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Biological characterization of isolates of the *Rhizoctonia solani* fungus in rice (*Oryza sativa*) from Karanganyar District, Indonesia

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Abstract. *Itsnaini NR, Supyani, Gutomo HS. 2019. Biological characterization of isolates of the* Rhizoctonia solani *fungus in rice (*Oryza sativa*) from Karanganyar District, Indonesia. Bioteknologi 16: 74-81.* This research aims to study the biological character of *Rhizoctonia solani* J.G.Kühn isolates, evaluate the virulence of *R. solani* isolates, analyze the relationship between biological characters and the virulence level of the collected *R. solani* isolates, and study the biological character of *R. solani*, which has a low virulence level. This research was conducted at the Laboratory of Plant Pests and Diseases Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia, at an altitude of 99 m above sea level, while *Rhizoctonia solani* fungus in rice (*Oryza sativa*) was collected from Karanganyar District, Indonesia. The researchers designed this research as exploratory research in the field and conducted it in the laboratory and greenhouse. Research variables included growth rate, colony phenotypic character (including color, surface structure, and the presence or absence of air hyphae), the diameter of damage to apples, percentage of spot area, and disease intensity. Data analysis of test results on the growth rate, virulence test on apples, and percentage of spot area using the mean and standard deviation presented in graphical form while the analysis of disease intensity on the host plant using analysis of variance based on the F test at 5% level and if there was a significant difference, it was continued with Duncan's test. The results showed that the *R. solani* isolates from the Karanganyar area had a variety of biological characteristics (color, air mycelium, colony profile, and colony diameter) and virulence levels. Isolates that were categorized as hypovirulent were isolates K+ (P6); P28; P17; P16; and P26, each caused a spot area of 35.93%; 36.94%; 42.16%; 45.28%; 45.99% in host plants and the isolate which was considered virulent was isolate P7 which caused 56.57% of the spot area. The disease intensity in isolate K+ (P6); P28; P17; P16; and P26, respectively, were 53.33%; 46.67%; 55.55%; 59.26%; 62.96%, and the disease intensity of P7 isolate, which was virulent was 73.33%.

Keywords: Biological characterization, *Oryza* sativa, *Rhizoctonia solani*

INTRODUCTION

The need for rice is increasing as the population increases. Therefore, it causes the high fulfillment of rice consumption needs. Rice is the staple food for most of Indonesia's population. However, disturbances from pests and diseases that attack rice plants can hinder the efforts to fulfill rice consumption needs.

Midrib blight is an important disease in rice plants. Rice midrib blight causes plants to fall easily. This disease causes the grain to be less filled or even empty. Midrib blight usually occurs when the plant begins to form tillers until just before harvest. However, this disease can also occur in young plants when the rice plants are 20 DAP (Days After Planting). The *Rhizoctonia solani* J.G.Kühn fungus causes this disease with early symptoms of pale white oval or round spots on the midrib. In humid conditions, this disease can reach the flag leaf. Pathogens survive and spread with the help of resistant structures called sclerotia (Center for Research and Development for Food Crops 2007).

Rhizoctonia can be found in all parts of Indonesia (environmental conditions allow) where the host plant is located. The severity of the infection can vary; for heavily infected patches, the severity can be very detrimental to the farmer. Some of these consequences are large yield losses (from 25% to 100%), increased soil tare (due to soil adhering to fungal mycelium), and poor quality of industrial plants based on increased sodium, potassium, and nitrogen levels. In addition, the host number of pathogen attacks causes various consequences and harms various plants. Midrib blight caused by this pathogen is the second deadliest disease after the rice blast (Molla et al. 2013).

High temperature, humidity, nitrogen fertilizers, and planting of susceptible varieties strongly favor disease development in the field. In addition, wet leaves and inter-plant and leaf-to-leaf contact favor the spread of disease. Pathogens can also be spread through irrigation water or soil displacement during planting preparation. In addition to rice, the pathogen can survive on citrus, cabbage, vegetables, beans, pumpkin, peanuts, chilies, carrots, soybeans, cotton, barley, celery, tomatoes, sorghum, wheat, tulips, and corn. (Suparyono 2009).

Plant pathogenic fungi (including *R. solani*) have many strains in the field. These strains have different virulence levels, including the damage or loss caused. For example, *R. solani*, which attacked rice during the planting season in 1997 at South Sulawesi, Indonesia; caused 0.5 ha of damage (high category) in Tana Toraja District, in 1997-1998, it caused damage of 1 ha (light category) in Soppeng District, during the 2000 planting season, this fungus caused damage of 4 ha in Enrekang District and 11 ha in

Tana Toraja District. During the 2001 planting season, this fungus caused 1 ha of damage in Luwu District (Syatrawati 2005). So far, only fungicides have been used to control damping off diseases.

