

Salicylic Acid effectiveness as Resistance Inducer of Rice Plant Against Sheath Blight Pathogen

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*This study aims to determine the effectiveness of salicylic acid in inhibiting the growth of *Rhizoctonia solani* in vitro and to determine the effect of salicylic acid in increasing rice plants' resistance to rice sheath blight disease. In the in vitro research stage, the antifungal activity of salicylic acid was assayed with 5 levels of concentration. The observed variable was the percentage of *R. solani* mycelium growth inhibition. The next stage of research was carried out in planta. The observed variables in the resistance assay were the pathosystem, growth, morphological, and physiological components. Based on the observed variables of pathosystem components, salicylic acid can reduce the intensity of rice sheath blight both in vitro and in planta. Based on observations of morphological and physiological components, the salicylic acid increased plant resistance by thickening the leaf epidermis and increasing the phenolic compounds.*

Keywords: induction of resistance, salicylic acid, rice sheath blight, rice plant

INTRODUCTION

Rice sheath blight disease is one of plant disease which reduce the production about 50-80%. The disease is caused by the soil-borne fungus *Rhizoctonia solani*. The fungal infections cause damage in the rice plant at any stages. It initiated with germination of pathogen propagules, sheath infection, and plant nutrient absorption. The infection develops and expand to the stem part causes plant rot (Inagaki, 2001). Inoculum of *R. solani* can survive in the soil for several years (Ritchie et al., 2013). Various controls were carried out, including biological control with vegetable fungicides, synthetic fungicides, and resistant varieties. But all of them have their own advantages and disadvantages.

The control effort of rice sheath blight disease can be conducted by the induction of plant resistance using chemical compounds such as salicylic acid (Leiwakabessy et al., 2017). It is further stated that salicylic acid is an intermediate compound in plants as a physiological and morphological response during infection by pathogens. Salicylic acid can accelerate the activation of resistance signals to produce various hormones for suppressing the rate of infection. Based on this, the



research was conducted to induce the resistance of rice plants to sheath blight disease using salicylic acid with the aim 1) determine the effectiveness of salicylic acid in suppressing the growth of *R. solani* in vitro, 2) determine the effect of salicylic acid application in the resistance induction of rice plants to *R. solani*.

RESEARCH METHOD

This research was carried out at the Plant Protection Laboratory and Greenhouse, Faculty of Agriculture, Jenderal Soedirman University from September to February 2021. The experimental design used was a Completely Randomized Design for the antifungal activity assay of salicylic acid in vitro and a Randomized Block Design for plant resistance assay in planta with 5 types salicylic acid concentration (0, 5 mM, 10 mM, 15 mM, 20 mM). Each treatment was repeated 5 times. The data obtained were analysed by the F test and continued with the Duncan Multiple Range Test (DMRT).

The isolate of *R. solani* from the exploration was used to the antifungal activity assay of salicylic acid in vitro. The antifungal activity of salicylic acid against *R. solani* was carried out by adding salicylic acid of various concentrations to 1 ml of potato dextrose agar (PDA) media and then inoculated by *R. solani*. The percentage of *R. solani* mycelia growth inhibition was calculated after 7 days with the formula according to El-Garhy *et al.* (2020).

$$\text{Percentage of the fungal colony inhibition} = \frac{\Delta d_0 - \Delta d}{\Delta d_0} \times 100\%$$

Δd_0 : The average of fungal colony diameter in control treatment, Δd : The average of fungal colony diameter in treatment

The in planta induce resistance examination was carried out on rice plants of the IR-64 variety. IR-64 seeds were soaked in water for 1x24 hours and then sown in a nursery tray. After the nursery was 21 days old, the plants were transferred to a 0.3 m polybag filled with 9.6 kg of soil. Plants were watered regularly and fertilized with NPK fertilizer at 14 and 21 days.

