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Antagonistic of some Trichoderma against Fusarium Oxysporum sp. f. cubense Tropical Race 4 (FocTR4)

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Abstract: Fusarium wilt of banana is a very important fungus that caused the destruction of banana trees in the tropical countries. Biological control is an alternative method to control fusarium wilt diseases such as *Trichoderma* has been known to be particularly active in the control of the plant pathogens. This study aimed to evaluate the ability *Trichoderma* isolates from suppressive soils in Malaysia to suppress Fusarium wilt of banana *in vitro*. Thirty one of *Trichoderma* isolates were tested their ability to inhibit the growth of *Foc*TR4 LJ27 strain. The isolates were screened *in vitro* by volatile compounds tested of *Trichoderma* isolates against LJ27 strain. Then eight *Trichoderma* spp. strains (TR10, T10v1, T1, Tveg2, TR102, TL5, Tveg1, T26) was produced the high toxic metabolites with strong activity against LJ27 strain, inhibiting the mycelia growth by 50.33%, 51.33%, 51.67%, 69%, 70.67%, 71.33%, 78%, 96% respectively. The result indicated to a high efficacy of *T. parareesei* T26 for inhibiting the growth of *Foc*TR4. But five isolates of *Trichoderma* such as *T. brevicompactum* (TL7), *T. reesei* (T658, TL102, and TL13552), and *T. harzianum* (TL21) were showing the very low effect on FocTR4. The volatile compounds can produce for the inhibiting of developing of *Foc*TR4 *in vitro*. This improves the high efficacy of *Trichoderma* to use as alternative methods in reducing the synthetic chemicals that are causing a toxic pollution for our environment.

Keyword: *Foc*TR4, *Trichoderma*, Biocontrol, Banana, Fusarium wilt

Introduction

Fusarium wilt of banana is a very important fungus that caused the destruction of banana trees in the wide world. This strain is forming high dangerous on the banana *Farming* in many tropical and subtropical countries such as Australia, Malaysia, Jordan, Oman, and Africa (Ploetz, 2006; Ploetz *et al.*, 2015). Fusarium wilt could not be controlled effectively, since its discovery. Many other groups of microorganisms have been proposed in the suppression of Fusarium wilts on other plants such as *Pseudomonas fluorescens* (Mohammed *et al.*, 2011; Al-Ani 2017), and *Trichoderma* spp. Many reports have indicated that *Trichoderma* spp. can suppress Fusarium wilt pathogens effectively (Calvet *et al.*, 1990) including Fusarium wilt of banana (Kidane and Laing, 2010). The biocontrol mechanisms of *Trichoderma* can be divided into mycoparasitism, competition, antibiosis, induced resistance, and action of cell wall degrading enzymes (Benítez *et al.*, 2004; Al-Ani 2018). Some of *Trichoderma* spp. have been described as having the ability to inhibit the growth of plant fungal pathogens by producing the volatile compounds (Raza *et al.*, 2013). *T. harzianum* T15 was able to inhibit growth the soil-borne plant pathogens including *Fusarium moniliforme*, *F. culmorum*, and *Gaeumannomyces graminis* var. *tritici in vitro* (Kucuk and Kivanc, 2004). Many strains of Trichoderma spp. produced the secondary metabolites having the toxic effect on the pathogen-host directly (Vinale *et al.*, 2014). *T. harzianum* was secreting volatile compounds showing high inhibition for the growth of *Fusarium oxysporum* f. sp. *melongenae* (Cherkupally *et al.*, 2017). Therefore, this study is very interesting to evaluate the efficiency of *Trichoderma* spp. in suppressing *Foc*TR4 by producing volatile metabolites.

Methods

Isolate *F. oxysporum* f. sp. *cubense* Tropical race 4

The isolate of *Foc*TR4 LJ27 strain was collected from Dr. Laith K.T. Al-Ani (School of Biological Science, Universiti Sains Malaysia, Malaysia), and re-cultured on Potato dextrose agar.

Isolates *Trichoderma* spp.

