

#### THE INDUCING COMPOUND OF FUSARIC ACID FOR RESISTANCE OF FUSARIUM WILT DISEASE IN FAMILY ORCHIDACEAE

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#### Abstract

Orchids are plants that belong to the Orchidaceae family. Orchid have charming flowers because they have various colors, shapes and sizes, and bloom perfectly and are long-lasting. In the process of growth, orchids can be attacked by several diseases, one of which is fusarium wilt caused by the fungus *Fusarium oxysporum*. *Fusarium oxysporum* produces a non-specific mycotoxin compound, namely fusaric acid. Fusaric acid at non-toxic concentrations can help induce the synthesis of phytoalexins, which is a form of plant response to inhibit pathogen activity. Based on the results of reviews from several journals, it can be concluded that fusaric acid compounds can be used as resistance compounds to fusarium wilt disease in orchids.

# Keywords : Fusaric acid, Fusarium Wilt, Orchidaceae, and Resistance

# Introduction

In Indonesia, orchids are one of the ornamental plants that are in great demand by the public. Orchids as ornamental plants have high economic value because of their aesthetic value (Ramadiana et al., 2008). One of the problems in the orchid growth process is Fusarium wilt caused by the fungus *Fusarium oxysporum*. In orchids, fusarium wilt will show symptoms in the form of yellowing leaves and stems, wrinkles, thin and curved, rotting leaf necks to the base of the stem (Soelistijono, 2015).

*Fusarium oxysporum* produces a non-specific phytotoxin compound, namely fusaric acid (AF). Nurcahyani (2013) states that one of the alternative ways to control disease that is safe, efficient, and effective on the environment is to use resistant varieties. The filtrate from fusarium culture or pure fusarium poison, namely fusaric acid (AF) can be used in the selection of resistance to fusarium wilt. The use of fusaric acid in in vitro selection is widely used because it is pathogenic and general to plants so that it can be applied to many plants. The purpose of this journal review is to find out whether fusaric acid can be used as a resistance compound against fusarium wilt disease in orchids.

# **Family Orchidaceae**



Orchid is one of the ornamental plants favored by the people of Indonesia because this plant has a variety of colors and flower patterns. Orchid plants are classified into the Orchidaceae family. Orchids are charming flowers because they come in various colors, shapes and sizes, and bloom perfectly and are long-lasting. In the last decade, the demand for several new orchid cultivars as cut flowers has increased. Because of the beautiful orchid flowers, this plant has a high economic value in national and international markets (Islam et al., 2015). In the growth process, orchid plants can be disturbed by pathogenic fungi, bacteria, or viruses that attack parts of the orchid plant's body, causing disease (Djatnika, 2012). One of the obstacles in the cultivation of orchids is fusarium wilt disease caused by the fungus *Fusarium oxysporum* (Panjaitan, 2005).

# **Fusarium Wilt Disease**

Fusarium wilt disease is one of the diseases caused by the fungus *Fusarium oxysporum* and can attack the quality and production of orchid plants (Palmer, 2011). Fusarium wilt disease is very detrimental because it can cause plant death and can reduce production by more than 50% and control with fungicides has not been able to overcome the disease (Wedge and Elmer, 2008). *Fusarium oxysporum* is a soil-borne pathogen that can spread through the soil or rhizomes of diseased plants, and can infect plants through wounds caused by seed transport, weeding, or by insects and nematodes. Mycelium will form on infected plants and grow until they reach the xylem vessels, so that nutrient transport is blocked and eventually causes the plants to wither (Semangun, 1996).

*Fusarium oxysporum* can infect plants through the roots, causing root rot, wilting and sometimes stem rot (Latifah et al., 2009), which can kill plants with systemic infections of the vascular system, creating severe nutrient and water shortages (Ichikawa et al., 2003). To increase resistance to *F. oxysporum*, selection is generally carried out on cell masses or budding nodules that have been treated with mutation induction and cultured on a medium containing selection components such as pure fusaric acid (AF) toxin or filtrate isolated from pathogens (Arceoni et al., 1987 in Lestari et al., 2006).

