TRICHODERMA ASPERELLUM AS A POTENTIAL BIOCONTROL AGENT AGAINST FUNGAL DISEASES OF CHILLI

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Abstract: Fungal diseases are one of the major risks in chilli production. Plant diseases are being controlled commonly by the application of synthetic fungicides. *Trichoderma* species found to be most effective antagonism of foliar and soil borne pathogens. In the present study, dual culture assay was conducted against phytopathogenic fungi of chilli in vitro and examined percent inhibition by the potential biocontrol agent. Dual culture plate test revealed that the percentage growth inhibition of *Colletotrichum gleosporoides*, *Fusarium oxysporum*, *Pythium apanidermatum*, *Curvularia lunata* and *Alternaria* sp by *Trichoderma asperellum* were in the range of 26.4 to 83.5 %.*T. asperellum*, a less studied fungus, is also a successful biological control agent against a wide range of plant pathogens. Thus, the aim of this research is to assess the antagonistic ability of *T. asperellum* towards chilli phytopathogenic fungi under in vitro conditions. The antagonistic efficacy of *T. asperellum* can be exploited in the disease management of chilli in field conditions.

Keywords: Biocontrol, *Trichoderma*, Chilli

Introduction

Chilli (*Capsicum annum*) is a major commercial crop grown as vegetable and spice having value addition in pharmaceuticals, cosmetics and beverages. In 2016, the world produced 34.5 million tonnes of fresh chilli and 3.92 million tonnes of dried chilli. (Raihana et al. 2018). Based on the 2020 crop statistics report supported by the Department of Agriculture Malaysia, chilli cultivation in Malaysia covered an area of 3,782 ha with a production of 32, 146 mt (DOA, 2020). Pests and diseases are major threats to chilli production causing significant losses. Many diseases can affect the chilli crop during its various stages of development including anthracnose, damping off, fusarium wilt, and alternaria and curvularia leaf spot. Chili can be severely infected with anthracnose disease (*Colletotrichum gloeosporioides)* with black swollen spots on fruit and necrotic spots on the leaves symptoms that may cause yield losses of up to 50% (Pakdeevaraporn et al., 2005). At the seedling stage, damping off caused by Pythium can be a disastrous disease causing up to 90% mortality at early growth of chili under nursery condition. It typically manifests as a pre-emergence loss where seedlings fail to emerge and a post-emergence loss where seedlings decay and collapse at their root. Fusarium wilt (*fusarium oxysporum*) interferes the water conducting vessel of the plant. The infection spreads up into stems and prevents water flow causing the plant to wilt and turned yellow. Leaf spot disease (*Alternaria* sp. and *Cuvurlaria lunata*) occurring on the foliage at any stage of the growth causing black lesion on the leaves and reducing the photosynthetic activity of infected plants which lead to yield loss.

Plant diseases are controlled through the use of synthetic fungicides. But the extensive use of fungicides resulted in the accumulation of residual toxicity, environmental pollution and killing the non-targeted microorganisms (Muthukumar et al. 2011). It is therefore essential to develop an effective, cost effective and environmentally safe method for the management of plant diseases. Biological control has been developed as an alternative to chemical fungicides and remarkable success has been achieved by employing antagonistic microorganisms for controlling soil-borne pathogens. Genus *Trichoderma* has been acknowledged since the last few decades due to its ability to control several deadly plant pathogens (de Medeiros et al. 2017). *Trichoderma* spp. are found ubiquitous in almost all soil types including cultivated soil, garden soil, fallow and pasture land and forest soil (Harman et al. 2006). They generally grow in their natural habitat on plant root surfaces and therefore, in particular, control root diseases Several modes of action have been investigated to explain the biocontrol of plant pathogens by *Trichoderma*; these include production of antibiotics and cell wall degrading enzymes, competition for nutrients, parasitism and

stimulation of plant defense mechanisms (Taghdi et al. 2015). *Trichoderma* spp. produces extracellular enzymes and have been implicated in the biological control of plant diseases (Nosir et al. 2016). As plant growth promoter and antagonist against plant pathogens, *Trichoderma* strains are considered as safe alternatives to hazardous fumigants and fungicides. *Trichoderma asperellum* is free-living, pervasive distributed fungus which is very common in the habitat of soil and root ecosystem and able to parasitize several soilborne phytopathogens (Verma et al. 2017).