Pesticides not only have a detrimental impact on human health and the environment but also on agricultural land and make agricultural products unsafe for consumption. In addition, the use of pesticides in agricultural ecosystems has resulted in various environmental damage and pollution, which impacts the destruction of species diversity in the ecosystem. Therefore, the impact that occurs requires limiting the use of pesticides and biological control to restore the ecological function of an agro-ecosystem.

This research was conducted to (i) study the biological character of *R. solani* isolates from Karanganyar District, Central Java, Indonesia, (ii) evaluate the virulence of *R. solani* isolates from Karanganyar, (iii) analyze the relationship between biological characteristics and the virulence level of *R. solani* isolates from Karanganyar, and (iv) studied the biological character of *R. solani* from Karanganyar which has a low virulence level.

MATERIALS AND METHODS

Materials

The main ingredients used were plant parts infected with the *R. solani*, collected from Karanganyar District, Central Java, Indonesia, and apples for comparison. The research was conducted at the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia, at an altitude of 99 m above sea level,

Research design

This research is designed as exploratory research in the field conducted in a laboratory and a greenhouse. The virulence test on apples was conducted in the laboratory. In contrast, the virulence test on the host rice plant was conducted in a greenhouse using a completely randomized design (CRD).

Collection of *R. solani* isolates

The *R. solani* isolates were collected from the endemic area of the *R. solani* in the Karanganyar area. Researchers collected these isolates by visiting the area. Rice plants that showed symptoms of midrib blight are oval spots on the midrib cut at the base. Parts of the plant, especially the stem and base of the stem, were examined for signs of disease, such as spots, wounds, or signs of disease in the form of sclerotia. Plant parts that showed symptoms or signs of the disease were then put in a cooler. Furthermore, the plant samples were immediately transferred to a refrigerator at 4°C to be further cultured in a PDA medium.

Culture of *R. solani* isolates on artificial medium

Cultured of *R. solani* isolates were conducted at LAF (Laminar Air Flow) according to the Streets (1972) method. Tissue surfaces showing symptoms of spots or sores were sterilized with 90% alcohol. A small section of the border area between diseased and healthy plant tissue was cut, removed, and placed in the center of a 90 mm diameter sterile petri dish containing 20 ml of PDA (Potato Dextrose Agar). The preparations were incubated on the experimental rack under standard conditions at 22-26°C for 7-10 days. All isolates were assigned an identification number according to the identity on the label when they were isolated from the field. Photographs for documentation were conducted when the culture was 1 week old.

At the time of the photo shoot, each isolate was stocked by culturing it on a regeneration medium in a 4 cm diameter petri dish, according to Hillman et al. (1990). Making this stock began with inoculating 3x3x3 mm cubic agar which was taken from the edge of the culture, then placed in the middle of the provided medium. The preparations were incubated on the experimental rack under standard conditions at 22-26°C for 1 week. After that, the preparation was stored in a refrigerator at 4˚C as stock for subsequent tests. Each of these stocks was assigned an appropriate identification number or derived from the isolated identity number on the PDA medium above.

Phenotype characterization of *R. solani* isolates

Morphological characterization was conducted following Hillman et al. (1990). The experiment was started by inoculating 3x3x3 mm^3 agar, taken from the edge of a 1-week-old stock culture, in the center of an 85 mm diameter petri dish containing 20 mL of PDA. The petri dish was then incubated on a test rack under standard conditions of 22-26°C. Cultures were observed on days 3, 5, 7, and 9. The observed and recorded characters were the colony growth rate and colony phenotype. If different characters were found in the observed isolates, such as colony growth rate indicated by smaller colony diameter, colony phenotype with a darker or lighter color, and non-smooth colony surface, then the isolates concerned were marked or selected and documented. These isolates had a high chance of low virulence (hypovirulence). Furthermore, the selected isolates were tested by the next test, which was the virulence test using apples and rice seeds.

Apple virulence test

The test was designed using Completely Randomized Design (CRD) and repeated three times. This virulence test used selected hypovirulent isolates based on the characterization of the isolates. The virulence test was conducted on selected isolates following the Elliston (1985) method. Ripe apples were disinfected with 90% alcohol. The researchers assigned 4 points around the fruit with a balanced spread position.

