http://dx.doi.org/10.21172/ijiet.142.01

Antagonism Of Endophytic Fungi Of Artemisia Against Cercospora Causal Agent Of Narrow Brown Leaf Spot Of Rice

Heni Hidayati[1], Hadiwiyono[2], Ahmad Yunus[3]
[1]*Department of Agronomy Graduate School of Sebelas Maret University Graduate School, Ir. Sutami Street 36 A, Surakarta (Solo) 57126, Indonesia*
[2,3]*Agrotechnology Major, Faculty of Agriculture, of Sebelas Maret University Graduate School, Ir. Sutami Street 36 A, Surakarta (Solo) 57126, Indonesia*

Abstract- Rice is the staple food of most Indonesians. One limiting factor in increasing rice production is the attack of plant pest organisms. The Cercospora leaf spot disease can lead to dry leaves before their time. The use of endophytic fungi in Artemisia is considered effective for controlling some plant diseases. This study aims to determine the presence or absence of endophytic fungi in Artemisia and to study the antagonism of Artemisia endophytic fungi against Cercospora oryzae. The study was conducted in September 2017 - May 2018 at the Laboratory C of Pest and Plant Disease and Greenhouse, Faculty of Agriculture, University of Sebelas Maret. This research started from exploration of Artemisia endophytic fungi up to in vitro and in vivo test. The research design used was completely randomized design with control, negative control and treatment with each replicated 3 times. The data will be analyzed based on a variety analysis or Anova. If the effect is real then the process was continued with 5% DMRT test. The results showed that endophytic fungi that was successfully isolated from the leaves of Artemisia annua were Penicillium sp., Phoma sp., Cladiosporium sp., Aureobasidium sp 1., and Aureobasidium sp 2. With in vitro assays, the isolate of endophytic fungi which have the highest inhibitory power against C. oryzae was Penicillium sp. with inhibitory power of 47.78% while with in vivo assays, the highest inhibitory power in isolate treatment was Aureobasidium sp 2.
Keywords – Rice, Cercospora oryzae, Artemisia Endophytic Fungi

I. INTRODUCTION

The average consumption of rice in Indonesia per capita a week in 2014 to 2016 tends to increase. In 2014 there were 1,626 Kg, 2015 totaling 1,631 Kg, in 2016 as many as 1,668 Kg [1]. One of the factors limiting rice production is the attack of plant disturbing organisms. Cercospora leaf spot disease or often called narrow brown patches can result in a decrease in yields of up to 10% [2] and also cause rice plant haste [3]. Endophytic fungi are one of the biological control agents that can be used to control narrow leaf spot disease in rice. Artemisia is a plant that can produce secondary metabolites. According to [4] metabolites produced by the Arteimisia annua L. plant found in Nanjing, China produce secondary metabolites in the form of 6-isoprenyl indolo, 3- carboxylic acid, 3β, 5α dihydroxy-6β-phenyl acetyloxy can be used to control diseases caused by Colletotrichum spp. The effectiveness of endophytic fungi in Artemisia needs to be studied to determine the antagonism of the fungus Cercospora oryzae.

II. EXPERIMENTAL SET-UP

Artemisia endophytic fungi isolated. In vivo tests were carried out with rice field media that had been sterilized on polybags. Endophytic fungal isolates were planted into bran media mixed with wood powder with a ratio of 1: 1 in the jar and then incubated for 2 weeks before being applied to the soil used in the in vivo test. Inoculation of C. oryzae was carried out by attaching C. oryzae to the healthy leaves selected as samples. Inoculation is carried out using an ose needle with a diameter of 5 mm. Isolates of C. oryzae used for inoculation are 8 days old. Spotting area was calculated on the 7th day after inoculation. The incubation period is calculated from the day of administration of C. oryzae inoculum until the day the symptoms appear. The disease index is calculated using a symptom of narrow leaf spot disease based on the area of the spot.

Table 1 Symptoms of Narrow Leaf Leaf Disease

Scales	Spotting
0	There are no spots
1	spots $0<x<2$
2	spots $2,1<x<4$
3	spots $4,1<x<6$
4	spots $6,1<x<8$
5	spots $x>8$

Infection rate is calculated using the regression formula: Y = a + bX, with Y = area of spotting and X = length of day. Biological Control Effectiveness (EPH) is calculated using the formula: EPH = (KP-P) / KP x 100%, with KP = the area of spotting from the treatment of C. oryzae inoculation and P = the area of the spot from the treatment of endophytic fungi inoculation.

