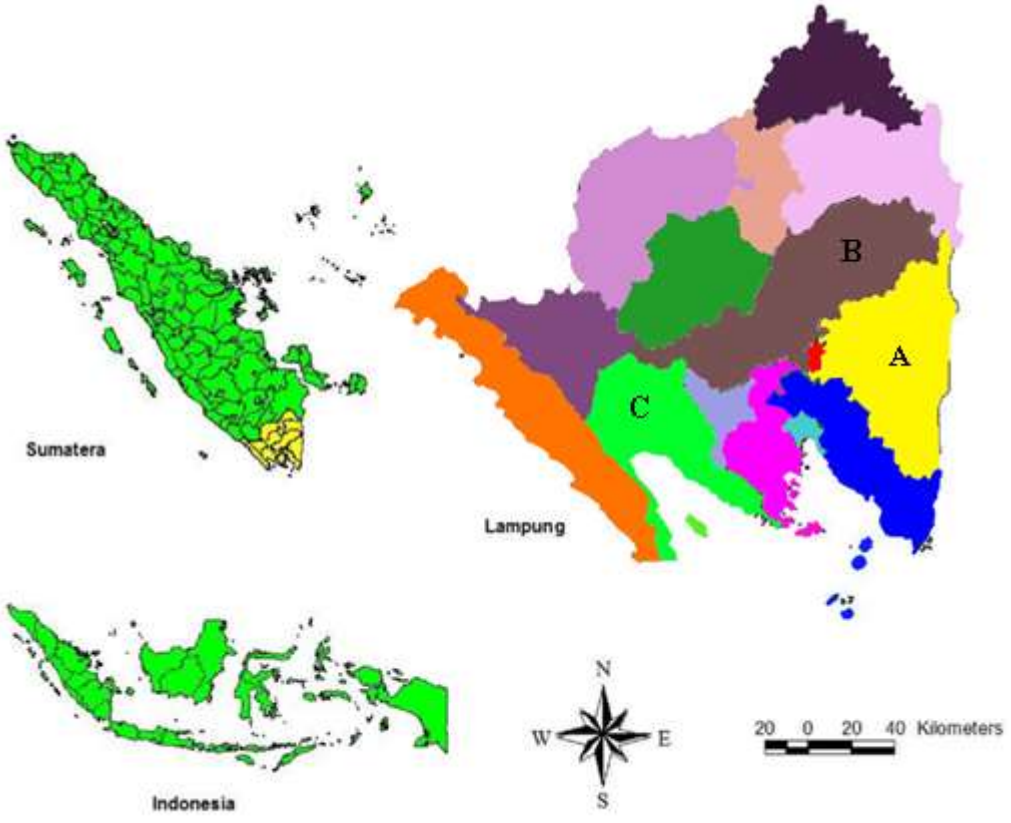
Identification is an important step to determine the genus or species of nematodes in an agroecosystem. By knowing the nematode species, it can be easily understanding the destructive behavior and ability. Based on this knowledge, the appropriate and effective control techniques can be determined (Bridge, 1987). Information regarding the diversity and abundance of nematodes in guava cultivations in Lampung is not yet available. This information is especially important for determining the initial steps for nematode management in guava plantations. This study aimed to understand the diversity and abundance of plant parasitic nematodes as well as the dominant nematodes in guava (*Psidium guajava* L.) cultivation in Lampung.

MATERIALS AND METHODS

Research Site. Soil samples were taken from the rhizosphere of guava plantations in 3 locations i.e. PT GGP Plantation Group IV (PG-IV), 5° 4'0.8652 S and 105° 41'33.3564 E (Lampung Timur); PT GGP Plantation Group I (PG-I), 4° 49'32.9412 S and 105° 13'55.5744 E (Lampung Tengah) and Gunung Alip District, 5° 21'2.8872" S and 104° 47'12.5988" E (Tanggamus) (Figure 1). Laboratory analysis were carried out at the Plant Pest Science Laboratory and Agricultural Biotechnology Laboratory, Faculty of Agriculture, Universitas Lampung. The research was conducted in December 2019 to July 2020.