Thirty one *Trichoderma spp*. were collected from Dr. Laith K.T. Al-Ani (School of Biological Science, Universiti Sains Malaysia, and Malaysia) and re-cultured on Potato dextrose agar.

Volatile metabolites test

In vitro inhibitory effects of *Trichoderma* spp. isolates against pathogenic LJ27 by the production of volatile metabolites were evaluated using the inverted plate method (Dennis and Webster, 1971) with some modifications. The plugs (5 mm in diameter each) of 31 isolates of Trichoderma were testing against plugs (5 mm) of LJ27 individually. A plug LJ27 of Petri dish inverted on fungal-free agar media using as a control factor. Three replicates were prepared for each npF. Colony diameters of the pathogen were measured at 7 days post incubation. Growth inhibition percentage was calculated as follows:

$$PIRG\% = \left[\frac{(Fp - Tt+p)}{Fp}\right] \times 100$$

Where:
PIRG, percent of growth inhibition;
FP, growth rate of pathogenic LJ27 control;
Tt+P, a growth rate of pathogenic LJ27 in treatment were combined with each the biocontrol factor of 31 *Trichoderma* isolates (El-Katatny *et al.*, 2011).

Results and Discussion

For evaluation and efficacy of *Trichoderma* spp. could confront and produce the volatile compounds affecting on the growth of *Foc*TR4. *Foc*TR4 was isolated from banana rhizosphere samples which were collected from random banana fields in Terong-Perak-Malaysia. All *Trichoderma* spp. were isolated from the rhizosphere and root and soil samples of the healthy banana plant. In this study, a total 31 strains of *Trichoderma* spp. are including *T. harzianum* (TL21, TL22, TL4, TL5, TL6, Tveg1, Tveg2, TR1031, TR1032 and T3), *T. reesei* (TL1, T31, T658, TL1355, TL13552, TL1322, TL101, T1 and TL102), *T. parareesei* (T6581, TL13551, TL261, T26, TL262, T10v1 and T2), *T. brevicompactum* (TL7), *T. koningii* (TR102), *T. atroviride* (TR10), *T. erinaceum* (TL3), and *T. capillare* (TL2), that isolated from banana healthy were testing against *Foc*TR4 both of dual culture and volatile compound tests. For volatile metabolites test, thirty one isolates of *Trichoderma* spp. showed different antagonistic effects against LJ27. The antagonism of the growth inhibition of the pathogen colony varied among strains of *Trichoderma* spp. The eight strains of *Trichoderma* spp. such as (TR10, T10v1, T1, Tveg2, TR102, TL5, Tveg1, and T26) was produced the high toxic metabolites with strong activity against *Foc*TR4, inhibiting the mycelia growth by 50.33%, 51.33%, 51.67%, 69%, 70.67%, 71.33%, 78%, and 96% respectively (Fig. 1).

The result shows the high role of *Trichoderma* in control the fusarium wilt disease and ability to parasite on hyphae of *Foc*TR4 that lead to degrading the full colony for this pathogen. Several *Trichoderma* spp. are having the ability to produce the antifungal compound that effects indirectly on fungal growth (Al-Ani, 2018). The volatile compounds are produced for the inhibiting of developing of *Foc*TR4 *in vitro*. The cell wall of *Foc*TR4 that contain chitin may possibly be as inducer factor for *Trichoderma* to produce the analysis metabolites that high affect on the fungal cell wall. Kucuk and Kivanc (2004) indicated for the ability of *Trichoderma* to produce the important metabolites that inhibit the mycelium growth. *Trichoderma* is secreting the secondary metabolites that related to the host. *Foc*TR4 may produce some secondary compounds that induce *Trichoderma* to attack *Foc*TR4 indirectly by producing several antifungal compounds. Therefore, they found some isolates of

Trichoderma producing the secondary metabolites very effect on the *Foc*TR4 growth but other did not affect on *Foc*TR4. Vinale *et al.*, (2009) indicated for the presence a relation between productions the secondary metabolites by *Trichoderma* with the pathogen-host.

