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HASIL PENILAIAN SEJAWAT SEBIDANG ATAU PEER REVIEW
KARYA ILMIAH : JURNAL ILMIAH INTERNASIONAL

Judul Jurnal Ilmiah (Artikel) : Analysis of Perodase Activity and Total Phenol from Spathogltis plicata BI Plantet Toward Fusarium Oxysporum

Penulis Jurnal Ilmiah : Endang Nurcahyani, Rochmah Agustrina, **Erdi Suroso**, Gardis Andari

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a	Orisinalitas (20%) (Memperlihatkan keaslian dan kebaruan gagasan)	8	6	4	5,8
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	Total (100%)	**40**	**30**	**20**	27,8

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Analysis of Peroxidase Activity and Total Phenol from *Spathoglottis plicata* Bl Plantlet Toward to *Fusarium oxysporum*

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Abstract: Ground orchid (*Spathoglottis plicata* Bl) is an ornamental plant widely appreciated by the public because it has a beautiful shape, colour and flower formation. One of trigger decrease of *S. plicata* production caused by Fusarium wilt which is caused the *Fusarium oxysporum*. Using resistant *S. plicata* cultivars are expected to be an alternative to control the disease. A resistant *S. plicatta* plantlet to *Fo* has been initiated by *in vitro* selection on medium countaining fusaric acid on different concentrations. This study aims to determine the peroxidase activity and total phenol of *S. plicata* plantlet. Research conducted at the *in vitro* Laboratory, Department of Biology, Faculty of Mathematics and Natural of Sciences, University of Lampung. The study design was Completely Randomized. Data analyzed using Analysis of Variance and if significantly different followed by Least Significant Difference (LSD) test 5% significance level. The results showed that increasing the concentration of fusaric acid, it also increases the peroxidase enzyme activity and total phenol in plantlet that resistant to *F. oxysporum*. At a concentration of 40 ppm, peroxidase activity and the highest total phenol content are 0.536 unit/mg/second and 10.33%.

Keywords: *Spathoglottis plicata*, Induced Resistance, *Fusarium oxysporum*, Peroxidase Activity, Total Phenol

1. Introduction

The cultivation of orchids have many obstacles, one of which is fusarium wilt which is caused by *Fusarium oxysporum* [10]. One of business that is efficient and safe to control *F. oxysporum*was by using eminent varieties which are resistant to the fungus [9]. Cultivars that are resistant to *F. oxysporum* infection can be identified by *in vitro* selection in a medium supplemented with fusaric acid [3]. Some of parameters that can be explained the mechanisms of plant resistance to pathogen infection include are increase of phenolic compounds, peroxidase enzymesactivity [17].

Fusaric acid (5-n-butylpicolinic acid) is a non-specific mycotoxin produced by *F. oxysporum* that causes symptoms of wilt and rot in plants [11]. There was a positive correlation between resistance to the toxin plantlets with plant resistance to Fusarium [2]. Using fusaric acid at a toxic concentration causes plant to be death, furthermore non-toxic concentrations (below 10^{-6} M) helped induce phytoalexin synthesis, a form of the response of plants to inhibit the activity of pathogenic [4]. Using selectors fusaric acid as the agent *in vitro* selection may produce mutant cell or tissue that is insensitive to fusaric acid, therefore after being regenerated into plants can produce strains that are resistant or tolerant to pathogen infection. This method had been carried out among others on the plant plantlets vanilla [9], showed resistance to fusarium wilt. Up to now, using fusaric acidin concentrations tolerant not conducted yet to induce resistance in plantlet of*S. plicata* against *F. oxysporum*. Therefore, this research needs to be conducted.

2. Material and Methods

2.1. Place and Time

This research was conducted in the in vitro Laboratory, Department of Biology, Faculty of Mathematics and Naturalof Sciences, University of Lampung, from January–March, 2016.