Soil Sampling and Nematode Extraction. This study was used survey methods for soil sampling. Soil samples were collected in three locations of guava crystals plantations: PT GGP PG4 (Lampung Timur), PT GGP PG 1 (Lampung Tengah), and Gunung Alip (Tanggamus). Prior to sampling, the planting plot was determined. At each sampling location, a plot of ± 2.5 ha was determined randomly. In each plot, ten sample plants were systematically selected following the diagonal line. In each sample plant ten sub-points were assigned, five sub-points positioned on a small circle with radius 30 cm from the plant and five sub-points positioned on the large circle with radius 60 cm from the plant (Figure 2) (Barker, 1985). At each sub-point, soil samples were taken using an auger or soil drill to a depth of about 20 cm. Soil samples from ten sub-points were composited then 1000 g were taken, put in a plastic bag, and labeled for further analysis in the laboratory.

Extraction of nematodes from the soil was carried out by decantation and centrifugation methods with sugar solution (Hooper *et al*., 2005). Sugar solution was prepared by dissolving 500 g of sugar (sucrose) in water until the volume of the solution becomes 1000 mL. As much as 300 mL of soil (which had previously been weighed to determine its weight) was put into a bucket, then 2000 mL of water was added and kneaded and left for 1 minute. The suspension was filtered using a 1 mm sieve and the soil was collected in a second bucket, then left for 3 minutes. After 3 minutes, the soil suspension in the second bucket was filtered again using a 53 µm sieve and the soil was collected in the third bucket. The soil that attached on the sieve was collected in a beaker. Furthermore, the soil suspension in the third bucket was filtered again using a 38 µm sieve. The soil suspension which attached on the sieve was added to the previous beaker. The soil suspension in the beaker glass was stirred evenly, then put into a centrifuge tube



Source: Dulbari *et al*. (2021)

Figure 1. Sampling locations. (A) Lampung Timur; (B) Lampung Tengah; (C) Tanggamus.

and centrifuged at 3500 rpm for 5 minutes. After that, the supernatant was removed, the sediment was added with sugar solution then stirred until evenly distributed, then centrifuged again at 1000 rpm for 2 minutes. Next, the supernatant which was a suspension of the nematodes in the sugar solution was rinsed under running water using a 38 μm sieve to clean the sugar solution. The nematode suspension then collected in a suspension bottle and labeled.

Nematode fixation used Golden X solution which was a mixture of 90 parts of distilled water, 8 parts of formalin, and 2 parts of glycerine (Gafur & Swibawa, 2004). Before fixation, the nematodes were killed by heating the suspension to a temperature of 60–70 °C. The nematode suspension was ajust into 3 mL, then Golden X solution was added until 10 mL, in this condition the nematode was in 3% formalin solution. Nematodes were counted using a hand counter under a binocular stereo microscope. The abundance of these nematodes was the number of individuals /300 mL of soil.

Nematode identification was carried out on permanent collection slides. Permanent collection slides were made by infiltrating glycerine into the nematodes using the Seinhorst Method (Seinhorst, 1959). Seinhorst I solution was prepared from a mixture of 20 parts of 96% alcohol, 2 parts of glycerin and 78 parts of distilled water, while Seinhorst II solution was made from 95 parts of 96% alcohol and 5 parts of glycerin. A total of 3 mL of nematodes in a Petri dish diameter 5 cm were added with 7 mL of Seinhorst I solution so that the volume became 10 mL, then put it in a desiccator containing 96% alcohol with a volume of 1/3 part and oven at 43 ºC overnight. Then the suspension was dried at 43 ºC for 4 hours until the volume was halved. Then the suspension was added with Seinhorst II solution so that it became 10 mL and put it back into the desiccator and oven for overnight at a temperature of 43 ºC, then dried again at 43 ºC for 4 hours. This process was carried out two times and ended by drying at 43 ºC for 48 hours. (Hooper *et al*., 2005). The nematode body that been infiltrated with glycerin and was ready to be used for making permanent collection slides on glass objects and covered with a cover glass.

The identification was carried out on 100 nematodes which were taken randomly. A total of 20 individual nematodes were picked up one by one using "nematodes pick" under binocular stereo microscope, then placed on a glass preparation which was previously dropped by glycerin solution and then covered with a cover glass. The side of cover glass was coated with clear nail polish which functions as an adhesive. Nematode identification was carried out to the genus taxonomic level using a compound microscope at magnification of 100–400 times based on morphological characteristics compared with the reference books Goodey (1963), Mai & Lyon (1975), and Smart & Nguyen (1988). Based on the genus taxon, the nematodes were grouped into plant parasitic nematodes and free-living nematodes (Yeates *et al*., 1993).