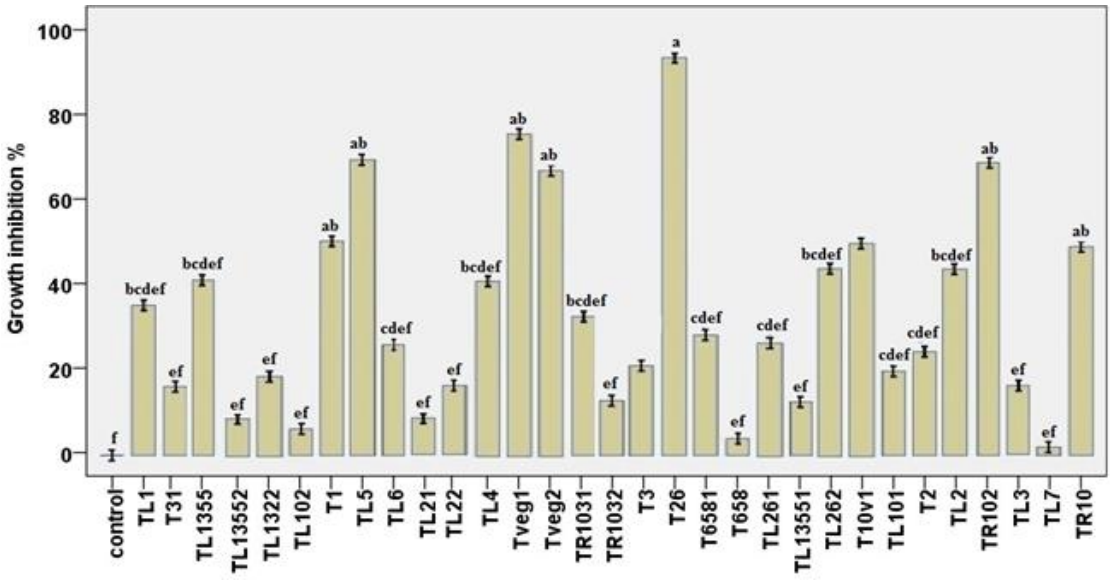

Figure 1**.** Effect of volatile metabolites produced by *Trichoderma* isolates towards LJ27 on PDA expressed as percentage of inhibition of LJ27 mycelia daily growth rate after 5 days of incubation. a) LSD = 0.055, b) LSD = 0.072, c) LSD = 0.063, d) LSD = 0.050, e) LSD = 0.051, f) LSD = 0.073, (Appendix E)

Conclusion

There are many methods useful for control the plant pathogens. Biological factor as *Trichoderma* is very important agent. It showed high efficacy in control *Foc*TR4. It could affect on the growth of pathogen *Foc*TR4 from distance. The T26 isolate of *T. parareesei* was showed high inhibition for the growth of *Foc*TR4 at 96%. But three species of *Trichoderma* spp. such as *T. harzianum* (Tveg1 and TL5) and *T. koningii* (TR102) showed a middle ability in growth inhibition of FocTR4 at 70.67%, 71.33%, 78%. While, five isolates of *Trichoderma* spp. such as *T. brevicompactum* (TL7), *T. reesei* (T658, TL102, and TL13552), and *T. harzianum* (TL21) didn't effect on mycelium growth of *Foc*TR4 that decrease the growth at range between 2% to 9%. It indicated for the high trait of some strains of *Trichoderma* in control of *Foc*TR4 by producing many secondary metabolites and inhibits the mycelium growth without contact between them. This result indicates that some those of secondary metabolites have the ability in degrading the hyphae of *Foc*TR4 as the antifungal.

Recommendations

This study is useful for controlling on *Foc*TR4 without using chemical pesticides. *Trichoderma* could produce the secondary metabolites impacting on the mycelium growth without contact between them. In additional, *Trichoderma* spp. were different in effect on the growth of *Foc*TR4. Therefore, it can detect the kinds of secondary metabolites that have antifungal activity against *Foc*TR4 in further future.

