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Interaction of Physiology Character - Secondary Metabolic of *Fusarium oxysporum* on Tomatoes of Fusarium Wilt Symptom

ABDUL Azis Ambar[1,a] HENNY Setyawati[2,b] NUR Ilmi[1,c]

[1]Department of Agrotechnology, Faculty of Agriculture,
Muhammadiyah University of Parepare, 91111, South Sulawesi, Indonesia

[2]Department of Biology, Education and Teaching Faculty
Muhammadiyah University of Parepare, 91111, South Sulawesi, Indonesia

[a]azisumpar1972@gmail.com; [b]hennys73@yahoo.co.id; [c]ilmi_261008@yahoo.co.id

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Abstract. *Fusarium oxysporum* (*F. oxysporum*) is the pathogen that caused fusarium wilt diseases on the tomatoes. The rise of the symptom was caused by secondary metabolic produced by *F. oxysporum*. The associated with the level of secondary metabolic pathogenesis that cause symptoms of wilt on tomatoes, but secondary metabolic excreted by *F. oxysporum* was not necessarily wilt in a plant. The phenomenon caused *F. oxysporum* producing secondary metabolic in the different concentration, either its quantity or quality. The nature of physiology being tested, observed by growing 4 isolates using a medium PDA on the temperature of 10, 15, 20, 25, 30, and 35 ^{0}C. The content of secondary metabolic measured on the four isolates using the Notz *et al.,* (2002) and analyzed by using *High-Performance Liquid Chromatography* (HPLC). The results of the analysis of the nature of the physiological (colour colonies) were that every isolate planted for 8 days shows the variation of white, white redness, white yellowness until purpleness. Based on *Methuen Handbook of Colour* [5], generally isolates tested shows a bright colour, signaled with A letter at every code. Analysis HPLC on the results of secondary metabolic, either quantity or quality, sequentially were: isolates BAR (3; 1,997 ppm); ENR (3; 5,105 ppm); SID (4; 2,135 ppm) and MAL (5; 2,065 rpm). If it was seen by the relationship between the colour of colonies with the production of the secondary metabolic compound, it seemed that the older or darker colonies' colour the more secondary compounds formed, but dark or old colonies' colour does not determine a high quality of acid fusaric produced.

Introduction

Fusarium oxysporum (*F. oxysporum*) is one of the edibilities of the cause disease almost in all plants, especially at the tomatoes. The disease caused by *Fusarium oxysporum* generally shows symptoms withering. Especially at the tomatoes, this disease causes harming, even puso has been reported that it can happen to seedling in the Enrekang district [2].

During the growth, *F. oxysporum* produced primary and secondary metabolic, one of secondary metabolics produced by *F. Oxysporum* was essential fusaric; compounds that cause wilting in the tomatoes (5-*butylpicolinic acid*).

The compounds associated with a pathogenesis that cause symptoms of wilt on the tomatoes [3], but fusaric acid excreted by *F. oxysporum* did not necessarily cause wilt in the plants. The phenomenon was caused by *F. oxysporum* that produced fusaric acid in the different concentration, in line with what Sheng Huang [8] stated that virulent pathogen correlated positively with the ability to produce the toxin, either quality or quantity, for example, the strain that virulent produces higher fitotoxin than strain avirulent. It was also suspected that the ability of *F. Oxysporum* to infect in plants not only one toxin, but there were some of the other toxins having a role in helping the process of infection.

This research attends to determine the correlation between the physiological properties and the ability to produce secondary metabolic of the *F. oxysporum* in quantity and quality. It is done as an effort of controlling the disease caused by *F. oxysporum*.

Research Methods

This research carried out in the Agrotechnology Laboratory of Agriculture Faculty of Universitas Muhammadiyah Parepare and Mycology Laboratory of Agriculture Faculty of UGM, from November 2017 - May 2018.

Stages of The Research

1) Test Colonies in The Different Temperature

All of the fungus grown on a medium PDA and incubated with the different treatment of temperature such as 15, 20, 25, 30, 35^{o}C. Each of the treatment was repeated for 5 times. The observation of the colonies' colour was conducted every day by seeing the development pattern of the colour of miselium air, it was observed on the bottom side of the petri cup. The colour of colonies was determined by using colour comparison in *Methuen Handbook of Colour* [5].