The measured variables was total individuals per sample, relative population (RP), absolute population (AP), relative frequency (RF), absolute frequency (AF), and population dominance. The relative population of the nematode genus was the number of individuals of the genus per 100 nematodes identified. The absolute population of the genus was calculated by multiplying the relative population of the genus by the total nematodes per sample. Absolute frequency (AF) was

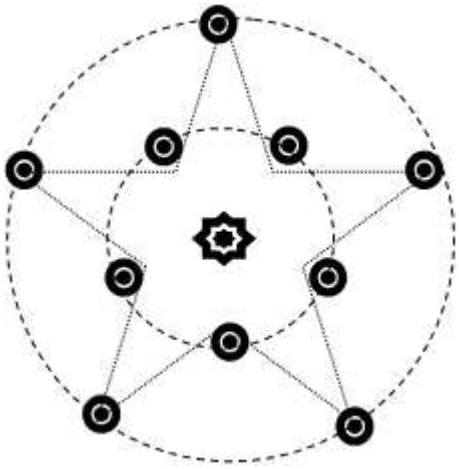


Figure 2. Soil sampling pattern at each sample plant. plant sample; site for collecting soil sampel.

how often a species found in samples. The relative frequency of the genus was determined based on the ratio between the frequencies of each species to the total frequencies. Nematode community data were analyzed to determine the dominant nematode genus using the genus prominence value (PV) with the following formula (Beals, 1960). The AF was calculated using formula :

$$AF = \left(\frac{\text{Number of samples containing a genus}}{\text{number of samples collected}} \right) \times 100\%$$

The RF was analyzed using formula :

$$RF = \left(\frac{\text{frequency of genus}}{\text{sum of frequency of all genus}} \right) \times 100\%$$

The PV was determined :

$$PV = AP \times \sqrt{AF}$$

RESULTS AND DISCUSSION

Nematode Community. A total of nine genera and one subfamily of nematodes were observed in the guava agroecosystem in Lampung (Figure 3, and Tables 1, 2, and 3). Not all genera were found in every sampling location, in PT GGP PG 4 (Lampung Timur) 6 genera and 1 subfamily were found, in PT GGP PG 1 (Lampung Tengah) there were 5 genera, and 5 genera and 1 subfamily was observed in guava plantations at Gunung Alip (Tanggamus). The nematodes found was consisted of both plant parasitic and free-living nematodes (Figure 3).

The plant parasitic nematodes that inhabited guava plantation in PT GGP PG 4 (Lampung Timur) were *Meloidogyne*, *Aphelenchus*, *Hemicriconemoides*, and *Aphelenchoides,* while the free-living nematodes were *Rhabditis*, *Dorylaimus*, and the subfamily Dorylaimine (Figure 3 and Table 1). The total individual of *Meloidogyne* was 585, the relative population was 58.50%, and the absolute population was 351.47 individuals/300 mL of soil. The relative frequency of this nematode was 33.33% and the absolute frequency was 100%. The genera *Aphelenchus*, *Hemicriconemoides*, and *Aphelencoides* had a lower population and frequency than *Meloidogyne* (Table 1). The total number of *Rhabditis* individuals reached 383 with a relative population of 38.30%, and an absolute population of 230 individuals/300 mL of soil, an absolute frequency of 100, and a relative frequency of 33.33%. *Dorylaimus*, and Dorylaimine showed a lower population and frequency than *Rhabditis* (Table 1).

On the other hand, PT GGP PG 1 (Lampung Tengah), 5 nematode genera were found, consisting of