The application of salicylic acid was carried out when the rice plants entered the early vegetative state (6 week after planting). Salicylic acid solution was prepared by dissolving powdered salicylic acid ((C₇H₆O₃) with 70% alcohol (Leiwakabessy *et al.*, 2017). This solution will become a stock solution. The stock solution was diluted into salicylic acid concentrations as follows: 0 mM, 5 mM, 10 mM, 15 mM, and 20



mM.

The application of salicylic acid was carried out by spraying salicylic acid solution on the leaves to the base of the stem as much as 10 ml for each test plant. Inoculation of *R. solani* was carried out after 3 days of salicylic acid being applied to rice plants according to Wasano et al. (1983).

The observed variables were included growth components, pathosystems, morphology, and physiology. Pathosystem components consist of incubation period, disease intensity and Area Under Disease Progress Curve (AUDPC). Measurement of disease intensity was determined by the standard formula for evaluation of the International Rice Testing Program (IRTP, 1988):

$$\text{Disease intensity of RSB} = \frac{4N_4 + 3N_3 + 2N_2 + 1N_1 + 0N_0}{4N} \times 100\%$$

N_4 = Number of tillers with a score of 7, N_3 = Number of tillers with a score of 5, N_2 = Number of tillers with a score of 3, N_1 = Number of tillers with a score of 1, N_0 = Number of tillers with a score of 0, N = Total Number ($N_4 + N_3 + N_2 + N_1 + N_0$).

The intensity of the disease observation used as the basic for calculating the Area Under Disease Progress Curve (AUDPC). According to Jeger & Rollinson (2001), AUDPC can be calculated by the formula as follows:

$$\text{AUDPC} = \sum_n^i \left(\frac{y + y_{i+1}}{2} \right) t_{i+1} - t_i$$

y : disease percentage at the time, t : days

The morphological component variables consisted of measuring the thickness of the leaf epidermis and calculating the stomatal density. The thickness calculation uses the formula according to Dewi et al. (2013).

$$\text{TK} = \text{TKM} \times K$$

TK : Thickness of cuticle and epidermis, TKM : Thickness of cuticle and epidermis in micrometer, K : Calibration (1mm = 0.0025 m)

Meanwhile, the observation of stomatal density was calculated using the following formula (Dama et al., 2020).

$$\text{Stomata density} = \frac{\text{number of stomata}}{\text{unit area of view}}$$

The physiological components observed consisted of a qualitative test of



saponins and tannins, and a quantitative test of total phenol content and salicylic acid levels. The content of saponins and tannins was observed qualitatively. The saponin test was carried out according to [Minarno et al. \(2016\)](#). The tannin test was carried out according to [Mainawati et al. \(2017\)](#). The total phenol content test was carried out using the Follin-Ciocalteu method using a spectrophotometer at a wavelength of 725 nm according to [Hapsari et al. \(2018\)](#) is calculated by the formula:

$$\text{Total phenol} = \frac{x \cdot V \cdot FP}{BS}$$

Note: x : Concentration ppm, V : Volume of extract solution (ml), FP : Dilution factor of sample solution, BS : Weight of extract (g)

The salicylic acid content test was carried out using the alkalimetric method according to [Safitri \(2017\)](#). Calculation of the percentage of salicylic acid using the following equation ([Rambe, 2018](#)):

$$\text{Salicylic acid (mg)} = \frac{V \times N}{0,1} \times 13,81$$

$$\% \text{ Salicylic acid} = \frac{\text{Salicylic acid content (g)}}{100 \text{ g}} \times 100\%$$

RESULT AND DISCUSSION

The antifungal activity of salicylic acid in vitro

In vitro antifungal activity assay showed that salicylic acid treatment with various concentrations (5 mM, 10 mM, 15 mM, and 20 mM) significantly affected the inhibition of *R. solani* mycelia (Table 1). The percentage of inhibition increased in accordance with the concentration of salicylic acid. According to [Mondol et al. \(2020\)](#), the application of salicylic acid (1-25 mM concentration) in PDA media was able to inhibit the mycelia growth of all types of pathogenic fungi. Salicylic acid has antifungal properties cause damage to the integrity and function of cell membranes, disrupt mitochondrial performance, and cell death. Furthermore, the growth of pathogenic fungal mycelia is inhibited ([Kong et al., 2021](#))

Table 1. Percentage of inhibition of growth of pathogenic *R. solani* by salicylic acid in vitro

Salicylic acid concentration	Inhibition percentage (%)
Salicylic acid 5 Mm	0,02a
Salicylic acid 10 Mm	0,08ab
Salicylic acid 15 mM	0,17b



Salicylic acid 20 mM

0,37c

Note: Numbers followed by the same letter in the same column show no significant difference according to DMRT at an error level of 5%.