Then, each point was inoculated with fungal isolates on the wounded area. Next, an inoculum was inserted into each wound in an inward position, then pressed with a sterile spatula until complete contact with the apple tissue was made. Then, the inoculated part was wrapped with

parafilm to prevent drying. Furthermore, the apples were incubated in a plastic tray of 35x25x7 cm at room temperature. The diameter of the lesion was measured on days 3, 5, 7, and 9.

Testing on host plants

A test on the host plant was conducted to determine the intensity and percentage of *R. solani* attacks on the real host. The selection of isolates to be tested was based on the results of the virulence test on apples. The virulence test for this host plant used the CRD (Completely Randomized Design).

From these treatments, there was 1 control as a comparison, namely a positive control (high virulence). Then, each stem was repeated 3 times, and each stem was taken from each midrib which was taken on average at the end of the observation. Researchers used Memberamo varieties of rice seedlings aged 36 DAP in this virulence test. This test will inoculate the selected fungal isolates by slipping the inoculated pieces between the rice midribs. Observations were made once a week after inoculation (MSI) for one month. The results of this virulence test show that the fungal isolates showed a lower virulence level (hypovirulent) than the control (virulent).

Preparation of planting media

The media used was latosol soil previously sterilized by autoclaving at a temperature of 121°C and a pressure of 1.5 atm for 2 hours. After sterilization, the soil was placed in a 20 cm diameter pot, which had previously been mixed with urea 1 g/plant and 6 g/plant of compost.

Nursery

Seedling of rice plants was to sow the seeds in a square container previously soaked for 24 hours so that seed imbibition occurred. Then, the soaked seeds were planted in square tubs that had been given sterile soil media and a mixture of compost in a ratio of 1:1 and sown for 14 days. After sowing, the rice seeds were transferred to the media provided.

Plant maintenance

Watering was conducted twice to maintain the humidity and temperature of the plants inoculated with the *R. solani* to prevent disease development. Fertilizer was given to meet the nutritional needs of plants, namely Urea, at a dose of 6 g/plant. Fertilizer was applied before planting, 21 DAP, 45 DAP, 60 DAP, and 90 DAP.

Observed variables

Growth rate

The growth rate of *R. solani* was observed by measuring the diameter of the fungal colonies on the 90 mm diameter petri dish. The growth rate measurement data is calculated with the standard deviation using the following formula:

$$SD = \sqrt{\frac{\sum(X_1 - \bar{X})^2}{n-1}}$$

Colony phenotype characters

The phenotypic characters of the fungal isolate colonies included color, surface structure, and the presence or absence of air hyphae.

Diameter of damage to apple

Observations were made by observing the diameter size of the lesion on the apple. Data on the diameter of the lesion in apples was calculated with the standard deviation using the following formula:

$$SD = \sqrt{\frac{\sum(X_1 - \bar{X})^2}{n-1}}$$

Disease intensity

Disease intensity was observed from the beginning of inoculation until harvest, starting two weeks after inoculation (MSI). Assessment to determine the intensity of plant disease using a scale of 0, 1, 2, 3, 4, and 5 as follows in Table 1.

Then, the plant disease intensity scale is used to calculate the disease intensity using the formula:

$$IP = \frac{\sum(n.v)}{N.V} x100\%$$

Where:

IP : Disease Intensity

n : the Number of plants that show symptoms with a certain category

v : scale value with a certain symptom category

N : Number of sample plants

V : The highest score for the symptom category

Data analysis

Growth rate and virulence test on apples: the data were analyzed with the mean and standard deviation presented as a graph. Virulence test to host plants: the virulence test data on the host plant used analysis of variance based on the 5% level F test, and if there was a significant difference, it was continued with Duncan's test.

Table 1. Plant disease intensity scale

Scale value	Symptoms of spotting
0	No spots/healthy
1	1-10% of spots
2	11-30% of spots
3	31-60% of spots
4	61-90% of spots
5	>90% or dead plants

RESULTS AND DISCUSSION

The *R. solani* isolate collection in the field

The location for collecting *R. solani* isolates in the field was Karanganyar Regency. The purpose of taking isolates

was to obtain information about the symptoms caused by the fungus as well as to study the biological character and level of damage to rice plants. Therefore, this information was used as a first step in determining isolates that were hypovirulent or high virulence. Moreover, the isolation results of hypovirulent fungi can be used as biological control agents for the disease.

