Inhibitory mechanism in vitro: Potential of bacterial consortium against shallot wilt disease caused by Fusarium oxysporum

by Tutik Kuswinanti

FILE	142.PDF (1.01M)		
TIME SUBMITTED	14-JUN-2020 09:52AM (UTC+0700)	WORD COUNT	1983
SUBMISSION ID	1343308072	CHARACTER COUNT	10676

IOP Conference Series: Earth and Environmental Science

PAPER · OPEN ACCESS

Inhibitory mechanism *in vitro*: Potential of bacterial consortium against shallot wilt disease caused by *Fusarium oxysporum*

To cite this article: T Kuswinanti *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **343** 012257

Inhibitory mechanism *in vitro*: Potential of bacterial consortium against shallot wilt disease caused by *Fusarium oxysporum*

T Kuswinanti, M Junaid, Baharuddin and Melina

Universitas Hasanuddin, Makassar, Indonesia

Email: koeswinanti@yahoo.com

Abstract. This study aimed to examine inhibitory effect of bacterial consortium formulated as bio-activator against wilt disease of shallot caused by *Fusarium oxysporum*. The study was split into three activities; bio activator preparation, isolation of *Fusarium oxysporum* and inhibitory test on solid and liquid culture. *Pseudomonas flourescens*, *Actinomycetes* sp. and *Streptomyces* sp. were obtained from collection of Biotechnology Laboratory and prepared as bio-activator in liquid form while *F. oxysporum* was collected from infected tissue at a highland in Enrekang district. The findings suggest that 2.0% and 2.5% per mL concentration of bio-activator shown to have a much greater rate of limiting filamentous growth of *F. oxysporum* in both medium. No different in inhibitory distance among trials except control, and a declined trend of all trials and control from time to time occurred. Bacteria with 2.5% concentration was consistent to have a much more considerable pressure of conidium development of *F. oxysporum*.

1. Introduction

Shallot (*Allium ascolanikum*) is an important horticultural commodity in Indonesia. In developing shallot industry, a better seed is the most important part of producing high yield [13,15] and this part is the highest cost, about IDR26 million per ha expenditure in one harvesting season [2]. A trend of shallot demand increases annually but the supply remains inadequate [1,13,15]. One of constrains is that in South Sulawesi due to a limited areal production [3]. So far, highland of Enrekang, together with Gowa, Bantaeng and Jeneponto areas is the main engine of shallot supplies to local markets. the other major constrain is *Fusarium oxysporum*, a soil-borne pathogen [6,14], which is a very economical impact to shallot industry with a wider range of commodity hosts [3,6,7,10,11]. Fusarium causes corn ears, injured kernels which links to produce mycotoxins [10]. In the shallot orchard, a wilt disease is an example of the cause of a significant declined yield and crop death [11,12,13] which its consequence is the increase of input cost in controlling disease.

Fusarium species is a superior Ascomycete pathogen as it performs inactive defense structure i.e. chlamydospore (tight wall) and able to perform stages of dormancy, parasite and saprophyte life cycle if it encounters extreme environment [4] and therefore, inoculum source will always exist and will out-break its population once environmental support and vulnerable host are available. To date, the only disease prevention is rational way to cease disease spread in orchards such as improving soil moisture and water drainage system [12,13,15]. Another is by using living microbial agent from *Achromobacter xylosoxidans* [5] and from bacterial formulated as bio-activator [1,2]. Testing bacteria as bio-activator in various commodities such as rice, potatoes and vanilla were undertaken to show a promising result

[1,2,9] and this was also developed as liquid fertilizer [7,8]. However, no scientific evidence is on fusarium wilt disease control on shallot orchard and therefore potential bio-activator consisting of microbes in-vitro in controlling *F. oxysporum* is proposed.

