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RICE SEED TREATMENT BY SOAKING IN WATER AND PESTICIDES TO CONTROL FOLIAR NEMATODE *Aphelenchoides besseyi*

Perlakuan Benih Padi dengan Perendaman Air dan Pestisida (Karbosulfan, Benomil dan Imidakloprid) untuk Mengendalikan Nematoda Aphelenchoides besseyi

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ABSTRACT

Aphelenchoides besseyi is one of the most important parasitic nematodes in rice plants, capable of dormancy in rice seeds. This study aims to determine the effects of soaking rice seeds in water and pesticides (carbosulfan, benomyl, and imidacloprid) on the mortality of *A. besseyi*, seed viability, and symptoms in rice seedlings. The experiment was conducted on a rice variety of Pak Tiwi-1 that was attacked by *A. besseyi*. Seed treatments include: (1) cold water treatment (cwt) 5-10˚C 24 hours + hot water treatment (hwt) 52˚C 10 min; (2) hwt 52˚C 10 min; (3) hwt 57˚C 10 (4) water immersion 25-30˚C 24 hours; seed dressing with (5) carbosulfan 25 ST 2%; (6) benomyl 50 WP 0.2%; and (7) imidacloprid 5 WP 2%. Cold water treatment (cwt) at 5-10˚C for 24 hours followed by hot water treatment (hwt) at 52˚C for 10 minutes caused 51.17% *A. besseyi* mortality yet reduced the seed viability. Soaking the seed in the water at 25-30˚C for 24 hours, followed by hwt at 57˚C for 10 minutes and hwt at 52˚C for 10 minutes, could cause 48.67%, 39.18%, and 33.05% nematode mortality, respectively. Seed dressing with imidacloprid 5WP (2% by seed weight) caused 47.25% *A. besseyi* mortality, whereas benomyl 50WP (0.2% by seed weight) and carbosulfan 25ST (2% by seed weight) caused only 27.61% and 11.34%. Low nematode mortality in all treatments (less than 60%) showed symptoms of *A. besseyi* infection in rice seedlings 21 days after planting. Based on the study's results, no effective treatment controlled the nematode *Aphelenchoides besseyi* on rice seeds.

Keywords: hot water treatment, mortality, seed dressing, seed viability, symptoms

INTRODUCTION

Aphelenchoides besseyi is a seed-borne nematode causing a "white tip" disease and decreasing grain number and viability (Duncan & Moens 2013). The primary host plants of *A. besseyi* are rice and strawberries, but *A. besseyi* is found in other crop plants, such as maize, chili, soybean, sweet potato, and onion (EPPO 2023). *A. besseyi* has been found in most rice cultivation areas worldwide (EPPO 2023), including Indonesia (Diana 2018). The yield loss in rice crops due to the *A. besseyi* attack might reach 10 to 50% (Singh *et al.* 2013). *A. besseyi* spreads primarily through seeds but can be locally distributed through irrigation water in paddy fields and seedlings of infected nurseries (Bridge *et al.* 2005). Nematodes are found under rice seed husks and will last 2-3 years on dried grains (Duncan & Moens 2013).

Controlling *A. besseyi* nematodes can be done using treatments on seeds, such as physical or chemical treatments. For physical treatment, soaking rice seed in water at 25˚C followed by drying may cause up to 72.4% nematode mortality (Hoshino & Togashi 2000). Soaking in cold water for 18-24 hours then immersing it in water at 51-53˚C for 15 minutes is also an effective treatment for seeds (Bridge *et al.* 2005). Hot water treatment by soaking bulbs in water for 30 min at 57°C is the most efficacious method for *Polianthes tuberosa* (Cuc *et al.* 2010). Chemical treatments that may increase *A. besseyi* mortality are the applications of benomyl, carbofuran (Gergon & Prat 1993), diazinon, and fipronil (Cuc *et al.* 2010) Dipping *P. tuberosa* with carbosulfan 25EC can reduce *A. besseyi* population up to 67% (Bala *et al.* 2016).

In Indonesia, the management of *A. besseyi* has not been applied by farmers. Studies on managing *A. besseyi* with various physical and chemical seed treatments have been conducted in various countries, such as India, Japan, and Vietnam. Still, a study is needed to ensure that farmers in

Indonesia can carry out seed treatments effectively and efficiently. This study aims to determine the effective seed treatment for controlling *A. besseyi*.

