Efficacy of application time of Penicillium sp. suspension on White Root Fungus (Rigidoporus lignosus) in Nutmeg (Myristica fragrans)

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Abstract. Cultivation of nutmeg (Myristica fragrans) is running into several problems, one of these is caused by white root fungus (Rigidoporus lignosus) which causes losses to nutmeg farmers. This fungus is known to be controlled by biological control using microorganisms such as Penicillium sp. This research was conducted to determine the efficacy of application time of Penicillium sp. crude extract against white root fungus (Rigidoporus lignosus) in nutmeg plants. The design used in this study was a non-factorial completely randomized design with the main factor being the application time of Penicillium sp. crude extract with 4 treatments. Each treatment on 7 replicates. Control without suspension application Penicillium sp. (PK0), Application of Penicillium sp. 7 days before inoculation of Rigidoporus lignosus (PK1), Application of suspension of Penicillium sp. concurrently with Rigidoporus lignosus (PK2) inoculation, Application of suspension of Penicillium sp. 7 days after Rigidoporus lignosus (PK3) inoculation. Total are 28 experimental units. In this case, the test plants used are 11 months old nutmeg. The research parameters consisted of the incubation period, disease incidence and attack intensity. The results showed that the treatment at one-time application of the suspension of Penicillium sp. significantly affect the intensity of attacks and the incidence of infectious disease pathogens Rigidoporus lignosus. Application time for Penicillium sp. 7 days before the suspension of Rigidoporus lignosus is the best offer in the attack of Rigidoporus lignosus disease.

1. Introduction

Nutmeg (Myristica fragrans) is one of Indonesia native plants that has great potential as a trading commodity. For a long time, the nutmeg plant has been known as a spice plant and has an important position as a source of essential oil which is needed in various industries, such as the food industry, medicine, perfume, cosmetics, and others. Indonesia has occupied one of the top positions in the world nutmeg producer. Indonesian nutmeg products are favored by foreign markets because they give a distinctive aroma and have a high oil yield (Rukmana, 2004). South Aceh district is one of the centers for nutmeg production in Indonesia. Nutmeg production from this region contributes greatly to meeting domestic and foreign nutmeg needs.

The area of the nutmeg plantations in South Aceh Regency in 1994 reached 11,245 ha with an average productivity of whole nutmegs of 8.2 tonnes/ha. In 2003, the total area decreased to 9,843 ha, and the average total productivity decreased to 1.1 tonnes/ha. In 2014 the average productivity value...
of Acehnese nutmeg decreased to 0.7 tonnes/ha (South Aceh Regency Dishutbun, 2014). In the cultivation of nutmeg there are problems that make nutmeg plants in various regions of Aceh die. One of them is caused by a white root fungal disease caused by the Rigidoporus lignosus. This disease has attacked the nutmeg plant in South Aceh since 1990. Symptoms of this disease attack are yellowing and wilting leaves starting from the top of the shoot, continuing from one branch to another, then the leaves fall completely and the plant dies withering. As a result of this disease attack can reduce the production of nutmeg by up to 70% (Harni and Trisawa, 2011).

Biological control using antagonistic fungi is expected to be more effective in controlling white root fungi, because it has several advantages, including: naturally living in the soil so that it is easy to adapt, generally functions as a decomposer of organic matter, and does not cause environmental pollution. Some of them can be as siderophore and enzyme producer (Chaiharn et al, 2019). Several genera of antagonistic fungi that have been known to inhibit the development of Rigidoporus sp. and has the potential to control white root disease, one of which is Penicillium (Kaewchai and Soytong, 2010). Penicillium sp. is a group of fungi that produce antibiotic compounds, one of which is Penicillin. Penicillin is characterized by the presence of a β-lactam ring and is produced by various types of fungi (eukaryotes), namely Penicillium, Aspergillus, and certain prokaryotes (Madigan et al., 2000). Penicillium sp. has the ability to inhibit the growth of pathogenic fungi due to competition and the release of several alkaloid compounds such as agroklavine and ergometrine which have antifungal properties (Haggag and Hala, 2007). It is also strong heterolytic and can degrade chitin (Gandjar et al., 1999).

Based on the results of research by Khoirunnisya (2009), isolates of Penicillium spp. which produced the most effective metabolite compounds in inhibiting the growth of Ralstonia solanacearum in the dual culture test was P. brevicompactum with an incubation time of 3 weeks and at a concentration of 1.5% the metabolite compound was able to suppress the population of Ralstonia solanacearum in liquid media. Based on the description above, it is important to conduct a study on the time effectiveness of giving Penicillium sp. in controlling white root fungal disease caused by Rigidoporus lignosus in nutmeg (Myristica fragrans).

