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Short Communication

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Wilt disease symptoms in red leaf lettuce (*Lactuca sativa* L.) after inoculated with *Trichoderma longibrachiatum*

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ABSTRACT

Trichoderma is a fungal genus that is widely reported to cause beneficial impacts to crop plants but rarely detriment. The present study was conducted to assess the effect of inoculation of *Trichoderma longibrachiatum* strain UPMT14 on red leaf lettuce (*Lactuca sativa* L.) under growth room conditions. A culture of UPMT14 was grown on potato dextrose broth to produce inoculum. This liquid inoculum was injected through the stem of red leaf lettuce seedlings 22 days old with five replications, and then plant growth progress was monitored for vegetative responses. Red leaf lettuce plants began to exhibit foliar symptoms on day 36, such as chlorosis, wilt, and drying out, before total collapsed on day 45 in comparison to untreated control red leaf lettuce plants. In conclusion, the wilting incidence in red leaf lettuce was observed after *T. longibrachiatum* inoculation. Further studies are needed in future to understand the pathogenesis of *T. longibrachiatum*.

KEYWORDS: *Lactuca sativa*, Lettuce, Plant wilt disease, *Trichoderma longibrachiatum*

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most popular vegetables in the world. In Malaysia, the annual production of leaf lettuce from 2014 to 2018 on average is about 56,000 metric tonnes and the planted area is about 2,700 hectares (DOA, 2018). Lettuce contains high fibre, iron, folate, and vitamin C and few in calories, fat, and salt (Rodrigo-García *et al*., 2019). Lettuce is also rich in manganese, iron, calcium, potassium, zinc, magnesium and phosphorus, which greatly varies based on their nutrient composition and lettuce varieties and all these components are good for human health (Kim *et al*., 2016). *In vitro* and *in vivo* studies have revealed that the bioactive chemicals in lettuce have anti-inflammatory, cholesterol-lowering, and anti-diabetic properties (Kim *et al*., 2016; Shi *et al*., 2022). One of the problems that can mess up the production of lettuce is a disease attack. Without crop rotation practices in the field, lettuce can be affected with severe vascular diseases caused by phytopathogens and leading to economic losses (Zavatta *et al*., 2021). The inoculation of fungal spores into plants can have positive or negative effects because not all fungal spores and infections cause symptoms of diseases (Deng & Cao, 2017; Modrzewska *et al*., 2022). Some fungi that could defend the host from rhizosphere pathogens such as *Trichoderma* spp. are mostly beneficial and are known to protect agricultural crops from phytopathogens attack (Zhang *et al*., 2021). The responses due to fungal inoculation to plants are sometimes not well understood. Plant parts such as leaves, buds, stems and roots are considerably easily inoculated with fungi (Kusari *et al*., 2014). It is worth exploring more about the responses from fungal strains infecting various crop plant species. This effort would be helpful to understand and provide more information about potential responses due to fungal inoculation against agricultural crops especially when the fungal inoculation caused disease. The fungal inoculation through injection method for plant tests on soil is commonly used (Posada *et al*., 2007). The best way to inoculate fungus on plants was the assessment of whole plant conditions (Clement *et al*., 2015). Our knowledge of the phytopathogenic of this species is still limited. The present study aimed to investigate the responses of red leaf lettuce after

Trichoderma longibrachiatum strain UPMT14 inoculation by injection method, red leaf lettuce plants were grown in sterile soil under a controlled growth room environment.

MATERIALS AND METHODS

Trichoderma longibrachiatum strain UPMT14 was previously isolated from diseased stems of black pepper vines collected from a pepper farm (unpublished data, Dr Franklin Ragai Kundat, personal communication) and identified based on internal transcribed spacer gene with the GenBank accession number MT634694. The culture of *T. longibrachiatum* strain UPMT14 was maintained on potato dextrose agar medium and stored at the Laboratory of Microbiology, Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus. Red leaf lettuce seeds were purchased from Green World Genetics Sdn. Bhd. The seeds were sown on water-saturated, two-layer laboratory tissue paper for seven days. Germinated red leaf lettuce seeds were then transplanted into a sterile soil mix (1:1:1, soil: sand: peat moss) and let it grow for 22 days under controlled growth room conditions. On day 22, UPMT14 inoculum was injected into the lower stem of red leaf lettuce plants while sterile normal was injected onto the stem of other individual red lettuce plants and served as untreated control plants. UPMT14 inoculum was prepared under room temperature after orbital shaking culture grown in potato dextrose broth for 48 h to achieve more than 10^5 spore/mL. The growth of red lettuce plants with respective treatments was followed daily until significant visual vegetative changes to UPMT14 inoculation were observed. The observation and qualitative-quantitative assessment were made by determining the plant detrimental percentage (%) associated with wilt symptoms observed every two days after inoculation on a scale from 0 to 5 based on the percentage of leaf area affected: 0 = 0%; 1 = 1 - 10%; 2 = 11 - 30%; 3 = 31 - 70%; 4 = 41 - 90%; and 5 = 91 - 100%.