2) Production, Extraction, and Purification of Secondary Metabolic

Isolate obtained was used as a sample for testing the production of the secondary metabolic compound. The monospora was taken by using cook borer with the size 5 mm as much as 2 discs, then it was put in the erlenmeyer flask that had been filled with 120 ml of czapek synthetic medium. The erlenmeyer flask was incubated at temperatures 25^{O}C for a week in the dark condition while it was shaken using shaker at the speed of 180 rpm. When the incubation time was finish, the results of the incubation of all the centrifugated isolates with the speed of 5.000 rpm for 15 minutes used the centrifuge tool (*precision vary-hi-speed centric one* 67390), therefore it was obtained 2 layers. The supernatant layer was taken and then it was filtered by using a microfilter with the size of 0,8 μm (*sartorius type* SM 11304), and its filtrate accommodated in the container then it was stored on the temperature 2^{O}C (*freezer*) [6], if it was not analyzed further.

A total of 40 ml filtrates were taken and acidified until pH 2 with adding ± 320 μl HCl 1,96 M, then it was mixed with 40 ml etilasetat 100% in funnel dividers, and then it was shaken for 1 minute. From the mixture, it was obtained 2 phases which were organic and liquid. The organic phase was drained by using rotavor .

3) Tes Quantity and Quality of Metabolic Secondary

The residue obtained from the rotavor tube was dissolved into 1 ml methanol, then it was analyzed with *high-performance liquid chromatography* (HPLC) (Jasco International Co. Ltd., Tokyo) which was equipped with reverse phase columns packed with 120-5-C18 nukleosil and set at temperature 50^{O}C. Samples (20 μl) was using metanol as the eluent, with the gradient from 25-100% in 0,43% acid *o*-fosforit for more than 12 minutes [6].

Secondary metabolic was detected by observing high peak and wide area produced by chromatogram at the A_{270} wavelength. The retention time was 2 minutes with the pace of phase flow rate of the car was 0,7 ml/minute. The number of peak appearing indicated a lot of secondary metabolic produced by *F. oxysporum.*

Result and Discussion

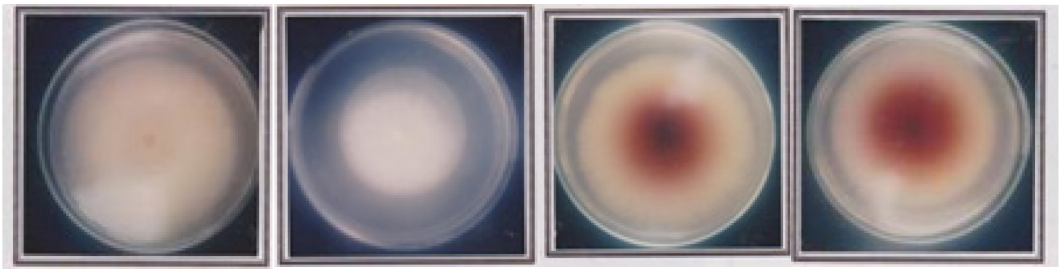
a. Testing Colonies in The Different Temperature

The isolation results of roots and stems from some areas of tomatoes, Bar and Enr (Enrekang Regency); Sid (Sidrap Regency); and Mal (Gowa Regency), showed variations in the form of colour after it was observed in some different temperature (Table 1).