The resistance examination of plants infected with *R. solani* with salicylic acid

Pathosystem component

The incubation period for rice sheath blight with various concentrations of salicylic acid shows longer than the control treatment (Table 2). This phenomenon is in line with the research conducted by Luo et al. (2012). Salicylic acid has the ability to induce plants through signalling and elicitation pathways. Resistance induction through salicylic acid signalling pathway expresses resistance genes on the cell wall, while the elicitor pathway can accelerate the necrosis response and mechanical damage. Furthermore, it prevents the pathogens infecting plants and reducing the incubation period of the disease (Nawangsih et al., 2014).

Table 2. Incubation period of rice sheath blight in salicylic acid treatment.

Treatment	Disease Incubation period (days after inoculation)
Control	6,60a
Salicylic acid 5 mM	6,80ab
Salicylic acid 10 mM	7,40b
Salicylic acid 15 mM	7,00ab
Salicylic acid 20 mM	6,60a

Note: Numbers followed by the same letter in the same column show no significant difference according to DMRT at an error level of 5%.

Salicylic acid concentrations of 15 mM and 20 mM had no significant effect on plant resistance compared to salicylic acid concentrations of 5 mM and 10 mM (Table 3). This is presumably because the high concentration of salicylic acid cannot give a significant effect in suppressing plant diseases. This statement is in line with the research results of Silero et al. (2012) on faba beans (*Vicia faba* L.) infected with rust and *Aschochyta* blight pathogens. It is possible that high concentrations of salicylic acid have no effect on suppressing the intensity of the disease because it can cause phytotoxicity. This is consistent with the generalization of Silero et al. (2012). Barilli et al. (2010) added that salicylic acid concentration >10 mM can cause phototoxicity symptoms and does not increase the systemic resistance of legumes to leaf rust.

A good concentration of salicylic acid to increase plant resistance to a disease based on a number of studies is in the range of 2-10 mM, according to research



conducted on potato plants infected with *Rhizoctonia solani* Hadi & Balali (2010), citrus fruits infected with *Penicillium digitatum* Sacc. and *P. italicum* (Iqbal et al., 2012), and tomato plants infected with wilt-causing pathogens (Jabnoun-Khiareddine et al., 2015).

Table 3. Intensity of rice sheath blight on salicylic acid treatment.

Treatment	Disease Intensity (%)				
	5 dai	10 dai	15 dai	20 dai	25 dai
Control	0,21b	0,27c	0,33c	0,40c	0,45c
Salicylic acid 5 mM	0,16a	0,21ab	0,29bc	0,33b	0,37ab
Salicylic acid 10 mM	0,14a	0,18a	0,22a	0,28a	0,32a
Salicylic acid 15 mM	0,14a	0,20ab	0,25ab	0,29ab	0,32ab
Salicylic acid 20 mM	0,16a	0,21b	0,28b	0,33b	0,38b

Note: Numbers followed by the same letter in the same column show no significant difference according to DMRT at an error level of 5%.

The Area Under Disease Progress Curve (AUDPC) shows that the highest AUDPC value is in the control treatment and the lowest is in the 10 mM salicylic acid treatment. This is in accordance with the results of research by Leiwakabessy et al. (2017) that 10 mM concentration of salicylic acid in Ciherang variety produced the lowest AUDPC value against bacterial leaf blight (BLB) pathotype VIII.

