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Research Article

Detection and Development of Infestation Rate of *Aphelenchoides besseyi* on Various Rice Seed Varieties

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ABSTRACT

Aphelenchoides besseyi is a seed borne nematode caused white tip disease. Infested rice seeds are beneficial inoculum source for *A. besseyi* widespread. The objective of this research was to determine the occurrence and development of symptom level caused by *A. besseyi* in rice seed varieties. The research was done by detecting *A. besseyi* in rice seed using Hoshino and Togashi method and counting the nematode population in the seed; observing the incubation time and measuring disease incidence every week. The result revealed that *A. besseyi* was found in 16 of 17 varieties, i.e. R1 IR64, R2 IR64, R3 IR64, R1 Situbagendit, R2 Situbagendit, Inpari 33, R1 Memberamo, R2 Memberamo, R3 Memberamo, R1 Way Apo Buru, R2 Way Apo Buru, Mekongga, Pepe, Ketan, Sintanur, and Ciherang. *A. besseyi i*nfection increased every week in many varieties depend on quantitative (statistics) and qualitative (symptom) indicators. There were 4 varieties have to be investigate further because they were infested by *A. besseyi* >30 nematodes/100 seeds, i.e. R1 Way Apo Buru, R1 Situbagendit, Ketan and R3 IR 64. A practice that is also required is the elimination of *A. besseyi* inoculum in rice seed before cultivate. .

Keywords: *Aphelenchoides besseyi*, development of infestation rate, occurrence detection

INTRODUCTION

Rice is an important crop in Indonesia. In 2014, 14.30 million hectares produced 71.33 million tons of dry milled (Sembiring, 2015 *cit* Habibi *et al*., 2016). In rice cultivation, *Aphelenchoides besseyi* come as an obstruction that carried and lives as ectoparasites on seeds and causes white tip (Tulek *et al*., 2015). This nematode was originally classified as OPTK A2 and has changed its status to become regular pest (regulated pest) since 2018 (Mentan RI, 2018).

A. besseyi morphology is seen from its slim and long body shape and has stomato stylet type. The size of the median bulb is three-quarter of body width. There is an overlapping between the esophagus and the intestine of *A. besseyi*. Another characteristic of *A. besseyi* is that there is a mucro at the tip of the tail (Efendi, 2016). An understanding of the characteristics morphological and biology of plant parasitic nematodes is important step in the detection and management of plant parasitic nematodes.

Rice seeds infested by *A. besseyi* are the main inoculum in its deployment in the world (Pashi *et al*., 2017). While in the grain (between the grain skin and rice seeds), *A. besseyi* is in dormant condition until three years after harvest. After the rice seeds were spread and germinate, *A. besseyi* will reactivate and move towards the growing point on pseudostems or leaves and eat plant tissue ectoparasitically (Azizah, 2017).

Seeds infested by *A. besseyi* form different shapes and bear black spots on the skin. Not only on seeds, symptoms also found in plants which can be seen at the beginning of growth that shows white tip in the newly emerged leaves. The tips of the leaves dry, curl and twist, while the other leaves remain normal. The incidence of white tip disease tends to elevate along with the increase of the initial nematode population per gram of seeds and could lead to yield loss (Tulek *et al*., 2015).

Yield loss due to *A. besseyi* has been widely studied. On infested land, yield losses generally range from 10–30%. On land where all crops were attacked, yield loss reaches 70% for susceptible cultivars and 20% for resistant cultivars (Prot, 1992). The amount of yield loss varies depending on cultivar, plant age, temperature, farming method, and other factors (Tulek & Cobanoglu, 2010).

Research on the detection and development of *A. besseyi* attack rates on various rice seed varieties was carried out to obtain the certainty about cultivar and the development of *A. besseyi* attack rates every week.

MATERIALS AND METHODS

Sampling

Rice seeds were taken randomly from one distributor in Karanganom, Polanharjo, and North Klaten District, which are thought to be the centers of market seeds by the farmers. Samples were taken 4–8 varieties of rice seeds randomly from each distributors, with 250 grams per each variety.

Detection of the Existence of Aphelenchoides besseyi *in Seeds*

Seeds that have been obtained from the field were then detected to observe the presence or absence of *A. besseyi*. The number of observed samples was 100 seeds with *A. besseyi* symptoms. Detection of the presence of nematodes in seeds was done by Hoshino and Togashi method (1999). Seeds were cut lengthwise and put in a 1 ml tip pipette (7 cm long, 1 mm in diameter and bottom 7.4 mm). A pipette tip filled with 6 ml of water was incubated at 25°C for 24 hours. Next, the water in the vial and tip pipette (nematode suspension) were transferred in a counting dish to count the number of nematodes and count the abundance of *A. besseyi* in the seeds.