# **Induced Resistance**

Plants have a natural defense stimulus or as an increase in expression by biotic and abiotic agents to ward off pathogen attack, this is referred to as induced resistance (Edvera 2004). Induction of resistance causes physiological conditions that can stimulate natural resistance mechanisms in host plants (Rahmawati et al. 2014). The addition of certain chemicals (eg elicitor plants), non-pathogenic microbes, avirulent pathogens, incompatible pathogenic races, and virulent pathogens that fail to infect due to unfavorable environmental conditions can trigger the induction of resistance in plants (Vallad and Goodman, 2004). In vitro induced resistance to Fusarium sp. The use of pure fusaric acid toxin has been used in several plants such as kepok banana nurseries (Damayanti, 2010), vanilla (Nurcahyani et al., 2012), Spathoglottis plicata (Nurchayani et al., 2016), and cassava (Nurcahyani et al., 2019).



#### **Fusaric Acid Compound**

*Fusarium oxysporum* produces a non-specific mycotoxin compound, namely fusaric acid (5n-butylpicolinic acid) which can cause wilting and rot in plants (Remmoti et al., 1997). The use of fusaric acid at non-toxic concentrations (below 10-6 M) can help induce the synthesis of phytoalexins, which is a form of plant response to inhibit pathogen activity, but fusaric acid can also cause death in plants with toxic concentrations that can inhibit the oxidation of cytokines and the process of respiration in the mitochondria, can reduce ATP in the plasma membrane and reduce the activity of polyphenols thereby inhibiting the growth and regeneration of cultures (Bouizgarne et al., 2006).

Fusaric acid as a selection agent in in vitro selection produces mutant cells or tissues that are insensitive to fusaric acid, so that after being regenerated into plants they can produce strains that are resistant or tolerant to pathogenic infections (Nurcahyani, 2013). Disease control with this alternative method does not have a negative impact, such as the use of fungicides by using superior varieties that are resistant to *Fusarium oxysporum* (Nurcahyani et al., 2012).

The effect of giving AF compound as an inducer can be known through the chlorophyll content, one of which is in *P. amabilis* plantlets. The chlorophyll content of *P. amabilis* plantlets was observed by comparing plantlets without AF (0 ppm) and plantlets induced by AF with concentrations of 10 ppm, 20 ppm, 30 ppm, and 40 ppm. Analysis of chlorophyll content in this study used the Harborne method (1987). The results of the analysis of the chlorophyll content of *P. amabilis* plantlets revealed an increase in the content of chlorophyll A, chlorophyll B, and total chlorophyll, this occurred along with the increase in the concentration of AF given. The results showed that the highest content of chlorophyll A, chlorophyll B, and total chlorophyll was at a concentration of 40 ppm AF. The results of the analysis of the analysis of the content of chlorophyll A, chlorophyll B, and total chlorophyll A and by plantlets by planting on VW media added with AF with various concentrations are presented in Table 1.

Fusaric Acid	Chlorophyll A content	Chlorophyll B content	Chlorophyll total
Concentration	(mg/g tissue)	(mg/g tissue)	content
(ppm)			(mg/g tissue)
0 (control)	$0,053\pm,003180^{b}$	$0,067\pm,002728^{b}$	$0,119\pm,006028^{b}$
10	$0,262\pm,018889^{a}$	$0,122\pm,018448^{a}$	0,397±,025621ª
20	$0,244\pm,026577^{a}$	$0,129\pm,005044^{a}$	$0,404\pm,028416^{a}$
30	0,293±,016737 <sup>a</sup>	0,137±,004177 <sup>a</sup>	0,429±,020851ª
40	$0,324\pm,015875^{a}$	$0,164\pm,004177^{a}$	0,488±,021835 <sup>a</sup>

Table 1. Chlorophyll Content of P. amabilis Planlets Induced by Fusaric Acid

Chlorophyll is a very important part of plants because it plays a role in the process of photosynthesis, with the main function of utilizing solar energy and processing it into carbohydrates. In theory, healthy plants will continue to produce chlorophyll as the plant



ages, but due to several factors the presence of chlorophyll will decrease (Nurcahyani et al., 2019).

In addition, in Andari's research journal (2018), there is an effect of administering fusaric acid on the chlorophyll content of soil orchid plantlets (*Spathoglottis plicata*) grown on Vacin & Went (VW) medium with the addition of various concentrations of fusaric acid, which is presented in Table 2.

