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Research

Plants Wilt Disease of Red Leaf Lettuce (*Lactuca sativa* L.) After Colonized by *Trichoderma longibrachiatum*

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ABSTRACT

Trichoderma longibrachiatum rarely reported can cause disease in plants. The present study investigates the pathogenicity of *T. longibrachiatum* isolate UPMT14 on the red leaf lettuce (*Lactuca sativa* L.) plants grown in sterile soil under a controlled growth room environment. The fungal isolate was initially characterized morphologically as *Trichoderma* sp. and was then further characterized by (ITS) region sequencing and BLAST comparison identified as *T. longibrachiatum*. To observe the response of *Trichoderma* isolate UPMT14 when imposed on lettuce plants. The injection was made and repeated five times, and then the lettuce growth followed for 36 days. On day 36, the present study found that the red leaf lettuce plants expressed foliar symptoms that began as chlorotic, reduced plant height, reduced leaf length and diameter, wilt, and dried up before it collapsed at day 45 compared to untreated control lettuce plants. Microscopic observation on lettuce roots showed that the *Trichoderma* spores invading the root system by mass sporulation and spatial competition possibly impaired plant water uptake and eventually caused plant wilting. Therefore, this study indicates that *T. longibrachiatum* is among the causal agents of wilt disease in the lettuce plant.

Key words: Internal transcribed spacer, *Lactuca sativa*, lettuce, plant wilt disease, *Trichoderma longibrachiatum*

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INTRODUCTION

Lettuce is rich in phytoconstituents such as folate, carotenoids, ascorbic acid, and polyphenols (Rodrigo-García *et al*., 2019). *Trichoderma* sp. is rarely reported attacked in the agriculture sector related to vegetables (Hatvani *et al*., 2012; Colavolpe *et al*., 2015), and *Trichoderma* sp. is treated as an economically important fungus for the environment and agriculture (Jaklitsch & Voglmayr, 2015; Waghunde *et al*., 2016). Most studies demonstrated the beneficial impact of *Trichoderma* spp. to suppress or against phytopathogens (Karima & Nadia, 2012; Salas-Marina *et al*., 2015; Innocenti *et al*., 2015b). However, a lack of information is available about *Trichoderma* sp. that causes disease in plants (Sarsaiya *et al*., 2020). *Trichoderma* sp. is rarely reported and can have a detrimental effect on the agriculture industry (Hatvani *et al*., 2012; Colavolpe *et al*., 2015). Reported for the first time that *Trichoderma longibrachiatum* (ZF05) infected and caused black circular spots on *Dendrobium nobile* in China (Sarsaiya *et al*., 2019; 2020). Based on our knowledge, the information on the phytopathogenic of this species is still limited, and the attack on the red leaf lettuce (*Lactuca sativa* L.) is novel.

Therefore, the investigation observation of the pathogenesis of this fungus was established under the control room. The morphological characteristics of *Trichoderma longibrachiatum* isolate further characterized

by internal transcriber spacer region sequencing were determined. To study of the pathogenesis of *T. longibrachiatum* isolate injected into red leaf lettuce plants grown in sterile soil under a controlled growth room environment was conducted.

MATERIALS AND METHODS

A collection culture was obtained from the Laboratory of Microbiology, Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus. A total of seven of these strains were grown and maintained on NA media. Plant pathogenic *T. longibrachiatum* (UPMT14) was subcultured and maintained on potato dextrose agar (PDA). The observation was made further on the mycelial radial growth pattern and phenotype when grown on PDA, Czapek-Dox (CZDA), and NA at 28±1 °C for seven days. Microscopic morphological characteristics of *T. longibrachiatum (UPMT14)*on PDA prepared from older colonies of two weeks culture (Lunge & Patil, 2012). This fungal strain was further identified based on a molecular approach using primer pair by sequencing of ITS region. The rDNA sequence of ITS region using universal primers; ITS1-ITS4 (Chakraborty *et al*., 2010). The sequence for ITS1 and ITS4 were shown as the following: ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') Inglis and Tigano, 2006) for species identification.

Trichoderma isolate UPMT14 was introduced on the red leaf lettuce (*Lactuca sativa* L.) plants by injection (Posada *et al*., 2007) grown in sterile soil under a controlled growth room environment for pathogenicity test. The injection was made and repeated five times, and then the lettuce growth followed for 36 days. Foliar symptoms were 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%. The final evaluation of disease incidence was conducted 45 days after injection. Observation on *T. longibrachiatum* UPMT14 colonization on lettuce roots by light microscopic analysis reconfirming the virulent infection and then for enzyme production ability test analysis, of *T. longibrachiatum UPMT14*, on three different types of media slightly modification were prepared, to investigate the type of enzyme produced by this fungus to penetrate plant cells: cellulose (Habib *et al*., 2005), casein (Brown & Smith, 2014) and chitin agar (Joe & Mapana, 2017). Parameters recorded following artificial inoculation include length of the root, plant height, the mass of the root, the mass of the upper part plant, dried root weight, weight of the fresh upper part plant, and total water content of roots, the total content of upper part plant and total content of the whole plant of red leaf lettuce were measured and record at day 45 included for dry weight of the shoot and root samples. The experiments were conducted in Completely Randomized Design (CRD) with five replicates. Recorded data were analyzed with SAS® 9.4 software for Windows.

RESULTS AND DISCUSSION

The color, colony appearance, and growth pattern of collected fungal isolate in the present study were examined as shown in Figure 1. The fungal isolate was characterized as belonging to *Trichoderma* sp. The matured green of conidia production was denser from the center towards the margin. A dark green pustule grew on the matured green mat of conidia distributed throughout the plate (Figure 1A). Micromorphological characteristic was observed as shown in Figure 1 (B) and (C). Hyphae of *Trichoderma* sp., macrospores bearing conidiophore, macroconidia (spores) (Figure 1B), and conidia of *Trichoderma* sp. UPMT14 (Figure 1C). This was in agreement with the previous reports made by Samuels *et al*.(2002), Phookamsak *et al*. (2019), and Recio *et al*. (2019). Shape size (length and width) of macroconidia (globose) and conidia (ellipsoid) were also measured at 4.789 × 5.035 µm and 3.131 × 2.771 µm, respectively. The colony appearance of *Trichorderma* sp. was dark green on PDA

The observation was made further on the mycelial radial growth pattern and phenotype when grown on PDA, CZDA, and NA at 28±1 °C for seven days (Figure 2 & Figure 3). The mycelial growth of *Trichoderma* sp. UPMT14 on three different media was trending higher from day 1 to day 7 with radial growth ranging from the lowest at 8.22±0.37 mm to the highest at 86.96±0.37 mm. The radial growth of *Trichoderma* sp. UPMT14 on three different media started to differentiate when grew after day 3. On day 4, the radial growth of strain UPMT14 grown on CZDA was significantly (p<0.05) bigger (65.24±0.40 mm) than PDA (53.74±0.22 mm), and the radial growth was smaller when strain UPMT14 grown on NA (41.66±0.23 mm). The growth trend was similar until day 6 and when reached day 7, the radial growth of strain UPMT14 on CZDA (86.96±0.37 mm) and PDA (86.42±0.44 mm) were significantly similar. However, the radial growth of strain UPMT14 on NA (49.82±0.41 mm) was significantly far smaller than both on CZDA and PDA. The slow growth rate of fungus on NA due to the lack of sucrose source, as medium supporting the growth of a wide range for most bacteria-type microorganisms contain peptone provides organic nitrogen and sodium chloride that gave the mixture proportions similar to those found in the cytoplasm of most organisms (MacFaddin, 2000; Downes & Ito, 2001).

On the phenotypic observations of *Trichoderma* sp. UPMT14 growth over 7 days (Figure 3), when grown on PDA, the UPMT14 formed 1-2 concentric rings with whitish conidial production. The

conidia production was denser in the center on the 3rd day. On day 5th, conidia *Trichoderma* sp. UPMT14 appeared in pale yellowish and distributed. An irregular yellow zone with conidia was present, and some off-white pustules were also found growing on the yellowish mat of conidia. On day 7th, *Trichoderma* sp. UPMT14 presented greenish and yellowish conidia with more than two irregular concentric rings present throughout the plate.