2. Methodology
This study used a non-factorial completely randomized design (CRD). The main factor is the application time of the Penicillium sp. Against Rigidoporus lignosus in nutmeg seeds with 4 treatments. Each treatment consisted of 7 replications. Thus there are 28 experimental units. PK0 Control (without Penicillium sp. Suspension application), PK1 Penicillium sp. Suspension application. 7 days before inoculation of Rigidoporus lignosus, PK2 Application of suspension of Penicillium sp. concurrently with Rigidoporus lignosus inoculation, PK3 Application of suspension of Penicillium sp. 7 days after inoculation of Rigidoporus lignosus.

2.1 Isolate Rejuvenation and Propagation
The rejuvenation and propagation of isolates were carried out in the Laboratory of Horticultural Food Crops in Nagan Raya. Isolate of white root fungus (Rigidoporus lignosus) and Penicillium sp. is a collection of the Laboratory of the Faculty of Agriculture, Teuku Umar University. Rigidoporus lignosus and Penicillium sp. rejuvenated on PDA medium. Rejuvenation is carried out according to Michailides (1991) with modifications. The mycelium of these two fungi was planted on PDA media using an inoculation needle (ose), then incubated at 25 °C in Laminar Air Flow until the mycelium filled the plate. The isolate obtained was then used as a source of inoculum for treatment.

2.2 Provision of Nutmeg (Myristica fragrans)
The commercial nutmeg seeds used are 11 months old. The seeds come from a nursery in Tapak Tuan, South Aceh Regency. The nutmeg seeds used in the study are assumed to be uniform because they come from the same parent tree where it grows, namely in Tapak Tuan, South Aceh Regency.
2.3 Preparation of Penicillium sp.
Colonies grown on solid media were taken using a 1 cm² spatula and mashed, then mixed with 50 ml of sterile water, the work was carried out in LAF and shaken for 5 minutes so that it was evenly distributed.

2.4 Application of White Root Fungus and Penicillium sp.
The roots of the nutmeg plant are dug until the roots of the plants are visible, then the roots of the plants are cleaned with 70% alcoholic cotton and then neutralized by giving sterile water. The root was injured using a sterile needle as much as 5 holes and then given a suspension according to the treatment. This paper will use the SLR approach to review research on the Naïve Bayes algorithm with the problem of attribute independence assumptions. Systematic Literature Review (SLR) is a process for identifying, assessing, and interpreting all available research with a view to providing answers to specific RQs[15].

2.5 Research Parameters
2.5.1 Incubation Period.
The incubation period was observed on the 4th day after inoculation until onset the first symptoms in plants.

2.5.2 Disease incidence. Disease incidence was observed on days 14, 21, 28 after inoculation and calculated using the following formula (Achmad et al. 2012).

\[ K = \frac{n}{N} \times 100\% \]

Remarks:
K: Disease incidence (%)
n: Number of wilting plant
N: Number of plant observed

2.5.3 Disease Intensity. Disease intensity was observed on the 28th day after inoculation. The development of white root fungus was observed by dismantling the polybags and observing the roots of the nutmeg seedlings after treatment. The effectiveness of the Penicillium sp. determined based on the scale of attacks in Table 1

| Scale | Symptom |
|-------|---------|
| 0     | There is no white root fungus mycelium attached to the roots of the seedlings |
| 1     | There is a white root fungus mycelium attached to the seedlings |
| 2     | there is a JAP mycelium attached to the roots seeds and has penetrated into the tissue; |
| 3     | The deep network will be black and rot |
| 4     | The tissue at the root rot completely |

Furthermore, the intensity of disease is determined by the following formula with reference to the intensity score in table 1 (Townsend and Heuberger, 1943 in Sinaga, 2006):

\[ IP = \frac{\sum n_i v_i}{Z N} \times 100\% \]

Remarks:
IP: Disease Intensity
n: plant number ith certain scoring value
v: scoring value each plant
Z: the highest value
N: total plant observed

3. Result and Discussion

3.1. Incubation Period

The incubation period of white root disease after inoculation with various applications of Penicillium sp. in the nutmeg plant is presented in Table 2.

Table 2. Incubation period for Rigidoporus lignosus at various times of application of Penicillium sp. on nutmeg seeds

| Treatments | Incubation period (day) | Score value |
|------------|-------------------------|-------------|
| PK0        | 4                       | 1           |
| PK1        | 8                       | 1           |
| PK2        | 7                       | 1           |
| PK3        | 4                       | 1           |

Table 2 shows the fastest incubation period found in the treatment PK3 and PK0 are 4 days. Where in both treatments Rigidoporus lignosus has infected and inhabited plant root tissue, in the absence of Penicillium sp. so that plants attacked more quickly and raises attack symptoms. Omorusi (2012) reported that Rigidoporus lignosus infection through rhizomorphs that is firmly attached to the roots, can then penetrate into the roots and cause rot, become soft and sometimes appear wet. The longest incubation period was found in PK1 treatment. This is thought to be Penicillium sp. already dominating the root zone of plants so that when Rigidoporus lignosus was present Penicillium sp. able to suppress the attack of Rigidoporus lignosus which is indicated by the length of the incubation period.

