Antagonism of Endophytic Fungi Isolates Artemisia Annua Towards Rhizoctonia Solani, Causal Agent Of Rice Sheath Blight

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Abstract- Rice sheath blight caused by Rhizoctonia solani Kühn decrease rice productivity. One of biological controls which can be used is antagonistic endophyte fungi from medical plants, i.e Artemisia annua L. Endophytic fungi can increase plant resistance. This research aimed to obtain fungi from A. annua leaves and evaluate its potential in inhibiting R. solani in vitro and in vivo. The study was conducted using a Completely Randomized Design (CRD) with 1 factor and 3 replications. A total of 11 fungi was successfully isolated, then a pathogenicity test showed that 5 fungi did not cause necrosis, namely Penicillium sp., Phoma sp., Cladosporium sp., Aureobasidium sp. 1, and Aureobasidium sp. 2. The result showed that Phoma sp. had the best inhibitory ability in vitro by 66.7%. Application of Phoma sp. gave the best inhibition on the lesion length by 11.33 mm and infection rate by 1.85 day units-1 with the effectiveness of disease control (EDC) 5.95% in vivo. The inhibition of rice sheath blight preinoculated with endophytic fungi appears to be a characteristic of induced resistance.

Keywords – Biological control, in vitro antagonism, in vivo antagonism

I. INTRODUCTION

Rice is Indonesia's main commodity with production 81.37 million tons in 2017 and widely consumed by its people. Rice productivity in Indonesia from 1980-2017 has increased by 1.24% per year. Rice consumption in households and outside households from 1981-2016 decreased by 1.664% and 1.02% per year. The volume of rice imports from 1983-2017 increased sharply by 483.83% per year [1].

Pests and plant diseases are biotic stresses that can reduce yields and can even cause crop failure [2]. Rice sheath blight is caused by R. solani become one of the problems in rice plants because it decreases the production. The yield losses can reach 20% in Indonesia [3]. Global yield losses are 10-30% and can even reach 50% [4]. These pathogens cause the breeding to fail, less fully filled grain even empty, plants become easy to fall down, etc. [5]. According to [6], one of the biological controls that can be used is antagonistic endophytic fungi.

Endophytic are microorganisms that live in plant tissues during their life cycle without causing symptoms [7]. The role of endophytes in plant tissues is to induce resistance in host plants [8]. Endophytes are symbiotic with many medicinal plants [9], one of them is Artemisia sp. which is able to inhibit several plant pathogens [10]. Some endophytes are generalists which are able to infect various plant hosts, and specialist which are limited to one or several plant hosts [11]. The endophytic fungi of isolates artemisia obtained are expected to be developed as biological control agents against R. solani, causal agent of rice sheath blight. Endophytic fungi information is also important as a source of diversity of biota.

II. EXPERIMENTAL SET-UP

This study was conducted in September 2017-July 2018 at Laboratory of Plant Diseases and Greenhouse C Faculty of Agriculture UNS. The material used was explants of A. annua leaves, R. solani isolates, Potato Dextrose Agar (PDA), rice 4 WAP. The tools used are autoclave, LAF, and glass equipment. Data was analyzed with F test and Duncan test level 5%.

Endophytic fungi isolation used A. annua leaf explants. Leaf explants were sterilized with 70% alcohol, 5.00% NaOCl for 30 seconds, and sterilized water 3 times, then isolated on PDA. The fungi found were purified to obtain a single isolate, then tested for pathogenicity. Endophytic fungi are identified and checked to the Illustrated Genera of Imperfect Fungi [12].

In vitro antagonism test was carried out by a dual culture method between endophytic fungi against R. solani. The percentage of inhibition is measured by:

Percentage of inhibition = $\frac{R1-R2}{R1} x\ 100\%$

with R1 = pathogenic fingers away from the antagonistic fungus, R2 = pathogenic radius approaches the antagonistic fungus.

Endophytic fungi are propagated using sawdust and bran. The test was carried out by CRD with 6 treatments. Application was given between rice sheath (25g / plant) at 2 weeks before R. solani pathogenic inoculation. Pathogen inoculation was carried out by patch method and observed for 7 days. The variables observed were the time of spotting, lesion length, infection rate, Disease Control Effectiveness (DCE), disease index with scoring from [13].