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References

Al-Ani, L.K.T. (2017). PGPR: A Good Step to Control Several of Plant Pathogens. In: Singh, H.B., Sarma, B.K. and Keswani, C. (Eds), Advances in PGPR Research. CABI, UK, pp.398-410.

Al-Ani, L.K.T. (2018). *Trichoderma*: beneficial role in sustainable agriculture by plant disease management. In: Egamberdieva D., Ahmad P. (eds) Plant Microbiome: Stress Response. Microorganisms for Sustainability, vol. 5. Springer, Singapore, pp. 105-126.

Benítez, T., Rincón, A.M., Limón, M.C. and Codón, A.C. (2004). Biocontrol mechanisms of Trichoderma strains. International Microbiology, 7:249-260.

Calvet, C., Pera, J. and Bera, J.M. (1990). Interaction of Trichoderma spp. with Glomus mossaeae and two wilt pathogenic fungi. Agricultural Ecosystem and Environment, 29: 59-65.

Cherkupally, R., Amballa, H. and Reddy, B.N. (2017). In vitro antagonistic activity of *Trichoderma* species against *Fusarium oxysporum* f. sp. *melongenae*. *International Journal of Applied Agricultural Research*, 12(1):87-95.

Dennis, C. and Webster, J. (1971). Antagonistic properties of species- group of *Trichoderma* II. Production of volatile antibiotics. Transactions of the British Mycological Society, 57: 41-48.

El-Katatny, M.H., El-Katatny, M.S., Fadi-Allah, E.M. and Emam, A.S. (2011). Antagonistic effect of two isolates of Trichoderma harzianum against postharvest pathogens of tomato (Lycopersicon esculentum). *Archives of Phytopathology and Plant Protection*, 44(7): 637-654.

El-Katatny, M.H., El-Katatny, M.S., Fadi-Allah, E.M. and Emam, A.S. (2011). Antagonistic effect of two isolates of Trichoderma harzianum against postharvest pathogens of tomato (Lycopersicon esculentum). Archives of Phytopathology and Plant Protection, 44(7): 637-654.

Kidane, E.G. and Laing, M.D. (2010). Integrated Control of Fusarium Wilt of Banana (Musa spp.). Acta Horticulturae, 879: 315-321.

Kucuk, C. and Kivanc, M. (2004). *In vitro* antifungal activity of strains of *Trichoderma harzianum*. *Turkish Journal of Biology*, 28:111-115

Mohammed, A.M., AL-Ani, L.K.T., Bekbayeva, L. and Salleh, B. (2011). Biological Control of *Fusarium oxysporum* f. sp. *cubense* by *Pseudomonas fluorescens* and BABA *in vitro*. *World Applied Sciences Journal*, 15(2):189–191.

Ploetz, R., Freeman, S., Konkol, J., Al-Abed, A., Naser, Z., Shalan, K., Barakat, R. and Israeli, Y. (2015). Tropical race 4 of Panama disease in the Middle East. Phytoparasitica, 43: 283–293.

Ploetz, R.C. (2006). Fusarium wilt of banana is caused by several pathogens referred to as Fusarium oxysporum f. sp. cubense. Phytopathology, 96: 653-656.

Raza, W., Faheem, M., Yousaf, S., Rajer, F.U. and Yameen, M. (2013). Volatile and non-volatile antifungal compounds produced by Trichoderma harzianum SQR-T037 suppressed the growth of Fusarium oxysporum f. sp. niveum. Science Letters, 1(1): 21-24.

Vinale, F., Ghisalberti, E.L., Sivasithamparam, K., Marra, R., Ritieni, A., Ferracane, R., Woo, S. and Lorito, M. (2009). Factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens. *Letters in Applied Microbiology*, 48:705–711.

Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Woo, S. L., Nigro, M., Marra, R., Lombardi, N., Pascale, A., Ruocco, M. and Lanzuise, S. (2014). Manganiello, G.; Lorito, M. Trichoderma secondary metabolites active on plants and fungal pathogens. *The Open Mycology Journal*, 8:127−139.

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