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Effect of *Trichoderma harzianum* against *Fusarium oxysporum* in resistant and susceptible tomato cultivars

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Abstract: Fusarium wilt disease presents a substantial challenge to tomato production, especially in an open field environment. The peroxidase (POD) activity and total phenolic compounds (TPCs) play a crucial role in measuring the antioxidant capacity of plants. Understanding the variations in the POD and TPC levels during disease-induced stress becomes important for effectively managing Fusarium wilt and enhancing tomato production. This study investigates the impacts of *Trichoderma harzianum* inoculation through the root drip method on five tomato cultivars. It compares these cultivars to their non-treated counterparts when they are subjected to infection by *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*). The results showed that the level of resistance to *Fol* is based on the specific tomato cultivar. Notably, 'MT26' exhibited the lowest disease severity index (DSI), indicating a strong response, whereas 'CLN3682F' showed notable susceptibility. Regarding the POD and TPC activity, its exhibition differed in compatibility with the response of each tomato cultivar to Fusarium wilt disease. The resistant cultivars increased the POD activity after the *Trichoderma* induction before the *Fol* inoculation, and this activity was further boosted when exposed to disease conditions. Consequently, enhancing the POD and TPC levels during the initial stages could potentially serve as a systemic defence mechanism of tomatoes against the Fusarium wilt disease.

Keywords: breeding for resistance; fungi; protection; tomato resistant source

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The tomato (*Solanum lycopersicum*.) is the most important global vegetable in Thailand; it is widely grown for both domestic and export markets. Tomato fruits are rich in nutrients, such as vitamin A, carotenoids, flavonoids, and lycopene. Several pathogens attack tomato plants and cause a significant production reduction. In Thailand, the soil-borne and host-specific pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) (Ozbay, Newman 2004; Inami et al. 2014), causing Fusarium wilt disease of tomatoes, is very difficult to manage. The upper leaves of the infected plants become yellow and wither, and the hypocotyl vessels become brown and die due to long-term infections (Olivain, Alabouvette 1999), which are unable to recover because the pathogen spreads within the vascular tissues, causing the plant to die (Srinivas et al. 2019). Chemical pesticides are widely used to control soil-borne diseases. However, it has potential risks to human health and increases environmental pollution, such as affecting the beneficial function of microorganisms living in the soil and root ecosystem (Akrami, Yousefi 2015). Recently, there has been a preference for using ecologically friendly technologies in plant disease management to meet the rising demand for high-quality, safer produce and environmental protection. Biological control methods and the use of resistant cultivars are approaches to disease management that are not only safe and environmentally friendly but also widely applied in controlling soil-borne diseases in vegetables (Naranjo et al. 2015). *Trichoderma* spp. has a multifaceted role in agriculture, as evidenced by their capabilities in controlling pathogens and pests (Vinale et al. 2008; Galarza et al. 2015; Morán-Diez et al. 2020). These beneficial fungi enhance the plant nutrient use efficiency (NUE) (Fiorentino et al. 2018) and contribute to the synchronisation of increased photosynthetic capacity and carbohydrate metabolism (Woo et al. 2014; Pascale et al. 2017). Furthermore, they have been found to promote plant growth, improve yields and enhance the quality of agricultural produce (Harman et al. 2004; Stewart, Hill 2014; Colla et al. 2015). Two species of *Trichoderma*, specifically *Trichoderma longibrachiatum* and *Trichoderma atroviride*, are widely recognised as common saprophytic fungi in the soil. These species are environmentally mild and pose no toxicity risks to human health (Kareem et al. 2016). *Trichoderma* strains are known for boosting the plant's defence mechanisms, specifically by increasing the levels of enzymes and pathogenesis-related (PR) proteins that are integral to a plant's immunity (Yedidia et al. 1999). An example of such proteins is β-1,3-glucanase (PR-2), which is known to inhibit the growth of several plant pathogens (Kauffmann et al. 1987). Additionally, the response to common plant diseases often involves the action of peroxidase (POD) enzymes and the production of phenolic compounds. Plant phenolics are secondary metabolites that constitute one of the most common and widespread groups of substances in plants. Phenolics biologically arise from the shikimate phenylpropanoids-flavonoids pathways (Lattanzio et al. 2006). The plant needs phenolic compounds for pigmentation, growth, resistance to pathogens, and for many other functions. However, the phenolic response in a plant, depending on the plant (cultivar, age and the part of the plant), in a resistant cultivar was reported to be associated with resistance to pest infection and the resistance gene. Therefore, the objectives of the present study were to investigate the response of both resistant and susceptible tomato cultivars to *Trichoderma harzianum* inoculation in incompatible and compatible interactions with the *Fol*