Development of Aphelenchoides besseyi *Infestation Rate*

Seeds that were indicated infested with *A. besseyi* were planted on sterile soil and manure at a ratio of 1:1. Planting was done in a specific seedling pot with one seed hole. The soil was watered one to two times a day depending on the weather. Observations were carried out every day until the plant reached 21 days after planting (DAP). Thirty seeds from each variety of each district were planted with 3 repetitions.

Parameters addressed in incubation time and disease incidence. Observations were made every day and the incubation time of the *A. besseyi* attack was recorded. Observation on nematode attack rate carried out on seeds planted in a greenhouse on the 7th, 14th and 21st DAP. The disease incidence rate was obtained by dividing the number of attacked plants with the total number of plants and then multiplied by 100%.

Data Analysis

The abundance of *A. besseyi* population that found in various rice seed varieties were then averaged and determined the safeness of each variety to be cultivated with the consideration that more than 30 nematodes per 100 seeds would be able to cause yield loss (Fukano, 1962), thus the seeds are not safe to plant. If *A. besseyi* nematode was found less than 30 nematodes per 100 seeds, it means that the seed variety is still safe to be planted. Data on the infestation rate per week (data per variety and time) were analyzed using a one-way analysis of variance (ANOVA) ($\alpha = 0.05$). If there were real differences, the data will further be tested using Duncan's New Multiple Range Test (DMRT) ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Detection of *A. besseyi* in seeds are difficult to be done with bare eyes due to not all symptomatic seeds were infested by the nematode. *A. besseyi* which was found to have slender shape, set off the mouth, short stylet, the median oval–shaped bulb that range ¾ body width (Figure 1A), has a mucro at the end of the posterior (Figure 1B), and overlapping esophagus.

Tables 1 and 2 explained that from 17 varieties with symptoms, only 16 varieties were infested with *A. besseyi*. This implies that certified seeds are not assured free from *A. besseyi* infestation. Similar research conducted by Diana *et al*. (2018) also stated that seeds from inbreeding upland rice, paddy rice, local varieties and excellent varieties of sticky rice can be infected by *A. besseyi*. In addition, *A. besseyi* also be found not only in rice seeds that show symptoms which is shape changes and bear black spots on the seed coat but also in seeds with no symptoms (Ahmad, 2017).

From 100 symptomatic seeds, there were only 1–9 seeds infested with *A. besseyi*. Nematode populations found in 100 symptomatic seeds were ranging from 0 in the R3 Situbagendit to 116 in Ketan varieties. Fukano (1962) determined 30 nematodes per 100 seeds as the limit population of *A. besseyi* to have cause yield loss. Because *A. besseyi* infestation in the rice seeds observed in this research were less than 30 nematodes per 100 seeds, this revealed that seeds were still safe to be cultivated, except for R1 WAB, R1 Situbagendit,

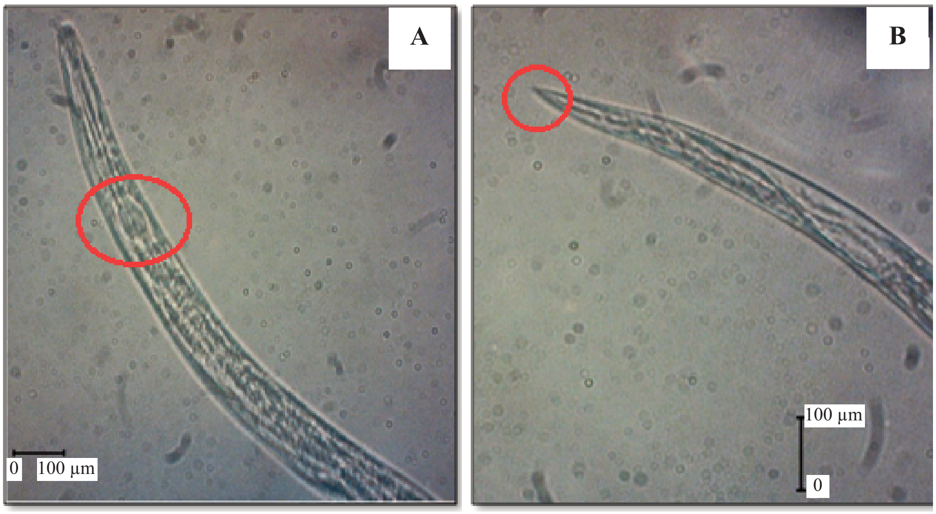

Figure 1. Morphology of *Aphelenchoides besseyi* which was found in the infested rice plants: (A) large median bulb (three–quarter of body width); (B) mucro on the tail tip

Table 1. The detection on the presence of *Aphelencoides besseyi* in various rice seed varieties