2.2. Preparation of Materials

Orchid plantlets of *Spathoglottis plicata* Bl aged 6 months sterile in culture bottle. This material had propagated by in vitro and had selected by fusaric acid concentration of 0 ppm, 10 ppm, 20 ppm, 30 ppm and 40 ppm in the preliminary study. Fungal isolates *F. oxysporum* obtained from the personal collection of Dr. Endang Nurcahyani, M. Si.

2.3. Inoculation and Test of Resistance Fusarium oxysporum in plantlets of Spathoglottis plicata Bl

Inoculation of *Fusarium oxysporum* directly conducted on plantlets. It was conducted in a culture bottle according to the method [6] with a density of 1.7 x 10^4 spores per ml is dripped onto the plantlets 1-2 drops, then incubated at room temperature for 24 hours. Observations carried out starting the third day after inoculation for four weeks by observing and counting the number of leaves that show symptoms of wilting or yellow.

2.4. Characterization of Orchid Plantlets of Spathoglottis plicata Bl Resistant to Fusarium oxysporum

The Characters of *S.plicata* plantlets related to resistance to *F. oxysporum* could be viewed by the peroxidase enzyme activity and total phenol

2.4.1. Analysis of Peroxidase Activity

Peroxidase enzyme activity was analyzed by [13]. It was created a mixture of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract from the leaves of plantlets of *S.plicata*, and 0.5 ml of 1% H_2O_2. The mixture was precipitated at room temperature and put in a cuvette size 0.5 ml. Spectrophotometer set with a wavelength of 420 nm and readable from zero. In the initial phase was added 100 ml of 1% H_2O_2 in cuvette which had contains a mixture of sample and read for 5 minutes. The enzyme activity was calculated by U/mg/ min. One unit of activity is changing optical dencity (OD) 420 nm on a spectrophotometer per minutes.

2.4.2. Analysis of Phenol Total

Material for analysing of phenolic compounds used ground orchid plantlets having been scanned with fusaric acid compared to controls. Analysis of total phenolic compounds used methods [1].

2.4.3. Preparation of Standard Calibration Curve Phenol Compound

Gallic acid was used as a standard solution. Pure concentration of gallic acid used were 10, 20, 30, 40 ppm in distilled water. Each concentrations was taken as 0.5 ml and added to 100 ml volumetric flask, then add 2.5 ml reagent Folin-Ciocalteau and 2 ml of 7.5% sodium carbonate after mixed inserted into the cuvette and seen the value of absorption at a wavelength of 765 nm, use distilled water as a control. After result had known, in the next steps standard curve must be created, and seen the regression equation between the concentration of gallic acid and uptake value.

2.4.4. Preparation of the Samples Plantlets

Ground orchid plantlets with weight 2 g crushed using a glass mortar and dissolved in 25 ml of 80% ethanol (Sigma Chemical Co). Furthermore, centrifuged the solution at 13,000 rpm for 15 minutes. After centrifuge the supernatant was taken.

2.4.5. Measurement of Total Phenols

The supernatant was taken as 0.5 ml was added to 100 ml volumetric flask, then added 250 ml of Folin-Ciocalteu reagent, after settling for 5 minutes and then add 1 ml of Na_2CO_3. When blended, was added to 5 ml volume cuvette premises and observed absorption value at a wavelength of 765 nm using a spectrophotometer, use distilled water as a control. From the absorption value determined content of phenolic compound gallic acid regression equation of the connection between the absorption value plantlets ground orchid extract and gallic acid concentrations series.