MATERIAL AND METHODS

Plant materials. This experiment was conducted in a greenhouse of the Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand, from March to July 2022. Five tomato cultivars were selected for this study based on their response to Fusarium wilt disease (*Fusarium oxysporum*). 'Seedathip 4' was used as the susceptible control, while the four others ('MT26', 'MT27', 'MT39', and 'CLN3682F') were resistant (authors' unpublished data). All the genotypes were sown in polystyrene transplant flats filled with a peat moss-based commercial media. Seedlings were identified for treatment ten days after germination, at the point when they began to form true leaves.

Experimental design and *Trichoderma* treatment. The experiment was conducted in a factorial randomised complete block design (RCBD) with two treatments and three replications. *Factor A* consisted of five tomato cultivars, and *Factor B* involved the *Trichoderma* treatments. We selected 60 seedlings (at 10 days old) from each tomato cultivar and divided them into two groups for the study. The first

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group was treated with *Trichoderma harzianum* at one-week intervals, four times. In contrast, the second group remain untreated as a control. We prepared a solution using an antagonistic commercial fungal isolate of *Trichoderma harzianum* for the *Trichoderma* treatment. The solution was prepared from powder. The powder was dissolved at 50 g/20 L in distilled water. Each tomato seedling in the treatment group received 3 mL of this solution weekly, applied directly via watering.

Preparation of the *Fol* inoculation, soil infestation, and evaluation. The virulent isolation of *Fol* race 1, isolate TFPK 401, was collected from tomato fields at Sakon Nakhon province. The pathogen was purified by the Department of Agriculture and Resources, Faculty of Natural Resources and Agro-Industry, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon, Thailand. For the inoculation, the *Fol* isolate was cultured on potato dextrose agar (PDA) and grown for 10 days. The peat moss media used for the seedling growth was sterilised by autoclaving at 121 °C for 20 minutes. Twenty-one-day-old seedlings were inoculated with a spore suspension of the *Fol* isolate, achieving a concentration of 1×10^6 spores/mL by the root drip method. The disease severity was subsequently evaluated at 7, 14, 21 and 28 days after inoculation using a scoring system on a scale of 0 to 4, with the following criteria: 0 – no symptoms (HR – highly resistant); 1 – one leaf wilted or starting to slightly yellow; 2 – two or three leaves wilted or yellow; 3 – half the plant wilted or/and yellow; 4 – all the leaves (whole plant) wilted or/and a yellow or a dead plant.

Peroxidase activity (POD). Tomato leaf samples were collected four weeks after inoculation with *Fol.* The POD was analysed and adapted from Malik and Singh (1980) with slight modifications. Approximately 0.4 g of either the second or third leaves from the apex of the plants in each treatment group was harvested. These samples were subsequently homogenised in a chilled 1.5 mL of 100 mM sodium phosphate buffer (pH 7.0). Following the homogenisation, the mixture was centrifuged at 10 000 rpm for 30 min at 5 °C. The supernatant (20 µL) was carefully collected and mixed with 2.98 mL of sodium phosphate buffer (pH 6.0), 250 µL of guaiacol, and 306 µL of H_2O_2. The reaction mixture's absorbance was measured at 470 nm by measuring the decrease in absorbance per minute, which facilitated the calculation of the POD activity.