III. RESULTS AND DISCUSSION

3.1 Identification of endophytic fungi

The results of isolation of Artemisia endophytic fungi from the leaves obtained as many as 5 endophytic fungal isolates. The success of isolation of endophytic fungi is determined by the sterilization of the surface and the parts of the plant under study [5]. According to [6], the identification of macroscopically is done by observing the shape of the colony, texture and color of the colony, while microscopically observed the shape, color, number (one or many) and the position of the conidiophores or sporangiophores.

The result from isolating Artemesia annua plant from a leaf, obtained abouf 5 isolated endophytic fungi. This success on isolating endophytic fungi which inside a plant tissue was determined by sterilization of the surface and other studied parts of the plant [5]. An isolate fungi that have succeed from isolated then will be observe through a colony morphology observation either with mascroscopic (Tabel 1) or microscopic way.

Tabel 1 Morphology characteristic of isolate endophytic fungi

No	Endophytic Fungi	Morphology characteristic			
		Shape	Edge	Elevation	Colour
1	EA1	Round	slippery	flat	White, green on center
2	EA2	L shaped	Like a wool	emerge	White, brownish
3	EA3	Round, with spreading edge	branched	flat	White, with spreading green spot
4	EA6	Yarn shaped	curved	Emerge	Bone white
5	EA7	Round	curved	Emerge	Bone white

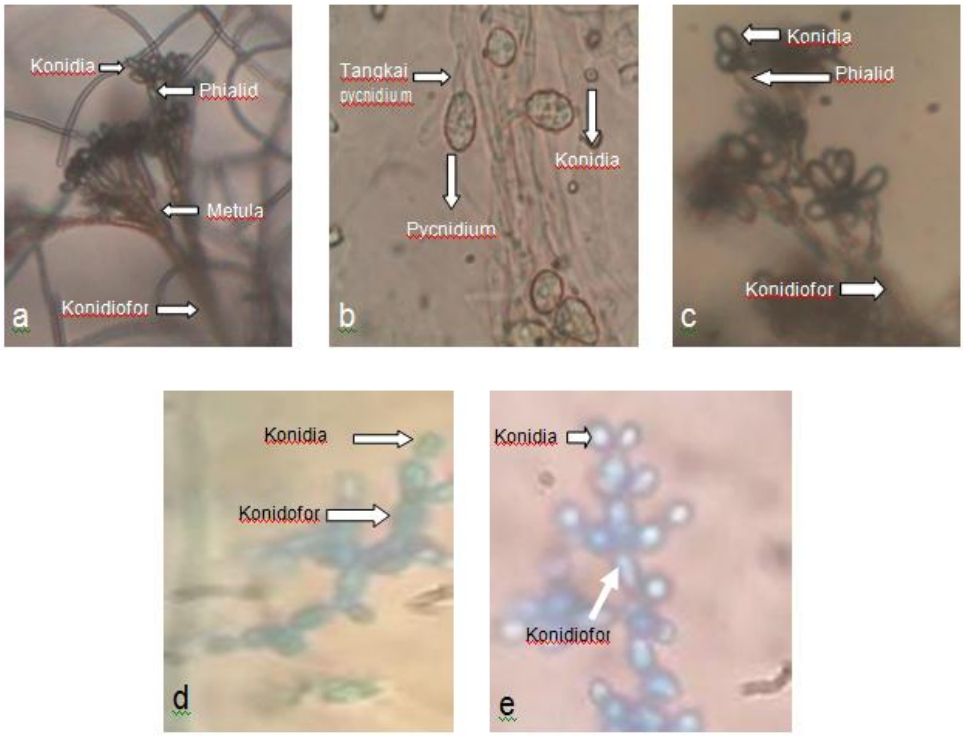


Figure 1 Microscopic form of endophytic fungus morphology; a. isolate EA1 1000x magnification, b. EA2 isolates 1000x magnification, c. EA3 isolates 1000x magnification, d. isolate EA6 magnification 400x, e. EA7 isolates 400x magnification.