No.	Variety	Producer	Certificate status	Presence
1.	R1 IR-64	PB. Sari Bumi	√	+
2.	R2 IR-64	PB. Trubus Super	√	+
3.	R3 IR-64	PB. Usaha Tani Group	√	+
4.	R1 Situbagendit	PB. Sari Bumi	√	+
5.	R2 Situbagendit	PB. Anugerah Tani Maju	√	+
6.	R3 Situbagendit	PB. Trubus Super	√	-
7.	R1 Memberamo	PB. Sari Bumi	√	+
8.	R2 Memberamo	PB. Trubus Super	√	+
9.	R3 Memberamo	PB. Kerja	√	+
10.	R1 Way Apo Buru	PB. Sari Bumi	√	+
11.	R2 Way Apo Buru	PB. Anugerah Tani Maju	√	+
12.	Ciherang	PB. Usaha Tani Group	√	+
13.	Inpari 33	PB. Usaha Tani Group	√	+
14.	Ketan	PB. Usaha Tani Group	√	+
15.	Mekongga	PB. Aditama Ponorogo	√	+
16.	Pepe	PB. Tani Mas	√	+
17.	Sintanur	PB. Usaha Tani Group	√	+

Remarks: (+) = infested, (-) = not infested, PB = seed company, R1, R2, R3: sample code for same variety but different producer

Ketan, and IR 64 (Table 2). The incubation time of *A. besseyi* attacking various varieties was varied from the 3rd to the 8th DAP. This is not much different from EPPO (2013) which stated that the first symptoms of *A. besseyi* attack on rice can be observed on the 6th DAP (Table 2).

A. besseyi provide typical symptoms at the beginning of growth, that is chlorosis at the tip of young leaves with a length of 1 cm (Figure 2A), then the chlorosis expands to 2 cm in the first week (Figure 2B), and reach 5 cm in the second week (Figure 2C). Then the tips of the leaves dried and wrinkled (Figure 2D) in the third week. The same results were also obtained by Sari (2017) and Azizah (2017) who mentioned the symptoms due to the infestation of *A. besseyi* were dried buds, curled, and wrinkled. *A. besseyi* attacks on rice plants causing stunted plant cell growth which lead to the absence of chloroplasts and hence leads to chlorosis of leaf buds. This nematode also damaged phloem tissue by disrupting the distribution of nutrients in plants which could be seen in interfered plant growth (Fortuner & Williams, 1975).

Table 2. The abundance of *Aphelenchoides besseyi* population on various rice seed varieties

No.	Variety	Number of infested seed	Population	Incubation time (DAP)
1	R1 IR 64	1	1	6
2	R2 IR 64	2	15	5
3	R3 IR 64	4	43	7
4	R1 Situbagendit	5	32	5
5	R2 Situbagendit	1	1	7
6	R3 Situbagendit	0	0	8
7	R1 Memberamo	5	12	7
8	R2 Memberamo	1	2	7
9	R3 Memberamo	6	19	3
10	R1 Way Abu Buru	7	40	6
11	R2 Way Apo Buru	1	3	6
12	Ciherang	4	23	0
13	Inpari 33	1	3	5
14	Ketan	9	116	0
15	Mekongga	3	15	0
16	Pepe	1	2	5
17	Sintanur	2	11	7

Remarks: The number of samples observed was 100 seeds with infested symptoms; DAP = day after planting.

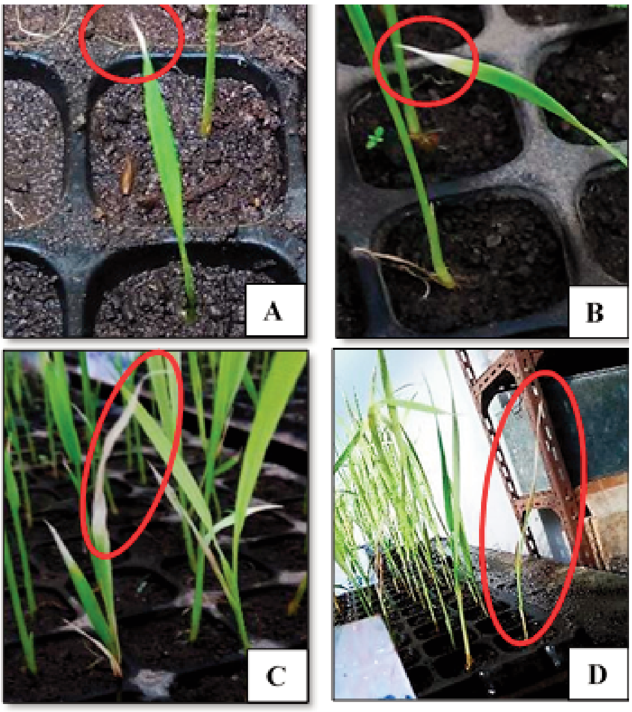

Figure 2. Variation in the development of symptoms of *Aphelenchoides besseyi* infestation: (A) the first symptom is 1 cm long of chlorosis leaf tip; (B) the first week symptom shows 2–3 cm long of chlorosis leaf tip; (C) the second week symptom shows that the chlorosis form up to half the length of the leaf followed by the seond leaf experiencing chlorosis; (D) the third week symptom shows that leaf is dry out