MATERIAL AND METHODS

The variety of rice seeds used in this study was Pak-Tiwi 1 rice infected with *Aphelenchoides besseyi*. The treatments used were physical treatment with variations in temperature and chemical treatment with types of pesticides. Physical treatment was carried out using three methods: hot water treatment (hwt), cold water treatment (cwt), and immersion in normal-temperature water. Seeds are placed on gauze and tied with string. In the P1 treatment, the seeds were soaked in water at 5-10˚C for 24 hours in cold storage, then soaked in hot water at 52˚C for 10 minutes. In the P2 and P3 treatments, the seeds were soaked in hot water at 52 ˚C for 10 minutes (P2) and 57 ˚C for 10 minutes (P3). In the P4 treatment, the seeds were soaked in water at room temperature, namely 25-30˚C for 24 hours. The seeds are then dried in the open air.

Chemical treatment was carried out using the seed dressing method. In treatment P5, the carbosulfan pesticide (Masrhal® 25ST, PT Bina Guna Kimia, Jakarta, Indonesia) weighed as much as 2% of the total weight of the seeds and then mixed with the seeds. In the P6 treatment, the benomyl pesticide (Benlox® 50WP, PT Dharma Guna Wibawa, Jakarta, Indonesia) was weighed as much as 0.2% of the total seed weight, added 10 ml of water/1.5 g of pesticide and then mixed with the seeds. In the P7 treatment, the imidacloprid pesticide (Confidor® 5WP, PT Bayer Indonesia, Jakarta, Indonesia) was weighed as much as 2% of the total seed weight, added 10 ml of water/1.5 g of pesticide, then the pesticide and seed were mixed evenly in plastic.

This study consisted of three assays: mortality of *A. besseyi*, seed viability, and the number of infected rice plants. The seeds used in each parameter are different from one another.

Mortality Assay

Each treatment consisted of 30 rice seeds with three replications. The experiment used a Completely Randomized Design. Nematodes were extracted in rice seeds using the pipette tip method (Hoshino & Togashi 1999). Rice seeds were cut lengthwise and put in a 1 ml pipette tip. Pipette tips holding a grain of rice (one rice seed) each were placed in a vial glass containing 6 ml of water and incubated in a dark room at ± 25˚C for 8 hours. Furthermore, the water in a glass vial and pipette tips were transferred to the counting dish and observed using a stereoscopic microscope. The calculation of nematode mortality was based on the percentage of living nematodes (% Mortality = number of dead nematodes/ total number of nematodes x 100%). The surviving nematodes moved actively, while the dead nematodes did not move after a dormant period.

Seed Viability

The treated rice seeds were germinated for seven days in a petri dish using a Completely Randomized Design of 25 seeds with three replications. The viability of seeds was measured through the germination rate (Germination rate (%) = number of seeds that germinate/ number of seeds germinated x 100%).

Number of Leaves with Foliar Nematode Symptoms

The treated rice seeds were planted in pots in the greenhouse. The design used a completely randomized design of 30 rice seeds for each treatment with three replications. The observation was done at 21 days after planting by calculating the sample of rice plants in each treatment that showed symptoms of *Aphelenchoides besseyi* (chlorosis and leaves forming whips). The symptoms of *A. besseyi* infection could be seen after the 16th day of post-germination (Nandakumar et al. 1975)

Data Analysis

Data were analyzed by one-way analysis of variance. Mortality data were transformed using arcsin $\sqrt{(x)}$. A significant difference between treatments led to the use of Duncan's New Multiple Range Test (DMRT) $P < 0.05$ to distinguish differences between means. The statistical software for analyses was Rstudio.

RESULTS AND DISCUSSION

Mortality of *Aphelenchoides besseyi*

Physical and chemical treatments were able to cause nematode mortality (Table 1). P1 (cwt at 5-10˚C for 24 hours) was the treatment that caused the highest mortality of 51.17%. The chemical treatment that effectively increased nematode mortality was P7 (imidacloprid 5 WP 2%) which was 47.25%. Other treatments that were quite effective were P2 (hwt 52˚C 10 min), P3 (hwt 57˚C 10 min), and P4 (soaking with water 25-30˚C 24 hours). Dead nematodes are characterized by damage to their body wall (Figure 1).