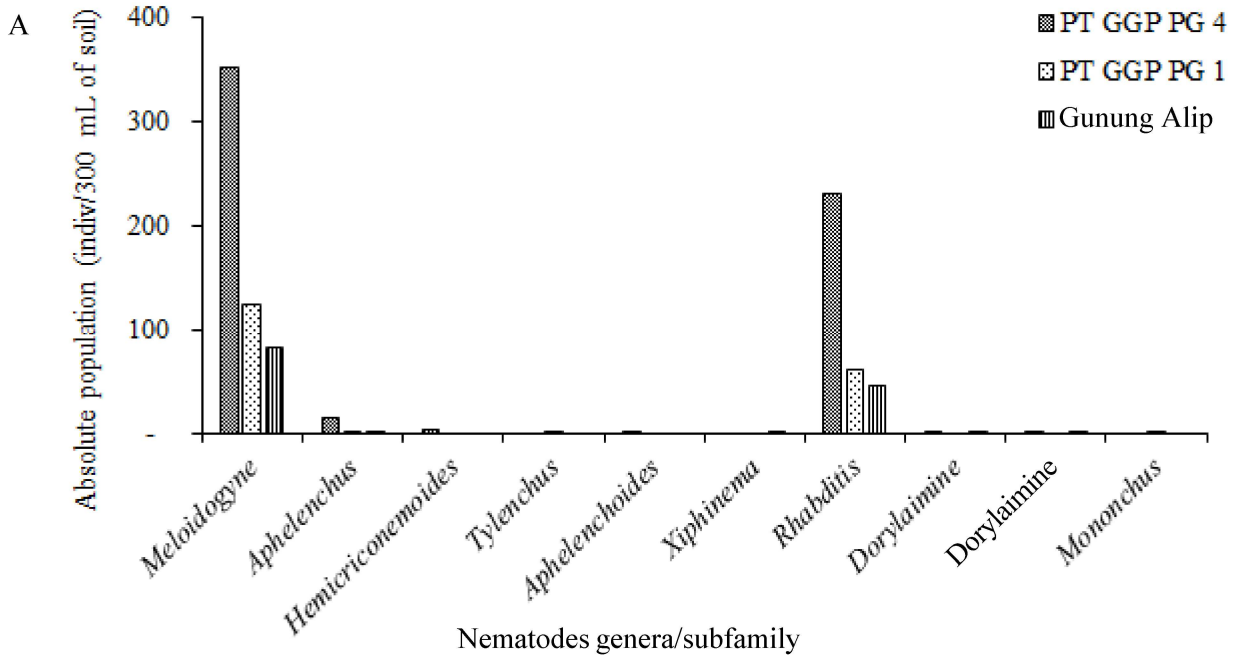


Figure 3. Absolute and relative populations of genera/subfamily of nematodes inhabited guava agroecosystem in Lampung, (A) Absolute population, (B) relative population.