Materials and methods

Isolation and identification of biocontrol agent and plant pathogen

Soil samples were collected from the healthy chilli plant rhizosphere cultivated in MARDI Organic Farm, Serdang for isolation potential biocontrol agent. Stock solutions of the soil sample were prepared by mixing 10 gram of soil sample into 90 ml of distilled water and were diluted into 101, 102, 103, 104, and 105 times. One ml of each dilution was cultured on Rose Bengal Medium (RBA) on a petri dish at 28± 1oC (Zhou et al. 2020). After 72 hours, *Trichoderma* colonies were picked and transferred onto new potato dextrose agar (PDA) medium. Chili plants showing characteristic symptoms as fusarium wilt, pythium rot and spotted leaves diseases were identified and brought into the laboratory for further steps. The plant tissues were washed under running tap water to remove surface soil, dust and other contaminants. Plant tissues infected with disease were cut from the edge of lesion and were surfaced sterilized in 10% sodium hypochlorite for 5 minutes. It was rinsed in sterile distilled water and air dried on sterile filter paper. The dried pieces were cut into smaller pieces, plated onto potato dextrose agar (PDA) and incubated at 25o C. Trichoderma isolates and pathogen isolated was subjected to molecular analysis for species confirmation.

Dual culture assay of *T. asprellum* against the pyhtopathogen

An isolate of *T. asperellum* were choose and evaluated for its capability against chilli phytopathogenic fungi by dual culture method. In the dual culture screening, an agar disc (0.5 cm) of the antagonist, *T. asperellum*, was placed 2 cm from the periphery of the petri dish, and a pathogen agar disc of the same size was placed 2 cm from the edge of the petri dish opposite *T. asperellum*. As control, agar disc of the pathogen was placed in a similar manner on a fresh PDA plate. As a control, a pathogen agar disc was placed in a similar manner on a fresh PDA plate. All pairings were carried out in 5 replicates and incubated at 28℃. After three days of incubation, antagonistic activity was measured by measuring the radius of the pathogen colony in the direction of the antagonist colony (R2) and the radius of the pathogen colony in the control plate (R1). The values were transformed into percentage inhibition of radial growth (PIRG) using the formula developed by Skidmore and Dickinson (1976).

$$\text{Where PIRG} = \frac{R1-R2}{R1} \times 100$$

Where PIRG = percent inhibition of mycelial growth (%), R1 = radial growth of pathogen in control plates (cm), R2 = radial growth of pathogen in dual culture (cm).

Results and discussion

The suppression of the pathogen by *T. asperellum* (Figure. 1) was observed and measured up to 10 days by using dual culture assay method (Figure. 2). In the present study, an isolate of *T. asperellum* exhibited antagonistic effect toward of *C. gloeosporioides*, *F. oxsporum*, *P. aphanidermatum*, *C. lunata* and *Alternaria* sp. by inhibiting mycelial growth of the pathogen. The mean percentage of mycelia growth inhibition by *T. asperellum* was 83.5% for *C. gloeosporioides*, 63.2 % in *F. oxsporum*, 26.4% in *P. aphanidermatum*, attempted 79.9 % in *C. lunata* and 75.5% for *Alternaria* sp. (Figure. 3). The potential of *T. asperellum* in controlling plant diseases are reported in numerous studies. According to findings by Ibarra-Medina et al. (2010) antagonistic isolates can be considered as potential biocontrol agent that 70% colonized the growth of pathogen. In a study by Reynaldo et al. (2018), less inhibition was achieved by *T. asperellum* T2-10 with only 22.5% inhibition against *C. gloeosporioides*. Petra at al. (2019), reported *T. asprellum* inhibited 88.3% of mycelia growth of *F. oxysporum* isolated from chilli. Narayana Bhat et al. (2016) reported other species of *Trichoderma*, *T. harzianum* and *T. viride* inhibition toward *F. oxysporum* was highest at 28.6% while *T. hamatum* did not overcome inhibition posed by *Fusarium*. Damping-off caused by *Pythium* species contributed more than 60 per cent mortality of seedlings both in nursery and main field as reported by Muthukumar et al. (2011). Eight isolates of *Trichoderma*