On the Czapek's dox agar (CZDA), *Trichorderma* sp. UPMT14 showed a scattered granular, yellowish conidia production on the 3rd day. White cottony mycelia of *Trichorderma* sp. UPMT14 presented to grow scarcely toward the edge on day 5th, some concentric matured green and yellowish conidia were also found on the plate, and there were no concentric rings formed. On day 7th, green conidia dispersed on the whole plate but appeared dense near the center of the plate. Some cottony mycelia appear growing on the green mat of conidia.

On the nutrient agar (NA), *Trichoderma* sp. UPMT14 formed an almost irregular concentric ring of conidial on 3rd day. On the 5th day, 1-2, irregular concentric rings were formed with yellowish conidia. Small white pustules were present growing on the yellowish mat of conidia. On the 7th day, greenish and yellowish conidia were present with some white pustules.

Microscopic morphological characteristics of *Trichoderma* sp. culture on PDA prepared from older colonies of two weeks culture (Lunge & Patil, 2012) or after one week for conidiophores and conidia observation (Jang *et al*., 2017) were suggested for proper visualization of the characteristic features of fungus, especially for examining the character of spore (Lunge & Patil, 2012). Different types of media to culture *Trichoderma* sp. such as on Czapek's dox agar, potato dextrose agar, and nutrient agar could produce different traits on colony morphology, colony color, colony growth rate, conidia, and macroconidia (Shah *et al*., 2012). The growth rate for *Trichoderma* sp. asexual life cycle presented differs depending on the type of media that they are cultured on (Lin *et al*., 2016).

Cellulose, Chitin, and Casein Test are considered the main enzymes produced by fungus for them to penetrate plant cells, in the present study some biochemical test was also conducted. *Trichoderma* sp. UPMT14 culture was spot inoculated on the different test media to test the presence of cellulose-, chitin-, and casein-producing activities. After 24 h growing on the test media under ambient and dark conditions. The clearing zone was observed and measured and considered positive (Figure 4).

Figure 5 shows the diameter of the clearing zone produced by different test media after inoculation with strain UPMT14 which demonstrates the level of cellulose, chitin, and casein production. The present study found that the UPMT14 produced significantly different among test media (p<0.05) with casein (33.2±0.6 mm) the highest over chitin (30.6±0.5 mm) and cellulose (21.8±0.8 mm) was the lowest. *Trichoderma* sp. can release volatile organic compounds, secondary toxic metabolites, and extracellular enzymes to adapt and survive in competitive environments (Mumpuni, Sharma, & Brown, 1998; Patil *et al*., 2016). *Trichoderma* sp. was recorded as capable of producing cellulase, proteases, and chitinase that caused damage to cellular components (Harman *et al*., 2004; Martinez *et al*., 2008; Seidl *et al*., 2009; Schmoll *et al*., 2010), followed by penetration inside the host and cause detrimental on the tissue that left host to die (Sarsaiya *et al*., 2019, 2020; Aydoğdu *et al*., 2020). The interaction between the host and pathogenic fungus includes recognition of the host, attack, and subsequent penetration and killing by secretion of cell wall degrading enzymes (CWDE) that hydrolyze the cell wall of the host (Rao *et al*., 2015).

ITS Gene Sequence Analysis, *Trichoderma* sp. UPMT14 was subjected to confirmation and speciation. Primers ITS1 and ITS4 with approximate sizes of 450-800 base pairs were used (Figure 6). Analysis of the generated ITS1/ITS4 nucleotide sequence (Figure 7) obtained in this study matched with *Trichoderma longibrachiatum* isolate S13G (Accession number = MT634694.1) at 99.79 % similarity after employing BLAST comparison. Therefore, *Trichoderma* sp. UPMT14 belonged to *Trichoderma longibrachiatum* strain UPMT14.

ITS nucleotide sequence of the strain UPMT14 was positioned on the phylogenetic tree. The phylogenetic tree was constructed using the equal-joining method. The phylogenetic analysis for this study was focused on the relationship of UPMT14 with *Trichoderma* strains that are associated with beneficial properties and with the one that is associated with phytopathogens. The tree construction was made by retrieving the selected ITS sequences of *Trichoderma* spp from GenBank Databases. A total of ten ITS-Trichoderma sequences were retrieved and used to construct a phylogenetic tree.

The present study found that the strain UPMT14 was positioned on a similar branch with *Trichoderma longibrachiatum* strain ZF05 (Figure 8). According to Sarsaiya *et al*. (2019; 2020), the strain ZF05 was reported to cause a deleterious effect on orchid plants. From this branch to farther branch that positioned the *Trichoderma viride* strain F122 formed a major cluster that shared similar traits as reported having phytopathogens. Therefore, the number of sequences that formed a major cluster was nine out of a total of 11 sequences on this phylogenetic tree. *Trichoderma longibrachiatum* strain UFT204 (dos Santos *et al*., 2021) and *Trichoderma asperellum* strain TaspHu1 (Yu *et al*., 2021) that out grouped were recently reported to have beneficial properties or that had a positive impact on plant growth. Based on phylogenetic tree analysis indicates that UPMT14 may be associated with the pathogenesis because of is closely related to phytopathogenic *Trichoderma* spp. *Trichoderma harzianum* strain KRCF131,

Trichoderma citrinoviride strain KRCF305, *Trichoderma longibrachiatum* strains (KRCF306, T13-Iraq and NFCF081) and *Trichoderma atroviride* strain KRCF660 that these pathogenic fungi had previously reported causing detrimental on mushroom industry related to fungi kingdom (Miyazaki *et al*., 2009; Yun *et al*., 2016; Al-Rubaiey & Al-Juboory, 2020), *Trichoderma longibrachiatum* strains ZF05 caused a pathogenic effect on orchid plant industry (Sarsaiya *et al*., 2019, 2020).

Assessing The Response to *Trichoderma longibrachiatum* Inoculation in Red Lettuce Plants, Visual observations of red leaf lettuce plants injected with *T. longibrachiatum* UPMT14 where the plants had reached a height of about 12-13 cm (Figure 9) after 22 days of growth. All plants were at normal growth until day 34 when the diseased plants started to exhibit wilting at older leaves (Figure 10a) with a lesion at the edge of the leaves (Figure 10b). However, Sarsaiya *et al*. (2019) reported for the first time that the *Trichoderma longibrachiatum* attacked *Dendrobium nobile* and exhibited leaf black circular spots.

Plant growth continued to decline in the following days, especially after 37 days of growth (Figure 9) until completely collapsed after 45 days of growth (Figure 9; Figure 10c). Plants without disease continue to show normal growth until they reach a height of 16-17 cm on the 45th day (Figure 9; Figure 10). Figure 11 shows the healthy or normal red lettuce plant as compared to diseased plants infected with *T. longibrachiatum* UPMT14. The symptoms observed were as wilt disease in red lettuce plant caused by *T. longibrachiatum* UPMT14. Based on present knowledge, *T. longibrachiatum* UPMT14 presented is the first report of disease incidence occurring in red lettuce plant. Limited information is available on the pathogenesis of *T. longibrachiatum* such as the strain ZF05 was recently reported to cause pathogenicity in *Dendrobium officinale* and *Dendrobium nobile* (Sarsaiya *et al*., 2020).

Trichoderma group is widely reported as an essential fungus in agriculture and the environment (Jaklitsch & Voglmayr, 2015). Fungi in the genus *Trichoderma* are also described as biocontrol agents to suppress phytopathogen growth (Samuels, 1996; Patil *et al*., 2016). *Trichoderma* spp. are rarely reported to attack crops, especially vegetable types. Some more examples of cases associated with *Trichoderma* sp. were *Pleurotus* sp. (Shah *et al*., 2012) and *Lentinula edodes* (Wang *et al*., 2016), known as a green mold disease capable of degrading edible mushrooms (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). *Trichoderma aggressivum* f. *europaeum* also causes Agaricus green mold in Hungary (Hatvani *et al*., 2007). In Hungary, *Trichoderma aggressivum* f. *europaeum* and *Trichoderma aggressivum* f. *aggressivum* (Th4) have also been reported to cause Agaricus green mold (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 percent mortality of *P. nigra* seedlings was described (Li Destri Nicosia *et al*., 2014). This finding was also in agreement and consistent with the claim that this culture (UPMT14) previously caused disease in pepper, *Piper nigrum* (Dr. Franklin Ragai Kundat, Personal communication, unpublished data).