Table 2. Content of chlorophyll a, chlorophyll b, and total chlorophyll in the leaves of the *Spathoglottis plicata* plantlet

Fusaric Acid	Chlorophyll A content	Chlorophyll B content	Chlorophyll total
Concentration	(mg/g tissue)	(mg/g tissue)	content
(ppm)			(mg/g tissue)
0 (control)	$1,722 \pm 1,130 \mathrm{x10^{-2a}}$	$0,585 \pm 2,553 \mathrm{x10^{-2a}}$	$2,309 \pm 2,750 \times 10^{-2a}$
10	$2,157 \pm 4,739 \mathrm{x10^{-2b}}$	$1,470 \pm 1,323 \mathrm{x10^{-2a}}$	$3,625 \pm 1,050 \mathrm{x10^{-1b}}$
20	$2,834 \pm 5,819 \mathrm{x10^{-3c}}$	$2,582 \pm 1,830 \mathrm{x10^{-3b}}$	$5,413 \pm 8,159 \mathrm{x10^{-3c}}$
30	$3,297 \pm 2,952 \times 10^{-4d}$	$3,966 \pm 9,140 \times 10^{-3c}$	$7,258 \pm 6,136 \mathrm{x10^{-3d}}$
40	$3,957 \pm 1,889 \mathrm{x10^{-2e}}$	$5,642 \pm 3,674 \mathrm{x10^{-1d}}$	$9,592 \pm 2,226 \times 10^{-10}$

Based on table 2, it can be seen that the addition of fusaric acid in VW medium with various concentrations significantly affected the chlorophyll a, chlorophyll b, and total chlorophyll content of *S. plicata* plantlet leaves at concentrations of fusaric acid 10 ppm, 20 ppm , 30 ppm, and 40 ppm respectively. significantly different from the control. BNT test at 5% significance level showed that the content of chlorophyll a, chlorophyll b and total chlorophyll of ground orchid plantlet leaves at 40 ppm AF concentration was different from the control.

In addition to research on orchids, there are research journals on banana trees affected by fusaric acid. Infection by *Fusarium oxysporum* resulted in distinct damage to the chloroplast and membrane systems of the lower and upper leaves (Fig. 1). The development of Fusarium wilt coincides with the senescence process because fusaric acid can accelerate the development of Fusarium wilt and the fusaric acid content in the lower and upper leaves is different, fusaric acid plays a more important role in the aging of the lower leaves. Aging of infected banana plants induced by Fusarium infection with fusaric acid production and different leaf position composition strongly contribute to certain aging processes (Dong et al., 2014)





**Figure 1**. Shows the injection of a suspension of conidia (a) and pure fusaric acid (b) into the veins of the upper leaves. The themasophyll structure of the upper leaves began to show waterlogged wounds (c), while the lower leaves showed chlorosis (d). Arrow indicates injection position

#### Conclusion

Based on the results of the review that has been carried out, it can be concluded that fusaric acid compounds can be used as resistance compounds to fusarium wilt disease in orchids.

#### References

- Andari, G, Nurcahyani, E. (2018). Analisis Kandungan Klorofil Hasil Ketahanan Terimbas Fusarium oxysporum Terhadap Spathoglottis plicata Secara In Vitro. Musamus Journal of Animal Livestock Science, Vol. 1 No.1
- Arceoni S, M Pezzotti and F Damiani F. (1987). *In vitro* selection on alfalfa plants resistant to *Fusarium* f.sp. medicaginis.*Theor. Appl. Genet.* 74, 700-705.
- Bouizgarne, B., El-Maarouf, B.H., Frankart, C., Reboutier, D., Madiona, K., Pennarun AM, Monestiez, M., Trouverie, J., Amiar, Z., Briand, J., Brault, M., Rona, JP., Ouhdouch, Y & El Hadrami, I. (2006). Early physiological responses of *Arabidopsis thaliana* cells to fusaric acid : Toxic and signallling effects. *New Phytopathologist* 169: 209 218.
- Djatnika, I. (2012). Seleksi Bakteri Antagonis Untuk Mengendalikan Layu Fusarium pada Tanaman Phalaenopsis. J. Hort. 22 (3): 276-284.
- Damayanti, F. (2010).Peningkatan Ketahanan Pisang Kepok (*Musa paradasiaca* L.) Hasil Kultur Jaringan Terhadap Penyakit Layu Fusarium Melalui Seleksi Asam Fusarat. *Jurnal Ilmiah Faktor Exacta*. 3(4):3 10-319.
- Dong, X., Xiong, Y., Ling, N., Shen, Q., & Guo, S. (2014). Fusaric acid accelerates the senescence of leaf in banana when infected by Fusarium. World Journal of Microbiology and Biotechnology, 30(4),1399-1408



