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Review Article

Plant Parasitic Nematodes in Agricultural Ecosystem of Indonesia

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ABSTRACT

The plant-parasitic nematode (PPN) is often not recognized as important limiting crop production in Indonesia. This is largely caused by non-specific and non-dramatic above-ground plant disease symptoms, their microscopic nature, and partly caused by inadequate demonstration of the economic importance of this hidden pathogen. However, change in agricultural practices to meet the ever-increasing food demand along with global climate change may increase the risk of PPNs on crop productivity in the future. This paper reviews PPN inventory in Indonesia during the last three decades. Thirty-three genera of PPNs were found to associate with 25 host plants. Some genera were present at the densities that are considered as damaging levels in other countries. Results among surveys are difficult to compare because of differences in crop cultivar surveyed, cultivation practices, sampling unit and method, and nematode extraction techniques. Lack of field supporting data did not permit a valid assessment of nematode risk on a particular crop. The first record of several quarantined species has been reported, but not all of them have been validated molecularly. Challenges and opportunities to improve the future field survey are presented in this paper.

Keywords: diversity; inventory; nematode, plant disease; potential threat

INTRODUCTION

Plant-parasitic nematodes (PPNs) are soil-borne pathogens that primarily parasitize root system. When its population exceeds economic threshold, a significant reduction in agricultural productivity can occur. Although widely distributed and infested various crops in Indonesia, their importance as the pathogen is often not recognized (Mustika, 2005). Therefore data on crop damage caused by PPNs are scarce or outdated. Not only in Indonesia, data on the economic impact caused by PPNs in other developing countries are also incomprehensive (De Waele & Elsen, 2007; Nicol *et al*., 2011). Unawareness of the existence of PPNs in the soil can be caused by (1) the above-ground non-specific disease symptom; (2) slow, non-dramatic disease progress and non-lethal effect on infected plant; (3)

patchy distribution of disease symptoms in the field; and (4) microscopic size of PPNs which requires soil extraction and microscopic examination in laboratory (Kalshoven, 1950; Mustika, 2005; De Waele & Elsen, 2007; Nicol *et al*., 2011; Schouteden *et al*., 2015).

Several reports on nematode inventory in Indonesia were published during the Dutch East Indies era. *Heterodera javanica* (later renamed to *Meloidogyne javanica*) was firstly described from a sugarcane field in Cirebon, West Java (Kalshoven, 1950). Later, various nematodes were reported to damage important crops. In 1889 root lesion nematode *Pratylenchus coffeae* damaged coffee plantation in Java and abaca in Deli, North Sumatera; burrowing nematode *Radopholus similis* was reported to be associated with yellowing disease of black pepper in Bangka and also in West and South Kalimantan; and in 1904, *Tylenchus oryzae* (renamed to *Hirschmanniella oryzae*) was known to associate with 'mentek' symptom on paddy rice in Java (Kalshoven, 1950). After this period, there has been no prominent nematode cases until the first report of rice root-knot nematode (RKN), *Meloidogyne graminicola*, damaging paddy rice planted in permeable soil in the Special Region of Yogyakarta (Netscher & Erlan, 1993) and the outbreak of golden cyst nematode or potato cyst nematode (PCN) *Globodera rostochiensis* in potato fields in Kota Batu, East Java (Mulyadi *et al*., 2003). Up to 71% potato yield losses were reported due to PCN (Hadisoeganda, 2006b). This species has been suspected to exist in Indonesia since 1989, but no further studies were carried out since until the outbreak (Mustika, 2010).