B

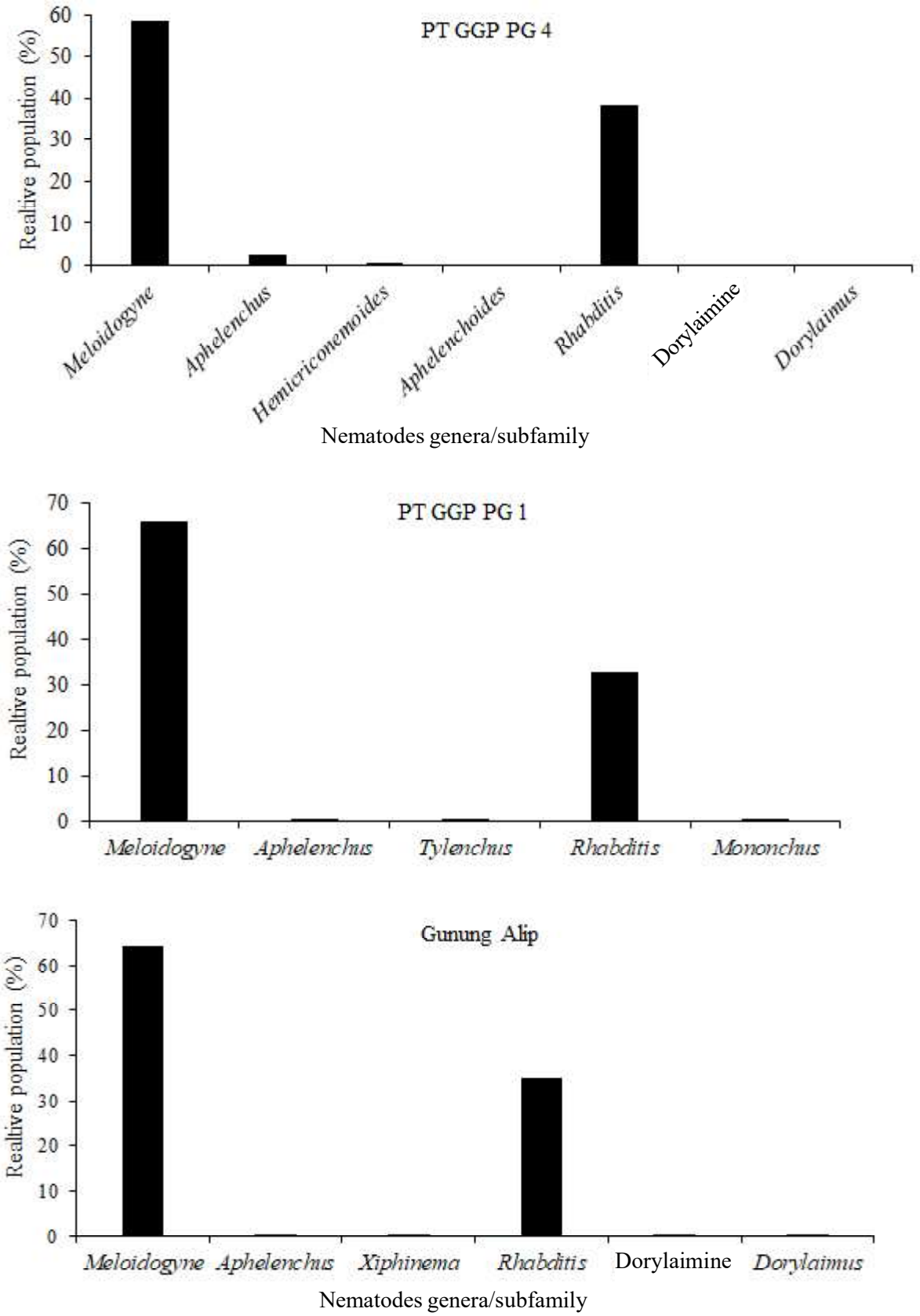


Figure 3. Absolute and relative populations of genera/subfamily of nematodes inhabited guava agroecosystem in Lampung, (A) Absolute population, (B) relative population (continue).

3 plant parasitic nematodes and 2 free-living nematodes (Table 2). The plant parasitic nematodes were *Meloidogyne*, *Aphelenchus*, and *Tylenchus*, while the free-living nematodes were *Rhabditis* and *Mononchus*. The total number of *Meloidogyne* individuals was 661 with a relative population of 66.10% and an absolute population of 124.27 individuals/300 mL of soil. The relative frequency of this nematode was 34.48% and the absolute frequency was 100%. The abundance and frequency of *Aphelenchus* and *Tylenchus* were lower than that of *Meloidogyne* (Table 2). The total number of *Rhabditis* individuals was 328 with a realistic population of 32.80% and an absolute population of 61.66 individuals/300 mL of soil. The relative frequency of

Table 1. Total number of individu, population dan frequency of nematode at guava cultivations area in PT GGP PG 4 (Lampung Timur)

Genus/Subfamily	Total individual	Relative population (%)	Absolute population/ 300 mL soil	Relative frequency (%)	Absolute frequency (%)
Plant parasite					
Meloidogyne	585	58.50	351.47	33.33	100
Aphelenchus	23	2.30	13.82	16.67	50
Hemicriconemoides	4	0.40	2.40	6.67	20
Tylenchus	0	0.00	0.00	0.00	0
Aphelenchoides	1	0.10	0.60	3.33	10
Xiphinema	0	0.00	0.00	0.00	0
Free-living					
Rhabditis	383	38.30	230.11	33.33	100
Dorylaimine	1	0.10	0.60	3.33	10
Dorylaimus	3	0.30	1.80	3.33	10
Mononchus	0	0.00	0.00	0.00	0

Tabel 2. Total number of individu, population dan frequency of nematode at guava cultivations area in PT GGP PG 1 (Lampung Tengah)

Genus /Subfamily	Total individual	Relative population (%)	Absolute population/ 300 mL soil	Relative frequency (%)	Absolute frequency (%)
Plant parasite					
Meloidogyne	661	66.10	124.27	34.48	100
Aphelenchus	6	0.60	1.13	13.79	40
Hemicriconemoides	0	0.00	0.00	0.00	0
Tylenchus	3	0.30	0.56	10.34	30
Aphelenchoides	0	0.00	0.00	0.00	0
Xiphinema	0	0.00	0.00	0.00	0
Free-living					
Rhabditis	328	32.80	61.66	34.48	100
Dorylaimine	0	0.00	0.00	0.00	0
Dorylaimus	0	0.00	0.00	0.00	0
Mononchus	2	0.20	0.38	6.90	20

this nematode was 34.48% and the absolute frequency was 100%. The genus *Mononchus* had an abundance and lower frequency than *Rhabditis*.