Symptoms in the field, namely on the surface of the midrib necrotic wounds, appeared like an ellipse. At first, these patches were oval, gray-green in color, and measured 1-3 cm in length. Then, the center of the spot became grayish white with brown edges. In severe cases, spotting may occur on leaves, including flag leaves. Plants that were attacked by this fungus easily fell and produced empty grain.

Then the researchers isolated the fungus based on the information found in the field. The isolating method of the *R. solani* was to take 1/3 of the diseased plant parts, and the healthy parts grew in PDA media. However, PDA media was still mixed with other fungi, so pure culturing was necessary. In pure culture, the researchers selected fungi that were believed to have the morphology and characteristics of *R. solani*. The isolation results produced 45 isolates which were then observed and cultured in PDA media.

The isolation results produced 31 isolates whose biological characteristics were studied, such as growth rate, presence or absence of air mycelium, and their virulence to the host plant. From these results, it will be known how the relationship between morphological characters to the level of pathogenicity in the host plant and the lowest level of damage to the host plant.

Macroscopic characterization of *R. solani* isolates

Some *R. solani*, which are pathogenic to rice, can produce sclerotia with thick outer walls so that they can float and survive in water. This fungus also survives as a mycelium employing a saprophyte colonizing soil organic matter, especially due to plant pathogenic activity. Sclerotia and/or mycelia, which are in the soil or plant tissue, grow and form hyphae that can attack several types of plants. This pathogen is suitable for poor soil structure and high soil moisture (Ceresini 1999).

According to Yulianti and Suhara (2010), the first step to determining effective control methods is to study the bio-ecology of these fungi. This morphological appearance can be used as an initial grouping of these isolates. Macroscopic observations of *R. solani* isolate colonies were conducted on the 3rd to 7th day after isolation. Macroscopic observations were conducted directly by looking at the development of each colony, such as colony diameter, colony color, air mycelium, and colony profile. This macroscopic observation determined the character of isolates with high or low virulence levels. The observation results of *R. solani* isolates are presented in Table 2.

Table 2. Macroscopic characterization of *R. solani* isolates on the 3rd day

Isolates	Colony diameter (cm)	Colony color	Air mycelium	Colony profile
P1	7.85	Dirty white	Plentiful	Like a ring and already formed sclerotia, rough
P2	7.90	Dirty white	Plentiful	Circlet, rough
P3	8.05	Dirty white	Plentiful	Sclerotia have formed, like a circlet
P4	7.70	Dirty white	Plentiful	like a circlet, rough
P5	7.30	Dirty white	Few	Circlet, rough
P6	7.60	Dirty white	Plentiful	Circlet, rough
P7	8.20	Dirty white	Few	Plenty of sclerotia, rough
P8	7.65	Dirty white	Plentiful	Circlet
P9	7.25	Dirty white	None	Smooth as it settled
P10	7.25	Dirty white	Plentiful	Sclerotia began to appear
P11P12	0	-	-	-
	6.30	Dirty white	Plentiful	Irregular wavy colony
P13	3.45	Green white	Plentiful	Rough
P14	8.15	Beige	Plentiful	Circlet
P15	3.65	White in the middle of the green	Plentiful	Rough
P16	8.25	Dirty white	Plentiful	Circlet, rough
P17	8.15	Dirty white	Plentiful	Circlet
P18	7.20	Dirty white	Plentiful	The circlet had formed sclerotia
P19	3.00	Green white	Plentiful	Rough
P20	7.05	Dirty white	Plentiful	Rough
P21	6.5	Dirty white	Plentiful	Rough
P22	6.55	Dirty white	Plentiful	Rough
P23	7.15	Dirty white	Plentiful	Rough, settled
P25	5.45	Dirty white	Plentiful	like a circlet, rough
P26	5.70	Dirty white	Plentiful	Formed a circle like a ring
P28	4.80	Dirty white	Plentiful	Rough, formed a circle
P29	4.95	Dirty white	Plentiful	Rough
P31	5.00	Dirty white	Plentiful	Rough, formed a circle
P32	2.35	Dirty white	Sparse	Rough, a light-dark circle was formed
P33	5.20	Dirty white	Plentiful	Rough, like a circlet
P39	5.1	Dirty white	Plentiful	Rough

This morphological appearance can be used as an initial grouping of these isolates. The isolation results in Table 2 show various variations in the morphological characters of the *R. solani* isolates, which were then identified according to color, hyphae, and profile. The results of the isolation produced 45 isolates, and 31 isolates were selected, which were suspected to be the *R. solani* for further identification. The results of this study found the diversity of *R. solani* isolates.