The change in agricultural practices such as monoculture cropping system without fallowing and cultivation expansion to dry, marginal areas to meet the ever-increasing food demand can promote population increase of certain nematodes which are currently in low population density and balance state in nature to become new emerging pathogens (Nicol *et al*., 2011). The outbreak of PCN in Indonesia is one example which has drawn specific attention by government and led to an international collaboration between Indonesia and Australia (Dawson *et al*., 2005). *M. graminicola* is an example of tropical species that potentially can increase in population due to habitat change. This species, currently also reported to infest rice field in West Java and South Sulawesi (Nurjayadi *et al.*, 2015; Mirsam & Kurniawati, 2018), can be managed by flooding rice fields (Dutta *et al.*, 2012). However, water shortage due to water use competition with industrial sector and climate change are predicted to increase the risk of *M. graminicola* on rice crop (De Waele & Elsen, 2007). With its short life cycles, high fecundity, and some species also reproduce parthenogenetically, continuous and high population growth of PPNs can sustain under the tropical climate

(Mateille *et al*., 2008). Crop yield loss by PPN in tropics is predicted to increase along with the global warming temperature and continuous, monoculture crop cultivation without fallowing (Mateille *et al*., 2008; Nicol *et al*., 2011).

PPN studies in Indonesia are generally lagged behind the nematode research in the other developing countries as well as behind other class pathogens. Low research priority compared to other biotic factors as well as the scarcity in skilled nematologists due to retirement or other reasons are the main factors of this setback. To attract research funding, the risk of plant-parasitic nematode infestation on crop production must be properly estimated. Nematode surveys can provide initial information on the predominant damaging genus and species. This paper reviews PPN inventory in agricultural ecosystem of Indonesia and propose the strategies to improve the future field surveys. This paper is expected can raise the awareness on the potential threat posed by PPN on agricultural productivity in Indonesia.

NEMATODE DIVERSITY AND DENSITY

Examination of 35 references about field surveys performed in 54 regencies of 16 provinces published in 1990 until 2021 indicated that numerous plant-parasitic nematode genera were associated with various crops at varying population densities. At least 33 genera were associated with 25 crop species. Root-knot nematode *Meloidogyne* is the most widely distributed genus and is associated with 21 crop species. The ring nematode (*Criconemoides* [syn. *Criconemella*]) is in second place, followed by the lesion nematode (*Pratylenchus*), reniform nematode (*Rotylenchulus*), *Rotylenchoides*, and dagger nematode (*Xiphinema*) in the third-place (Figure 1). Higly damaging nematode such as PCN and *Radopholus* that infect crops of high economic value such as potato (PCN), coffee and blackpepper (*Radopholus*) have narrow distribution. Nematode such as the cyst nematode *Heterodera*, ring nematode *Criconema* and *Hemicriconemoides*, pin nematode *Gracilacus*, cystoid nematode *Meloidodera*, needle nematode *Paralongidorus*, *Radopholoides*, lesion nematode *Zygotylenchus*, *Longidorella*, and *Tetylenchus* were rarely detected (Wati *et al.*, 2015; Hasanah *et al.*, 2016; Swibawa *et al.*, 2019).

Eleven nematode species were recorded for the first time in Indonesia (Table 1). Except for *Heterodera zeae*, the identity of the remaining species have been confirmed by molecular analysis. *M. fallax*, a quarantined species in Europe (Elling, 2013), is suspected of entering Indonesia via imported contaminated seeds (Halimah *et al.*, 2013). The rice white type nematode *A. besseyi*, previously mentioned by Kalshoven (1950), was “re-discovered”

on rice grain in Bogor at a density of 60 to 160 nematodes per g grain (Kurniawati *et al.*, 2017). Field surveys by Japanese nematologists in the 1970s reported soybean cyst nematode *H. glycine* infesting soybean fields in Central Java, but this finding has never been confirmed (Chaerani & Herman, 1992).

Comparison of nematode prevalence and diversity among surveys on the same crop is difficult to make because of differences in cultivar surveyed, cultivation practice, sampling technique (purposive vs. random), soil sample unit measurement (volume vs. weight), and extraction method (Baermann funnel vs. centrifugal floatation). Only living and active nematodes can be retrieved by using the Baermann funnel method, whereas all nematodes including the dead and sluggish ones such as Criconematidae can be obtained by centrifugal floatation technique. Nevertheless, about 290 nematodes/100 ml soil and 3 to 5900 nematodes/100 g soil have been recorded. Endoparasitic nematodes such as *Meloidogyne* and *Pratylenchus* can present from 70 to 320 nematodes per 10 g roots.