Table-1 Index scale of lesion length of rice sheath blight

Scale	Lesion lenght (mm)
0	No lesion
1	Infection without lesion
3	Lesion 20<x<30
5	Lesion 40<x<50
7	Lesion 50<x<60
9	Lesion x>60

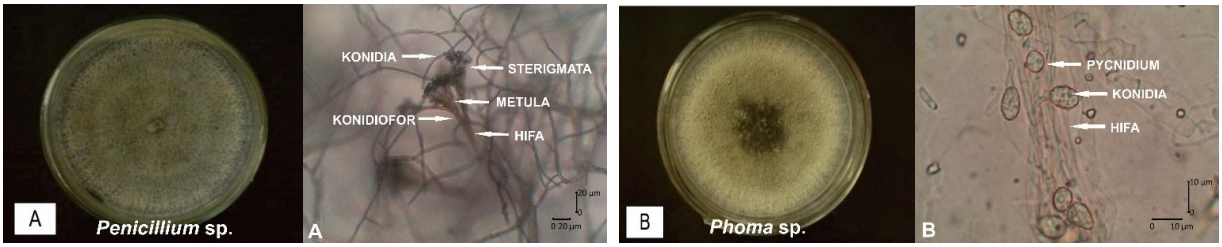
III. RESULTS AND DISCUSSION

Endophytic fungi isolated were Penicillium sp., Phoma sp., Cladosporium sp., Aureobasidium sp. 1, and Aureobasidium sp. 2. Isolate Penicillium sp. has a rounded colony shape with slippery edges or flat and flat elevation. Isolate is white on the third day and starts green in the middle on the seventh day. Isolate of Penicillium sp. consists of 5 parts namely conidia measuring 5.9x3.7 µm, sterigmata, metula, conidiophores, and hyphae that are septate (Figure 1A). According to [14], the size of the conidia of the fungus Penicillium sp. is about 3-6 µm, ellipsoidal to cylindrical, pale yellow to light brown. [15] showed that Penicillium sp. forming parallel conidiospores called coremia or synnemata.

Isolate Phoma sp. has an L shape with edges like wool and arise elevation. Isolate Phoma sp. white on the outside, green in the middle and brown on the seventh day. Figure 2B showed that isolate Phoma sp. forming pycnidium, round, measuring 10.3x5.6 µm, having short conidiophores, and having small, ovoid conidia and measuring 1.7x2.4 µm (Figure 1B). According to [16], conidia size of Phoma sp. is about 2-2.5 µm. [17] showed that Phoma sp. forms pycnidial conidiomata which has different shapes and sizes, but mostly globose or subglobese. Pycnidia has a color which varies depending on environmental conditions and age of the isolate.

Isolate Cladosporium sp. has a round shape with edges that spread and branch, and flat elevation. Isolate Cladosporium sp. has white and green in the middle on the seventh day. Microscopic observation, the fungus had hyphae, conidiophores, ovoid conidia, and was 17x10.5 µm (Figure 1C). According to [14], the size of conidia of Cladosporium sp. is around 15-25x7-10 µm. [12] showed that Cladosporium sp. have conidia that form chains or can be single-celled and shaped lemon-like.

Isolate Aureobasidium sp. notched edges, raised elevations and white bones. Form of Aureobasidium sp. 1 is macroscopically threaded, while the form of Isolate Aureobasidium sp. 2 round shape with edges arising. Microscopically Isolate Aureobasidium sp. do not have conidiophores, conidia form groups and are located along hyphae, conidia continue to multiply by budding and form blastoconidia, which distinguishes isolates of Aureobasidium sp. 1 and isolates of Aureobasidium sp. 2 is the size of the conidia of Aureobasidium sp. Isolates. 1 is 12.5 x 2.9 µm and has a threaded form (Figure 1D), whereas E11 isolates are 8.6 x 2.9 µm and are circular in shape with a raised edge (Figure 1E). According to [18], conidia from Aureobasidium sp. are hyaline, cell-celled, ellipsoidal, and vary in shape and size, and what distinguishes aureobasisium isolates is the pigment isolates.