RESULTS AND DISCUSSION

Disease-symptomatic red leaf lettuce plants were observed after 32 days of growth on the UPMT14-treated plants as compared to the untreated control of red leaf lettuce plants (Figure 1). The disease-symptomatic red leaf lettuce plants were shown as the wilting leaf. When red leaf lettuce plants were injected with *T. longibrachiatum* UPMT14, the plants reached a height of about 12-13 cm after 22 days of growth. All plants were at normal growth until day-29 when the diseased plants started to exhibit wilting (Figure 1) at older leaves with a lesion at the edge of the leaves and a black circular spot (Figures 2 & 3). The similar incidence was reported by Sarsaiya *et al.* (2019) who reported for the first time that the *T. longibrachiatum* attacked *Dendrobium nobile* that exhibited leaf black circular spots. In the present study, the disease incidence *T. longibrachiatum* caused red leaf lettuce plants to be in a devastated state where injected plants on the 45-day-old present in wilt and wither states. Some negative cases associated with *Trichoderma* sp. were *Pleurotus* sp. (Shah *et al.*, 2012) and *Lentinula edodes* (Wang *et al.*, 2016), known as a green mould disease capable of degrading

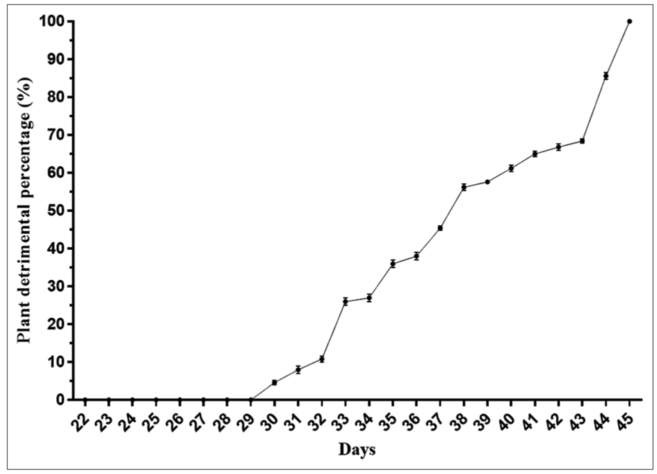

Figure 1: Plant detrimental percentage (%) associated with plant wilt symptoms damage versus days of growth

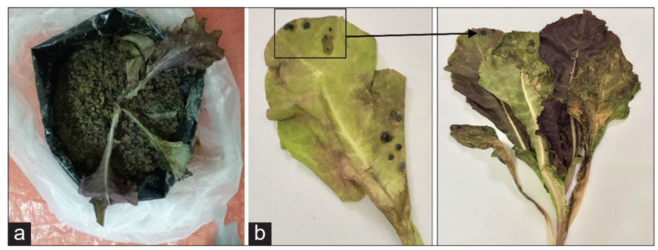

Figure 2: Visual observations on the red leaf lettuce plant in a polybag after 37-45 days of inoculation. Leaves of the plant were wilted (a) with a black circular spot on the leaf edge (b)

Figure 3: Red leaf lettuce plants after 45 days of growth. A) normal, untreated red leaf lettuce plants and B) symptomatic red leaf lettuce plants

edible mushroom (Singh *et al.*, 2006; Shah *et al.*, 2012; Wang *et al.*, 2016) and cellulolytic filamentous fungus that can often contaminate mushroom substrates (Colavolpe *et al.*, 2015). In Hungary, *Trichoderma aggressivum* f. *europaeum* and *Trichoderma aggressivum* f. *aggressivum* (Th4) have also been reported to cause Agaricus green mould (Hatvani *et al.*, 2007; Aydoğdu *et al.*, 2020). The mushroom growing industry and Croatian mushroom farms (Croatia) faced high detrimental quality caused by this fungus (Samuels *et al.*, 2002; Hatvani *et al.*, 2012; Colavolpe *et al.*, 2015). *T. viride* and *T. harzianum* reported can damage *P. nigra* seedlings. Following inoculation with *T. viride* for two years about 30 to 80 per cent mortality of *P. nigra* seedlings was described (Nicosia *et al*., 2014). *T. atroviride* strain KRCF660 that a pathogenic fungus had previously been reported to cause detrimental on the mushroom industry related to the fungi kingdom (Miyazaki *et al*., 2009; Yun *et al*., 2016; Al-Rubaiey & Al-Juboory, 2020), *T. longibrachiatum* strains ZF05 caused a pathogenic effect on orchid plant industry (Sarsaiya *et al*., 2019, 2020).

All the examples of plant-disease events and of the present study demonstrated therefore, it shows clearly that the plants that are infected by fungi can suffer severe harm, which has negative effects on the economy and environment. To determine which fungal species are most likely to spread disease, it is vital to conduct infection tests on plants, especially those that are significant to agriculture and efficient ways to manage and prevent fungal infections. In addition, in ways to increase food security and lessen the negative effects of chemical pesticides, it is also necessary the creation of disease-resistant crop types.

CONCLUSION

This study shows the importance of evaluating fungal species that are likely to cause disease through infection tests on plants, especially plants that are important in agriculture. In conclusion, the wilting incidence in red leaf lettuce was caused by *T. longibrachiatum*. Further studies are needed in future to understand the pathogenesis of *T. longibrachiatum*.

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