Most of the field surveys were performed locally in one or few regions and were often not focused on a certain nematode genus. One report described a survey across provinces on certain nematode species (Hadisoeganda, 2006a).

NEMATODE INFESTATION AND CROP YIELD LOSS

The high soil population density of PPN is not always manifested as obvious plant damage or significant yield loss (Stanton & Stirling, 1997). Complex interaction among nematode, host, environment, and the presence of other organisms determine the outcome on the host plant. Environmental condition is the most influential factor because under optimum growth plants are tolerant to nematode infestation (Stanton & Stirling, 1997).

From nematode density experiments, *Meloidogyne* spp. at the minimum density 500 J2/100 g soil can reduce potato yield by 12% (Hadisoeganda, 1981). RKN infestation in potato soil in Sumatera and Java far exceeded this threshold, but the nematode effect on potato yield reduction was not obvious (Hadisoeganda, 2006b). As much as 114 J2/100 g soil or 2-675 cysts/100 g soil of PCN were reported (Hadisoeganda, 2006b; Lisnawita *et al.*, 2012; Nugrahana *et al.*, 2017; Syafi'i *et al.*, 2018). At 100 J2/100 g soil, PCN can cause 11% potato yield loss in Western Europe (Hadisoeganda, 2006a). In another example, as much as 123 *Criconemella* per 200 cm^3 was present in soil planted with various groundnut accessions (Zulchi *et al.*, 2019). This number is almost triple to the population threshold of *C. ornata* on groundnut in the US, which is 71 nematode/200 cm^3 (Barker *et al.*, 1982).

Rough estimates on nematode impact on crop yield have been obtained by relating nematode infestation and crop productivity or interviewing growers. Up to 42% banana yield reduction was attributed to high population density (4900 to 5900 nematodes/100 g soil) of *Pratylenchus, Radopholus,* and *Meloidogyne* (Suyadi & Rosfiansyah, 2017). However, this data was not based on a comparison of nematode-infested and non-infested-field. Potato growers claimed that 42% to 71% yield loss occurred during the first PCN outbreak (Hadisoeganda, 2006b), whereas carrot growers estimated a range of 15% to 95% yield reduction under *Meloidogyne* spp. infestation (Supramana & Suastika, 2012).

NEMATODE DISEASE COMPLEX

Disease complex caused by nematode and another class of pathogens can exacerbate disease symptoms and increase yield loss compared to a single infection by each pathogen. Nematode's contribution in disease complex are (1) their feeding activity on root system creates entry points for other soil-borne pathogens, (2) increase in nutrient content in root exudate activate the dormant stage and stimulate the pathogenicity of a pathogen, (3) during its presence in plant tissue, nematodes induce the synthesis of several compounds which support pathogen growth, (4) defeat plant immune system by producing a toxic compound and inactivating harmful enzymes to the pathogen (Marwoto, 1996).

In several field surveys, the observed severe plant disease symptoms were reported as the result from interaction of fungal or bacterial pathogens with PPNs. The most reknown nematode disease complex is the interaction between Fusarium yellowing symptom of black pepper in Bangka and Kalimantan with *Meloidogyne* or *Radopholus* (Mustika, 1990; Munif & Sulistiawati, 2014; Suryanti *et al*., 2017). Other examples are wilt symptom on pineapple with *P. brachyurus* and *P. coffea* (Lisnawita *et al.*, 2011), bacterial wilt of banana with *Pratylenchus*, *Radopholus,* and *Hoplolaimus* (Indarti *et al*., 2011), and banana wilt caused by *Fusarium oxysporum* f.sp. *cubense* with *R. similis* (Sitepu *et al.*, 2014). The synergistic interaction between nematode and fungal or bacterial disease results in the use of fungal or bacterial resistant varieties being meaningless unless the crop is resistant to nematode infection.