species tested for the mycelial growth of *P. aphanidermatum* where isolate TVC3 from chilli rhizosphere exhibited maximum growth inhibition of *P. aphanidermatum* (88.0%) compared with the control, followed by THC1 (83.9%) and TVC5 (80.0).

A study by Ibrahim and Riad in 2019 found that an isolate *Trichoderma asperellum* can inhibit the growth of P. aphanidermatum MF356677 which is isolated from tomatoes with a refraction rate of 80.2 %. However, the inhibition by *T. asperellum a*gainst *P. aphanidermatum* isolated from chilli in the MARDI study was lower at only 26.4%. While in a study by Mbarga et al. (2012) revealed that *T. asperellum* suppresses the growth of another species of *Pythium; P. myriotylum* by more than 60% in Taro. For the control of *C. lunata*, *T. asperellum* in the present study managed to inhibit the growth of this pathogen at 79.9%. These results were found to be equivalent to a study by Iftikhar et al. (2017) which found that another species of Trichoderma; *T. atroviride* was also able to inhibit the growth of *C. lunata* at a rate of 80.0%. For *Alternaria* blight, *T. asperellum* TH is able to inhibit the growth *of Alternaria* sp. by up to 56% in dual culture tests as reported by Muhamad Usman et al. (2019). However, these results were found to be lower than *T. asprellum* in the present study which managed to inhibit the growth *of Alternaria* sp. by up to 75.5%. When it comes to the treatment of disease, a biocontrol agent's rapid growth rate and aggressive processes are seen to be crucial characteristics. *Trichoderma* is a rapidly growing fungus that spreads quickly on culture media. The release of hydrolytic enzyme such as 1-3-glucanase, chitinase, protease, and cellulase contributed to the growth inhibition of the pathogen by *Trichoderma* (Ezziyyani et al., 2004), which are a key step for initiating the pathogen cell wall degradation during mycoparasitism (Rey et al., 2000).

Figure 1: 7 days of Trichoderma asperellum on a PDA cultured medium

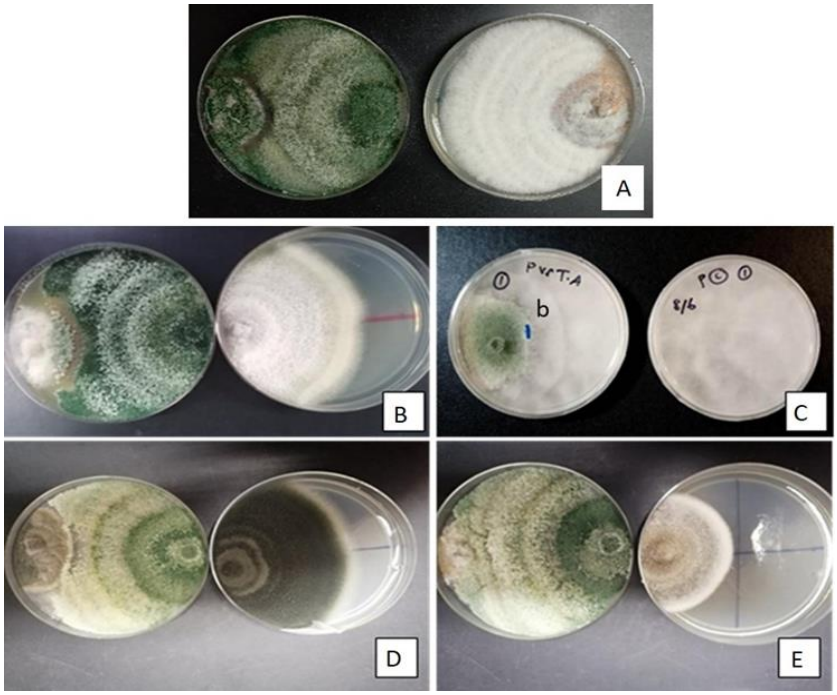

Figure 2: Dual culture assay of *T. asperellum* against A) *C. gleosporoides*; B) *F. oxysporum*; C) *P. aphanidermatum*; D) *C. lunata* and E) *Alternaria* sp.