Plant growth measurements show the impact of injecting *T. longibrachiatum* UPMT14 on the growth of red lettuce plants. Most of the shoot and root parameters were negatively affected by disease after 45 days of growth. On day 45, the plant height of the red lettuce plant was not able to be measured due to complete collapse by the disease as compared to the normal untreated red lettuce plant which reached a height of over 17 cm. The same happened on the root part of the plant where the normal plant was recorded as about 12 cm in length. A similar phenomenon occurred in other parameters of shoots and roots such as the mass of the upper plant part and roots, the total water content of the upper plant part and roots, and the total water content of the whole plant. However, the upper plant part dry weight reacted differently where the weight was significantly higher in diseased lettuce plants (2.1 g) than normal healthy lettuce plants (1.06 g). This could be due to the overproduction of mycelia or sporulation that colonized the upper plant part tissue which contributed much of the weight of the total dry mass of the upper plant part of the red lettuce plant (Maharshi *et al*., 2021).

Root colonization of *T. longibrachiatum* UPMT14 on red lettuce roots by using light microscopic analysis, and microscopic observation using a light microscope was conducted on roots sampled from diseased red lettuce plants and normal red lettuce plants of 45 days of growth. The present study found that spores of UPMT14 were present (Figure 13a) and no spore was detected on normal red lettuce plant roots (data not shown). The presence of conidia inside root tissues was an indication that the strain was able to penetrate the root tissue endophytically for internal colonization and able to control the host root system for space and food sources (Rahman *et al*., 2021). The ability of the strain to produce enzymes is among the mechanisms that aid the penetration into the root tissues (Rao *et al*., 2015).

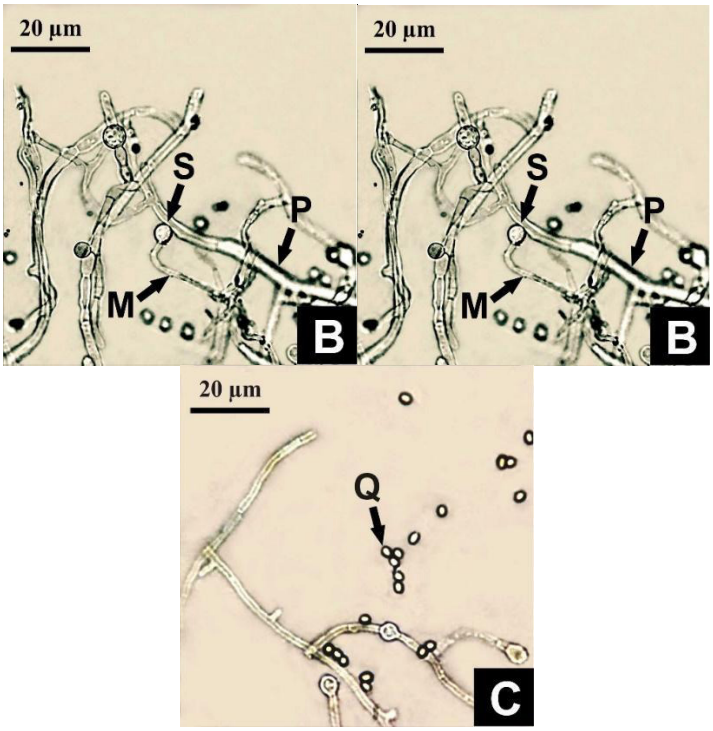

Fig. 1. After 7 days at 28±1 °C, *Trichoderma* sp. UPMT14 was grown on potato dextrose agar (PDA) (A). Macrospores bearing conidiophore (M) and Macroconidia (spores) (S) (at 100x magnification under a compound microscope). Conidia of *Trichoderma* sp. UPMT14 (Q) (at 100x magnification under a compound microscope) (as shown in Plate (B) and (C)).

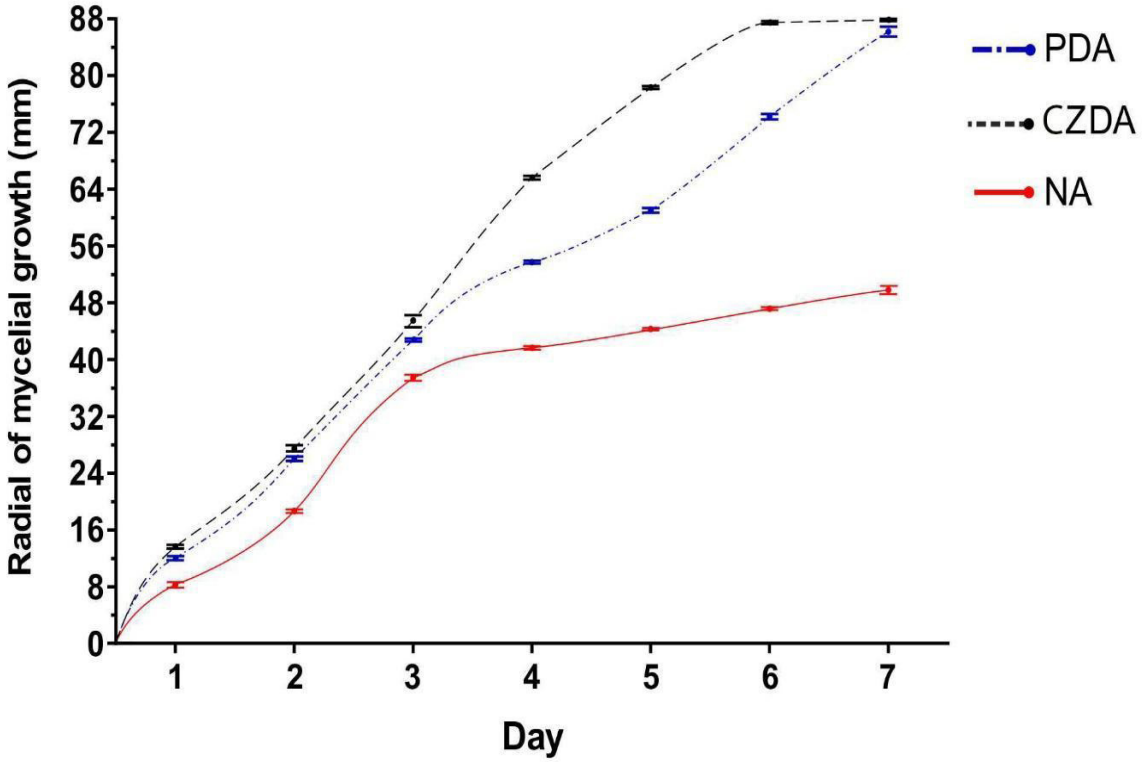

Fig. 2. Radial growth of *Trichoderma longibrachiatum UPMT14* mycelia on Czapek's dox agar (CZDA), potato dextrose agar (PDA), and Nutrient agar (NA) for 168 hr under ambient conditions.