MEASURE OF NEMATODE BIODIVERSITY AND ITS RELATIVE RISKS ON CROP SPECIES

The prevalence and importance of a nematode is commonly based on frequency (the number of locations where the species is found) and abundance ($\log_{10}$ of the average number

of the species in the sample) (Fortuner & Merny, 1979). A method to determine the potential damage of nematode to a certain crop based on nematode abundance and frequency was established by Fortuner and Merny (1979) following survey on nematodes associated with cultivated rice in Senegal and Gambia, West Africa. This method was adopted for nematode assessment on other crops (Sawadogo *et al*., 2009). A nematode was regarded as abundant if it present at ≥1.3 (=$\log_{10}$ [20 individuals/g of roots]) or ≥2.3 (=$\log_{10}$ [200 individuals/L of soil]), and frequent if detected in at least 30% of soil or roots samples (Fortuner & Merny, 1979). Based on abundance and frequency data, four groups of nematodes can be distinguished by sectioning plots of abundance by frequency into quadrants based on the threshold values (Figure 2) (Sawadogo *et al*., 2009). The nematode group within the upper right quadrant is of high prevalence because its abundance and frequency values are above the assigned thresholds (Sawadogo *et al*., 2009). Based on this method of assessment, the nematode survey data in Indonesia indicated that *Pratylenchus* is abundant because it has exceeded the limits (i.e., 3.2 in soil and 1.5 in root), whereas *Meloidogyne*, *Rotylenchulus*, *Pratylenchus*, *Criconemella, Helicotylenchus*, and *Xiphinema* are frequently detected because they are present in at least 35% of soil samples.

Diversity indices have become common quantitative tools for describing the type and distribution patterns of plant-parasitic nematodes. In addition to abundance, other indices include richness, evenness, and dominance are also frequently used to describe nematode status (Zeng *et al*., 2012; Palomares-Rius *et al*., 2015; Fleming *et al*., 2016). These indices can be used to estimate the effect of environmental data (such as soil type, host composition, and crop yield) on nematode population (Table 1) and to assess the relative risk of a nematode on a plant species (De Waele & Elsen, 2007; Sawadogo *et al*., 2009).

Statistical test method such as Kruskal-Walis and multivariate analysis by principal component analysis can also be used for assessing nematode status and its potential as crop yield reducer (De Waele & Elsen, 2007). In the Kruskal-Walis test method, the probability of a nematode as a crop yield reducer is tested by association of the rank of crop production centers against the frequency of nematode occurrence and average population density of each predominant species (De Waele & Elsen, 2007).

DNA-BASED IDENTIFICATION METHOD

Morphological identification is almost free, i.e. it requires no sophisticated equipment and expensive consumables, but requires high skills and intensive training. Additionally, morphological identification is impossible for specimen available only in the form of egg or

juvenile stage. The difficulties in identifying nematodes include their small size, high diversity, and the absence of specific morphological features (Blok & Powers, 2009). The advanced polymerase chain reaction (PCR)-based diagnostic technique has circumvented the complexity of morphological-based nematode identification.

Molecular identification relies on the occurrence of polymorphisms in DNA sequences among groups of nematodes, especially in the ribosomal DNA (rDNA) repeating unit, including 18S (small subunit of rDNA or SSU); 28S (or large subunit rDNA, LSU); 5.8S coding genes and the spacer regions (internal transcribed spacer [ITS], external transcribed spacer [ETS] and intergenic spacer [IGS]); and mitochondrial DNA (mtDNA) (Cunha *et al*., 2018). The D2-D3 expansion region of LSU has been used for designing diagnostics primers for nematode species identification (Blok & Powers, 2009; Cunha *et al*., 2018). DNA sequencing of ITS region is widely used protocol for nematode species identification, although limited sequence polymorphism in the ITS sequences can occur among species complex sharing gene lineages such as *M. incognita*, *M. javanica,* and *M. arenaria* (Blok & Powers, 2009). For RKN, specific sequenced characterized amplified region (SCAR) primers designed based on rDNA sequences and random amplified polymorphism DNA (RAPDs) have been developed (Blok & Powers, 2009).