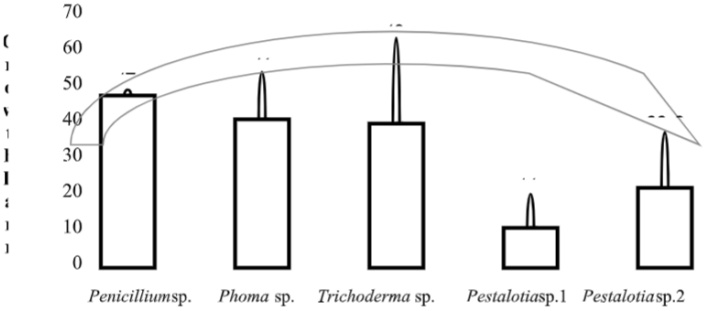
According to [7] morphology of fungal isolates influenced by nutrition, temperature, light, and humidity. The results of observations carried out macroscopically and microscopically can be identified that the EA1 isolate is Penicillium sp. Usually bersepta, fruit body shaped like a broom followed by sterigma / phialid and conidia arranged in chains [8]. EA2 isolate is Phoma sp. it is characterized by white colony colors and gradually turns gray, velvety texture, and microscopically hyaline brown, has pycnidium and single-celled. EA3 isolate is Cladiosporium sp., Has microscopic characteristics of green hyphae, short fialid stalks, greenish conidia, globuse (round) growth on the tip and conidium formed in light green clusters on the surface of conidiophores. Fialid has a length of ± 11.1µm and conidiophores are ± 13.4µm long [9]. EA6 isolates were Aureobasidium sp 1., and EA7 isolates were Aureobasidium sp 2. The morphological characteristics of conidia were 84,6-96,8µm x 26,7-333,5µm and consisted of 5 lined cells. The end cell or apical cell (first cell) hyaline is rather elongated or narrowed to the tip while the base cell or basal hyaline cell is rather cylindrical. All parts of hyaline conidiospores, apical cells, basal cells, and setula, are easily deformed, ie slightly wrinkled when stored for a long time [10].

3.2 In vivo antagonism

The results of in vitro antagonism showed that Artemisia endophytic fungi isolates had inhibitory effect on C. oryzae (Figure 6). The highest inhibition percentage was produced by EA1 (Penicillium sp.) Mushroom isolates at 47.78% and the lowest was by EA6 fungal isolates (Aureobasidium sp1.). Penicillium sp. produces 34 bioctive metabolites which function as antifungal [11]. The inhibition of the growth of these pathogens can be caused by nutritional competition [12] and also the presence of secondary metabolites produced by endophytic fungi that cause lysis of pathogenic fungi [13]. Bioactive compounds resulting from the isolation of endophytic fungi have activity in fighting pathogenic microbes [14], [15].

3.3 In vivo antagonism

Four Artemisia endophytic fungi isolates were able to suppress C. oryzae infection in vivo (Table 2). EA7 fungal isolates had a controlling effect on C. oryzae at 40.86% with symptoms appearing at 3.6 days after inoculation, 3.70 mm2 spotting area, 2.67 disease index and 0.75 unit / day infection rate. EA2 fungal isolates did not have effectiveness in controlling C. oryzae as indicated by the appearance of symptoms faster than the control treatment and the spotting area was also greater than the control treatment. This can occur because the Phoma sp. less able to associate with plants and develop in their new environment. According to [16] the activity of pathogens, host susceptibility and their interactions are influenced by the environment. Appropriate method and application concentration of biocontrol agents which is inappropriate can affect the effectiveness in suppressing disease growth [17].



ENDOPHYTIC FUNGI OF ARTEMISIA

Explanation: The same numbers that are follow with letters, shows there is no differentiation when conducting the Duncan test of 5%

Figure 2 Histogram of power obstacle from Artemisia sp endophytic fungi towards Cercospora oryzae.

Endophytic Fungi	Time of symptom appearance (DAI*)		Spotting Area (mm2)		Disease Index		Infection rate (unit day-1)		EPH (%)	
EA5	2,93±0.23	bc	5.00±0.72	bc	3,00±0.00	ab	0,86±0.05	b	19,35±11.63	ab
EA6	2,20±0.20	b	6.40±0.40	c	3,67±0.58	b	0,65±0.07	a	0,00±6.45	a
EA7	2,40±0.20	b	5.40±0.87	c	3,00±0.00	ab	0,77±0.08	ab	12,90±14.06	a
EA10	2,40±0.20	b	5.70±1.03	c	3,33±0.58	ab	0,70±0.18	ab	8,60±16.55	a

EA11	3,60±0.91	c	3.70±1.29	b	2,67±0.58	a	0,84±0.06	b	40,86±20.74	b
KP (Pathogen)	2,40±0.23	bc	6.20±0.92	c	3,33±0.58	ab	0,74±0.03	ab		

Note: numbers followed by letters that do not match the real difference in the Duncan test of 5%, the EPH value is the result of data transformation.