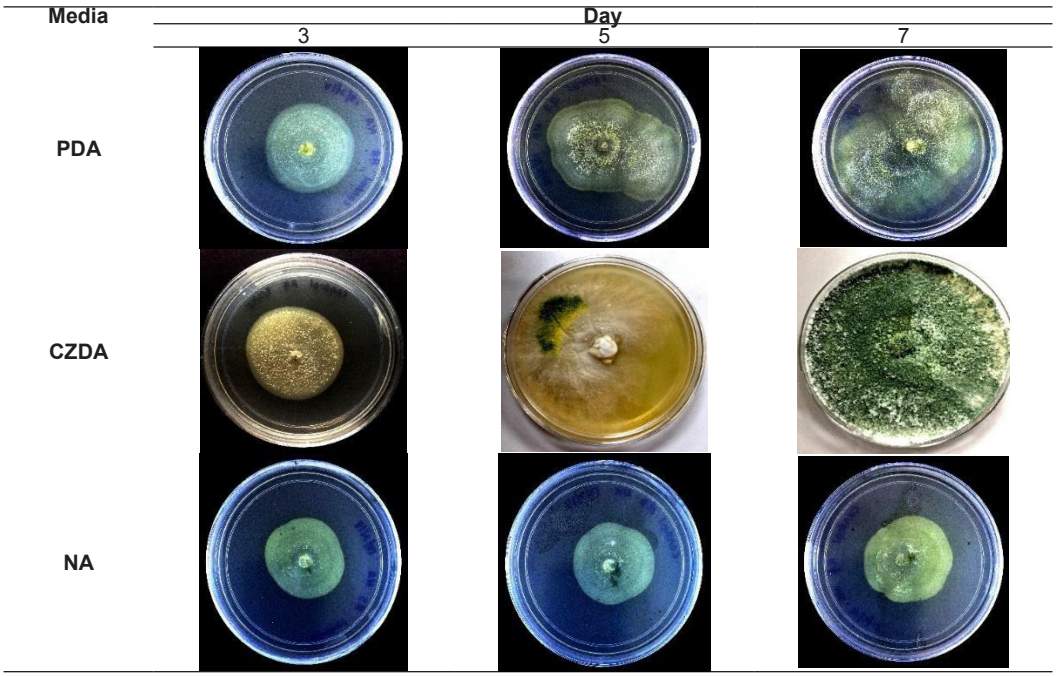

Fig. 3. Colony appearance of *Trichoderma longibrachiatum* UPMT14 grown for 168 h at 28±1 °C on Czapek's dox agar (CZDA), potato dextrose agar (PDA) and Nutrient agar (NA).

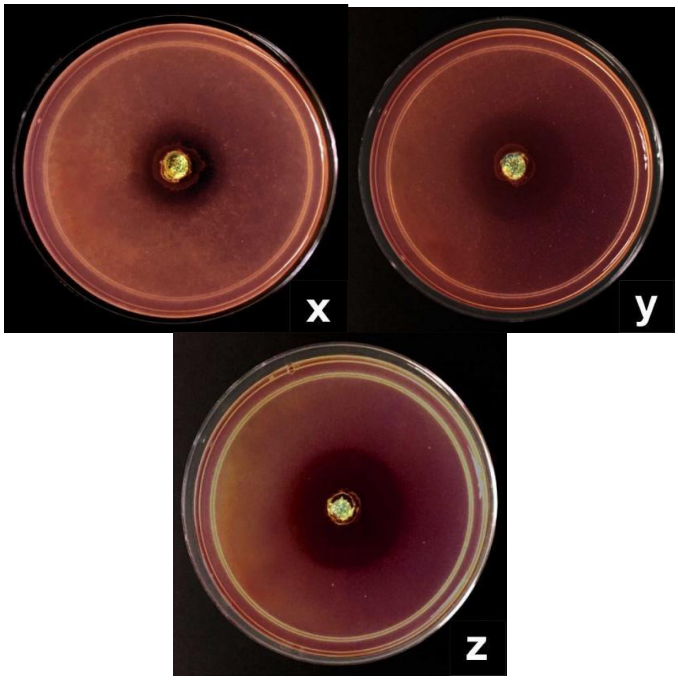

Fig. 4. *Trichoderma* sp. UPMT14 was grown on cellulose (x), chitin (y), and casein (z) media for 8 days at 26±2 °C under the dark condition with a clear zone surrounding the colony (dark zone due to the dark background in use).

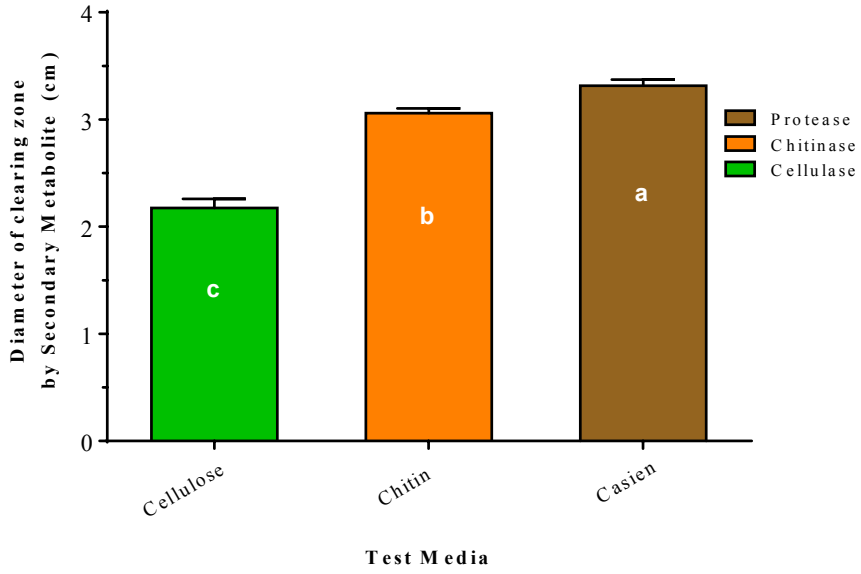

Fig. 5. Diameter of clearing zone (cm) by *Trichoderma* sp. UPMT14 on different test media after 24 h incubation at 26±2 °C. Means with different alphabet was significantly different at $P<0.05$ (Tukey's Range Test).

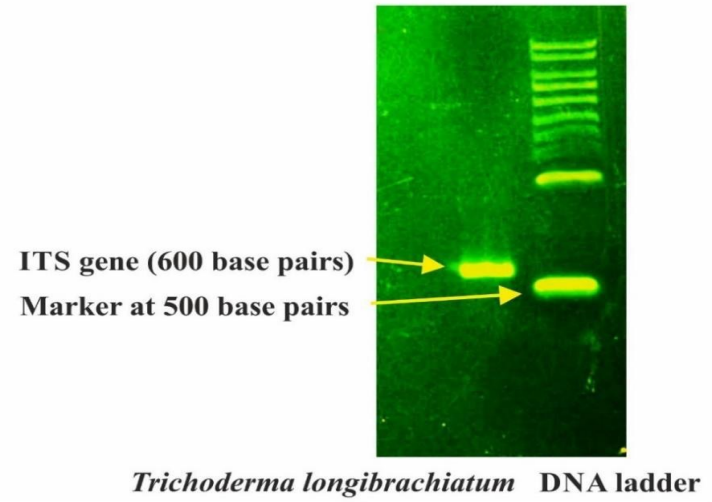

Fig. 6. PCR amplification detection of ITS genes from *Trichoderma longibrachiatum* isolate. M represent VC 1kb DNA ladder (Vivantis). For ITS1 and ITS4 primers, the amplifier product was around 450-800 bp in size.

GCTGTGGCCGCGCCGCGCTCCCGGTGCGAGTGTGCAAACTACTGCGCAGGA
GAGGCTGCGGCGAGACCGCCACTGTATTTCGGGGGCGGCCCGGTGAGGGGC
CGATCCCCAACGCCGACCCCCCGGAGGGGTTCGAGGGTTGAAAATGACGCTC
GGACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAAGATT
CGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCT
TCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTCATTTTCGA
GACGCCCGCTAGGGTCGCCGAGAAAGGCTCAGAGCAAAAATAAAACAGAGC
CGCGACGGGAGCCGCGACGGAGAGAAAAAAAGAGTTTGGAGTTGGTCCTCC
GGCGGGCGCCATGGGATCCGGGGCTGCGACGCGCCCGGGGCAAGAGAATCC
CGCCGA