Total phenolic content (TPC). TPC was determined using the Folin-Ciocalteu method (Dadáková et al. 2020) with some modifications. Tomato leaves were harvested and dried at 60 °C. Fifty (50) mg of dried leaves were used for extraction with 80% methanol by vortexing for 1 min, sonicating for 10 min, and incubating at 750 rpm for 30 min at 25 °C. One hundred fifty (150) µL of the Folin-Ciocalteu reagent was added to 300 µL of the extract, followed by 100 µL of a sodium carbonate solution (5 g/L) and 2 250 µL of water after 3 minutes. The mixture was incubated at 50 °C for 16 minutes. Then, the absorbance was measured at 765 nm and compared to that obtained from the blank.

Data analysis. The disease severity index (DSI, %) of each cultivar was calculated as a percentage of the disease incidence by the following formula:

$$\text{DSI (\%)} = \Sigma\,[(n \times v)/(N \times V)] \times 100\%$$

where: DSI – disease severity index; n – class frequency; v – score of the rating class; N – total number of plants; V – maximal disease incidence.

A factorial in RCBD with three replications was analysed. The means of each trait studied were compared using an analysis of variance (ANOVA), and the treatment mean was compared with LSD (least significant difference) ($P = 0.05$) using Statistix version 10 software.

RESULTS AND DISCUSSION

Effect of *Trichoderma* antagonists against the Fusarium wilt pathogen. The responses of five tomato cultivars in the plants treated with and without *Trichoderma harzianum* (*Trichoderma*) to *Fol* are given in Table 1. Based on the Fusarium wilt disease symptoms, the tomato cultivars showed significantly different responses. 'CLN3682F' and the susceptible check 'Seedathip 4' exhibited the highest susceptibility to *Fol* in the first week after inoculation, with a disease severity index (DSI) of 60.58% and 44.79%, respectively. 'MT26', 'MT27', and 'MT39' exhibited a lower DSI of 30.75%, 29.21% and 33.61%, respectively. In the 2nd to 4th weeks after inoculation, all the tomato cultivars showed significant differences in the disease progression. 'CLN3682F' showed the highest susceptibility to the disease, while 'MT26' showed the lowest susceptibility. This evidence suggests that the tomato cultivar is the main fac-

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Table 1. Fusarium wilt disease severity index, peroxidase activity and total phenolic content of five tomato cultivars under *Trichoderma harzianum* treatment in untreated and treated conditions

Treatments	Fusarium wilt disease severity index (%)				Peroxidase activity (min/g)					Total phenolic content (mg/g fresh weight)				
	weeks after treatment and inoculation													
	1	2	3	4	pre-1	pre-2	post-1	post-2	post-3	pre-1	pre-2	post-1	post-2	post-3
Cultivar														
'Seedathip 4'	44.7^{ab}	52.39^{b}	60.73^{b}	58.12^{b}	0.18^{c}	0.12^{bc}	0.52^{c}	0.71	1.31^{a}	1.70	3.02^{ab}	2.23	2.10^{b}	2.43^{b}
'MT26'	30.75^{b}	32.65^{b}	40.61^{c}	38.63^{b}	0.42^{b}	0.23^{a}	0.74^{ab}	0.35	0.49^{b}	1.74	3.04^{ab}	1.87	2.42^{a}	2.74^{a}
'MT27'	29.21^{b}	36.25^{b}	51.24^{bc}	49.72^{b}	0.72^{a}	0.17^{ab}	0.86^{a}	0.41	1.18^{a}	1.79	3.28^{a}	2.19	2.57^{a}	2.77^{a}
'MT39'	33.61^{b}	30.57^{b}	42.13^{c}	39.48^{b}	0.27^{c}	0.04^{c}	0.56^{bc}	0.44	0.61^{b}	1.57	2.90^{ab}	2.39	2.34^{a}	2.63^{ab}
'CLN3682F'	60.58^{a}	80.15^{a}	82.14^{a}	81.05^{a}	0.21^{c}	0.11^{bc}	0.41^{c}	0.75	0.51^{b}	1.45	2.62^{b}	2.40	2.38^{a}	2.81^{a}
F-test	**	*	*	*	*	*	*	ns	*	ns	*	ns	*	*
Trichoderma harzianum														
Untreated	37.48	43.77	59.41	56.98	0.22^{B}	0.10^{B}	0.52^{B}	0.36^{B}	0.68^{B}	1.50	2.79^{B}	2.20	2.24	2.65
Treated	42.10	49.03	51.33	49.81	0.49^{A}	0.17^{A}	0.71^{A}	0.70^{A}	0.96^{A}	1.74	3.15^{A}	2.23	2.49	2.70
F-test	ns	ns	ns	ns	*	*	*	*	*	ns	*	ns	ns	ns