In general, the colony color of all isolates was dirty white. However, if the incubation period of each isolate on PDA media were extended, the color would be brown, and a sclerotium-like structure would form the stroma. According to Holliday (1989) cit. Irawati (2010), the color of the colonies of the isolates was related to the melanin content produced by the isolates. Melanin usually does not play a role in growth but increases endurance and competitiveness, especially in fungi.

According to Yulianti and Suhara (2010), the hyphae pigment of *Rhizoctonia* sp. generally varied, with the main color brown. Young colonies on artificial media are usually white or close to white, but with increasing age, the colonies will become dark brown. According to Danersen and Rasmussen (1996) cit. Irawati (2010), the formation of sclerotium is not the main feature of the *Rhizoctonia* genus. The formation of sclerotium is often influenced by the type of substrate in which this fungus grows, whereas monyloid cells are the precursors of sclerotia formation in the Rhizoctonia genus. The results of this study (Table 2) showed that, in general, the isolates grown in PDA formed sclerotium.

The research observations (Table 2) showed that all isolates had a lot of air mycelium. This result follows the opinion of Sneh et al. (1991) cit. Irawati (2010), that mushrooms grown in bright conditions will have more air mycelium than in other conditions. It is due to fungal hyphae growing following the direction of light (phototropy). Light plays an important role in the formation of the teleomorph phase of the *Rhizoctonia* sp. Sporulation occurs at night, and light stimulates hymenium formation but inhibits basidium maturation.

The effect of light on the growth of fungal vegetative hyphae is usually in the form of inhibiting or triggering their growth. According to Semangun (1988), mushrooms are generally hyaline pigmented (colorless). If the fungus is colored, then the fungus is pigmented, generally a melanin pigment bound to the cell wall of the hyphae.

The *R. solani* isolates grown in the PDA medium had different colony appearances from one another. The results showed that the isolates had the appearance of rings, rough and smooth. These results follow Irawati (2010), that light may affect the concentration of pigment production. With these two light-influenced factors, if a fungal culture is treated with alternating dark and light conditions, a colony morphology will form light-dark concentric circles. Therefore, concentric circles generally formed in continuous darkness and light-dark conditions are not visible. The isolation results found the presence of concentric circles such as rings with dark and light.

Observation of the colony growth rate showed that the colony growth rate of each isolate was different. Observation of this growth rate by growing isolates on PDA media was observed and measured on days 3, 5, 7, and 9 DAI (Days after Isolation). In general, the growth of *R. solani* was very fast. One isolate could grow to cover a 90 mm petri dish in three days. This fungus can live for several years by producing sclerotia in soil and plant tissues. The results of observations of colony growth rates of *R. solani* isolates are presented in Figure 1.