Fig. 7. Nucleotide sequence of ITS gene of *Trichoderma sp.* UPMT14

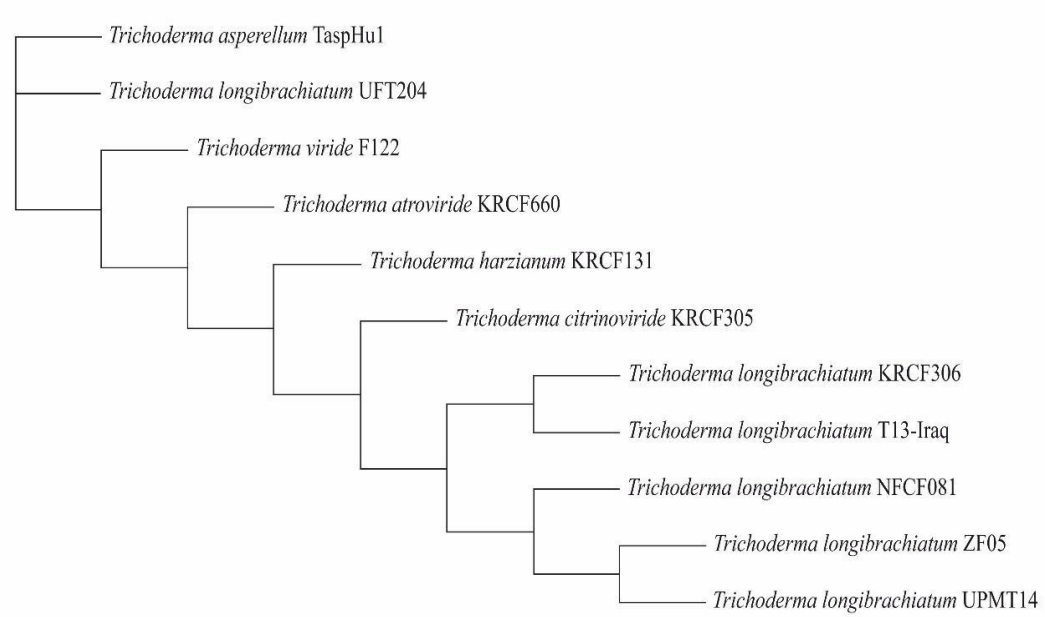

Fig. 8. Phylogenetic tree showing the relationship of closely related *Trichoderma* species with newly identified strain, *Trichoderma longibrachiatum* UPMT14, constructed using the equal-joining method based on ITS gene sequences.

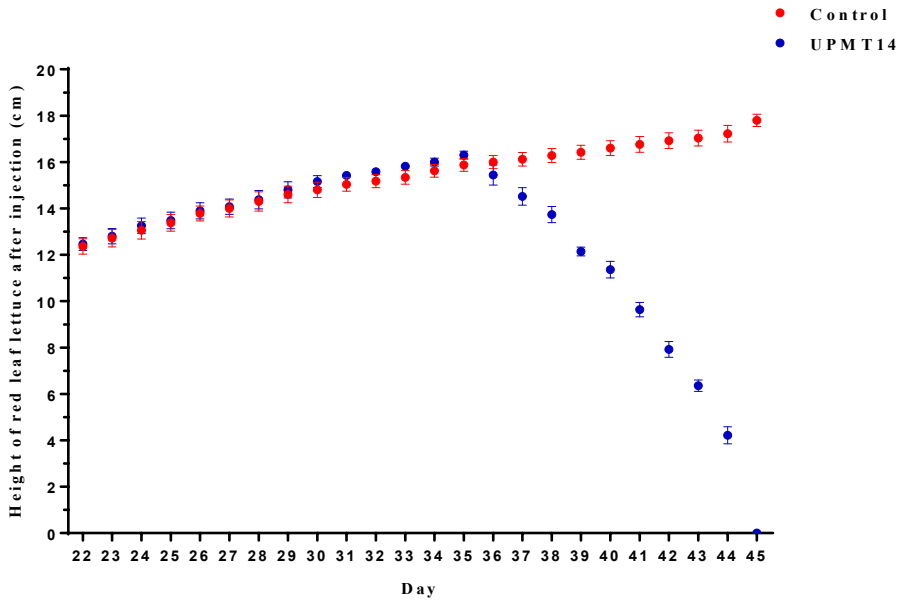

Fig. 9. Height of red lettuce after injection with *Trichoderma longibrachiatum* isolate versus days of growth.

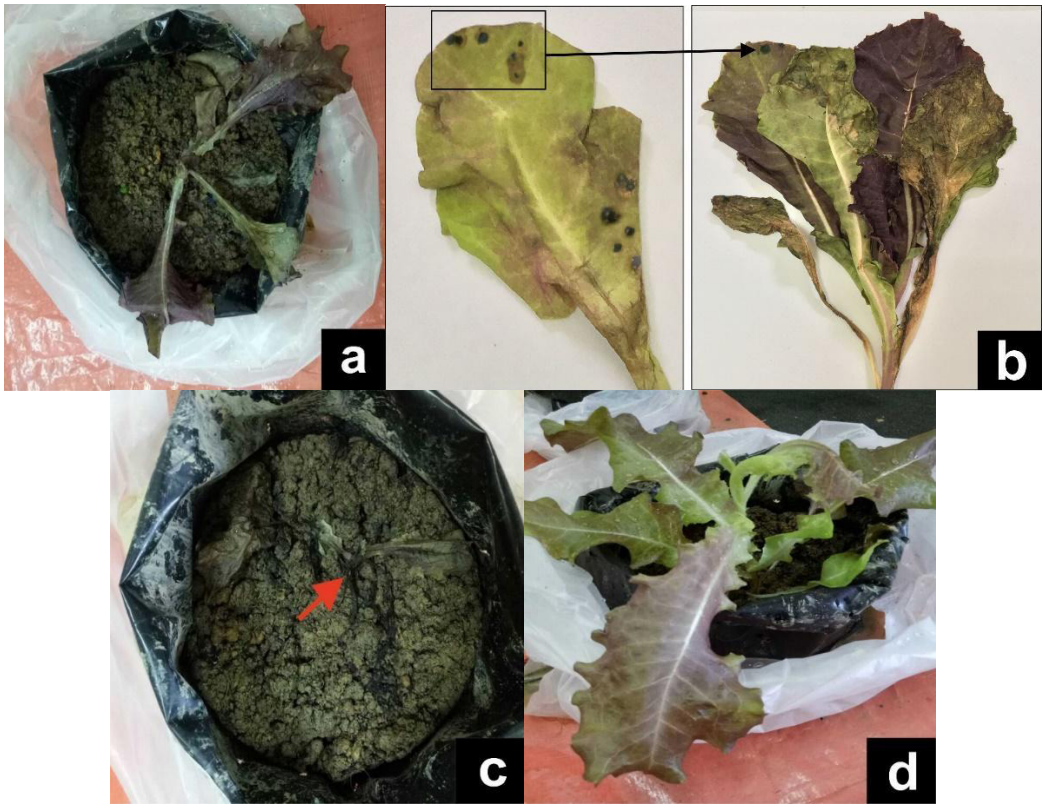

Fig. 10. Visual observations on the red leaf lettuce plant in a polybag after 37-45 days of inoculation. Leaves of the plant were wilted (a) with a black circular spot on the leaf edge (b). The infected plant eventually collapsed on day 45 (c). Plate (d) shows the untreated control plant.