pre-1, 2 – pre-inoculation week 1, 2; post-1, 2, 3 – post-inoculation week 1, 2, 3; ns – non-significant

*,**significance at 0.05, 0.01 probability level, respectively

$^{a–c}$values with different lowercase letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT); $^{A–B}$values with different capital letters are significantly different at $P \leq 0.05$ between treatment groups

tor influencing the resistance to this disease (Chitwood-Brown et al. 2021). However, limited information on the resistance mechanisms responsible for host-pathogen interactions is available. In resistant plants, these mechanisms play a crucial role in preventing infection and colonisation by fungal diseases or recognising them (Chitwood-Brown et al. 2021). Many barriers to infection exist in the root zone, including root exudates, the tissue structure, and the timing and magnitude of the response to the invasive growth of a pathogen (Baetz, Martinoia 2014; Gordon 2017). In this study, we found new lines of resistance to Fusarium wilt disease. Specifically, the resistant cultivars 'MT26' and 'MT27' originate from 'Hawaii7996' and 'R-3034-3-10-N-UG', respectively, which are selected based on their good adaptation to the environment in Thailand. These lines were sourced from The World Vegetable Center in Taiwan and have been reported to have resistance to bacterial wilt disease. Interestingly, during the initial week of treatment, the tomato plants treated with *Trichoderma* showed a higher DSI (42.10%) than the untreated plants (7.48%), although this difference was not significant. However, by the final week, the DSI was different to the initial week. Figure 1 shows the response of each tomato cultivar to the Fusarium wilt disease. In all the cultivars, responding in a similar direction and treating the plants with *Trichoderma* decreased the DSI. This suggests that *Trichoderma* may improve the resistance to Fusarium wilt disease (Houssien et al. 2010). Moreover, Hibar et al. (2007) reported that *Trichoderma* has an impact on inducing systemic resistance in plants against this plant pathogen, because *Trichoderma* activated the resistance to Fusarium wilt disease, such as through the polyphenols, pathogenesis-related (PR) proteins (Metraux 2001). Therefore, we suggest using a resistant cultivar combined with the applied *Trichoderma* to manage tomato Fusarium wilt disease under open field conditions. The optimal application of *Trichoderma* for soil preparation should occur approximately 14 days prior to transplanting. This timing is crucial as it allows the *Trichoderma* mycelium ample opportunity to establish itself within the plant root zone. This colonisation process plays a pivotal role in enhancing the root growth and ultimately boosting the overall plant productivity (Harman 2000).

Analysis of the peroxidase (POD) activity. The reaction of the POD activities of five tomato cultivars to *Trichoderma* treatments and infection of *Fol* is given in Table 1. The difference in the tomato cul-