2. Materials and method

The study was split into three activities; bio-activator preparation, isolation of *Fusarium oxysporum* and inhibitory test on solid and liquid culture. Bio-activator was prepared from indigenous bacteria *Pseudomonas flourescens*, *Actynomycetes* sp. and *Streptomyces* sp. (as collection of Agricultural Biotechnology lab.) while *F. oxysporum* was collected from highland of Enrekang. Infected shallot tissue was cut into small pieces with 2 cm square, sterilized using 70% alcohol, rinsed with distilled water, before grown on the sterile filter paper in the petri dish. The growing pathogen was subsequently identified under microscope. After the pure culture obtained, the bacteria as bio-activator commenced to grow and was transferred to Potato Dextrose Agar (PDA) media using a preparatory needle, and afterwards the pathogen was transferred into new PDA media using cork-borer. In addition, for inhibitory test on the subculture, the media was poured into 20 jars and each jar was filled with 20 ml. The all media was autoclaved for 15 minutes at 121^{o}C. Once the media gently warmed, bio-activator suspension in different concentration was loaded and while *F. oxysporum* was laid on the medium surface with 5 mm diameter. First observation commenced in 7th day post inoculation (dpi). For statistical analysis, every trial consisted of 4 different concentration with control and every trial was designed with 4 replications. The parameters were conidia density/ml and wet and dry weight of mycelium. The subculture was vortexed and spore was pipetted before a drop was loaded into hemocytometer square and measured as follows; $\mathbf{c} = [\frac{t}{n\,x\,0{,}25}]\,\boldsymbol{x}\,10^{6}$ which C= Conidia/ml concentration; t= number of conidia calculated in hemocytometer square column; n= Number of square column observed.

To measure fresh weight of the pathogen, mass mycelium was laid into filter paper and substrate flow through the paper was discharged while filaments deposited on the filter paper was measured its moist weight. For dry weight, the mycelium was put into the oven for 24 hours at 100^{o}C, and then the dry weight calculated.

For inhibitory test, bacteria colony was scratched in the media using a preparatory needle, in accordance to the concentration trial. Pure culture of *F. oxysporum* was transferred to a new PDA media using cork-borer and laid its position (figure 1), i.e. 2.5 cm from the edge of the petri dish, 2.5 cm from the point for bacteria colony and 4 cm away from the right edge of the petri dish. The fungal disk and bacteria were then incubated for one week at room temperature. For statistical analysis, environmentally randomized complete design was used, consisting of 4 concentration trials and control and each trial with 4 replications as follows; Control, 1%, 1.5%, 2% and 2.5% of mL concentration. The inhibitory bacteria against *F. oxysporum* formulation was commonly used as following $I = \left[\frac{A-B}{A}\right] x\ 100\%$ which I= Inhibitory bacteria (%), A = The range of the Fusarium colony to the petri dish edge, B= the range of Fusarium mycelium to bacteria.

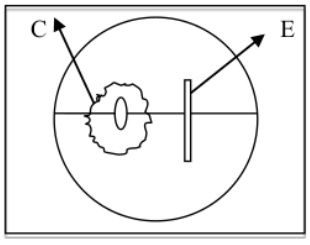

Figure 1. Dual culture scheme of bio-activator colony and *F. oxysporum* on PDA.
E = bacterial colony; C= *F. oxysporum*

3. Results and discussion

3.1. Dual culture test in vitro

Figure 2 performs that overall, inhibitory rate against F. oxysporum was much more effective concentration in 2.0 % and 2.5% trials from 60%, 40% and 30% respectively than others while 1.5% concentration shown less effective about 30% in all day observation. Although all trials shown to decrease a trend during observation, their inhibitory role was a much greater significant than control.