- Edvera, A. (2004). A novel strategy for plant protection: Inducedresistance. J. of Cell and Molecullar Biol. 3:61-69.
- Ichikawa, K., Kawasaki, S., Tanaka, C., Tsuda, M. (2003). Induced resistance against Fusarium diseases of *Cymbidium* species by weakly virulent strain HPF-1 (Fusarium sp.) *Journal of General Plant Pathology*. 69: 400-405.
- Islam, T,Bhattacharjee, B, Islam, SMS,Uddain, J, dan Subramaniam, S. (2015). Axenic Seed Culture And In Vitro Mass Propagation Of Malyasian Wild Orchid Cymbidium finlaysonianum Lindl. Pak. J. Bot, 47(6): 2361-2367
- Latifah, Z, Hayati, MZN, Baharuddin, S & Maziah, Z. (2009). Identification and pathogenicity of Fusarium species associated with root rot and stem rot of Dendrobium. *Asian J. Plant Pathol*, vol. 3, no. 1, pp. 14-21.
- Lestari, E.G, Mariska, I, Roostika, I, dan Kosmiatin, M. (2006). Induksi Mutasi dan Seleksi In Vitro Menggunakan Asam Fusarat untuk Ketahanan Penyakit Layu pada Pisang. *Berita Biologi*, Volume 8, Nomor I.
- Nurcahyani, E. (2013). Karakterisasi Planlet Vanili (Vanilla planifolia Andrews) Hasil Seleksi *In Vitro* dengan Asam Fusarat Terhadap *Fusarium oxysporumf.sp. vanillae*. Universitas Gadjah Mada.Yogyakarta. Disertasi.
- Nurcahyani, E., Issirep, S., Bambang, H. dan Suharyanto, E. (2012). Penekanan Perkembangan Penyakit Busuk Batang Vanili (*Fusarium Oxysporum F.Sp.Vanillae*) Melalui Seleksi Asam Fusarat Secara *In Vitro. J. HPT Tropika*. 12 (1): 12 – 22.
- Nurcahyani, E., R. Agustrina, T. T. Handayani. (2016). The Protein Profile of the Plantlets of Spathoglotti splicata Bl. Induced Resistance to Fusarium oxysporum. Journal of Plant Sciences. Vol. 4, No. 5, pp. 102-105.
- Nurcahyani, E., Sumardi., Hardoko, I. Q., Asma, P. and Sholekhah. (2019). Analysis of Chlorophyll *Phalaenopsis amabilis* (L.) Bl. Results of the Resistance to Fusarium oxysporum and Drought Stress. *IOSR Journal of Agriculture and Veterinary Science* (*IOSR-JAVS*). 12 (11) Ser. I. PP 41-46.
- Palmer, GD 2011, The control of orchids, acsessed 25 Mei 2021, <u>http://www.ehow.com/info\_8525784\_control-fusarium-wilt-orchids.html#ixzz1RIMQAIMJ</u>
- Panjaitan, E. (2005). Respons Pertumbuhan Tanaman Anggrek (*Dendrobium* sp.) Terhadap Pemberian BAP dan NAA Secara In Vitro. Jurnal Penelitian Bidang Ilmu Pertanian. Vol.3. No. 3. Pp: 45-51.
- Ramadiana, S., A.P. Sari, Yusnita dan D. Hapsoro. (2008). Hibridisasi, Pengaruh Dua Jenis Media Dasar dan Pepton Terhadap Perkecambahan Biji dan Pertumbuhan Protokorm Anggrek Dendrobium Hibrida secara In Vitro. *Prosiding Seminar Nasional Sains dan Teknologi-II Universitas Lampung*.17-18 Agustus.



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.

Semangun, H. (1996). Pengantar Ilmu Penyakit Tumbuhan. UGM Press. Yogyakarta

- Soelistijono. (2015). Kajian Efektifitas Rhizoctonia sp Mikoriza Dataran Rendah dan Sedang pada Tingkat Keparahan Penyakit (Dsi) Phalaenopsis amabilis terhadap Fusarium sp. Biosaintifika.7 (2).
- Vallad, GE and Goodman, RM. (2004). Systemic acquired resistance and induced systemic resistance in conventional Agriculture. *Crop Science Society of America*. vol. 44, no. 6, pp. 1920-34
- Wedge, DE & Elmer, WH (2008), Fusarium wilt of orchids, ICOGO Bull., vol. 2, no. 3, pp. 9-10.