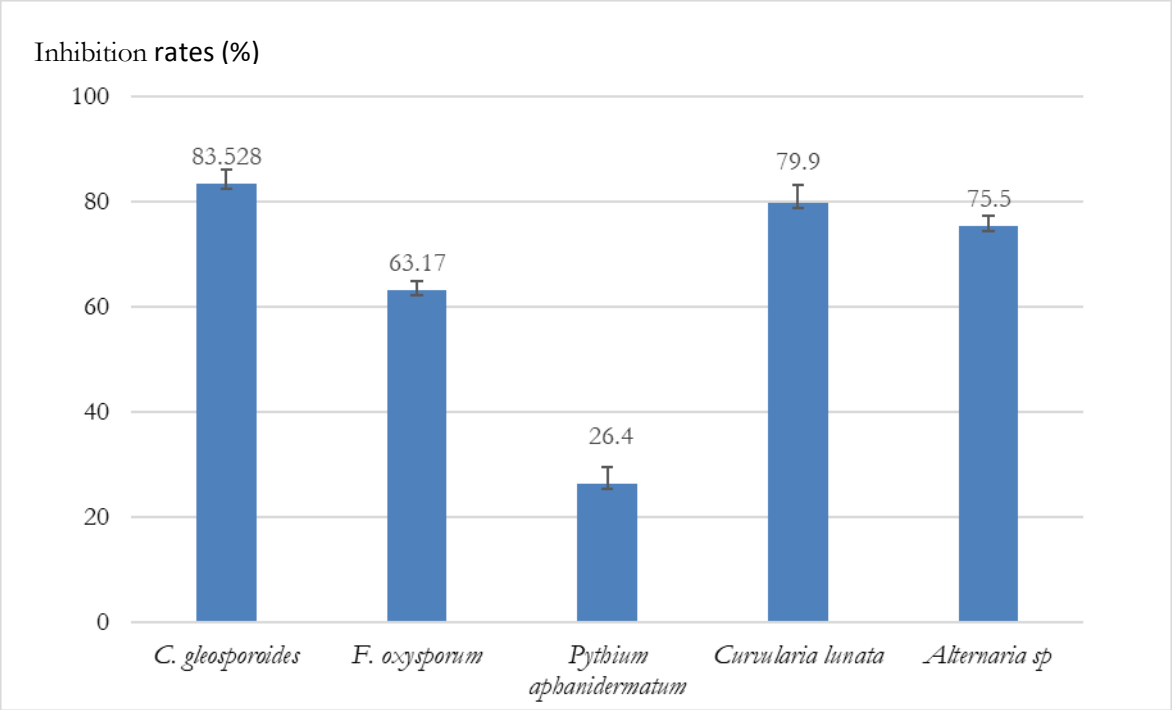

Figure 3: The effects of *T. asperellum* on the mycelia growth of pathogen in dual culture assay against *C. gleosporoides*, *F. oxysporum*, *P. aphanidermatum*, *C. lunata* and *Alternaria* sp.

Conclusion

Antagonistic efficacy of *T. asperellum* species against *C. gleosporoides*, *F. oxsporum*, *C. lunata*, *Pythium aphanidermatum* and *Alternaria* sp. revealed that the isolates of *T. asperellum* have highly effective against all the tested pathogens. *T. asperellum* have demonstrated the existence of biological alternative for the control of phytopathogens of chili in laboratory conditions. Therefore, it is suggested to carry out evaluations on the *T. asperellum* based product on the field with greater diversity of microorganisms and environmental factors. As per results, it can be concluded that antagonistic characteristic of *T. asperellum* can be exploited as an eco- friendly effective tool in controlling plant pathogens.

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