Table 3 showed that the nematode community in the crystal guava agroecosystem in Gunung Alip (Tanggamus Regency) was consisted of 6 nematode genera; 3 genera were plant parasitic nematodes and the other 2 genera and 1 subfamily were free-living nematodes. The plant parasitic nematodes found were *Meloidogyne*, *Aphelenchus*, and *Xiphinema*. The free-living nematodes that inhabited the agroecosystem were *Rhabditis*, *Dorylaimus* and Dorylaimine. The total number of *Meloidogyne* individuals was 641 with a relative population of 64.10%, an absolute population of 82.18 individuals/300 mL of soil. The relative frequency of this nematode was 38.46% and the absolute frequency was 100%. *Aphelenchus* and *Xiphinema* were of lower abundance and frequency than *Meloidogyne* (Table 3). The total number of *Rhabditis* individuals was 350 with a relative population of 35.00% and an absolute population of 44.87 individuals/300 mL of soil. The relative frequency of this nematode was 33.33% and the absolute frequency was 100%. Dorylaimine and *Dorylaimus* showed an abundance and lower frequency than *Rhabditis*.

Nematode Dominance. Nematotode dominance was measured using prominence value (PV). *Meloidogyne*, a plant parasitic nematode was dominant in the crystal guava agroecosystem in three planting locations (Table 4). The PV of *Meloidogyne* in the planting location at PT GGP PG4 (Lampung Timur) was 3514.68, at the location of PT GGP PG 1 (Lampung Tengah) was 1242.68, and at Gunung Alip (Tanggamus) was 821.76. Another plant parasitic nematode PV in nematode communities on guava cultivations area at the three sampling sites was much smaller than the *Meloidogyne*.

Rhabditis was the dominant free-living nematodes in the nematode community that inhabited the guava agroecosystem in the three planting locations. At PT GGP PG 4 (Lampung Timur), the PV of *Rhabditis* was 2301.6, at PT GGP PG 1 (Lampung Tengah) the PV was 616.64 and at Gunung Alip (Tanggamus) was 448.70. Other free-living nematodes were found to have lower PV than *Rhabditis* (Table 4).

Nine genera and 1 subfamily of nematodes consisting of 6 plant parasitic nematodes and 4 free-living nematodes were found in guava cultivation agroecosystem (Tables 1, 2, and 3). Twelve genera of nematodes consisting of 9 plant parasitic nematodes and 3 free-living nematodes had been reported on guava plantations in West Bengal, India (Khan *et al*., 2007). Pradhan *et al*. (2020) reported that 13 genera of nematodes consisting of ten plant parasitic nematodes and three free-living nematodes were associated with guava cultivation in Bhubaneshwar, India. Several genera were found in all three sampling locations, while other genera were only found at one location where crystal guava was grown. The plant parasitic nematodes

Tabel 3. Total number of individu, population dan frequency of nematode at guava cultivations area in Gunung Alip (Tanggamus)

Genus/Subfamily	Total individual	Relative population (%)	Absolute population/ 300 mL soil	Relative frequency (%)	Absolute frequency (%)
Plant parasite					
Meloidogyne	641	64.10	82.18	38.46	100
Aphelenchus	6	0.60	0.77	10.00	30
Hemicriconemoides	0	0.00	0.00	0.00	0
Tylenchus	0	0.00	0.00	0.00	0
Aphelenchoides	0	0.00	0.00	0.00	0
Xiphinema	1	0.10	0.13	3.33	10
Free-living					
Rhabditis	350	35.00	44.87	33.33	100
Dorylaimine	1	0.10	0.13	3.33	10
Dorylaimus	1	0.10	0.13	3.33	10
Mononchus	0	0.00	0.00	0.00	0

that inhabited all three locations were *Meloidogyne* and *Aphelenchus*, while free-living nematodes that inhibited all three locations were *Rhabditis*. Other nematodes were only found at one or two planting sites of crystal guava.

Based on the PV, *Meloidogyne* was the dominant plant parasitic nematode and *Rhabditis* was the dominant free-living nematode at the three crystal guava planting sites in Lampung. These two genera of nematodes had the highest PV in the nematode communities of plant parasites and free-living nematodes, respectively (Table 4). This finding was different with the similar report by Pradhan *et al*. (2020) which reported that, in fruit crops including guava in India the plant parasitic nematode that had the highest PV was *Rotylenchulus reniformis* while the free-living nematode that had highest PV was *Dorylamid*.