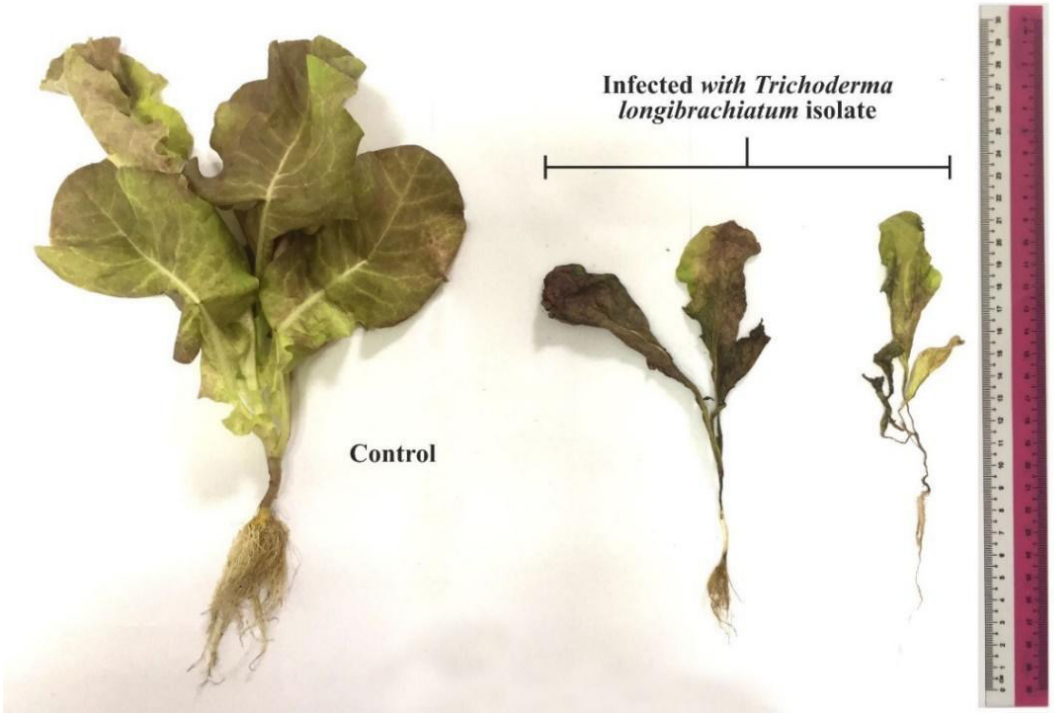

Fig. 11. Visual symptoms of red leaf lettuce infected with *Trichoderma longibrachiatum* UPMT14 (right side) versus healthy red leaf lettuce (left side).

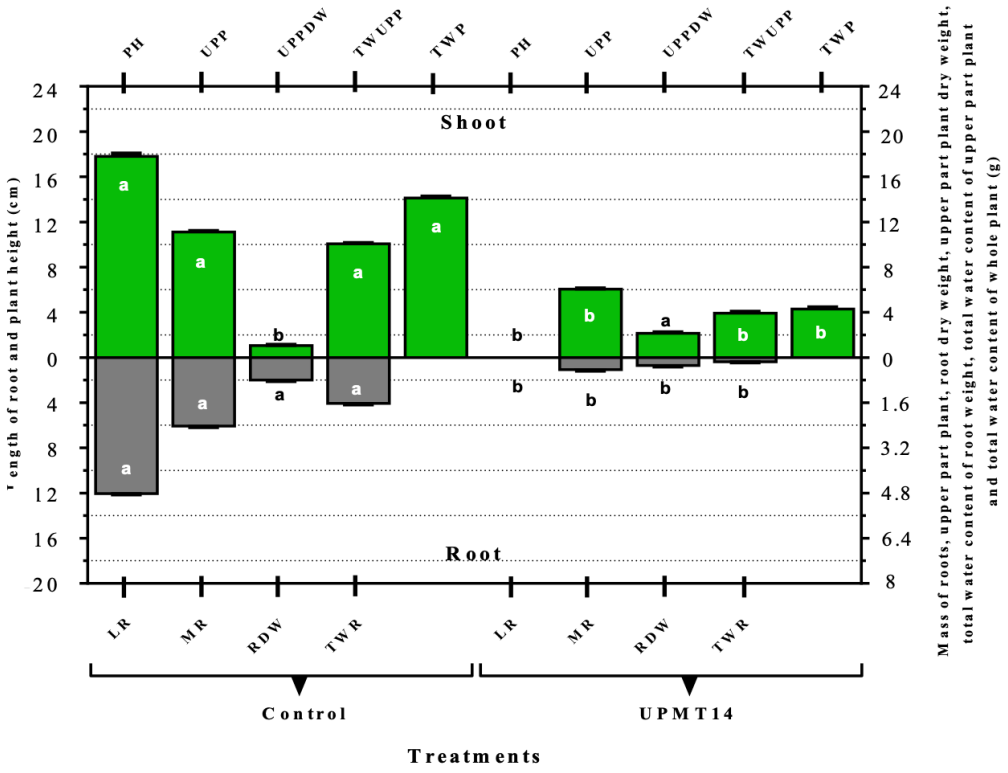

Fig. 12. Length of root (LR), plant height (PH), mass of roots (MR), upper part plant (UPP), root dry weight (RDW), upper part plant dry weight (UPPDW), total water content of root weight (TWR), total water content of upper part plant (TWUPP) and total water content of whole plant (TWP) after 45 days of growth. The Tukey's Range Test revealed that means with a similar alphabet were not significantly different at $P<0.05$.

CONCLUSION

In conclusion, the morphological characteristics of *T. longibrachiatum UPMT14*UPMT14 were successfully cultured and determined on different types of growth media (PDA), (CZDA) and (NA) resulting in different characteristics and growth patterns. On the PDA, *Trichoderma* sp. UPMT14 was observed at the early stage with 1-2 concentric rings with whitish conidial production to greenish and yellowish conidia with more than two irregular concentric rings visible throughout the plate. On the CZDA, *Trichoderma* sp. UPMT14 showed a scattered granular, with yellowish conidia production to green conidia, spread on the whole plate but demonstrated density near the center of the plate. While on the NA, *Trichoderma* sp. UPMT14 formed a single irregular concentric ring of conidial at the early stage and subsequently formed 1-2 an irregular concentric ring with yellowish conidia. The growth pattern on NA eventually turned greenish and yellowish of conidia present with some white pustules at the final sampling date (day 7). The growth rate of *Trichoderma* sp. UPMT14 on PDA, CZDA, and NA were different with UPMT14 growth on NA starting to slow from day 3 while on the PDA and CZDA on uprising obviously until day 6. The ITS gene was extracted from *Trichoderma* sp. UPMT14 and identified as *Trichoderma longibrachiatum* strain UPMT14. Plant assay was conducted by injecting *T. longibrachiatum* strain UPMT14 spore suspension into red leaf lettuce (*Lactuca sativa* L.) plants and found that the strain can cause wilting from day 35 of growth. The test plant collapsed on day 45 of growth. As far as is known, this is a new report on *T. longibrachiatum* strain UPMT14 that causes wilt disease in red leaf lettuce. Besides that, the pathogen might use enzymatic mechanisms such as cellose, chitin, and casein, to invade and colonize the endophytic environment of plant roots. This finding provides updated information on lettuce wilt disease caused by *T. longibrachiatum* strain UPMT14 and helps to understand the disease and explore suitable control techniques for the pathogen.