Other symptoms of the attack are flag leaf bending, panicles emerging inhibition, reducing rice grains, sterile flowers, seed distortion, and weight reduction in 1000 seeds. In the fields, economic losses of 0–70% depend on variety, year, and country (Todd & Atkins, 1958 *cit.* Teng *et al*., 1994; Yoshii & Yamamoto, 1995; Todd & Atkins, 1958 *cit.* Teng *et al*., 1994).

The development of the symptoms started from the leaf buds to the base. White tip disease will expand followed by necrosis. When the seeds were sown,

Table 3. The development of *Aphelenchoides besseyi* infestation rate on various varieties of plants over 3 weeks observation

Week	Treatment	Infestation (%)	
3	R3IR64	100.00	a
3	R3Memberamo	100.00	a
3	Pepe	100.00	a
3	Sintanur	100.00	a
3	R1Inpari33	100.00	a
3	R1IR64	100.00	a
3	R1Memberamo	100.00	a
3	R1WAB	100.00	a
3	R2Memberamo	100.00	a
3	R2Situbagendit	100.00	a
3	R2WAB	100.00	a
2	R2Situbagendit	97.78	a
2	R1IR64	93.33	ab
2	R3Memberamo	91.36	abc
2	R2IR64	89.73	abc
2	R1Memberamo	88.49	abc
2	R1Situbagendit	87.18	abc
2	R1Inpari33	85.13	abcd
2	R3IR64	82.92	abcd
2	R2Memberamo	81.41	bcd
2	R3Situbagendit	78.60	bcd
2	R1WAB	74.97	cde
2	R2WAB	70.83	de
2	Pepe	62.20	ef
2	Sintanur	61.94	ef
3	R2IR64	58.60	ef
3	R1Situbagendit	53.31	f
3	R3Situbagendit	49.72	f
1	R2Situbagendit	20.56	g
1	Inpari 33	18.89	g
1	R2WAB	12.96	gh
1	Pepe	11.44	gh
1	Sintanur	11.27	gh
1	R1Memberamo	9.40	gh
1	R1WAB	7.65	gh
1	R2IR64	7.27	gh
1	R3Memberamo	6.73	gh
1	R1IR64	1.44	h
1	R3IR64	1.39	h
1	R1Situbagendit	1.14	h
1	R2Memberamo	1.11	h
1	Ciherang	0.00	h
1	Ketan	0.00	h
1	Mekongga	0.00	h
1	R3Situbagendit	0.00	h
2	Ciherang	0.00	h
2	Ketan	0.00	h
2	Mekongga	0.00	h
3	Ciherang	0.00	h
3	Ketan	0.00	h
3	Mekongga	0.00	h

Remarks: Values followed by the same letter were not significantly different according to DMRT ($\alpha = 0.05$).

A. besseyi anabiosis immediately and attracted by the meristematic. At the initial growth, *A. besseyi* will be in the leaf fronds in a small population. This nematode will parasite the host plants ectoparasitically around the apical meristem area (Luc *et al*., 1990).

The development of the infestation rate of *A. besseyi* on plant varieties increased every week. The highest attack in the first week occurred on the R2 Situbagendit variety (20.93%) although they were not different from the Inpari 33. The incidence strongly increased in the second week with the highest infestation that occurred in the R2 Situbagendit where the value was nearly equal to the 3rd-week attack in several varieties. The peak of the attack occurred in the third week, although the number of attacks in each variety was the same except in the R1 Situbagendit, R3 Situbagendit and R2 IR 64 varieties, where the number of attacks in the second week was higher than the number of attacks in the third week (Table 3).

The increasing percentage of *A. besseyi* infestation rate in various rice seed varieties due to the transmission of nematodes from one plant to another. The same result also obtained by Efendi (2016) and Mahdavian & Javadi (2012) which stated that transmission between plants can occur when planted in the same area. Infested symptoms decreased in Situbagendit R1, R2 IR 64, and Situbagendit R3 presumably because the plant was able to regenerate immediately hence the plant grows normal again. Masked symptoms (plants not showing any symptom even infected by *A. besseyi*) can also occur in infected plants. The number of nematodes did not affect the symptoms that appeared in plants. A high number of the nematode population does not always cause symptoms in plants but can lead to yield loss (Feng *et al*., 2014).

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