Table 1. Colonies colour of *F. oxysporum* in PDA medium that comes from the different location in variated temperatures. The eighth day after the inkulation based on *Methuen Handbook of Colour.*

Isolate	10^{O}C	15^{O}C	20^{O}C	25 ^{O}C	30 ^{O}C	35 ^{O}C
BAR	6A2 (white orange)	7A3 (pale red)	9A2 (reddish white)	5A2 (whitish orange)	12A2 (reddish white)	2D3 (olive green)
ENR	6A2(white orange)	6A2(white orange)	4A3 (pale yellow)	4A2 (yellowis	A2h white) 5A2 (orange white)	6B4 (gray orange)
SID	3A2(yellowish white)	8B3 (red dull)	9A5 (pastel red)	9F7 (reddish brown)	9A2 (reddish white)	5A2 (whitish orange)
MAL	4A2(white orange)	9B5(gray red)	13A2 (reddish white)	9E8 (reddish chocolate)	17A2 (purplish white)	5A3 (pale orange)

The Colonies' colour in every isolate planted for 8 days showed a variation from white, reddish white, yellowish white, until purplish (Figure 1). Based on *the Methuen Handbook of Colour* [5], isolates tested generally shows a bright colour, which it was symboled with the letter A at every code.



(A) (B) (C) (D)

Figure 1. Colonies' colour *F. oxysporum* in medium PDA that comes from the location of BAR (A); ENR (B); SID (C); and MAL (D).

Variation of *F. oxysporum* colonies' colour found was the same as the research conducted by Ambar *et al*. [1] from some isolates of the *F. oxysporum* in Indonesia and Rozlianah & Sariah [7] from some isolates in Malaysia. The temperature difference in laboratory testing will affect the colonies' colour [1], similarly in the difference and the availability of nutrients in a medium also affect the colour of colonies [7], therefore there was an alleged difference from the correlated with the temperature and nutrients, consequently a variation of colours happened.

The colour difference of colony can happen because the ability of the fungus produced a variety of secondary metabolic. If it was connected with fusaric produced by each isolate (Figure 1), the difference of colonies colour can be seen (Figure 2) shows the content difference of fusaric acid, but the results of the research showed that the more dark colonies' colour did not mean the more high content of the fusaric acid.

b. Test Quantity and Quality of Metabolic Secondary

Based on the observation, the relationship between the concentration and the area can be seen, therefore the equation line of regressing linear formed. The equation of linear line was used as a formula to determine the concentration of fusaric acid produced by the *F. oxysporum*.

The standard analysis result of synthesis fusaric acid by using HPLC can be seen in Figure 2.

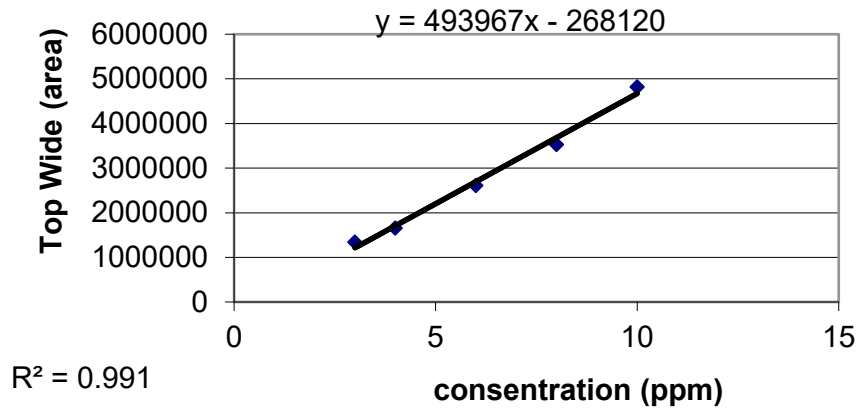


Figure 2. The regression line of the relationship between the standard fusaric acid's concentration (ppm) with the peak area (area)

The analysis result of the secondary compound of 4 isolates, either in quality or quantity. Production of the secondary metabolic and fusaric acid of 4 isolates was served in Figure 3.

Figure 3 shows that the fungus' isolate of *F. oxysporum* from different locations tofography shows the results of secondary metabolic, either quantity (the number of peaks detected, after analyzing it with HPLC) or the quality (concentration of fusaric acid detected after analyzing it with HPLC), regularly were: isolates BAR (3; 1,997 ppm); ENR (3; 5,105 ppm); SID (4; 2,135 ppm) and MAL (5; 2,065 ppm).

Analysis result shows that the secondary metabolic produced in quantity was in range 3 to 5 secondary metabolic, even though from the result of this research indicated that many secondary metabolic that was produced. It was shown by the MAL isolate, then SID.