Table 1. The effects of soaking rice seeds in water and pesticides on the mortality of *Aphelenchoides besseyi* and the number of leaves with symptoms on the 21st day after planting

Treatments	Mortality (%)	Number of Leaves with Symptoms
Control	2.68^{d}	22.33^{b}
Cwt 5-10˚C 24 hours + hwt 52˚C 10 min	51.17^{a}	22.00^{b}
Hwt 52˚C 10 min	33.05^{ab}	21.67^{b}
Hwt 57˚C 10 min	39.18^{ab}	16.67^{c}
Soaking with water 25-30˚C 24 hours	48.67^{ab}	26.33^{a}
Carbosulfan 25 ST 2%	11.34^{cd}	21.00^{b}
Benomyl 50 WP 0,2%	27.61^{bc}	22.67^{b}
Imidacloprid 5 WP 2%	47.25^{a}	23.67^{ab}

Note : Cwt = cold water treatment, hwt = hot water treatment. Common letter in the same column are not significantly different according to Duncan's Multiple Range Test at P = 0.05

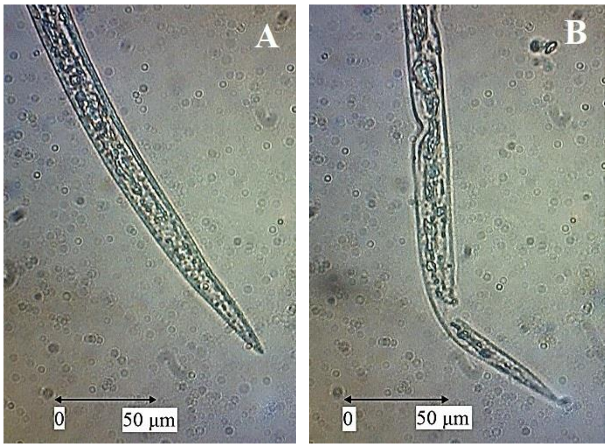

Figure 1. The effect of hot water treatment on the body of *Aphelenchoides besseyi* (A) live nematodes (B) dead nematodes. Dead nematodes are characterized by the damage on the body wall.

Seed Viability after Treatment

Some treatments resulted in a seed viability decrease (Table 2). P1 (Cwt 5-10˚C 24 hours + hwt 52˚C 10 minutes) was a treatment that reduced the germination rate of rice seeds. The germination rates of rice seeds in other treatments (P2, P3, P4, P5, and P6) were insignificant with the control,

Table 2. The effects of soaking rice seeds in water and pesticide on seed viability

Treatments	Germination rate (%)
Control	72.00^{a}
Cwt 5-10˚C 24 hours + hwt 52˚C 10 min (P1)	39.33^{b}
Hwt 52˚C 10 min (P2)	67.67^{a}
Hwt 57˚C 10 min (P3)	57.33^{ab}
Soaking with water 25-30˚C 24 hours (P4)	69.00^{a}
Carbosulfan 25 ST 2% (P5)	65.33^{a}
Benomyl 50 WP 0,2% (P6)	61.00^{a}
Imidacloprid 5 WP 2% P7	67.00^{a}

Note : Cwt = cold water treatment, hwt = hot water treatment. Common letter in the same column are not significantly different according to Duncan's Multiple Range Test at P = 0.05

meaning those treatments did not affect seed viability. Viability of seeds infected with nematodes tended to be low, seed viability in the control treatment only showed 72%.

Number of Leaves with Foliar Nematode Symptoms

The effect of treatments on rice growth after germination was observed by looking at the symptoms of *A. besseyi* in plants. The tips of leaves infected by *A. besseyi* turned white, dried and curled, forming the shape of a whip (Figure 2). All treatments showed symptoms of *A. besseyi* infection (Table 1). Hot water treatment at 57˚C for 10 minutes was the only treatment capable of reducing the symptoms of infection on rice seeds. Other treatments did not show any significant difference when they were compared with controls.

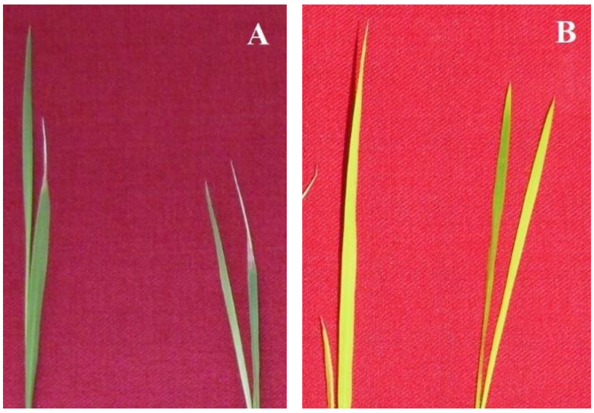

Figure 2. (A) Symptoms of foliar nematode *Aphelenchoides besseyi* on 21-day old rice leaves, the tips of the leaves turn white and form a whip (B) leaves without symptoms

Discussion

The effectiveness of physical and chemical treatments to suppress the spread of *A. besseyi* on rice seeds was evaluated through *A. besseyi* mortality, seed viability, and the number of infected rice plants. Treatment is considered effective if the treatment could show significant differences with the control on these three parameters. Seed viability was measured by germination rate. Germination is a measure of potential viability, which indicates the ability of seeds to grow under optimum conditions and grow normal plants.