Both ITS rDNA primers and species-specific primer have been used in Indonesia for nematode identification (Table 1) and monitoring the spatial and temporal distribution of PCN (Nugrahana *et al*., 2017). For nematodes that have been identified morphology (Table 2), species confirmation by the molecular protocol is necessary because accurate identification is essential for implementing management strategies, i.e., for designing a crop rotation scheme.

CONCLUDING REMARKS

A list of nematode taxa associated with a crop is of limited utility if it does not indicate which PPN species are predominant and potentially damaging (De Waele & Elsen, 2007). In order to assess nematode status, a nationwide field survey supported is suggested. Host plant, environmental factors, including temperature and soil water content affect nematode population density, whereas soil characteristics including type and texture influence PPN migration and reproduction, and soil chemistry (pH and nutrient content) affect nematode prevalence and diversity (Palomares-Rius *et al*., 2015; Fleming *et al*., 2016). Therefore, the availability of ecological data, crop history, and crop yield data under low and high nematode infestation will allow valid evaluation of nematode prevalence and their

relative risk on crop productivity. Bioassay should follow for prevalent and potentially damaging nematodes to determine their population or economic thresholds, of which information are currently unavailable or outdated. Those informations are then used for setting national nematode research priorities.

Compared to nematodes in temperate regions, not all nematode species in the tropics have been described (De Waele & Elsen, 2007). Among PPNs, *Meloidogyne* spp., *Pratylenchus* spp., *Radopholus* spp., and *Heterodera* spp. are of particular interest genera in tropical plant nematology discipline and their new species continue to be identified (De Waele & Elsen, 2007). For example, the application of DNA marker analysis to support morphological observations has led to the description of *M. lopezi* n.sp. associated with coffee in Costa Rica (Humphreys-Pereira *et al*., 2014); *P. haiduongensis* n. sp. associated with carrot in Vietnam (Nguyen *et al*., 2017); *R. bridgei* from turmeric and *R. citri* from citrus in Indonesia (De Waele & Elsen, 2007); and *H. hainanensis* n.sp. from bamboo in the tropical region of China (Zhuo *et al*., 2013). Being situated in the tropical region with high plant biodiversity, it is predicted that Indonesia soils contain many new nematode species. Therefore, molecular diagnostic tool should be applied in every field survey to reveal nematode biodiversity in Indonesia. The information obtained will make a significant contribution to the tropical nematology discipline and narrow the gap in the knowledge of tropical nematology with temperate nematology.