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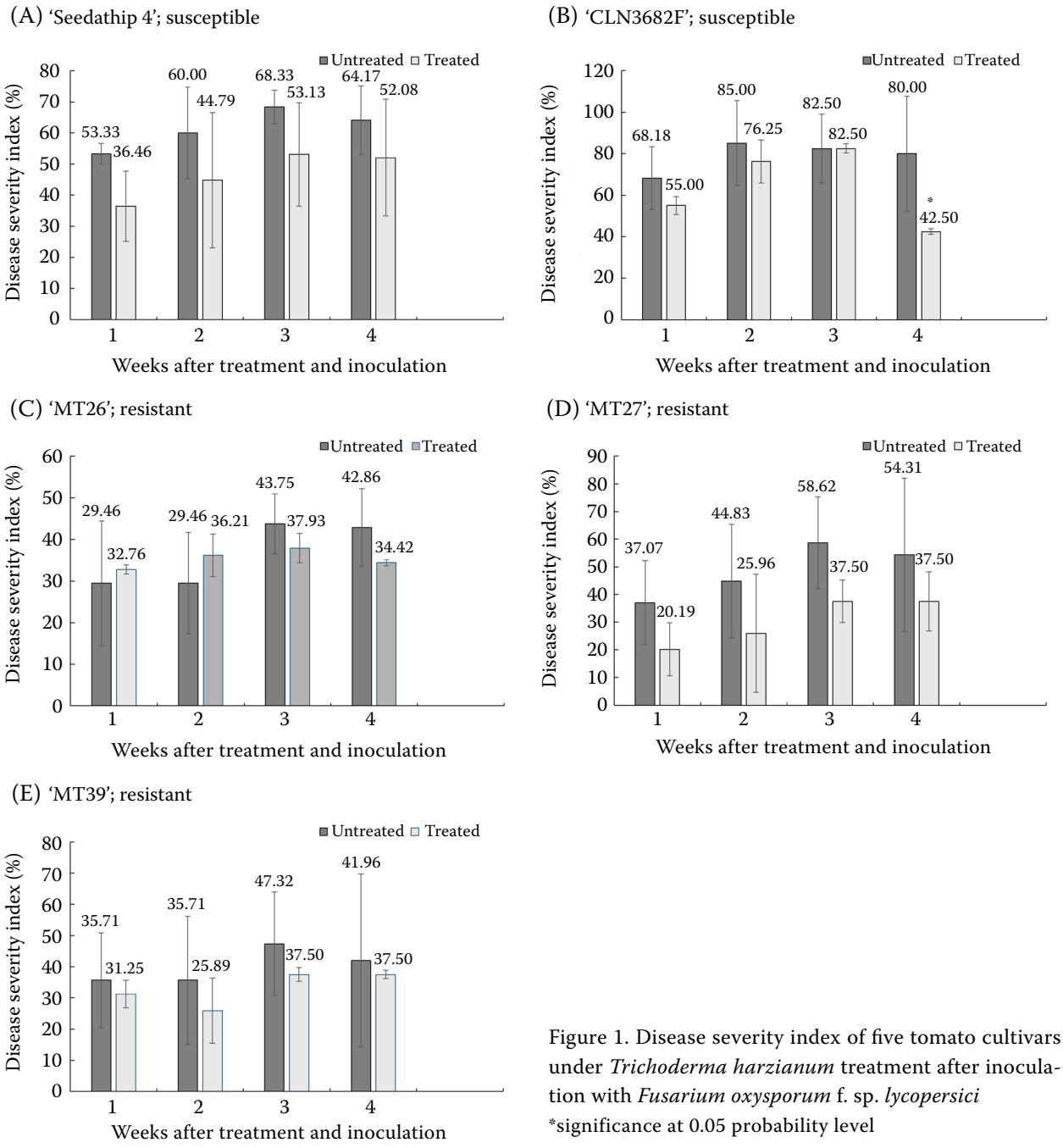