Doms and Gams (1972) reported when Penicillium sp. colonizing the roots of Aloe vera, the possibility of this plant being triggered to produce defense compounds against pathogenic attack mechanisms, so when inoculated by pathogens through similar attack mechanisms, the plants are more resistant to pathogens. Agrios (1997) added that the incubation period is the time interval from inoculation until symptoms of disease appear in plants. The longer the incubation period, the better the resistance of a plant.

3.2. Disease Incidence

The average incidence of white root disease caused by Rigidoporus lignosus at various times of application of Penicillium sp. shown in Table 3.

Table 3. Average incidence of white root disease caused by Rigidoporus lignosus at various times of application of Penicillium sp

| Parameters | PK0       | PK1     | PK2      | PK3       |
|------------|-----------|---------|----------|-----------|
| Disease incidence (%) | 90b       | 13,35a  | 77,23b   | 90b       |

Table 3 shows that the lowest incidence of disease was found in PK1 treatments which was significantly different from other treatments. This is thought to be Penicillium sp. has entered into the root tissue of plants so that plants have better resistance when Rigidoporus lignosus is present Penicillium sp. able to suppress the attack of Rigidoporus lignosus with an attack rate of 13.35% or the potential to reduce the incidence of disease by 85.16%. The ability of Penicillium sp. in suppressing attacks Rigidoporus lignosus possibly influenced by the chemical compounds present in Penicillium sp.

This is in line with what was reported by Haggag and Mohamed (2007), Penicillium sp. can be antagonistic through the mechanism of removing several alkaloid compounds such as agroclavine and
ergometrine which have anti-fungal properties against Botrytis cinerea, Fusarium solani, and Alternaria tenius. The PK3 and PK2 treatments showed Penicillium sp. unable to control pathogen attack when the pathogen has invaded plant tissue first. This is consistent with the research of Djarir (1993), species Penicillium sp. can release bioactive compounds that function as antibiosis, such as penicillin and riboxin, so that the utilization of Penicillium sp. as a biological agent, it is more appropriate to use bioactive products produced by Penicillium sp. not a colony because it is less able to compete against space.

3.3 Disease Intensity
The mean intensity of white root disease caused by Rigidoporus lignosus and various times of application of Penicillium sp. The nutmeg seeds are presented in Table 4.

Table 4. Average intensity of white root disease caused by Rigidoporus lignosus at various times of application of Penicillium sp.

| Parametres                  | Treatments |   |   |   |   |
|-----------------------------|------------|---|---|---|---|
| Disease intensity (%)       | PK0        | PK1| PK2| PK3|   |
|                             | 60c        | 6,92a| 38,65b| 49,29bc|

Note: A number followed by the same letter indicating significant differences at the LSD test of 0.05.

Table 4.3. shows that the lowest disease intensity was found in PK1 treatment and the highest was found in PK0 treatment. It is suspected that PK1 treatment causes plants to have better resistance to Rigidoporus lignosus attacks, so that when the pathogen attacks, plants already have antibiotics so that pathogens are unable to attack parts of plant tissue. In PK1 treatment the attack level Rigidoporus lignosus is only 6.92% or has the potential to reduce the intensity of attacks by 88.46%. The ability of Penicillium sp. in suppressing the development of Rigidoporus lignosus is thought to be affected by several chemical compounds such as agroklavine, ergometrine and griseofulvin.

According to Panda et al. (2005) the griseofulvin compound is able to inhibit fungal growth by disrupting the function of spindle threads and cytoplasmic microtubules, thereby inhibiting fungal cell mitosis. Nugroho et al, (2013) added that in addition to releasing alkaloid compounds, Penicillium also produces chitinase enzymes and cellulases. Chitinase enzyme produced by Penicillium sp. can hydrolyze the β-1,4 bonds between the N-acetylglucosamine (NAcGlc) subunits on the chitin polymer, so that it interferes with the process of forming the fungal cell wall. The lowest numerical value was found in PK1 treatment and the highest numerical value was found in PK3 and PK0 treatments with symptoms of root damage as seen in Figure 1.

![Figure 1. Symptoms of white root fungal disease](image)
(A). Treatment PK0: black tissue shows have been infected with Rigidoporus lignosus, (B) Treatment PK1: skin colored dots yellow indicates Rigidoporus lignosus entered the tissue (C) Treatment PK2: the tissue has been attacked Rigidoporus lignosus, (D) Treatment PK3: colored tissue brownish indicates that it has been infected with Rigidoporus lignosus, (E) Normal tissue plants that are not attacked by Rigidoporus lignosus.

It is suspected that PK1 treatment causes plants to have better resistance to the attack of Rigidoporus lignosus. So that when Rigidoporus lignosus attacks, the plant already has antibiotics so that the disease is unable to attack the plant tissue. In PK3 treatment Rigidoporus lignosus has attacked and inhabited the root zone of plants and made Penicillium sp. unable to control the pathogen attack when the pathogen has attacked the plant first.

4. Conclusion
Application time for Penicillium sp. 7 days before inoculation of Rigidoporus lignosus suspension is the best administration in controlling the attack of white rot disease.

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