IV. CONCLUSION

Artemisia endophytic fungi that were isolated were Penicillium sp, Phoma sp, Cladiosporiumsp., Aureobasidium sp 1. and Aureobasidium sp 2. The five endophytic fungal isolates had an antagonism towards C. oryzae in vitro, and in vivo Aureobasidium sp 2 isolates had a controlling effectiveness of 40.86% with a spotting area of 3.70 mm2 and an infection rate of 0.75 units / day. Further research is needed to study the metabolites released by endophytic fungi in inhibiting the growth of pathogens.

V. REFERENCE

[1] Central Bureau of Statistics Indonesia. "Average per capita consumption per week for a number of important foods from 2007 to 2015". Indonesian Central Statistics Agency. 2017.
[2] Prasetya MSH, Masnilah R, Wagiyana. "Study on the Intensity of Narrow Brown Spots (Cercospora oryzae) and Control Techniques in Rice (Oryza sativa L.) in Jember Regency". J Sci Agrotech. Vol. 3, no.2. 2017.
[3] Semangun H. "Food crop diseases in Indonesia". Yogyakarta (ID): Gajah Mada University Press. 2004.
[4] Tejesvi MV, Nalini MS, Mahesh B. "New hopes from endophytic fungal secondary metabolites". J Intern Bol Soc Quim Mex. vol.1, no. 1, pp. 19-26. 2015.
[5] Selim KA, El-Beih AA, AbdEl-Rahman TM, El-Diwany Al. "Biology of Endophytic Fungi". Res Environ Myco. vol. 2, no. 1, pp. 31-82. 2012.
[6] Cosoveanu A, Sabina SR, Cabrera R. "Fungi as Endophytes in Artemisia thuscula: Juxtaposed Elements of Diversity and Phylogeny". J Fungi. vol. 4, pp. 17. 2018.
[7] Visagie CM, Houbraken J, Hong SB, Klaassen CHW, Perrone G, Seifert KA, Varga J, Yaguchi T, Samson RA. "Identification and nomenclature of the genus Penicillium". J Myco. vol. 78, pp. 343-371. 2014.
[8] Purwantisari S, Hastuti RB. "Isolation and identification of indigenous rhizosphere fungi of potato plants from organic potato farms in Pakis village, Magelang". J Biome. vol. 11, no. 2, pp. 45-53. 2009.
[9] Suanda IW. "Morphological characterization of Trichoderma sp. JB isolate and antagonism to pathogens that cause sprout (Sclerotium rolfsii Sacc.) in tomato plants". Proceedings of the National Mathematics and Natural Sciences. 2016.
[10] Sutarman, Achmad, Hadi S. "Pinus merkusii seedling leaf blight disease in the nursery". J agritek. vol. 9, no. 4, pp. 1419-1427. 2016.
[11] Grijseels S, Nielsen JC, Nielsen J, Larsen TO, Frisvad JC, Nielsen KF, Frandsen RJN, Workman M. "Physiological Characterization of Secondary Metabolite Producing Penicillium Cell Factories". J Fungal Biol and Biotech. vol. 4, pp. 8. 2017.
[12] Amaria W, Rita H, Samsudin. "Evaluation of antagonistic fungi in inhibiting the growth of Rigidoporus microporus that causes white root fungus in rubber plants". J Industrial Freshener Plant. vol. 2, no. 1, pp. 51-60. 2015.
[13] Wang SL, Yen YH, Tsiao WJ, Chang WT, Wang CL. "Production of antimicrobial compounds by Monascus purpureus CCRC31499 using shrimp and crab shell powder as a carbon source". J Enzyme Micro Tech. vol. 31, pp. 337-344. 2002.
[14] Sandhu SS, Kumar S, Aharwal Rp. "Isolation and identification of endophytic fungi from Ricinus communis Linn. and their antibacterial activity". J intern Pharma Chemis. vol. 4, no. 3, pp. 611-618. 2014.
[15] Walker K, Croteau R. "Taxol biosynthetic genes". J Intern Phytoch. vol. 58, pp. 1-7. 2001.
[16] Agrios GN. "Plant pathology". United States of America (USA): Elsevier Academic Press. 2005.
[17] Susanna. "Utilization of antagonistic bacteria as a biocontrol agent of wilt disease (Fusarium oxysporum f.sp. cubense) in banana plants". J Floratek. vol. 2, pp. 114- 121. 2006.