Figure 1. Disease severity index of five tomato cultivars under *Trichoderma harzianum* treatment after inoculation with *Fusarium oxysporum* f. sp. *lycopersici*
*significance at 0.05 probability level

tivars and *Trichoderma* treatments had an impact on the POD activities (Figure 2). The POD activity was found to be highest in 'MT26' and 'MT27' (resistant cultivar) before and after inoculation. In the resistant cultivars ('MT26', 'MT27'), the POD activity increased sharply after the *Fol*-challenged inoculation, and there was a significant difference between the resistant and susceptible cultivars. This is the point of the resistant plant defence mechanism against pathogens. Subsequently, the POD activity decreased in the resistant cultivars, but increased in the susceptible cultivars. All the cultivars showed an increase in the 3rd week after the *Fol* infection. Therefore, the increase in POD at the early stage might have contributed more to the enhanced disease infection than the increase in the late stage (Ojha, Chatterjee 2012). However, after the disease seriously attacked the susceptible cultivars, they may have been prevented from producing the enzymatic defence system. In response to *Trichoderma* treatment in the 1st and 2nd weeks before inoculation with the *Fol*, the POD activ-

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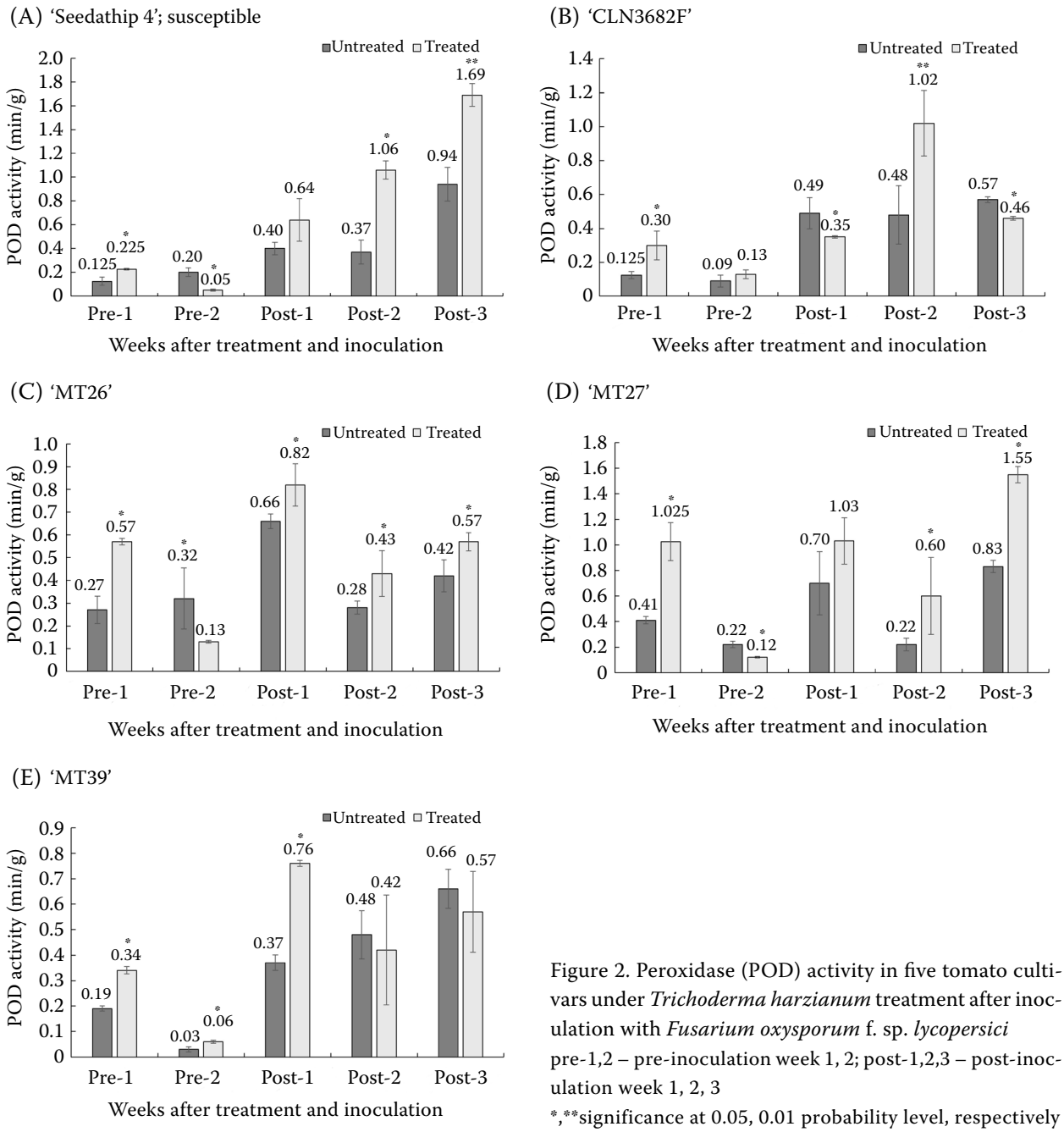

