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Influence of Growth Regulators and Hot Water on Rice Seeds and Seedlings (*Oryza sativa* L) Infected with Nematodes

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ABSTRACT
A. besseyi is a nematode species that causes white tip disease and is widespread in rice cultivation areas in Indonesia. *A. Besseyi* can be controlled by hot water treatment. But, using hot water treatment in seeds may decrease the percentage of germinated seeds. So, this study will examine the combination of hot water treatment to controlled nematodes and growth regulators as things that can maintain or improve the percentage of germinated seeds. The result shows that Water 55 ^{O}C + 100 % coconut water (P8), water 60 ^{O}C + 100 % coconut water (P9), water 55 ^{O}C + onion water 100 % (P11), and water 60 ^{O}C + onion water 100 % (P12) give 100 % of germinated seeds. have best value while water 60 ^{O}C (P3), water 60 ^{O}C + 1 ppm Rootone F (P6), water 60 ^{O}C + 100 % coconut water (P9), and water 60 ^{O}C + onion water 100 % (P12) is the best treatment in the parameters of the percentage of symptomatic seedlings that are mutually not significantly different from the lowest value in P3 which is 16.67 %.

Keywords:
Growth Regulators,
Pathogens,
Physical Control,
Plant Disease

INTRODUCTION

Seeds are plant material resulting from the generative propagation of rice plants that are used for crop production. There are several pathogens that cause rice plant diseases in the field that can be carried by seeds. One of these pathogens comes from the nematode group, *Aphelenchoides besseyi*. Information about the nematode species *A. besseyi* in Indonesia is still limited. [1] have reported the presence of *A. besseyi* in eight rice varieties in Bogor, West Java, and identified them based on morphological characters.

A. besseyi is a nematode species that causes white tip disease and is widespread in rice cultivation areas in Indonesia. *A. besseyi* is a migratory ectoparasitic nematode and can survive in rice seeds under anhydrobiosis conditions. When the rice seeds are sown, *A. besseyi* will be active again because of the presence of water moving towards the growing point and in line with plant growth will reach the tip of the leaf, causing white shoots [2]. Since the early 20th century, *A. besseyi* has been reported to cause serious yield losses in rice crops in Japan and parts of the United States. Nematode *A. besseyi* can reduce production by 17-54% in susceptible plants and 0-24% in resistant plants. Yield losses are 14.5-46.7% in Japan, 29-46% in Taiwan, and 20-60% in India [3].

One way to control *A. besseyi* is to use hot water treatment. Based on the results of research

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conducted by [4], mortality of *A. besseyi* reaches 100 percent at temperatures above 47 ^{O}C. However, when the temperature is increased to 50 ^{O}C, seed germination begins to decline. One method to maintain and increase the percentage of seed germination is to use growth regulators. Growth regulators are plant organic compounds that in low concentrations affect physiological processes, especially plant differentiation and development. But in the seeds sometimes the number is limited. Thus, the administration of exogenous growth regulators can be given as a treatment, especially on germination. Exogenous growth regulators act like endogenous growth regulators capable of causing stimulation and influence on plants, acting as precursors, namely compounds that precede the rate of other compounds in metabolic processes. So, this study will examine the combination of hot water treatment as physical control and administration of growth regulators as technical culture control.

METHOD

The research was conducted at the Plant Protection Laboratory, Faculty of Agriculture, University of Borneo Tarakan using the seeds of the Pak Tiwi-1 variety as the object of research and a mixture of sand and sterile soil in the same ratio as the planting medium. The study used a completely randomised design with 12 treatments which were repeated 3 times. The treatment of soaking the seeds for 20 minutes using a combination of hot water and PGR is described as follows:

P1 =Water 50 ^{O}C
P2 =Water 55 ^{O}C
P3 =Water 60 ^{O}C
P4 =Water 50 ^{O}C + 1 ppm Rootone F
P5 =Water 55 ^{O}C + 1 ppm Rootone F
P6 =Water 60 ^{O}C + 1 ppm Rootone F
P7 =Water 50 ^{O}C + 100% coconut water
P8 =Water 55 ^{O}C + 100% coconut water
P9 =Water 60 ^{O}C + 100% coconut water
P10 =Water 50 ^{O}C + onion water 100%
P11 =Water 55 ^{O}C + onion water 100%
P12 =Water 60 ^{O}C + onion water 100%