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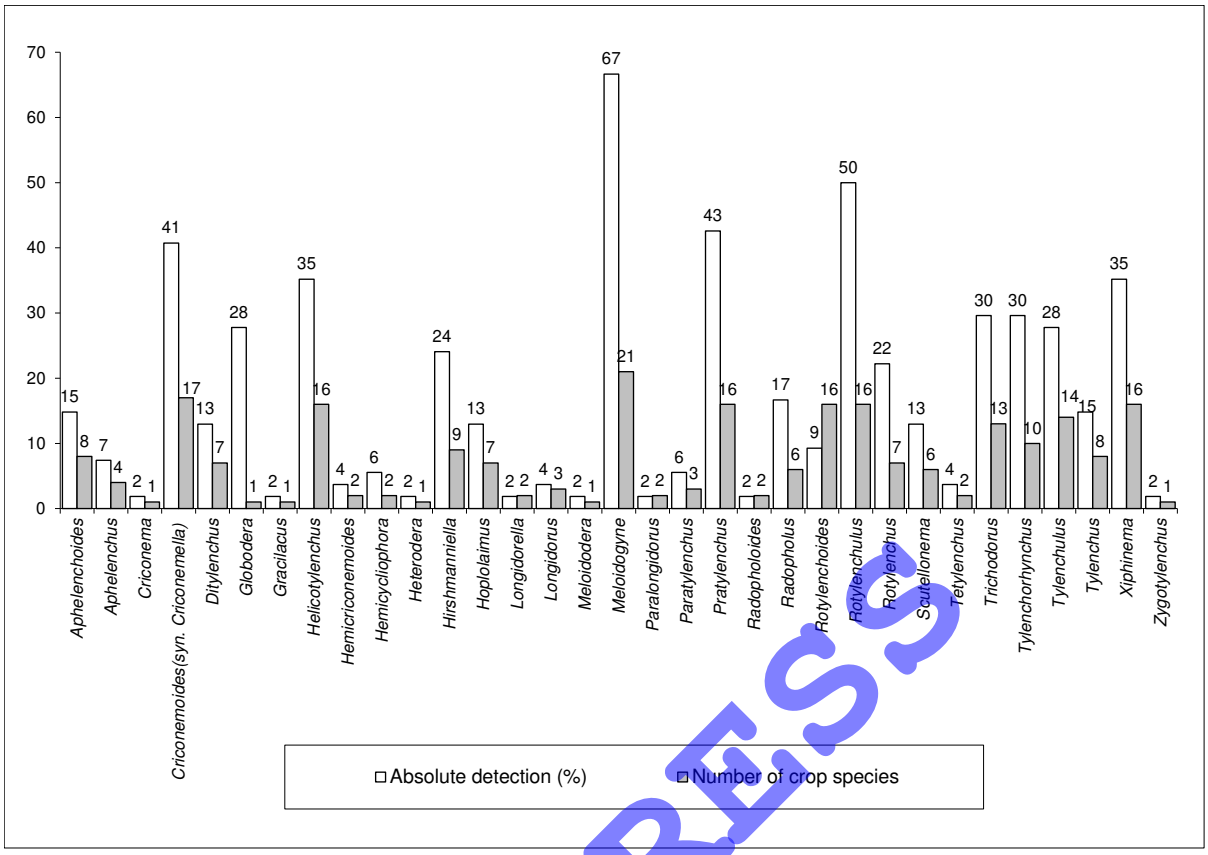

Figure 1. Frequency of plant-parasitic nematode detection in 54 regencies of 16 provinces (North, West, and South Sumatera; Lampung; Bangka; West, Central, and East Java; Yogyakarta; West and East Kalimantan; South, Central and South East Sulawesi; East Nusa Tenggara; and Papua) and the number of crop plant-associated (ananas, bitterweed [*Andrographis paniculata*], banana, black pepper, carrot, cassava, celery, chili pepper, cucumber, coffee, corn, eggplant, garlic, groundnut, guava, leafy vegetables, mungbean, potato, patchouli, rice, shallot, sugarcane, sweet potato, and tomato)