The total number of the secondary metabolic produced by both of isolates was alleged because the colour produced by the isolates during the incubation become darker than the others, therefore there was a hypothesis that the more dark colour produced by the isolate, the more secondary metabolic produced by fungal *F.oxysporum.*

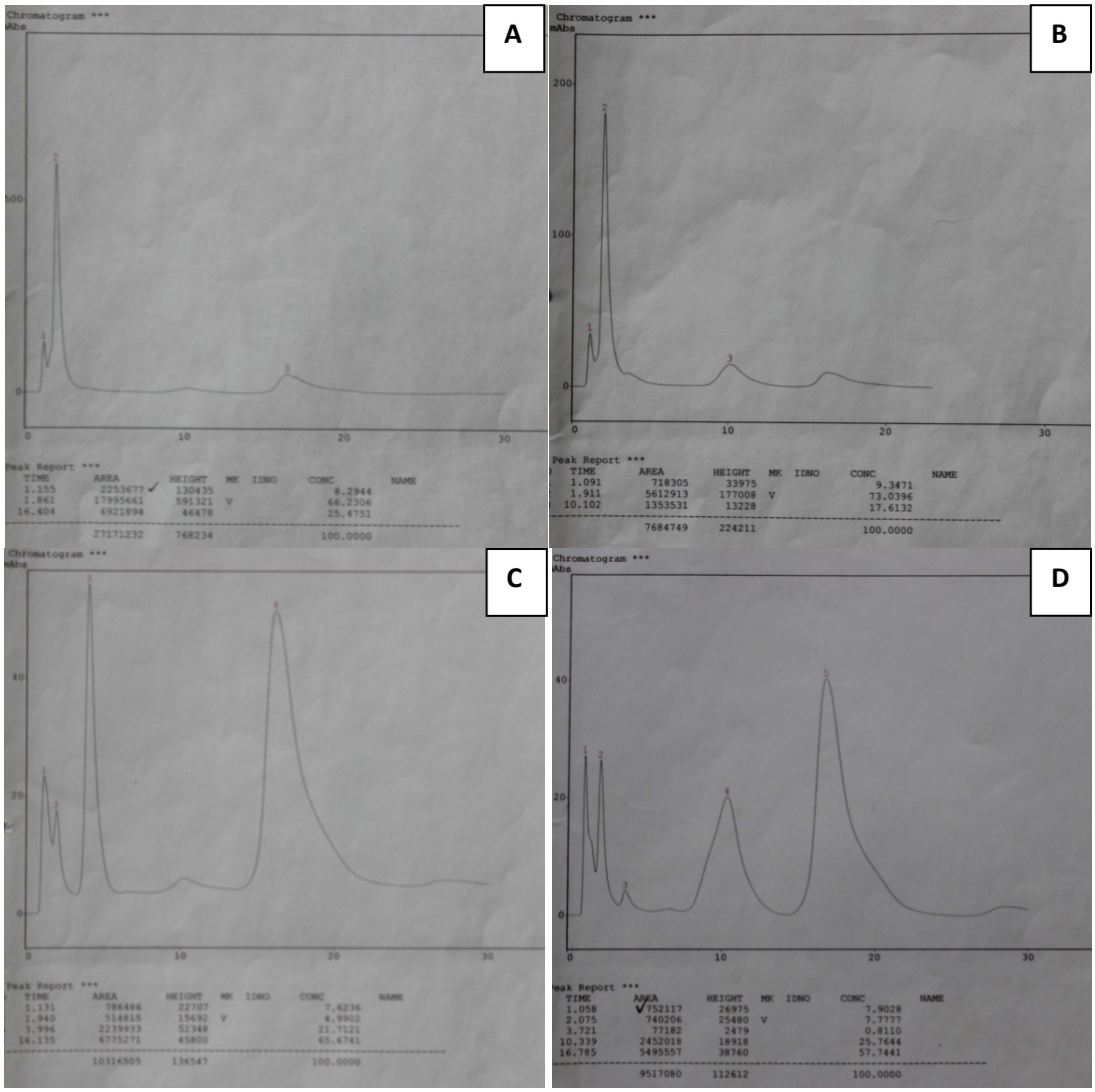


Figure 3. Histogram analysis result of HPLC showing quantity and quality of the secondary metabolic produced by BAR (A); ENR (B); SID (C); MAL (D) isolates