2.5. Data Analysis

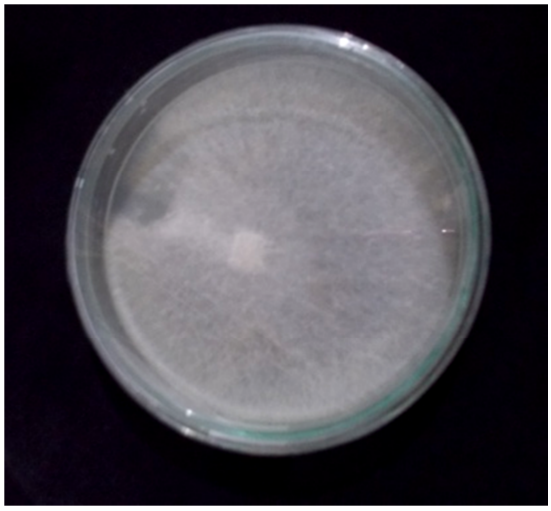
Data obtained from growth of ground orchid plantlets *S. plicata* during the selection with fusaric acid was quantitative and qualitative data. The qualitative data presented in the form of a comparative descriptive and backed photos. Quantitative data of each parameters analyzed by analysis of variant, performed on the 5% significance level and a further test with Least Significant Difference (LSD) at the 5% significance level.

3. Results and Discussion

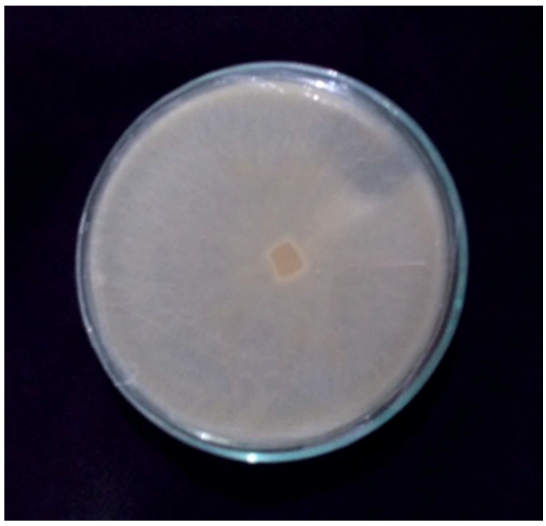
3.1. Inoculation and Testing Resistance Fusarium oxysporum on Plantlets of Spathoglottis plicata Bl

Fusarium oxysporum which was used in this study is in the form of monospora. *F. oxysporum* colonies grown on Potato Dextrose Agar (PDA) medium having white on the bottom and the top surface. Isolates monospora used to obtain a uniform and stable nature of *F. oxysporum* was expected to provide consistent results [18] on the test used ground orchid plantlets. Inoculation method used in the endurance test plantlets of *Spathoglottis plicata* which is given essences of fusaric acid was by directly inoculating microconidium of *F. oxysporum* with a density of 1.7 x 10^4 spores per ml in 1-2 drops plantlets in culture bottles were incubated at room with temperature (25°C) for 24 hours [6]. Observations were made every day for 4 weeks by counting the number of leaves that show symptoms of wilting plantlets. Based on the observations of ground orchid plantlets were scanned, symptoms of wilting leaves at the controls appear on day 4

after inoculation. Colonies of *F. oxysporum* monospore are presented in Figure 1.



A



B

A= upper surface, B=bottom surface

Figure 1.*Colonies of F. oxysporum.*

3.2. Characterization of Plantlets of Spathoglottis plicata Bl Resistant to Fusarium oxysporum

3.2.1. Peroxidase Enzyme Activity

Peroxidase activity as a resistance mechanism of *S. plicata* plantlets against *F. oxysporum* had been measured using the method [13], the orchid plantlets that have been selected with fusaric acid (concentrations of 10 ppm, 20 ppm, 30 ppm and 40 ppm) and controls. The results of analysis of peroxidase activity in ground orchid plantlets are presented in Table 1.

