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Suppression of Fusarium wilt of banana with an application of *Trichoderma asperellum* inoculants

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Suppression of Fusarium wilt of banana with an application of *Trichoderma asperellum* inoculants

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Abstract. Fusarium wilt is one of the most serious diseases of banana plants in Malaysia that now becomes a worldwide problem. It is caused by soil-borne pathogen *Fusarium oxysporum* Schlecht f. sp. *cubense* (E.F. Smith) Snyder & H.N. Hansen. In this study, *in vitro* and *in vivo* experiments were conducted to evaluate the effects of *Trichoderma asperellum* B01 inoculants on disease incidence of Fusarium wilt of banana. Results indicated that percentage inhibition of radial growth (PIRG) was observed in dual culture plates of *Trichoderma* isolates B01 by 84.85%. In addition, under plant house condition, the application of conidial suspension of *T. asperellum* B01 has significantly decreased the incidence rate of Fusarium wilt by up to 94.4% compared to control. In general, the application of *T. asperellum* B01 inoculants revealed a great potential for the control of Fusarium wilt disease in banana plants under plant house condition, which a good approach to start in agricultural field. For future study, new method must be focusing on optimize the production of *Trichoderma* spores to be using for agricultural purposes.

1. Introduction

Disease management of Fusarium wilt of banana caused by *F. oxysporum* f. sp. *cubense* (*Foc*) in the industry using synthetic fungicides. It is linked to the increase of frequency of some types of cancer and poses serious risks of health within the human food chain [1]. In Malaysia, to minimize the disease from spreading and making damage to the plantation, some approaches have been practiced including shifting cultivation practiced by farmers, intercropping with oil palm or rubber to achieve 3-4 harvests and the use of chemical fungicides [2]. Many types of fungicide have been applied worldwide to treat the plant disease [3]; however, they can be unsafe to the ecosystem.

Alternative approach in managing Fusarium wilt disease can be done by using biocontrol, which has no negative effect on plant, animal, human and environment as it is eco-friendly and can be obtained from the surrounding; for instance, microorganisms. Based on a study by [4], *Trichoderma* spp. and *Pseudomonas* spp. can act as potential biological controls of Fusarium wilt disease of banana. *Trichoderma* spp. are interactive in a soil, root and foliar environments. In the host, they produce various metabolites that induce localized or systemic resistance responses. Many researchers have used *Trichoderma* species as biological control agent in substituting chemical pesticides. Based on [5], the application of *T. harzianum* T73s has successfully inhibited Fusarium ear rot of maize under plant house condition. Additionally, a soil borne-pathogen of basal stem rot in oil palm, *Ganoderma boninense* can also be suppressed by *T. harzianum* FA1132 [6]. In order to ensure an eco-friendly cure of the disease, this study has focused on screening the natural enemies agent specifically *Trichoderma* species against pathogen.



2. Materials and Methods

2.1. Fungal isolates

Trichoderma asperellum isolate B01 originated from soil was revived from fungal collection at Department of Biology, Faculty of Science, Universiti Putra Malaysia. The isolate was identified based on phenotypic and genotypic characteristics by Sharifah Siti Maryam [7]. For a causal agent culture, *Foc* isolate 9888 that is pathogenic towards banana plant was obtained from Fusarium Collection Center, Plant Pathology Laboratory, Universiti Sains Malaysia.

2.2. Dual culture test

Trichoderma asperellum isolate B01 was challenged for its antagonistic properties against the pathogenic isolate of *Foc* under *in vitro* condition. The isolate was cultured on Potato Dextrose Agar (PDA) and incubated at $28\pm 2^\circ\text{C}$ for seven days. A disk of young mycelial of pathogen sized 0.5 cm was placed by 1 cm from the end plate and a disk of *Trichoderma* isolate sized 0.5 cm was placed also by 1 cm from the other opposite end side in PDA culture plate. For the control plate, a disk of pathogen sized 0.5 cm was placed in a similar position which was 1 cm apart from the plate end but without *Trichoderma* isolate. The inoculated plates were incubated at $28\pm 2^\circ\text{C}$ for five days and completed in four replicates.

The competency of *Trichoderma* isolate B01 to inhibit pathogen's growth was assessed by measuring the radial growth of pathogen in the presence of *Trichoderma* and its growth on the control plate. The fungal growth was converted into percentage inhibition of radial growth (PIRG) [8].