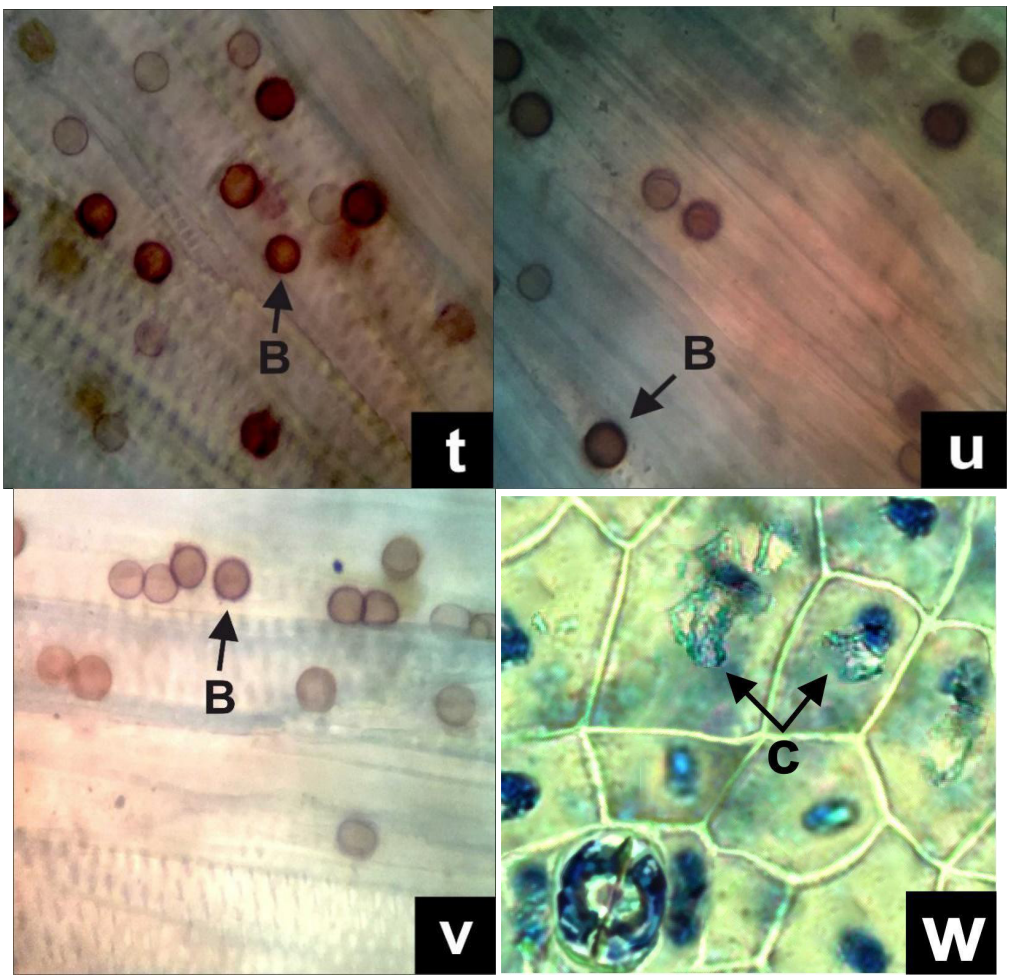

Fig. 13. Infected root (B) viewed under a dissecting microscope. t, u, and v observed from several cross-sectioned root samples of diseased red lettuce plant caused by *Trichoderma longibrachiatum* UPMT14 (at 100x magnification under a compound microscope) (as shown in Plate (t), (u) and (v)). (w) Dense colonies of *Trichoderma longibrachiatum* UPMT14 in phloem with spores (at 40x magnification under a compound microscope).

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ETHICAL STATEMENT
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES
Al-Rubaiey, W.L. & Al-Juboory, H.H. 2020. Molecular identification of *Trichoderma longibrachiatum* causing green mold in *Pleurotus eryngii* culture media. Plant Archives, 20(1): 181-184.

Aydoğdu, M., Kurbetli, İ., Kitapçı, A. & Sülü, G. 2020. Aggressiveness of green mould on cultivated mushroom (*Agaricus bisporus*) in Turkey. Journal of Plant Diseases and Protection, 127(5): 695-708. https://doi.org/10.1007/s41348-020-00328-8

Bakri, Y., Masson, M. & Thonart, P. 2010. Isolation and identification of two new fungal strains for xylanase production. Applied Biochemistry and Biotechnology, 162(6): 1626-1634. https://doi.org/10.1007/s12010-010-8944-x

Brown, A. & Smith, H. 2014. Benson's Microbiological Applications, Laboratory Manual in General Microbiology, Short Version. McGraw-Hill Education.

Chakraborty, B.N., Chakraborty, U., Saha, A., Dey, P.L. & Sunar, K. 2010. Molecular characterization of *Trichoderma viride* and *Trichoderma harzianum* isolated from soils of North Bengal based on rDNA markers and analysis of their PCR-RAPD profiles. Global Journal of Biotechnology & Biochemistry, 5(1): 55-61.

Colavolpe, M.B., Mejía, S.J. & Albertó, E. 2015. Efficiency of treatments for controlling *Trichoderma* spp. during spawning in cultivation of lignicolous mushrooms. Brazilian Journal of Microbiology, 45(4): 1263-1270. https://doi.org/10.1590/S1517-83822014000400017

DOA. 2018. Department of Agriculture Peninsular Malaysia (DOA). In: Crop Statistic (Food Crop Sub-Sector). p. 64.

dos Santos, J.L., Ribeiro, E.A., de Oliveira, R.S., Luz, J.H.D.S., Nunes, B.H.D.N., Oliveira, H.P.D., Sarmento, R.D.A., da Silva, R.R. & Chagas, A.F., 2021. Volatile organic compounds produced by *Trichoderma* sp. morphophysiologically altered maize growth at initial stages. Australian Journal of Crop Science, 15(2): 215-223. https://doi.org/10.21475/ajcs.21.15.02.p2605

Downes, F.P. & Ito, K. 2001. Compendium of Methods for the Microbiological Examination of Foods. Washington DC, USA: American Public Health Association. https://doi.org/10.2105/9780875531755

Habib Onsori, M.R.Z., Motallebi, M. & Zarghami, N. 2005. Identification of over producer strain of endo-b-1, 4-glucanase in *Aspergillus* species: Characterization of crude carboxymethyl cellulase. African Journal of Biotechnology, 4: 26-30.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. & Lorito, M. 2004. *Trichoderma* species-Opportunistic, avirulent plant symbionts. Nature Reviews Microbiology, 2(1): 43-56. https://doi.org/10.1038/nrmicro797

Hatvani, L., Antal, Z., Manczinger, L., Szekeres, A., Druzhinina, I.S., Kubicek, C.P., Nagy, A., Nagy, E., Vágvölgyi, C. & Kredics, L. 2007. Green mold diseases of *Agaricus* and *Pleurotus* spp. are caused by related but phylogenetically different *Trichoderma* species. PhytopathologyTM, 97(4): 532-537. https://doi.org/10.1094/PHYTO-97-4-0532

Hatvani, L., Sabolić, P., Kocsubé, S., Kredics, L., Czifra, D., Vágvölgyi, C., Kaliterna, J., Ivić, D., Đermić, E. & Kosalec, I. 2012. The first report on mushroom green mould disease in Croatia. Arhiv Za Higijenu Rada i Toksikologiju, 63(4): 481-486. https://doi.org/10.2478/10004-1254-63-2012-2220

Herath, H.H.M.A.U., Wijesundera, R.L.C., Chandrasekharan, N.V., Wijesundera, W.S.S. & Kathriarachchi, H.S. 2015. Isolation and characterization of Trichoderma erinaceum for antagonistic activity against plant pathogenic fungi. Current Research in Environmental & Applied Mycology, 5(2): 120-127. https://doi.org/10.5943/cream/5/2/5

Inglis, P. W. & Tigano, M. S. 2006. Identification and taxonomy of some entomopathogenic *Paecilomyces* spp. (Ascomycota) isolates using rDNA-ITS sequences. Genetics and Molecular Biology, 29, 132-136. https://doi.org/10.1590/S1415-47572006000100025

Innocenti, G., Roberti, R. & Piattoni, F. 2015. Biocontrol ability of *Trichoderma harzianum* strain T22 against Fusarium wilt disease on water-stressed lettuce plants. BioControl, 60(4): 573-581. https://doi.org/10.1007/s10526-015-9662-7

Jaklitsch, W.M. & Voglmayr, H. 2015. Biodiversity of *Trichoderma* (Hypocreaceae) in Southern Europe and Macaronesia. Studies in Mycology, 80: 1-87. https://doi.org/10.1016/j.simyco.2014.11.001

Jang, S., Jang, Y., Kim, C.-W., Lee, H., Hong, J.-H., Heo, Y.M., Lee, Y.M., Lee, D.W., Lee, H.B. & Kim, J.-J. 2017. Five new records of soil-derived Trichoderma in Korea: *T. albolutescens*, *T. asperelloides*, *T. orientale*, *T. spirale*, and *T. tomentosum*. Mycobiology, 45(1): 1-8. https://doi.org/10.5941/MYCO.2017.45.1.1

Joe, S. & Mapana, S.S. 2017. An efficient method of production of colloidal chitin for enumeration of chitinase producing bacteria. Journal of Sciences, 4(16): 37-45. https://doi.org/10.12723/mjs.43.4

Li Destri Nicosia, M.G., Mosca, S., Mercurio, R. & Schena, L. 2014. Dieback of *Pinus nigra* seedlings caused by a strain of *Trichoderma viride*. Plant Disease, 99(1): 44-49. https://doi.org/10.1094/PDIS-04-14-0433-RE

Lin, H., Travisano, M. & Kazlauskas, R. J. 2016. The fungus Trichoderma regulates submerged conidiation using the steroid pregnenolone. ACS Chemical Biology, 11(9): 2568-2575. https://doi.org/10.1021/acschembio.6b00376

Lunge, A.G. & Patil, A.S. 2012. Characterization of efficient chitinolytic enzyme producing Trichoderma species: A tool for better antagonistic approach. International Journal of Science, Environment and Technology, 1(5): 377-385.