Table 1. *Effect of fusaric acid concentration to peroxidase enzyme activity.*

Fusaric Acid Concentration (ppm w/v)	Peroxidase Enzyme Activity (unit/mg/second)
0 (control)	0,183 ± 1,177[a]
10	0,279 ± 2,032[b]
20	0,413 ± 2,230[c]
30	0,440 ± 7,510[c]
40	0,536 ± 2,893[d]

Table 1 shows indication of increased activity of peroxidase in increasing of fusaric acid compared with the control, peroxidase activity was 0.183 U/mg/ min. It shows that the ground orchid responds are better resistance after being given treatment fusaric acid. Vanilla plantlets showed that increasing of peroxidase activity on vanilla plantlets exposed to fusaric acid [9]. According to [4] peroxide is a compound that can trigger increased activity of the enzyme peroxidase. [16] reported that watermelon plants treated with *Bacillus subtilis* showed increased activity of peroxidase was 280% compared with controls.

3.2.2. Total Phenol Content Analysis

One of indicators mechanisms of resistance in *S. plicata* induced is increase of phenolic compound plantlets inoculated with *F. oxysporum* by in vitro. Phenol compound is result of plant metabolism which is formed by one of functions as an immune system chemical plants shown by the formation of a chemical compound that is capable of preventing the growth and development of pathogens [17]. Before observing phenolic compounds in plantlets of *S. plicata* for each treatment, measurements were taken by using a standard curve prior gallic acid to estimate the total quality phenol with a linear regression [1]. From measurement of the standard curve generated a linear regression equation (y= 0.001x + 0.002) and has a value of correlation (R^2= 0998) it shows the diversity is homogeneous between gallic acid concentration with absorbance. Based on the standard curve gallic acid, it can be searched total phenol content of each treatment based on the equation of the regression line (Table 2).

Table 2. *Analysis of total phenol content of ground orchid plantlets in several various concentrations fusaric acid.*

Fusaric Acid Concentration (ppm w/v)	The average content of total phenols (%)
0 ppm	10,13 ± 4,111[a]
10 ppm	27,19 ± 1,228[b]
20 ppm	31,33 ± 5,268[c]
30 ppm	34,53 ± 3,011[d]
40 ppm	35,53 ± 4,111[d]

Table 2 shows increase of total phenol content from10.13% in the control became 27.19% at 10 ppm and 35.53% at 40 ppm concentration fusaric acid. It proves that the higher fusaric acid concentration, the higher total phenol content generated. Increasing of total phenolic compounds in plantlets of *S. plicata* were induced by fusaric acid is other

evidences of increase resistance of plants to resist infection rate *F. oxysporum*. It correlates with the opinion of [17], which states that one of parameters creating increase in plant resistance to pathogens is increasing phenolic compound. The results are consistent with the results of research conducted by [9] on vanilla plantlets were induced by fusaric acid and vanilla produces plantlets that were resistant to *F. oxysporum* f. sp. *vanillae*. The total phenol content increases with increasing concentrations of fusaric acid which is given [7] on *Chicpea* plants infected with *Fusarium oxysporum* f. sp. *ciceris* found that there was an increase of about 16-17% of total phenols. Fusaric acid at non toxic concentrations (10^{-7}M) can induce phytoalexin synthesis, a form of inhibition of the plant against pathogens [4].

4. Conclusion

Results from induce of *Spathoglottis plicata* orchid plantlets against *Fusarium oxysporum* that have selected using fusaric acid at a concentration of 10 ppm, 20 ppm, 30 ppm and 40 ppm in medium VW are able to increase the activity of peroxidase and total phenol content in *Spathoglottis plicata* planlets compared with controls, peroxidase enzyme activity and total phenol content are corelate with increase of fusaric acid stress.

References

[1] Aberouman A. A and Deokule S. S. 2008. Comparison of Phenolic Compounds of Some Edible Plants of Iran and India. *Pakistan Journal of Nutrition* 7(4): 582-585.

[2] Arai M and Takeuchi M. 1993. Influence of Fusarium Wilt toxin (s) on Carnation cell. *Plant Cells, Tissue and Organ Culture* (34): 287–293.

[3] Bacon CW, Porter JK, Norred WP dan Leslie JF. 1996. Production of Fusaric Acid by *Fusarium* sp. *Applied and Enviromental Microbiology.* 62 (11): 4039-4043.