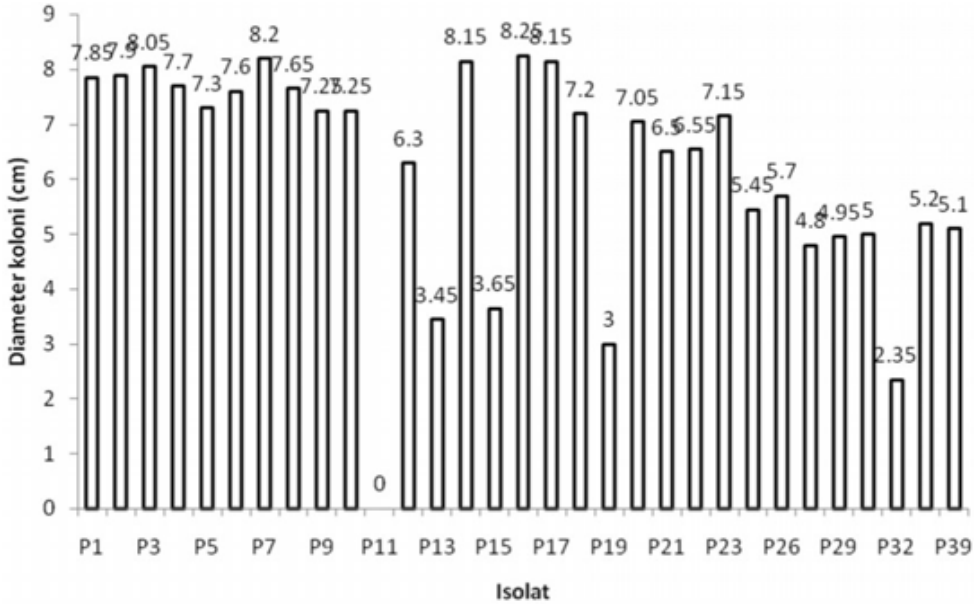

Figure 1. Bar chart of *R. solani* colony diameter on PDA day 3

The study's results on the colony growth rate (Figure 1) showed that each isolate had a different diversity of colony growth. The diameter measurement results of the observed isolates on the 3rd day showed that the highest isolate colony diameter was found in isolate P16 at 8.25 cm. In comparison, the lowest diameter of the isolated colony was found in isolate P11 at 0 cm. According to Irawati (2010), there is a relationship between the morphological character of the colony with the growth rate and level of pathogenicity of *Rhizoctonia*. The grouping of *Rhizoctonia* can be done based on the appearance of the colony morphology, but this is rather difficult to do because of the high diversity (variability) of this species.

The *R. solani* virulence assay on apples

The factors that influence the development of epidemic diseases are the level of virulence, the Number and type of inoculum approaching the host, the reproductive cycle (generation time), the environment in which the inoculum is formed, the resistance of the inoculum to the environment, the spread of the pathogen and its potential inoculum (Purnomo 2010)

The isolates that had been identified macroscopically were tested for virulence to determine the ability of the *R. solani* isolates to cause both symptoms and damage to apples. Testing on apples was the first step in selecting isolates suspected to have low virulence levels to be tested on host plants and one isolate suspected to have high virulence levels to be used as control. The parameter observed was the diameter of apple damage caused by *R. solani* isolates after inoculation. Virulence test observations were conducted on days 3, 5, 7, and 9. The results of virulence testing on apples were presented in the form of a bar chart with a standard deviation (Figure 2).

The observations of damage to apples on the ninth day after inoculation showed that the isolates of *R. solani* in each colony had different levels of infecting ability. These results indicate that the isolates tested gave the most serious damage to the apple fruit lesion in isolate P14 of 2.76 cm. Isolates that gave the lowest level of damage were isolates of P13, P22, P10, Control, P4, P7, and P16, each of which was 0 cm; 0.1 cm; 0.2 cm; 0.3 cm; 0.3 cm; 0.3 cm; 0.4 cm. Furthermore, several researchers, such as Syaifudin (2010), stated that fungi that received culture treatment would lose their pathogenicity after being transferred several times in the medium or after being stored for a long time.

According to Syaifudin (2010), various strains or origins (isolates) of a type of pathogen can vary in severity (virulence), depending on the genes contained in the nucleus or the material that acts as the nucleus. Furthermore, Gene arrangement can change due to various processes, so the virulence of a particular type of pathogen can change from time to time. For example, these changes can occur due to hybridization, heterochrony, and parasexuality. In addition, the pathogenicity of a pathogen is influenced by internal factors such as age and physical condition and external factors such as climate, environmental conditions, and agronomic measures, especially the use of antifungal and antimicrobial materials.

Furthermore, the researchers selected 20 isolates from the results of the virulence test on apples that were thought to have the lowest level of damage. One isolate with the highest damage rate as control was then tested on the host plant.