Sterilise Aquades and growing media using an autoclave at a pressure of 1 atm for 15 minutes. Take and clean the seeds from the remnants of dirt using a sieve and separate the empty seeds. Soak 20 seeds for each treatment for 10 minutes. The sterile planting medium was weighed as much as 3.5 kg and then poured into a container measuring 35 cm x 25 x 10 cm. Plant seeds that have been treated and watered using 250 ml of sterile water every day. The percentage of germinated seeds was seen at 20 day after planting by counting the number of seeds that grew and then calculated by the formula:

$$BK = \frac{K}{B} \times 100\%$$

Where:

BK = Percentage of germinated seeds (%)
K = Number of germinated seeds
B = Total seeds

The percentage of symptomatic seedlings was seen at 20 day after planting by counting the number of symptomatic seedlings and then calculated by the formula:

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$$BG = \frac{G}{L} \times 100\%$$

Where:

BG = Percentage of symptomatic seedlings (%)

G = Number of symptomatic seedlings

B = Total seeds

Calculating the length of symptoms was carried out by measuring the symptoms from the tip of the leaf to the apparent limit of symptoms on day 20.

Data were analysed using analysis of variance and continued with Duncan Multiple Range Test (DMRT) if different data were found due to the effect of different treatments.

RESULT AND DISCUSSION

Symptoms caused by nematode attacks on seeds are indicated by the presence of spots. While the seeds are marked by the death of tissue at the tips of the leaves which turn white as shown in the following picture.

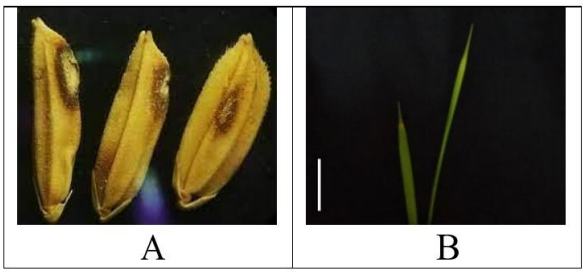

Figure 1. Symptoms of *A. Besseyi* attack (A. On Seed, B. On Plant)

Based on the results of the analysis of variance, the combination of hot water and PGR had a significant effect on the parameters of the percentage of symptomatic seedlings and symptom length, but had no significant effect on the parameters of the percentage of germinated seeds and seedling height. This can be seen in the comparison of the calculated F value and the table F at the 5% level in the following table:

Table 1. Comparison of calculated F values and 5% level tables for each parameter of observation

Parameters	F Value	F Table
Percentage of germinated seeds	1.86tn	2.22
Percentage of symptomatic seedlings	51.28*	-

Parameter data with the result that the treatment does not have a significant effect on the observations are presented in the graph as follows:

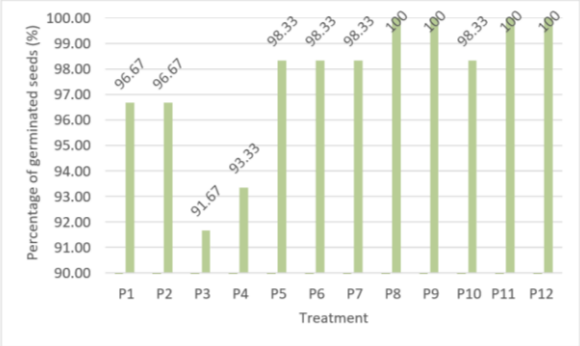

Figure 2. Percentage of germinated seeds (%)

Parameters that have a calculated f value greater than f table are then tested using DMRT to see which treatment has an effect on the observed parameters. The results of the DMRT test parameters for the percentage of symptomatic seedlings are shown in the following table:

Table 2. DMRT parameters for the percentage of symptomatic seedlings

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Treatment	The Percentage of Symptomatic Seedlings (%)
P1	100c
P2	96,67bc
P3	16,67a
P4	91,67b
P5	98,33bc
P6	18,33a
P7	91,67b
P8	100c
P9	21,67a
P10	95bc
P11	100c
P12	20a