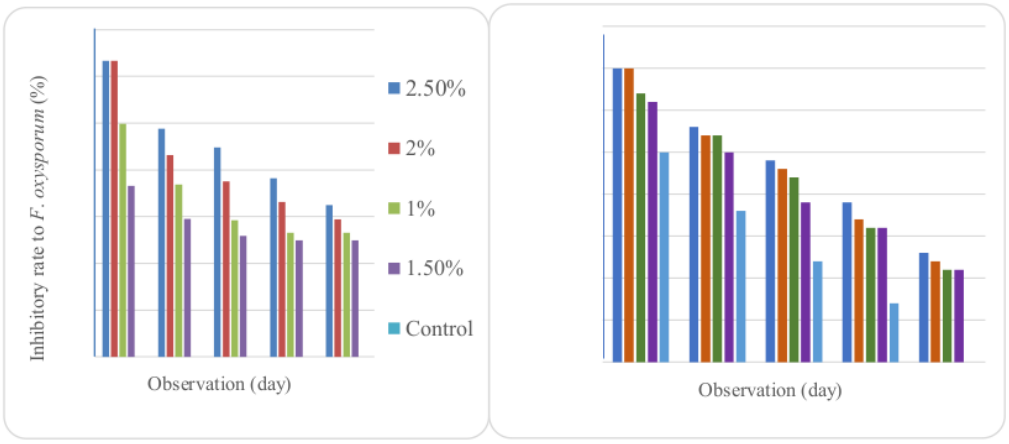

Figure 2. Percentage of inhibitory bacteria as bio-activator against growth of *F. oxysporum* on solid media.

Figure 3. Inhibitory distance of bacteria against mycelium growth of *F. oxysporum* in solid media.

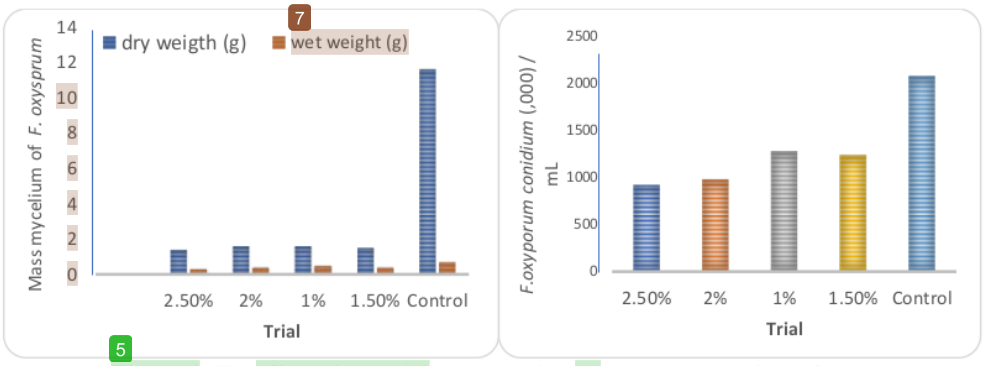

Figure 4. The effect of bacterial concentration on dry and wet weight of mass mycelium of *F. oxysporum*.

Figure 5. Effect of bacterial concentration on the formation of *F. oxysporum* conidium.

3.2. Inhibitory distance of bacteria against F. oxysporum mycelium growth in vitro

Figure 3 shows that overall trials of 2% and 2.5% concentration had a wider limiting distance from 3.5 cm (day 1), 2.8 cm (day 2), 2.4 cm (day 3), 1.9 cm (day 4) to 1.3 cm (day 5) respectively and almost all trials seemed to have no significant expression of inhibitory gap of mycelium growth of pathogen

on the solid media. To compare Control, the trials shown to have considerable distance of inhibitor. All trials and control have been shown a declined trend of inhibitory distance until end of observation.

3.3. Effect of concentration trials on developing mass mycelium and conidium of F. oxysporum on the liquid media.

Overall, dry and wet weight of filamentous pathogen is important parameter to understand the effective pressure of trials. The findings suggest that all trials shown to have much greater effect on limiting development of both filamentous pathogen and conidia than control (figure 4 and 5). In addition, bacteria with both 2.0% and 2.5%/mL concentration performed a greater limitation of developing conidia of wilt disease pathogen and overall, these concentration trials shown to perform much greater against F. oxysporum in all parameters.

4. Conclusion

Based on the result, it concludes that trials with 2.0% and 2.5%/ mL bacterial concentration had a much more consistent to perform inhibitory rate and distance and to limit development of filamentous and conidium *F. oxysporum in vitro* than other trials and control.