Figure 2. Peroxidase (POD) activity in five tomato cultivars under *Trichoderma harzianum* treatment after inoculation with *Fusarium oxysporum* f. sp. *lycopersici*

pre-1,2 – pre-inoculation week 1, 2; post-1,2,3 – post-inoculation week 1, 2, 3

*,**significance at 0.05, 0.01 probability level, respectively

ity in the treated plants was higher than in the untreated plants (Table 1, Figure 2). This indicates that *Trichoderma* alone can stimulate the POD enzyme activity. In addition, after the plants were infected with the *Fol*, they sharply increased their POD activity in both the untreated and treated plants. Nevertheless, the treated plants showed POD activity higher than the untreated ones. Therefore, applying *Trichoderma* to the growing media at the seedling stage can induce systemic resistance in tomatoes in susceptible and resistant cultivars. Moreover, POD can be stimulated by biotic stress, bio-inducer treatment, and plant development (Houssien et al. 2010; Ojha, Chatterjee 2012).

Analysis of the total phenolic compound (TPC) content. Figure 3 shows that TPC in all the tomato cultivars increased after treatment with *Trichoderma*, with the highest increase observed in the 1st week of pre-inoculation with the *Fol* (Table 1). In the 2nd week of pre-inoculation, the TPC content of the treated plants was significantly higher than that of the untreated plants. Among