A. besseyi is a seed-borne nematode. Taxonomically, this nematode belongs to the Aphelenchoididae group and is generally known as the white tip nematode of rice. The morphological characters of *A. besseyi* that are quite distinctive are the shape of the lips, median bulb, and tail shape. The seeds used are rice seeds of the Pak Tiwi-1 variety, which is known to carry the pathogen *A. besseyi*. This is in accordance with the opinion of [5] who succeeded in identifying *A. besseyi* in Pak Tiwi-1 seeds. Seeds that carry this pathogen are characterised by symptoms of sunspots on the grain (Figure 1A). Sunspot symptoms on rice seeds indicated that the seeds had a high infection rate due to *A. besseyi* attack and the population could reach 400 individuals per seed. These nematodes can survive for 2-3 years in dry seed conditions. *A. besseyi* will be active again when rice seeds are sown due to the presence of water. This nematode activity causes symptoms at the tips of rice seeds. Symptoms found in the vegetative phase were chlorosis in the leaf shoots and in some leaf shoots abnormal narrowing (Figure 1B).

The death of rice shoot tissue due to infection with *A. besseyi* is included in the symptoms of chlorosis or loss of chlorophyll. In addition to chlorosis, the leaf tips of rice plants infected with this nematode will experience malformations. Attacks of *A. besseyi* generally have typical symptoms, namely tissue death on white shoots accompanied by leaf curling. This is why the nematode *A. besseyi* is known as the white tip nematode. *A. besseyi* can move towards the growing point and along with the growth of the plant will reach the tip of the leaf and cause white shoot symptoms [6]. The discovery of different symptoms can be caused by an unsuitable environment. [5] stated that *A. besseyi* infection can show different symptoms in different environments and cultivars. These nematodes do not always show symptoms on infected plant parts. This does not rule out the presence of nematodes *A. besseyi* on seeds or plants that do not show symptoms.

The combination of hot water treatment and PGR did not have a significant effect on the percentage of symptomatic seed parameters. The percentage of germinated seeds in all treatments was above 90%. This indicates that the seeds have good viability. The high percentage of germinating seeds was due to the application of hot water to help break seed dormancy. This is in accordance with the opinion of [7] who stated that one method of breaking seed dormancy is by soaking in hot water. In Figure 2. It can be seen that the percentage of seeds germinating with PGR administration was generally higher than using only hot water. If averaged, the seeds given PGR

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had a percentage value of seed germination up to 98%, while without PGR only up to 95%. This is in accordance with the opinion of [8] which stated that PGR in general was able to increase the percentage of growing seeds. [9] in their research found that giving ZPT coconut water could increase the percentage of seeds growing up to 99%. Research conducted by [10] proved that soaking local rice seeds from Toraja in onion extract could increase the percentage of germination by up to 96%.

The significant effect on the application of a combination of hot water and PGR was seen in the parameter of the percentage of symptomatic seedlings. The best treatment for symptomatic seed percentage parameters was P3, P6, P9 and P12 where these four treatments were soaking the seeds at a temperature of 60 ^{O}C. The percentage value of symptomatic seeds ranged from 16% to 23%. This percentage was very low when compared to other treatments, where the percentage of infected seeds reached 100% (P1, P8 and P11). This indicated that the hot water treatment was able to eliminate the nematode *A. besseyi.* Soaking rice seeds in hot water is effective in reducing the number of nematodes in the seeds. Hot water treatment did not affect the seeds and effectively controlled nematodes. The low number of nematode inoculum was positively correlated with the percentage of symptomatic seeds. According to the disease triangle theory, a low inoculum reduces the possibility of pathogens infecting plants. In short, the symptoms of infection in plants indicated the low number of *A. besseyi* inoculum. The condition of low inoculum can occur due to several factors, namely the varying number of nematodes that are dormant on the seeds and the response of nematodes to hot water. Even so, hot water soaking treatment cannot eliminate 100% of nematodes [11].

CONCLUSION

Based on the results of the study, it can be concluded that the application of a combination of hot water treatment and ZPT has a significant effect on the percentage parameter of symptomatic seedlings but does not have a real effect on the percentage parameter of germinated seeds.

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