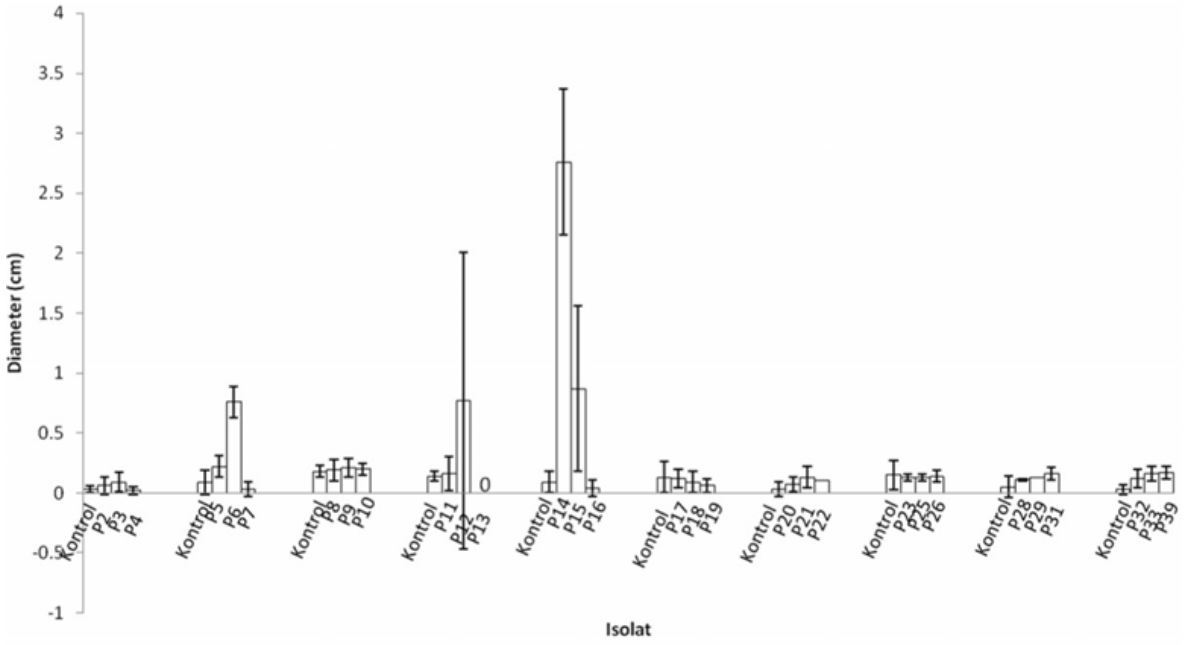

Figure 2. Diameter of damage to apples on day 9 (data were analyzed by means and standard deviation)

The *R.solani* virulence test on host plants

The results of the virulence test on apples were used as the first step in selecting isolates that were thought to have low virulence levels compared to positive controls. Then, 20 isolates from the test results on apples were selected to be tested on host plants. The test on the host plant was to determine the extent of damage to the host plant caused by the *R. solani* isolates, which had been characterized by color, hyphae, and colony diameter.

The virulence test on this host plant was conducted in a greenhouse with an average daily temperature of 25-40°C. This test used Memberamo rice varieties at 36 DAP (Days after Planting). The test on this host plant was to inoculate sclerotia on the rice midrib by inserting the sclerotia on the rice midrib by opening the rice midrib a little and then inserting the sclerotia clumps into it so that infection was expected between the pathogen and the host plant being tested. The data on the results of the virulence test on the host plant is presented in Figure 3.

Figure 3 shows that the isolates tested on the host plant had different pathogenicity in each isolate. This test on host plants aims to determine the relationship between morphological characters and the level of damage to the test plants. The results of virulence testing on 4 MSI (weeks after inoculation) host plants showed that the isolates suspected to be hypovirulent were isolates of K+ (P6); P28; P17; P16; and P26, each caused a spot area of 35.93%; 36.94%; 42.16%; 45.28%; 45.99%. While the isolate, which was initially suspected to be a hypovirulent isolate, when tested on the host plant, giving the highest spot area, which was isolate of P7 with 56.57%. Those follow Wakman (2004), who states that the extent of a disease attack is influenced by pathogens, hosts, and the environment. Pathogens with high pathogenicity that attack sensitive hosts with favorable environmental conditions will expand the symptoms of the attack. However, if one of these factors is not appropriate, the occurrence of the disease will be hampered.

According to Prayudi (2000), if the disease progresses to the flag leaf, the yield reduction can reach 20%. The higher the intensity of leaf midrib blight, the more it will disrupt yield stability. Efforts to control rice disease caused by *R. solani* are experiencing obstacles because the pathogen has a very diverse host and can survive for a long time in the form of sclerotium. In subtropical areas such as Japan, a sclerotium is a survival tool and a source of initial inoculum (X_0) for subsequent crops (Prayudi 2000). It happens because no other form of pathogen other than sclerotium can survive in winter. In the following season, sclerotium emerges on the soil surface from tillage and is ready to become a source of early inoculum for planting. In the tropics, other forms besides sclerotium are always available, so the role of sclerotium as a source of initial inoculum for planting is not dominant.

The *R. solani* that infects the host cell wall causes lesions on the midrib, which causes it to fall, and the exudate of the pathogen joins the flow of water transport, causing widespread attacks and increasing the intensity of the disease. This research follows Hadi et al. (1975) in Rosnawati (1991) cit. Winarni et al. (2004), where the high intensity of the disease was influenced by incubation, conidium density, and the ability of the pathogen to attack the bundle vessels in ginger plants which were closely related to water transport in plants because these pathogens were in the xylem and the conidium was transported by transpiration flow. The speed at which the transport is conducted also affects the speed at which the disease symptoms occur. The intensity of the disease is presented in Table 3.