[4] Bouizgarne B, Bouteau HEM, Frankart C, Reboutier D, Madiona K, Pennarun AM, Monestiez M, Trouverie J, Amiar Z, Briand J, Brault M, Rona JP, Ouhdouch Y, & Hadramu EI. 2006. *Early Physiological Responses of Arabidopsis thaliana Cells to Fusaric Acid*: Toxic and Signallling Effects. *New Phytologist* 169: 209–218.

[5] Goodman RN, Zoltan K, and Milton Z, 1986. *The Biochemistry and Physiology of Plant Disease.* D. Van Nostrand Company, Inc. New Jersey, Toronto, London, Melbourne.

[6] Hadisutrisno, B. 1995. *Taktik dan Strategi Perlindungan Tanaman Menghadapi Gangguan Penyakit Layu Fusarium*. Makalah Simposium Nasional I di Purwokerto, 2-3 Maret.

[7] Khan I. A, Alam S. S, Haq A, & Jabbar A. 2005. Biochemistry of Resistance in *Chicpea* Against Wilt Disease Caused by *Fusarium oxysporum* f. sp. *ciceris*. *Pak. J. Bot*. 37: 97-104.

[8] Landa BB, Cachinero-Diaz JM, Lemanceu P, Jimenez-Diaz RM & Alabouvette C. 2002. Effect of fusaric acid and phytoanticipans on growth of rhizobacteria and *Fusarium oxysporum*. *Canadian Journal of Microbiology* 48: 971-985.

[9] Nurcahyani E, Sumardi I, Hadisutrisno B, & Suharyanto E. 2012. Supression of Development of Vanilla Foot Rot Disease (*Fusarium oxysporum* f. sp. *vanillae*) Throught In Vitro Fusaric Acid Selection. *Journal of Tropical Plant Pests and Diseases.* Acreditation of DIKTI No.110/DIKTI/Kep/2009. ISSN: 1411-7525. Vol. 12 /No. 1: 12-22.

[10] Palmer GD. 2011. *The control of orchids*. http://www.ebow.com/info_8525784_control_fusarium-wilt-orchids html. Accesed on January 20, 2016.

[11] Remotti PC, Lofler HJM & Lotten-Doting LV. 1997. Selection of cell lines and regeneration of plants resistance to fusaric acid from Gladiolus x gradiflorus c.v 'Peteer Pear'. *Euphytica* 96: 237-245.

[12] Ruzin, SE. 1999. *Plant Microtechnique and Microscopy*. Oxford University Press, New York.

[13] Saravanan T, Bhaskaran R, and Muthusamy M. 2004. *Pseudomonas fluorescens* Induced Enzymological Changes in Banana Roots (cv. Rasthali) against *Fusarium* Wilt Disease. *Plant Pathology Journal.* 3: 72-80.

[14] Sticher L, Mauch-Mani B and Metraux JP. 1997. Systemic acquired resistance. *Annual Review Phytopathology* 35: 235-270.

[15] Toyoda H, Hasyashi H and Yamamoto K. 1984. Selection of resistant tomato calli to fusaric acid. *Ann. Phytopathol. Soc. Japan* 50: 538–540.

[16] Umamaheswari R and Mohanan K. V. 2009. A Study of the Association of Agronomic Characters in *Vanilla planifolia* Andrews. *International Journal of Plant Breeding and Genetics* 5 (1): 53-58.

[17] Vidhyasekaran P. 1997. *Fungal Pathogenesis in Plants and Crops, Molecular Biology and Host Defense mechanism. Marcel Dekker*. New York. 553 p.

[18] Windels CE. 1993. *Fusarium.* pp. 115-128 In: *Methods for Research on Soilborne Phytopathogenic fungi.* Singleton LL, Mihail JD & Rush CM. eds. APS Press. St. Paul, Minnesota.