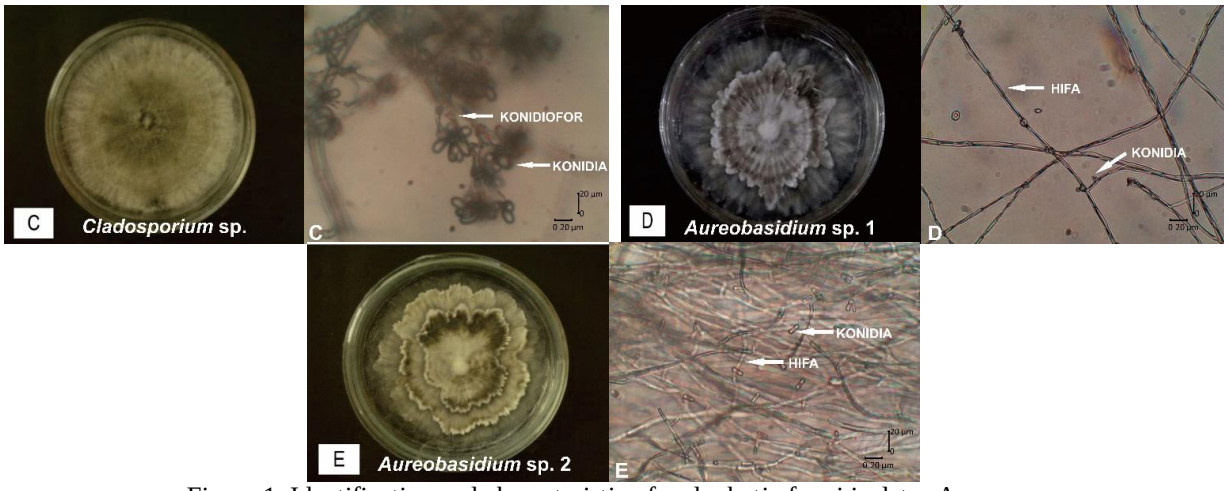


Figure 1. Identification and characteristic of endophytic fungi isolates A. annua.

The growth of R. solani causes of rice sheath blight disease can be inhibited by growth of endophytic fungi isolates artemisia with varying inhibitory ability in vitro (Figure 2). Isolate Phoma sp. has the highest ability to inhibit among other treatments, namely 66.7%. [19] also shows that Phoma sp. has the highest ability to inhibit Colletrotichum gloeosporioides by 53%. Isolate Penicillium sp. can inhibit R. solani by 60%. Penicillium sp. also has a high ability to inhibit Phytophthora capsici by 26% [20]. Isolate Cladosporium sp. and Aureobasidium sp. 2 has ability to inhibit R. solani by 54.4%. Cladosporium sp. also quite effective in inhibiting endophytic Trichoderma viride growth by 29% [21]. Isolate Aureobasidium sp. 1 has the lowest ability to inhibit R. solani by 24.4%. Endophytes Aureobasidium sp. originating from the leaves of Espeletia sp. has a fairly large antimicrobial and can inhibit the growth of pathogens Fusarium oxysporum, Phytophthora infestans, and R. solani [22]. Endophytic fungi are thought to have the ability to inhibit different pathogens, depending on the type of compound produced, the species of fungi, and the host from which the fungus was found.

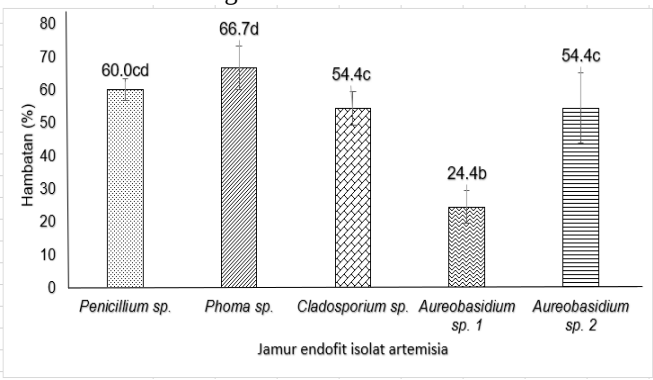


Figure 2. Inhibiory isolat R. solani by endophytic fungi isolates artemisia.