There was a different concentration of fusaric acid produced by 4 isolates because there was the diversity of the isolates tested. Each isolate has the different ability to grow, consequently, they produced a different secondary metabolic as well, either in quality and quantity, including the production of the fusaric acid compound [3]. The research of Bacon et al. [3] shows that the isolate of *Fusarium* sp. from the same plant but different location showed the different production ability. The 4 isolates tested come from the different location, namely BAR and ENR comes from Enrekang, SID comes from Sidrap and Mal comes from Gowa.

The establishment of the colour getting older along with the age of colony, meaning that the older age of colonies, the older or darker colour colonies looked. The colour change of colonies was probably caused by the number of the fungus conidium mass that grew on a czapek medium, consequently, nutrition available began to diminish. Reduced nutrients in a medium cause its growth began to hamper.

This was in line with Bu'Lock's [4] research stating that the gibrelin synthesis by *Giberella fujikuroi* decreased when the nutrients started to decrease/limit and the rate of growth starts to hamper. The rate of growth was hampered after nutrients up and being guardian factors, therefore, secondary metabolism occurred in these conditions.

Summary

The result of the research can be concluded as follows:

1. The nature of the physiological in the form colonies' colours of every *F. Oxysporum's* isolate formed was variated. Ranging from white, orange, and the reddish-brown.
2. The results of secondary metabolic formed in quantity were on a range 3 to 5, with the most results produced by MAL's isolate, while in the quality, such as the formation of fusaric acid was at the range 1,997 ppm to 5,105 ppm, with the highest production was ENR's isolate.
3. The more dark/old colonies' colour formed, the more quantity of secondary metabolic produced, but it did not affect the quality of secondary metabolic (fusaric acid) produced by the *F. Oxysporum's isolate.*

References

[1] Ambar, A.A., 2002. *Karakterisasi Fusarium oxysporum Penyebab Penyakit Layu pada Tomat.* (Disertasi tidak dipublikasi).

[2] Ambar, A.A., dan N. Ilmi, 2012. Peningkatan Ketahanan Bibit Tomat dengan Asam Fusaric terhadap Patogen Jamur *Fusarium oxysporum.* (Laporan hasil penelitian Hiba Bersaing)

[3] Bacon, C.W., J.K. Porter, W.P. Norred, & J.F. Leslie, 1996.Production of Fusaric Acid by *Fusarium* Species.*Applied and Environmental Microbiology* 62: 4039 – 4043.

[4] Garraway, M.O. & R.C. Evans., 1984.*Fungal Nutrition Physiology.*John Wiley & Sons, Inc.New York, Chichester, Brisbane, Toronto, Singapore.

[5] Kornerup, A. & J.H. Wanscher, 1978.*Methuen Handbook of Colour.*3th Edition. Colour Plates Printed in Denmark, Richard Clay, Chichester.

[6] Notz, R., M. Maurhofer, H. Dubach, D. Haas, & G. Défagoi, 2002. Fusaric Acid-Producing Strain of *Fusarium oxysporum* Alter 2,4-Diacetylphloroglucinol Biosynthetic Gene Expression in *Pseudomonas fluorescens* CHA0 *In-Vitro* and the Rhizosphere of Wheat. *Applied and Environmental Microbiology* 68: 2229 – 2235.

[7] Rozlianah, F.S., & M. Sariah, 2006.Characterization of Malaysian Isolates of *Fusarium* from Tomato and Pathogenicity Testing.*Research Journal of Microbiology* 3: 266 – 272.

[8] Sheng Huang, J., 2001. *Plant Pathogenesis and Resistance: Biochemistry and Physiology of Plant-Microbe Interactions*. Kluwer Academic Publishers, Dordrecht, Boston, London.