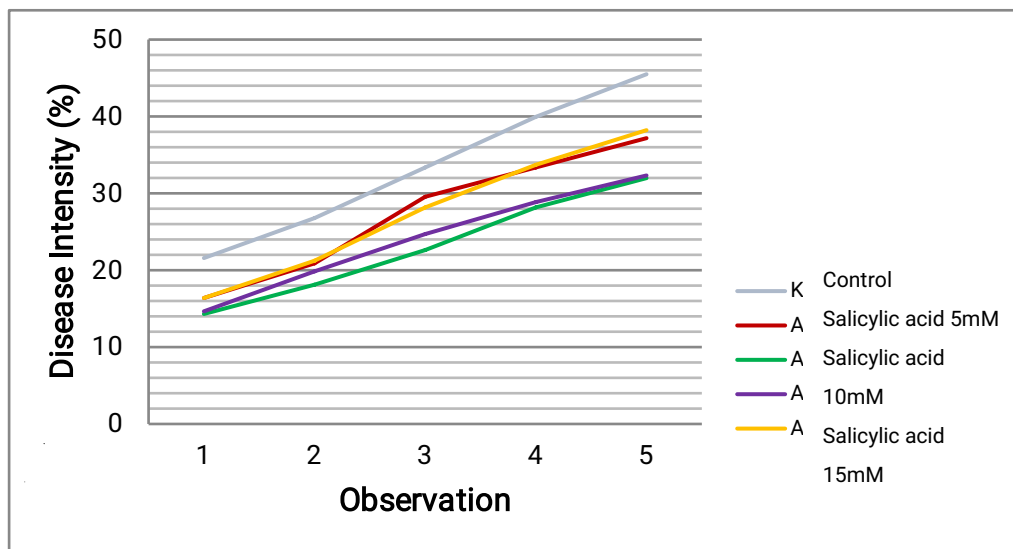


Figure 1. AUDPC of leaf sheath blight



Morphology Component

The salicylic acid treatment on the thickness of the upper and lower epidermis showed a significantly different response between treatments (Table 4). The results obtained that the largest leaf epidermis thickness was found in the 15 mM concentration of salicylic acid treatment.

Nurcahyani & Lindawati (2014) stated that salicylic acid can accelerate plant resistance signals in forming anatomical resistance, such as thickening of the epidermis, as a structural resistance response to pathogen infection. In contrast to the results of the analysis of leaf epidermal thickness, the results of the analysis of stomatal density variance showed that it was not significantly different from the control (Table 4). This is presumably because stomata density does not affect plant resistance to disease, but as a way of entry for pathogens to infect plants. This is in accordance with research conducted by Hasanah & Sembiring (2018).

Table 4. Leaf epidermis thickness and stomata density

Treatment	Leaf epidermis thickness (μm)		Stomata density (sel/mm^2)
	Adaxial	Abaxial	
Control	20,86a	41,14a	16,46a
Salicylic acid 5 mM	31,05ab	51,43ab	15,91a
Salicylic acid 10 mM	26,29ab	46,19ab	14,77a
Salicylic acid 15 mM	32,38b	59,33b	14,60a
Salicylic acid 20 mM	25,33ab	48,19ab	16,54a

Note: Numbers followed by the same letter in the same column show no significant difference according to DMRT at an error level of 5%.

Physiology component

The results of the qualitative test showed that the administration of salicylic acid had an effect on the levels of saponins and tannins in rice plants (Table 5). This is indicated by the formation of foam in the saponin test and brown color in the tannin test (Figure 2). The saponin test on the salicylic acid treatment with concentrations of 10 mM, 15 mM, and 20 mM had foam with the same thickness but thicker than the treatment with 5 mM concentration of salicylic acid and control. These results are in accordance with the research of Jirakiattikul et al. (2021) that the application of salicylic acid can increase the saponin content in plants.

In a qualitative test to determine the tannin content, it was found that the highest tannin content was found in the 10 mM concentration of salicylic acid



treatment with a darker brown color than the other treatments, while the lowest tannin content was found in the control test solution which had a light brown color. Godghate & Gogle (2018) stated that salicylic acid affects the tannin content in plants.