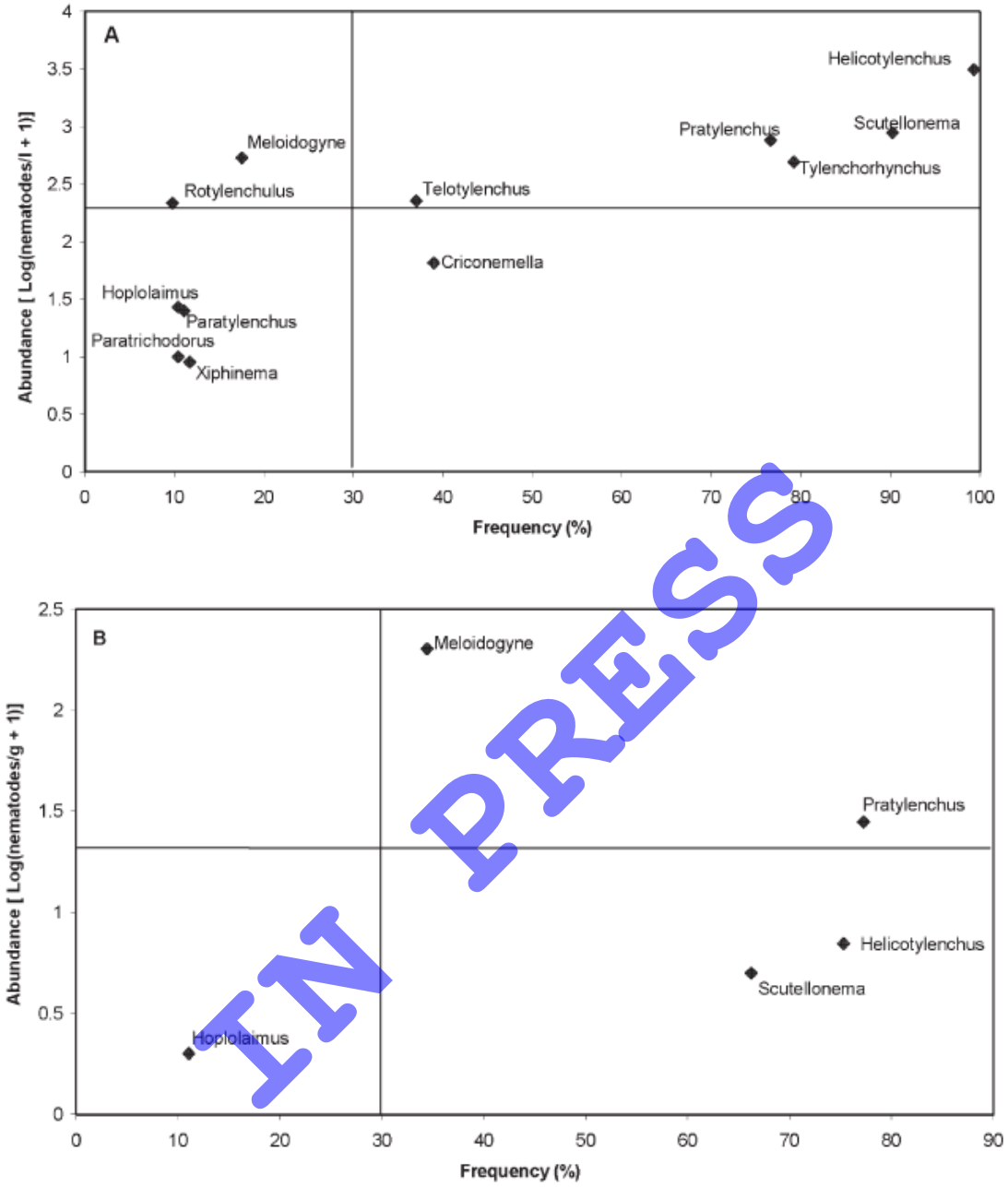

Figure 2. Method of plant-parasitic nematode relative importance classification based on frequency and abundance values. Examples shown are graphs of frequency (percentage of positive samples) and abundance (mean numbers per sample) of the plant-parasitic nematode genera associated with cowpea (*Vigna unguiculata*) in soil (A) and root (B) samples in a national survey of Burkina Faso (Sawadogo *et al.*, 2009). Nematodes on the upper right quadrant are of high prevalence because both values of abundance are above the assigned thresholds (≥1.3 (=$\log_{10}$ [20 individuals/g of roots]) or ≥2.3 (=$\log_{10}$ [200 individuals/L of soil]) and present in at least 30% of the samples