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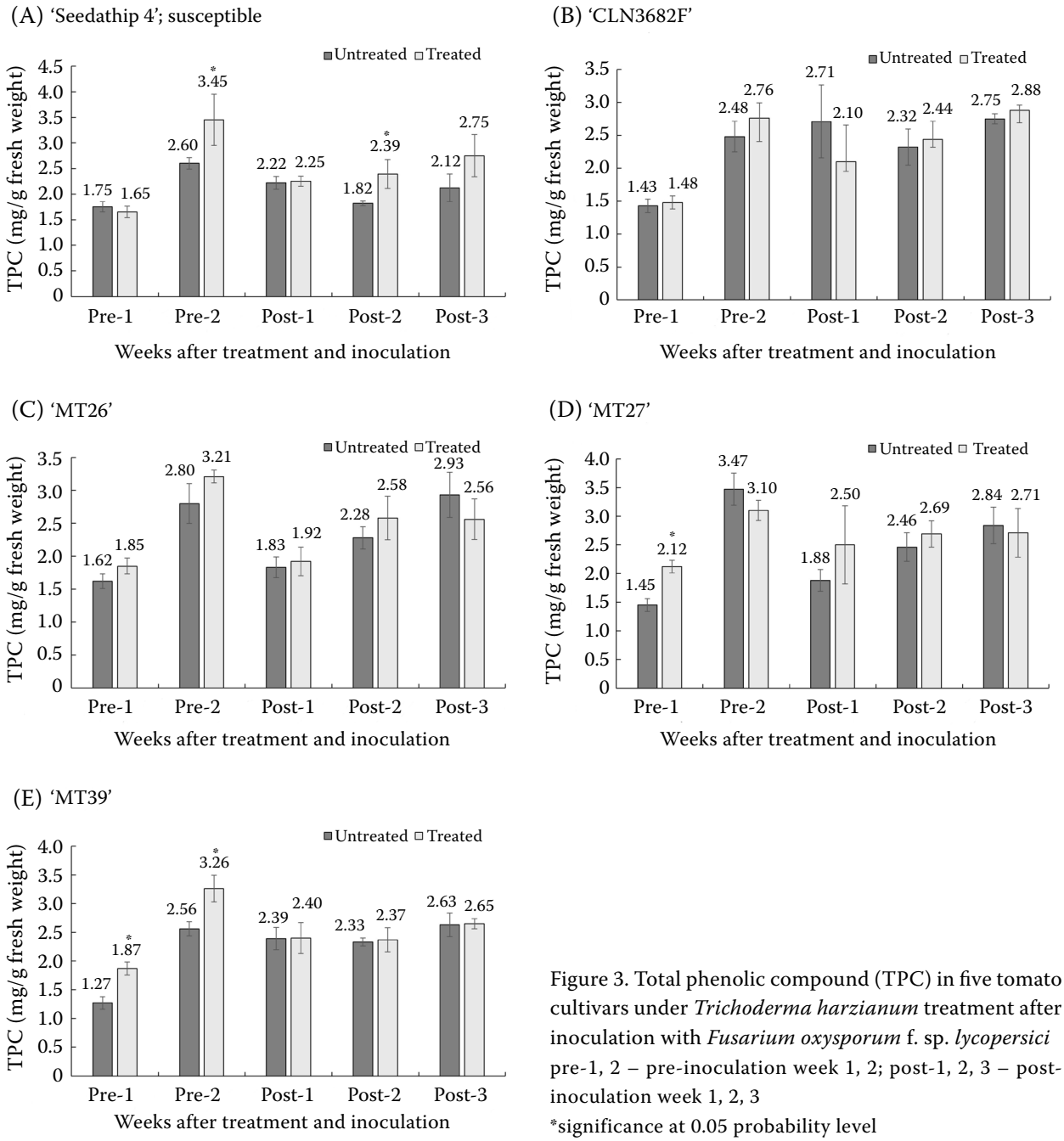

Figure 3. Total phenolic compound (TPC) in five tomato cultivars under *Trichoderma harzianum* treatment after inoculation with *Fusarium oxysporum* f. sp. *lycopersici*
pre-1, 2 – pre-inoculation week 1, 2; post-1, 2, 3 – post-inoculation week 1, 2, 3
*significance at 0.05 probability level

the five tomato cultivars, 'CLN3682F' had the lowest TPC content (2.62 mg/g fresh weight) as compared to the other cultivars, but after the plants were attacked by *Fol*, all the cultivars reduced the TPC content in both the treated and untreated plants with *Trichoderma*. However, in the resistant cultivar group ('MT26', 'MT27' and 'MT39'), the TPC content in the treated plants was higher than the TPC content in the untreated plants, while in the susceptible cultivar group ('Seedathip 4' and 'CLN3682F'), it was lower than in the untreated ones. This evidence could be explained by the fact that the phenolic compound is an antimicrobial agent involved in plant defence responses (Piao et al. 2013; Silva-Beltrán et al. 2015). Their elevated presence in resistant cultivars suggests better adaptation to biotic stress, as phenolics have been reported to function as an adaptive trait (Pedley, Martin 2003; Lattanzio et al. 2006). However, the TPC contents also vary on the basis of the plant genotype, development stage and environmental conditions (Dadáková et al. 2020).

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CONCLUSION

'MT26', 'MT27', and 'MT39' are newly developed breeding lines that are resistant to *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*). Treatment with *Trichoderma* has been shown to enhance resistance to *Fol* across all tomato cultivars. However, the defence mechanisms that play a pivotal role in controlling *Fol* infections are more pronounced in resistant cultivars in the early infection stages. These defence mechanisms include POD activity and phenolic compound production. Conversely, the susceptible tomato cultivars did not exhibit these defence mechanisms. Consequently, resistant cultivars have superior adaptive defences against biotic stress compared to susceptible cultivars.

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