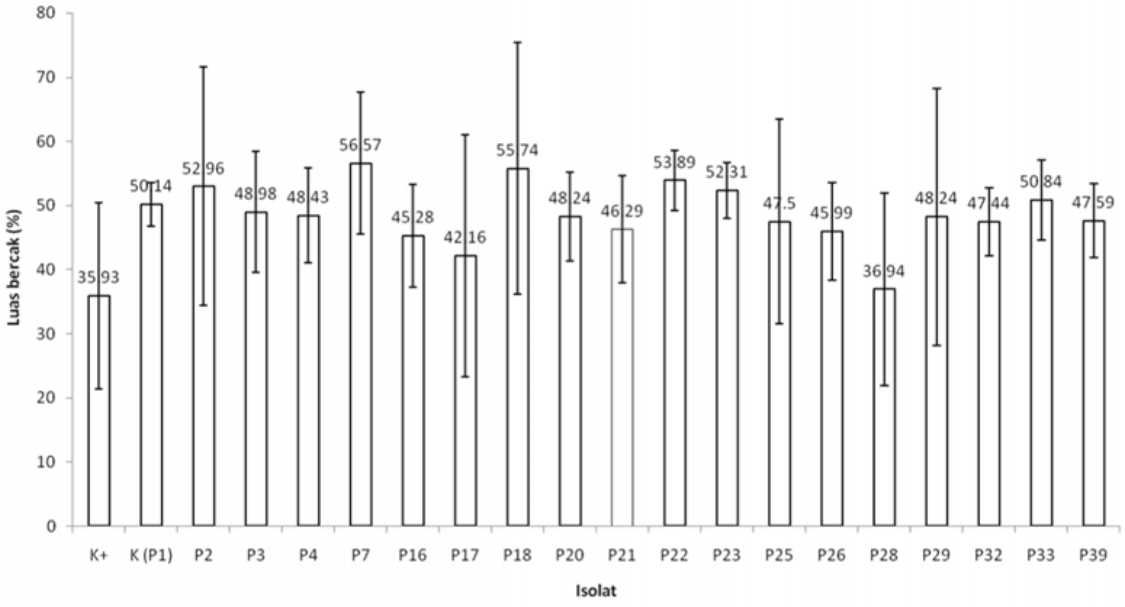

Figure 3. Percentage of *Rhizoctonia solani* spot area on host plants at 4 weeks after inoculation

Table 3. Disease intensity at 4 weeks after inoculation

Isolates	4th Week
K+	53.33ab
K (P1)	65.93 ab
P2	63.70 ab
P3	60.74 ab
P4	65.92 ab
P7	73.33 b
P16	59.26 ab
P17	55.55 ab
P18	69.63 ab
P20	59.26 ab
P21	62.96 ab
P22	63.70 ab
P23	65.18 ab
P25	62.22 ab
P26	62.96 ab
P28	46.67a
P29	62.96 ab
P32	62.96 ab
P33	69.63 ab
P39	62.96 ab

Information: Numbers followed by the same letter are not significantly different according to Duncan's Test 5 %

The results showed that the intensity of the disease caused by *R. solani* isolates fluctuated. The isolates suspected to be hypovirulent were isolates of K+(P6); P28; P17; P16; and P26 had disease intensity of 53.33%; 46.67%; 55.55%; 59.26%; 62.96%, respectively while isolate of P7 which was suspected to have high virulence had a disease intensity of 73.33%.

Research on *R. solani* isolates which were characterized macroscopically, virulence tests on apples and host plants were conducted to determine the virulence level of each isolate. The results of this study showed that isolates with relatively low virulence levels were found in isolates of K+(P6), P28, P17, P16, and P26 which had biological characteristics such as having a dirty white color, lots of air mycelium, a ring-like colony profile or forming concentric circles. Meanwhile, isolates with a high virulence level were found in isolate P7, which had biological characteristics such as dirty white color, little air mycelium, colony profile with lots of sclerotia, and roughness.

The results showed: (i) *R. solani* isolates from Karanganyar had a variety of biological characteristics (color, air mycelium, colony profile, and colony diameter). (ii) *R. solani* isolates from Karanganyar had various virulence levels. (iii) Isolates that were classified as hypovirulent were isolates of K+ (P6); P28; P17; P16; and P26, each caused a spot area of 35.93%; 36.94%; 42.16%; 45.28%; 45.99% in host plants and the isolate which was considered virulent was isolate of P7 which caused 56.57% of the spot area. (iv) Disease intensity in isolates of K+(P6); P28; P17; P16; and P26, respectively by 53.33%; 46.67%; 55.55%; 59.26%; 62.96%, and the disease intensity of P7 isolate which was virulent was 73.33%.

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