References

[1] Baharuddin, Nursaba and Kuswinanti 2005 Pengaruh Pemberian Pseudomonas dan EM4 dalam Menekan Penyakit Bakteri (*R. solancearum*). *Prosiding Sem. Ilmiah*. 195-200
[2] Baharuddin 2008 Introduksi Paket Bioteknologi Ramah Lingkungan Untuk Meningkatkan Usaha Perbenihan Bawang Merah Berbasis Kelompok Tani Di Kabupaten Enrekang. Lembaga Penelitian. Universitas Hasanuddin. Makassar
[3] BPS, 2016. https://sulsel.bps.go.id, accessed 30th July 2019
[4] Bullerman LB 2003 SPOILAGE: Fungi in Food – An Overview. In: Caballero B, editor. Encyclopedia of Food Sciences and Nutrition (Second Edition). *Oxford: Academic Press*. **55** 11-22
[5] Dhaouadi S, Wafa R, Mougou-Hamdane A and Nasraoui B 2018 Evaluation of biocontrol potential of Achromobacter xylosoxidans against Fusarium wilt of melon *Euro. J. of Plant Path*. 154
[6] Gonsalvels A K and S A Ferreira 2003 Fusarium oxysporum. Crop Knowledge Master. University of Hawai at Mamoa
[7] Knights E J 2004 CHICKPEA: Overview. In: Wrigley C, editor. *Encycloped. of Grain Sci*. 280-7
[8] Kuswinanti T 2001 Efektivitas 4 Jenis Limbah *Barassica* spp. Pada formulasi cair dan kompos terhadap pertumbuhan Fusarium oxysporum secara *in vitro* dan rumah kaca. *Laporan penelitian DPP/SPP*
[9] Kuswinanti T, Fitriani dan Baharuddin 2005 Isolasi dan Identifikasi Mikroba Terbawa Benih Kentang Di Penyimpanan. *Prosiding Seminar Ilmiah dan Pertemuan Tahunan XVI.PEI dan PFI Komisariat Sulsel* 173-177
[10] Munkvold G P, Arias S, Taschl I and Gruber-Dorninger C 2019 Chapter 9 - Mycotoxins in Corn: Occurrence, Impacts, and Management. In: Serna-Saldivar SO, editor. *Corn (Third Edition)*. Oxford: AACC International Press. 235-87
[11] Okungbowa F I, Shittu H 2014 Fusarium Wilts: An Overview. 83-104
[12] Rukmana R 2007 Budidaya dan Pengolahan Pasca Panen Bawang Merah dari Biji. Penerbit Aneka Ilmu. Semarang. 90
[13] Singgih W 1989 Budidaya Bawang Putih, Bawang Merah dan Bawang Bombay. Penebar Swadaya. Kakarta
[14] Subba Rao N S 1994 Mikroorganisme Tanah dan Pertumbuhan Tanaman. Universitas Indonesia Press, Jakarta.
[15] Sunarjono H 1989 Budidaya Bawang Merah. Sinar Baru, Bandung 24

Inhibitory mechanism in vitro: Potential of bacterial consortium against shallot wilt disease caused by Fusarium oxysporum

ORIGINALITY REPORT

%14	%12	%12	%11
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Universitas Hasanuddin Student Paper	%6
2	earchive.tpu.ru Internet Source	%3
3	www.semanticscholar.org Internet Source	%2
4	Submitted to Edgewood High School Student Paper	%1
5	R J Critchley, S Jezzard, K J Radford, S Goussard, N R Lemoine, C Grillot-Courvalin, G Vassaux. "Potential therapeutic applications of recombinant, invasive E. coli", Gene Therapy, 2004 Publication	%1
6	iopscience.iop.org Internet Source	%1
7	www.eisrjc.com Internet Source	%1

EXCLUDE QUOTES	ON	EXCLUDE MATCHES	< 5 WORDS
EXCLUDE BIBLIOGRAPHY	ON		