Table 5. Qualitative test for saponin and tannin levels on rice plants treated by salicylic acid

Treatment	Saponin	Tanin
Control	+	+
Salicylic acid 5 mM	+	+
Salicylic acid 10 mM	++	+++
Salicylic acid 15 mM	++	++
Salicylic acid 20 mM	++	++

Note: The + sign in the saponin test means foaming, ++ thick foaming, and +++ very thick foaming. The + sign in the tannin test means brown, ++ dark brown, and +++ blackish brown.

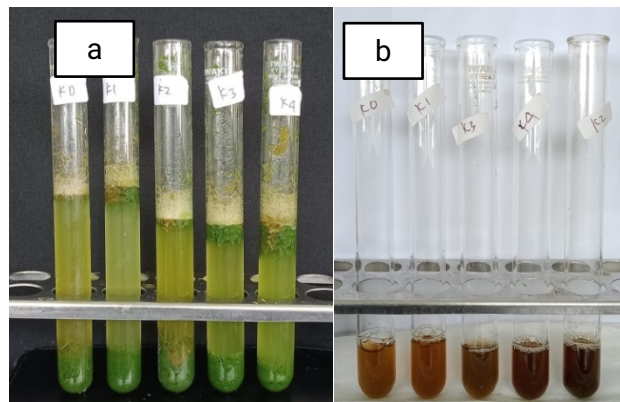


Figure 2. The results of the qualitative test of saponin (a) and tannin (b) levels on rice plants.

The quantitative test to determine the total phenol content (Table 7) showed that 5 mM and 10 mM salicylic acid treatment could not increase the phenol content in rice plants, while the 15 mM and 20 mM salicylic acid treatments were able to increase the phenol content of rice plants when compared to the control treatment. The increase in total phenol levels in the 15 mM and 20 mM salicylic acid treatment was in accordance with Ali's (2021) statement that exogenous application of salicylic acid was able to increase the activity and gene expression of the phenylalanine ammonia-lyase (PAL) enzyme. The increased activity of these enzymes causes the accumulation of phenolics. Preciado-Rangel et al. (2019) adds that the higher the concentration of salicylic acid, the higher the total phenol content, but the response of plants to various concentrations of salicylic acid is different due



to the influence of plant species, application method, and environmental conditions.

Table 7. Total phenol content on rice plants treated by salicylic acid

Treatment	Total Phenol (mg GAE/g sample)
Control	123,04
Salicylic acid 5 mM	76,67
Salicylic acid 10 mM	112,89
Salicylic acid 15 mM	163,62
Salicylic acid 20 mM	194,06

The content of salicylic acid in rice plants (Table 9) shows that salicylic acid treatment with concentrations of 5 mM, 10 mM, 15 mM, and 20 mM had an effect on increasing levels of salicylic acid in rice plants when compared to the control treatment. The content of salicylic acid increases as the concentration of salicylic acid increases. According to Tajik et al. (2019), exogenous application of salicylic acid can increase the content of phenolic compounds such as jasmonic acid and salicylic acid. Endogenous salicylic acid increases with increasing concentration of salicylic acid given. Khan et al. (2015) added that spraying salicylic acid on leaves plays a role in regulating biochemical and physiological processes that can increase the accumulation of secondary metabolites, especially phenolic compounds such as salicylic acid.

Table 9. Calculation of salicylic acid levels in rice plants in salicylic acid treatment.

Treatment	Salicylic acid content (mg)	Percentage of salicylic acid content (b/b)
Control	8,286	8%
Salicylic acid 5 mM	15,191	15%
Salicylic acid 10 mM	15,8815	16%
Salicylic acid 15 mM	16,572	17%
Salicylic acid 20 mM	17,953	18%

CONCLUSION

The conclusions obtained from this research are as follows:

1. Salicylic acid is effective in suppressing the growth of *R. solani* in vitro.
2. Application of salicylic acid affects the components of the pathosystem and can increase the resistance of rice plants morphologically and physiologically.

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