The most effective treatment in causing *A. besseyi* mortality was P1 (cwt 5-10˚C 24 hours + hwt 52˚C 10 minutes) which caused 51.17% mortality. Soaking in cold water for 18-24 hours and then immersing it at 51-53˚C for 15 minutes was an effective seed treatment (Bridge, Plowright & Peng De Liang 2005). Under IRRI standards, seed treatment to control *A. besseyi* nematodes is immersion in cold water for 3 hours, followed by immersion in hot water at a temperature of 52-57˚C for 15 minutes (Kulkarni, 2019). Heat treatment at a temperature of 50-100˚C for at least 0.1 seconds might result in ruptured cell walls, while a reduction in temperatures below freezing may cause similar injuries due to the crystallization of cell contents (Laguë *et al.* 2001). However, the P1 treatment caused a significant decrease in seed viability, and seed viability was the main parameter for seed quality. High mortality that was not followed by high viability will reduce seed quality. Treatments that have a negative impact on seed viability cannot be recommended for seed treatment.

Another physical treatment, P4 (soaking in 25-30˚C water for 24 hours followed by drying), caused 48.67% nematode mortality. Seed soaking in 25˚C of water followed by air drying might increase the mortality of *A. besseyi* up to 82% (Hoshino & Togashi 2000). This treatment (P4) did not reduce seed viability. In the cultivation of rice plants, the seeds will be soaked for 24 hours before seeding. However, after soaking, the seeds are then ripened in wet sacks and not dried in the air.

Based on this study, soaking treatments with cold water reduced seed viability, while hot water treatment did not (Table 2). Hot water treatment at 52˚C for 15 minutes was physiologically safe for rice seed and increased field seedling vigor and tiller number (Ventura & Garrity 1987). When the temperature was increased from 52˚C to 57˚C, seed viability decreased from 68% to 57%.

The chemical treatment that effectively increased nematode mortality was P7 (imidacloprid 5 WP 2%) which was 47.25%. Seed dressing with imidacloprid caused more significant mortality of nematodes than benomyl and carbosulfan. The treatments with benomyl and imidacloprid might increase *A. besseyi* mortality by 83.97% and 93.65% from controls on *Polianthes tuberosa* bulbs, respectively (Cuc *et al.* 2010). Benomyl, which belongs to the benzimidazole group, is a nematicide that influences the post-embryo stage and reproduction and inhibits the growth of larvae to mature (Mulyadi

2009). Carbosulfan is a carbamate pesticide that has the potential to control nematodes and influences the activity of several types of stages in the life cycle of nematodes (Stenersen 2004). However, the results of this study indicated that carbosulfan pesticides only caused 11.34% *A. besseyi* mortality. The effectiveness of pesticides is influenced by several factors, such as movement and mode of action. Carbosulfan as organophosphate nematicide diffuses through water, so its effectiveness depends upon its movement in water and level of adsorption (Mulyadi 2009).

Treatment with carbosulfan, benomyl, and imidacloprid did not inhibit rice germination. Diazinone and carbosulfan pesticides did not affect the germination of rice seeds (Meher et al. 2010; Moore & Kröger 2010). The germination rate in control was only 72%. Seeds infected with *A. besseyi* had low viability and delayed germination (Tamura & Kegasawa 1959). This proves that rice seeds infected with *A. besseyi* have low germination.

All treatments showed symptoms of *A. besseyi* attack. On the 6^{th} day after germination, 72 percent of the nematodes reached the leaf midrib, and 15 percent reached the leaf blade; the nematode population increased on the 16th day, leaving 7 percent of the nematodes in the seeds (Nandakumar et al., 1975).The visible symptoms are the tips of the leaves, which turn white and then dry and curl to form a whip. Bridge et al. (2005) found an identic symptom: chlorosis at the tip of the new leaf, then the tip will dry and curl. If related to the results of nematode mortality, no treatment caused 100% mortality. There was a possibility that nematodes could still attack and show symptoms. This result could indicate that the treatment was able to increase the mortality rate of nematodes but was not able to suppress the symptoms.

CONCLUSION

Based on three parameters (mortality, seed viability, and the number of infected rice plants), no effective treatment controled the nematode *Aphelenchoides besseyi* on rice seeds.

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