Table 1. Firstly reported plant-parasitic nematode species in Indonesia

No.	Species	Common name	Crop[a]	Locality	Method of identification	Reference[b]
1.	*Aphelenhoides fragariae*	strawberry crimp nematode	bitterweed (*Andrographis paniculata*) leaf	Bogor	morphology	3
2.	*Aphelenhoides varicaudatus*	-	garlic	Tegal (C. Java)	molecular	8
3.	*Ditylenchus dipsaci*	stem and bulb nematode	garlic leaf	Temanggung (C. Java)	morphology	6
			imported garlic bulb	Tanjung Priok (Jakarta)	morphology	10
4.	*Globodera pallida*	white potato cyst nematode	potato	Wonosobo and Banjarnegara (C. Java)	molecular	9
5.	*Globodera rostochiensis*	golden cyst nematode	potato	Kota Batu (E. Java)	molecular	5
6.	*Hemicriconemoides cocophillus*	-	coffee	Malang (E. Java)	morphology and molecular	2
7.	*Heterodera zeae*	corn cyst nematode	corn	Bangkalan (E. Java)	morphology	1
8.	*Hirschmanniella mucronata*	rice nematode	rice	Cangkringan (Yogyakarta)	morphology and molecular	7
9.	*Meloidogyne fallax*	false Columbia root-knot nematode	carrot	Cianjur (W. Java)	molecular	12
10.	*Meloidogyne hapla*	northern root-knot	carrot	Kota Batu (E. Java)	molecular	4
11.	*Meloidogyne graminicola*	rice root knot nematode	rice	Bantul (Yogyakarta)	morpohology	11

[a]Unless mentioned, nematodes were found in root or soil

[b]1=Baliadi (2008); 2=Budiman *et al.* (2020); 3=Djiwanti & Supriadi (2008); 4=Halimah *et al.* (2013); 5=Indarti *et al.*, (2004); 6=Indarti *et al.* (2018); 7=Indarti *et al.* (2020); 8=Kusuma *et al.* (2020); 9=Lisnawita *et al.*, (2012); 10=Muliya *et al.* (2018); 11=Netscher & Erlan (1993); 12=Supramana & Suastika (2012)

Table 2. Primers used in polymerase chain reaction (PCR)–based identification of plant-parasitic nematode in Indonesia

No.	Primer pair	Amplicon size (bp)	Species	Reference[a]
1.	D2A/D3B[b]	780	*Aphelenchoides varicaudatus*	6
		765	*Hemicriconemoides cocophillus*	2
		766	*Hirschmanniella mucronata*	5
		759	*Pratylenchus coffeae*	2
		694	*Hemicriconemoides cocophillus*	2
2.	Far/Rar	420	*Meloidogyne arenaria*	11,15
3.	Fjav/Rjav	720	*Meloidogyne javanica*	4, 17, 18
4.	ITS1/ITS2[b]	830	*Aphelenchoides besseyi*	3, 11, 14
5.	G*pa*-specific/universal primer 5.8 rDNA	391	*Globodera pallida*	7
6.	*Gro*-specific/universal primer 5.8 rDNA	238	*Globodera rostochiensis*	7
7.	JMV1/JMV2/JMV-hapla[c]	670	*Meloidogyne fallax*	15
8.	JMV1/JMV2/JMV-hapla[c]	440	*Meloidogyne hapla*	4, 15
9.	MI-F/MI-R	999	*Meloidogyne incognita*	1, 15, 17, 18
10.	MIGF/MIGR	750	*Meloidogyne incognita*	11
11.	PITSr3/ITS5	434	*Globodera rostochiensis*	10, 12, 13
12.	rDNA2/rDNA 1.58s[b]	500	*Meloidogyne graminicola*	8
13.	TW81/AB28[b]	941-952	*Pratylenchus coffeae*	2

[a]1=Aprilyani *et al.*, (2015); 2=Budiman *et al.* (2019); 3=Diana *et al.* (2018); 4=Halimah *et al.*, (2013); 5=Indarti *et al.* (2020); 6=Kusuma *et al.*, (2020); 7=Lisnawita *et al.*, (2012); 8=Mirsam & Kurniawati (2018); 9=Muliya *et al.* (2018); 10=Mulyadi *et al.* (2014); 11=Mutala'liah *et al.* (2019); 12=Nugrahana *et al.*, (2017); 13=Nurjanah *et al.* (2016); 14=Rahman *et al.*, (2018); 15=Supramana & Suastika (2012); 17=Tuminem *et al.* (2015); 18=Utami *et al.* (2017)

[b]DNA sequencing is performed following PCR amplification with universal primer

[c]Multiplex primer