MacFaddin, J.F. 2000. Biochemical Tests for Identification of Medical Bacteria. 3rd Edition. Lippincott Williams & Wilkins, Philadelphia. 527 pp.

Maharshi, A., Rashid, M.M., Teli, B., Yadav, S.K., Singh, D.P. & Sarma, B.K. 2021. Salt stress alters pathogenic behaviour of *Fusarium oxysporum* f. Sp. Ciceris and contributes to severity in chickpea wilt incidence. Physiological and Molecular Plant Pathology, 113: 101602. https://doi.org/10.1016/j.pmpp.2021.101602

Martinez, D., Berka, R.M., Henrissat, B., Saloheimo, M., Arvas, M., Baker, S.E., Chapman, J., Chertkov,

O., Coutinho, P.M. & Cullen, D. 2008. Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). Nature Biotechnology, 26(5): 553-560. https://doi.org/10.1038/nbt1403

Miyazaki, K., Tsuchiya, Y. & Okuda, T. 2009. Specific PCR assays for the detection of *Trichoderma harzianum* causing green mold disease during mushroom cultivation. Mycoscience, 50(2): 94-99. https://doi.org/10.1007/S10267-008-0460-2

Mumpuni, A., Sharma, H.S.S. & Brown, A.E. 1998. Effect of metabolites produced by *Trichoderma harzianum* biotypes and *Agaricus bisporus* on their respective growth Radii in Culture. Applied and Environmental Microbiology, 64(12): 5053-5056. https://doi.org/10.1128/AEM.64.12.5053-5056.1998

Patil, A.S., Patil, S.R. & Paikrao, H.M. 2016. Trichoderma secondary metabolites: Their biochemistry and possible role in disease management. In Microbial-mediated induced systemic resistance in plants. Springer, Cham. pp. 69-102. https://doi.org/10.1007/978-981-10-0388-2_6

Phookamsak, R., Hyde, K.D., Jeewon, R., Bhat, D.J., Jones, E.G., Maharachchikumbura, S.S., Raspe, O., Karunarathna, S.C., Wanasinghe, D.N. & Hongsanan, S. 2019. Fungal diversity notes 929-1035: Taxonomic and phylogenetic contributions on genera and species of fungi. Fungal Diversity, 95(1): 1-273. https://doi.org/10.1007/s13225-019-00421-w

Posada, F., Aime, M.C., Peterson, S.W., Rehner, S.A. & Vega, F.E. 2007. Inoculation of coffee plants with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales). Mycological Research, 111(6): 748-757. https://doi.org/10.1016/j.mycres.2007.03.006

Rahman, S.S.M.S.A., Zainudin, N.A.I.M. & Aziz, N.A.A. 2021. Evaluation of *Trichoderma asperellum* B1902 in controlling Fusarium Wilt of Cavendish Banana Cultivar. Sains Malaysiana, 50(9): 2549-2561. https://doi.org/10.17576/jsm-2021-5009-05

Rao, G.S., Reddy, N.R. & Surekha, C. 2015. Induction of plant systemic resistance in *Legumes cajanus* cajan, *Vigna radiata*, *Vigna mungo* against plant pathogens *Fusarium oxysporum* and alternaria alternata-a *Trichoderma viride* mediated reprogramming of plant defense mechanism. International Journal of Recent Scientific Research, 6: 4270-4280.

Recio, R., Meléndez-Carmona, M.Á., Martín-Higuera, M.C., Pérez, V., López, E., López-Medrano, F. & Pérez-Ayala, A. 2019. *Trichoderma longibrachiatum*: An unusual pathogen of fungal pericarditis. Clinical Microbiology and Infection, 25(5): 586-587. https://doi.org/10.1016/j.cmi.2019.02.006

Rodrigo-García, J., Navarrete-Laborde, B.A., Rosa, L.A. de la, Alvarez-Parrilla, E., Núñez-Gastélum, J.A., Rodrigo-García, J., Navarrete-Laborde, B.A., Rosa, L.A. de la, Alvarez-Parrilla, E. & Núñez-Gastélum, J.A. 2019. Effect of Harpin protein as an elicitor on the content of phenolic compounds and antioxidant capacity in two hydroponically grown lettuce (Lactuca sativa L.) varieties. Food Science and Technology, 39(1): 72-77. https://doi.org/10.1590/fst.20417

Samuels, G.J. 1996. *Trichoderma*: A review of biology and systematics of the genus. Mycological Research, 100(8): 923-935. https://doi.org/10.1016/S0953-7562(96)80043-8

Samuels, G.J., Dodd, S.L., Gams, W., Castlebury, L.A. & Petrini, O. 2002. *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. Mycologia, 94(1): 146-170. https://doi.org/10.1080/15572536.2003.11833257

Sarsaiya, S., Jain, A., Jia, Q., Fan, X., Shu, F., Chen, Z., Zhou, Q., Shi, J. & Chen, J. 2020. Molecular identification of endophytic fungi and their pathogenicity evaluation against *Dendrobium nobile* and *Dendrobium officinale*. International Journal of Molecular Sciences, 21(1): 316. https://doi.org/10.3390/ijms21010316

Sarsaiya, S., Jia, Q., Fan, X., Jain, A., Shu, F., Chen, J., Lu, Y. & Shi, J. 2019. First report of leaf black circular spots on *Dendrobium nobile* caused by *Trichoderma longibrachiatum* in Guizhou Province, China. Plant Disease, 103(12): 3275. https://doi.org/10.1094/PDIS-03-19-0672-PDN

Schmoll, M., Esquivel-Naranjo, E.U. & Herrera-Estrella, A. 2010. Trichoderma in the light of day-physiology and development. Fungal Genetics and Biology, 47(11): 909-916. https://doi.org/10.1016/j.fgb.2010.04.010

Seidl, V., Seibel, C., Kubicek, C.P. & Schmoll, M. 2009. Sexual development in the industrial workhorse *Trichoderma reesei*. Proceedings of the National Academy of Sciences, 106(33): 13909-13914. https://doi.org/10.1073/pnas.0904936106

Shah, S., Nasreen, S. & Sheikh, P.A. 2012. Cultural and morphological characterization of *Trichoderma* spp. associated with green mold disease of Pleurotus spp. In Kashmir. Research Journal of Microbiology, 7(2): 139. https://doi.org/10.3923/jm.2012.139.144

Singh, S.K., Sharma, V.P., Sharma, S.R., Kumar, S. & Tiwari, M. 2006. Molecular characterization of *Trichoderma* taxa causing green mould disease in edible mushrooms. Current Science, 427-431.

Waghunde, R.R., Shelake, R.M. & Sabalpara, A.N. 2016. *Trichoderma*: A significant fungus for agriculture and environment. African Journal of Agricultural Research, 11(22): 1952-1965. https://doi.org/10.5897/AJAR2015.10584

Wang, G., Cao, X., Ma, X., Guo, M., Liu, C., Yan, L. & Bian, Y. 2016. Diversity and effect of *Trichoderma* spp. associated with green mold disease on *Lentinula edodes* in China. Microbiologyopen, 5(4): 709-718. https://doi.org/10.1002/mbo3.364

Yu, Z., Wang, Z., Zhang, Y., Wang, Y. & Liu, Z. 2021. Biocontrol and growth-promoting effect of Trichoderma asperellum TaspHu1 isolate from Juglans mandshurica rhizosphere soil. Microbiological Research, 242: 126596. https://doi.org/10.1016/j.micres.2020.126596

Yun, S.-H., Lee, S. H., So, K.-K., Kim, J.-M. & Kim, D.-H. 2016. Incidence of diverse dsRNA mycoviruses in *Trichoderma* spp. causing green mold disease of shiitake *Lentinula edodes*. FEMS Microbiology Letters, 363(19): fnw220. https://doi.org/10.1093/femsle/fnw220