Information: Numbers followed by letters that are not same show a significant difference in the Duncan test 5%.
Based on Table 1. Control (+) treatment caused spots that appeared at 3.2 DAI similar to the treatment of Cladosporium sp. and Aureobasidium sp. 1 which are the fastest symptom appearance. The longest time for symptoms appeared in treatment Phoma sp. by 3.67 DAI. The second longest symptom appeared in treatment Penicillium sp. by 3.4 DAI, and the third treatment Aureobasidium sp. 2 is 3.33 DAI. This is in accordance with [23] and [24] that is the incubation period of rice sheath blight can be seen day 3 after inoculation by causing symptoms of grayish spots. The sheath blight disease index is <2%. According to [13], a disease index of <2% showed symptoms of spot of <20 mm, according to the spot area of <20 mm and showed mild symptoms. According to [25], the application of endophytes that have an effect on reducing the incidence of disease can be caused by competition of space and nutrients with pathogens and are induced by plant endurance because endophytes produce secondary metabolites.
The largest length of rice sheath blight appeared on Aureobasidium sp.1 by 20 mm, greater than pathogenenic control treatment which is only 17.53 mm. The smallest spot length appeared on Phoma sp. by 11.33 mm. [19] said

that this caused by ethyl acetate extract produced by Phoma sp. produce which has ability to inhibit pathogens, including pathogenic inhibitors Colletrotichum gloeosporioides. Isolate Cladosporium sp. has the second highest inhibiting ability that is able to inhibit the rice sheath blight by 12.67 mm, followed by Penicillium sp. by 15.60 mm, and Aureobasidium sp. 2 by 17.33 mm.

The highest infection rate occurred in the treatment of Aureobasidium sp.1 by 3.32 units day-1 and the lowest infection rate was treatment of Phoma sp. by 1.85 units day-1. [17] added that abundant conidia production with a relatively fast growth rate and the ability to invade many hosts and substrates are main reasons Phoma sp. capable of spreading widely and good in inhibiting pathogens. The highest isolate in suppressing R. solani is the treatment of Phoma sp. by 5.95%. Treatment of Cladosporium sp. was 4.33%, treatment of Penicillium sp. was 2.80%, the treatment of Aureobasidium sp.2 was 1.33%, and the treatment of Aureobasidium sp.1 was 0.67.

Table 2 Effect of isolates artemisia on time of symptom appearance, disease index, infection rate, lesion length, and effectiveness of disease control (EDC)

Treatment of endophytic fungi isolate artemisia and R. solani	Time of symptom appearance (DAI*)	Disease index (%)	Infection rate (unit day-1)	Lesion length (mm)	EDC (%)
Penicillium sp.	3,40±0,53	2,07±0,42 b	2,59±0,46 abc	15,60±2,62 abc	2,80±2,66
Phoma sp.	3,67±0,83	1,27±0,31 a	1,85±0,33 a	11,33±2,31 a	5,95±1,17
Cladosporium sp.	3,20±0,72	1,27±0,61 a	2,13±0,89 ab	12,67±5,51 ab	4,33±3,97
Aureobasidium sp. 1	3,20±0,20	2,40±0,20 b	3,32±0,36 c	20,00±2,65 c	0,67±1,15
Aureobasidium sp. 2	3,33±0,42	1,87±0,12 ab	2,75±0,11 bc	17,33±0,58 bc	1,33±1,55
Control (+)	3,20±0,72	1,93±0,23 ab	2,80±0,18 bc	17,53±1,29 bc	-

Information: *DAI is Day After Inoculation. Numbers followed by letters that are not same show a significant difference in the Duncan test 5%. The EDC value is transformed with $\sqrt{x+1}$.

IV. CONCLUSION

Endophytic fungi isolated from A. annua leaves were Penicillium sp., Phoma sp. Cladosporium sp., Aureobasidium sp. 1, and Aureobasidium sp. 2. Isolate Phoma sp. showed the best inhibition of R. solani in vitro with 66.7%. Application of Phoma sp. gave the best inhibition on the lesion length of rice sheath blight (11.33 mm) and infection rate (1.85 units day-1) with EDC of 5.95%.

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