2.3. In-vivo study on *Trichoderma* isolates against *F. oxysporum* f.sp. *cubense*

About 1.5 kg of the mixture topsoil, peat and sand in ratio 3:2:1 (v/v) was used as a medium. The medium was put into each polyethylene bag sized 10 x 10 cm covering half of the banana seedling (Cavendish variety) at age 4 weeks-old plant's depth. The experiment was conducted under plant house condition in which the space between each plant was 25 cm apart with 25 cm between rows. Nitrogen, phosphorus and potassium (NPK) fertilizer was given at week 0 - 6 after acclimatization following the manufacturer's recommendations. All other factors were controlled manually, and the plants were watered manually using tap water once a day [5].

The preparation of conidial suspension in concentration 1×10^7 spore/ml of *Trichoderma* isolate was completed following the method by Nur Ain Izzati and Abdullah [6]. Conidia of seven days-old *Trichoderma* isolate were harvested. The mycelia debris was removed by filtration using sterile double-layered muslin cloth. The conidial suspension was watered on the soil 500 ml/polybag for every two weeks after acclimatization. The inoculation of *Foc* isolate 9888 was introduced in week 3. The preparation of conidial suspension procedure was done similar to that mentioned above; nevertheless, the concentration was adjusted to 1×10^6 conidia/ml [7].

After seven days of inoculation, the plants' conditions were recorded and scored for the discoloration of the leaves and height of the plant. The scoring was conducted every week until week 20 using disease scale from 0 – 4 following the scoring scheme proposed by [9] with slight modifications for visible symptoms recorded.

2.4. Data analysis

Data of PIRG and disease severity index (DSI) of plants under plant house condition were analysed using IBM SPSS Statistics version 21.

3. Results and Discussion

Trichoderma asperellum isolate B01 inhibited the growth of the pathogen with the PIRG at 84.85%. Dual culture test is one of the conventionally quantitative methods in screening the efficacy of *Trichoderma* species to be used as biocontrol agent in managing plant diseases. This *in vitro* test can display complete antagonistic potential of agent [10] as it is mainly a predictive tool to determine growth

inhibition capability before carrying out time-consuming and more expensive study [11]. Prolonged incubation, the mycelia of all *Trichoderma* isolates were fully overgrown on the pathogen colony and sporulated on the pathogen colony for space to live.

For *in-vivo* study on *Trichoderma* isolates against *Foc*, after 20 weeks of pathogen inoculation, the symptoms of Fusarium wilt disease were observed and recorded. Figure 1 shows the symptoms of *Foc* experienced by basal parts of banana plants treated with *T. asperellum* isolate B01 on week 20 of post-inoculation. The suppression of *T. asperellum* against *Foc* isolate 9888 for this plant house condition was employed by direct mechanism as both *T. asperellum* and *Foc* as pathogens were directly applied on soil. Studies showed that many *Trichoderma* strains are very efficient in the biocontrol of several pathogens or in plant growth promotion via rhizosphere colonization [12] or in providing nutrients to the plant.



Figure 1. Basal parts (rhizome and stem) of control and treated plants on week 20 post-inoculation showed the internal symptoms of Fusarium wilt with the reddish-brown colour tissues indicating the lesion from *Foc* colonization in the plant. A: Negative control showed no symptom and healthy corm. B: Positive control of the corm became rotten (arrows) because of the disturbed vascular system by *Foc*. C: Plant showed disease suppression effect on corm (arrows), plant treated with *Trichoderma* isolate B01.

Fusarium oxysporum f.sp. *cubense* is known as one of invasive and lethal diseases of banana that become a major problem for the present banana crop plantation and industry [13]. Even though there are many holistic approaches that have been completed to overcome the threat of banana wilt disease [14], the circumstance did not show much firm promising results in treating diseases. Due to this condition, the local farmers decided to use extreme solution by using synthetic chemical pesticides or synthetic fungicides as a main tool to control the plant disease [11]. From this study, *T. asperellum* can be used as promising biological agent substituting chemical reagent in controlling plant disease. Using *Trichoderma* as biocontrol for plant disease is a good approach to start in agricultural field. However, to substitute *Trichoderma* as bio fungicide in controlling plant disease in plantation needs lots of budget

for formulating their spores. For future study, new method must be focusing on optimize the production of *Trichoderma* spores that can be used for agricultural purposes.

4. Conclusions

As a conclusion, *T. asperellum* isolate B01 in this study has showed its effectiveness and ability in inhibiting Fusarium wilt pathogen, *Foc* under *in vitro* and *in vivo* conditions.

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