Meloidogyne was dominant in the nematode community in crystal guava plantations in Lampung, indicating that this nematode was important in guava cultivation. This could occur because these nematodes were widespread, polyphagous (Muin, 2008), and had a large host range (Shurtleff & Averre , 2000). According to Luc *et al*. (2005) plant parasitic nematodes were considered important, because they were able to cause severe losses. Thus, *Meloidogyne* can become an important pest in crystal guava cultivation in Lampung. This finding was consistent with the report of Swibawa *et al*. (2018) which mentioned that, nematodes that attacked crystal guava plantations in East Lampung were *Meloidogyne incognita* and *M. javanica*. Some researchers also reported that *Meloidogyne* was an important pest in guava cultivation (Milan, 2007; Rahman *et al*., 2008; Razak & Lim, 1987).

Other parasitic nematodes in guava plantations in Lampung were *Aphelenchus*, *Hemicriconemoides*, *Tylenchus*, *Aphelenchoides*, and *Xiphinema*. The presence of some of these plant parasitic nematodes was not dominant and the population was low. Of all the parasitic nematodes found in guava plantations in Lampung, the *Aphelenchus* and *Xiphinema*, were also reported to be associated with guava crops in India (Khan *et al*., 2007).

Rhabditis was a dominant free-living nematode in guava plantations in Lampung. Based on the function in the ecosystem, this nematode feed organic matter degrading bacteria (Yeates *et al*., 1993). The presence of these nematodes which were dominant and abundant in guava plantations could be an indication that the agroecosystem conditions were rich in organic matter which became a substrate for bacteria. Luckyana *et al*. (2020) stated that, free-living nematode communities, which were dominated by bacterial-feeders

Tabel 4. The prominence value (PV) of nematode genus or famili inhibiting guava cultivation agroecosystem in Lampung

Genus/Subfamily	Location		
	PT GGP PG 4 (Lampung Timur)	PT GGP PG 1 (Lampung Tengah)	Gunung Alip (Tanggamus)
Plant parasite			
Meloidogyne	3514.68	1242.68	821.76
Aphelenchus	97.71	7.13	4.21
Hemicriconemoides	10.75	0.00	0.00
Tylenchus	0.00	3.09	0.00
Aphelenchoides	1.90	0.00	0.00
Xiphinema	0.00	0.00	0.41
Free living			
Rhabditis	2301.06	616.64	448.70
Dorylaimine	1.90	0.00	0.41
Dorylaimus	5.70	0.00	0.41
Mononchus	0.00	1.68	0.00
Total genus	7	5	6

nematodes, increasing the rate of organic matter decomposition the soil.

The abundance and diversity of nematodes on guava plantation in Lampung might affected by the climate conditions and soil types. PT GGP on PG 4 in Lampung Timur and PG-1 in Lampung Tengah had the same soil type and climate condition. The soil type in those plantations was Ultisol with sandy-clay texture and the climate condition as follow: rainfall was 2,971.56 mm/year and the temperature maximum 31.8°C, minimum 23.6 ºC, with average 27.3 °C and the Relative Hummidity (RH) was 89.05% (Nugroho, 2021; personal communication). The soil type of guava plantation in Gunung Alip (Tanggamus) was Inseptisol (Hafif *et al*., 2017), with climate condition as follow: rainfall was 1800–2000 mm/year, temperature was 26–30 °C, average 28 °C, and RH 80–88%, average 84% (https://sippa.ciptakarya.pu.go.id).

CONCLUSION

Based on the results, it could be concluded that the plant parasitic nematodes found in guava plantations in Lampung were *Meloidogyne*, *Aphelenchus*, *Hemicriconemoides*, *Tylenchus*, *Aphelenchoides*, and *Xiphinema*. The free-living nematodes were *Rhabditis*, *Dorylaimus*, Dorylaimine and *Mononchus*. *Meloidogyne* was a dominant and important plant parasitic nematode in Crystal guava cultivation in Lampung with PV = 3514.68 and an abundance of 351.47 individuals/300 mL of soil in PT GGP PG 4 (Lampung Timur), PV = 1242.68 and an abundance of 124.27 individuals/300 mL of soil in PT GGF PG 1 (Lampung Tengah), and PV = 821.76 and an abundance of 82.18 individuals/300 mL of soil in Gunung Alip (Tanggamus). The dominant free-living nematode in the three locations was *Rhabditis*.

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