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Local *Trichoderma* strains as a control strategy of complex black root rot disease of strawberry in Egypt

Farid Abd-El-Kareem, Ibrahim E. Elshahawy and Mahfouz M. M. Abd-Elgawad*

Abstract

Background: Economics and human safety to avoid health risks caused by fungicides are materializing new era of biological pest control. *Trichoderma* species ranked high among other agents to control complex black root rot disease of strawberry caused by *Fusarium solani*, *Rhizoctonia solani*, and *Pythium* sp. Our study aimed to document the efficacy of local strains representing *T. harzianum*, *T. viride*, *T. virinis*, and *T. koningii* against such a disease.

Materials/methods: These strains were cultured separately on potato dextrose broth medium to test their inhibitory effect against strawberry black root rot in vitro and in vivo. Strawberry growth and yield were also assessed relative to the untreated check and the fungicide Actamyl. Activity of peroxidase and chitinase were measured in plant leaves using spectrophotometer.

Results: Each of the antagonistic fungal strains significantly reduced growth area of all pathogenic fungi collectively causing the disease. *Trichoderma harzianum*, *T. viride*, and *T. koningii* reduced the growth area more than 90.6% for all tested pathogenic fungi. Each species significantly reduced disease incidence and severity under field conditions. The highest reduction in the disease incidence and severity, 83.3 and 88.5% respectively, was attained by mixture of the four species. This mixture increased the strawberry fresh and dry weight by 83.3 and 176.9%, respectively, and the yield by 117.1%. All *Trichoderma* species tested significantly increased the activity of two plant defense-related enzymes of strawberry plants against the pathogens. Their mixture attained the highest increase of peroxidase and chitinase activity by 150 and 160.9%, respectively.

Conclusions: While the fungal mixture could considerably increase the strawberry fresh and dry weight as well as the yield, it suppressed the incidence and severity of the disease. So, integrated pest management in ways that make these biocontrol agents complementary or superior to chemical fungicides should further be examined against this disease.

Keywords: Biocontrol, Black root rot, Enzyme activity, Strawberry, *Trichoderma*

Background

Black root rot is a complex disease because several fungi usually work together giving rise to its occurrence (Fang et al. 2012; Hutton et al. 2013; Juber et al. 2014). This complex disease is characterized by feeder rootlet killing, deterioration and blackening of the main root system, and a decline in vigor and productivity of the plant stand causing considerable reduction in the yield (Abdet-Sattar et al. 2008; Ceja-Torres et al. 2014).

Biological control agents rank high among other fungal management options given mounting care to lessen application of chemical fungicides with a clear aim at the avoidance of human health hazards and attaining pollution-free environment. *Trichoderma* spp. can be considered as ideal biocontrol agents for their good characteristics. Using *T. harzianum* and *T. viride* as seedling dressing to suppress root rot of strawberry and improve its yield components has long been tried (Sullivan 2004). Both fungi showed dual effect by producing growth regulators (Karlidag et al. 2012) and antioxidant effect, thus improving plant physiology and metabolism (Hernandez et al. 2011).

* Correspondence: mahfouzian2000@yahoo.com
Plant Pathology Department, National Research Centre, El-behooth St., Dokki, 12622, Giza, Egypt

Ahmed and El-Fiki (2017) reported that *T. harzianum*, *T. album*, *T. viride*, and plant guard were highly effective antagonists as shown by supporting strawberry plant survival against root rot disease. So, those treatments scored best fruit yields under field conditions. Moreover, application of these antagonists recorded the highest increase in total phenols, total nitrogen percentage, and total chlorophyll of strawberry in comparison with the untreated check. The mechanisms of *Trichoderma* action in plant protection have been recorded. For example, *T. harzianum* secretes many lytic enzymes like chitinase, glucanases, and proteases which help parasitism of plant pathogens. The chitin layer of the pathogen is dissolved via enzymatic activity. The hyphae of *T. harzianum* penetrate the pathogen, proliferate within the organism, and produce toxic metabolites. Likewise, *T. viride* produces antibiotics like trichodermin, dermadin, trichoviridin, and sesquiterpene heptalic acid which are involved in the suppression of the pathogens (Abd-Elgawad and Askary 2018).

Trichoderma album, *T. hamatum*, *T. harzianum*, and *T. viride* were reported to significantly reduce the mycelial growth of *Fusarium solani* and *Rhizoctonia solani*. In this respect, the activity of plant defense-related enzymes against pathogens such as chitinase, peroxidase, and polyphenol oxidase was increased in treated strawberry plants compared to untreated ones (Cherif et al. 2007; Latha et al. 2009; Prlak and Kose 2009; Saksirirat et al. 2009).

Currently, Egypt is experiencing a revival of the strawberry cultivation backed by its Mediterranean climate, fertile soils, and geographic location, which support high production and profitability of such a specialty crop. These factors can collectively offer early fruiting and long harvest season, good quality, low production costs, and closeness of export markets (Abd-Elgawad 2019). However, a few pests and diseases can cause considerable yield losses in the crop size and quality. Therefore, updated comprehensive revisions to control strawberry white grub or rootgrub *Temnorhynchus baal* (Shehata et al. 2019), strawberry leaf blight disease caused by *Phomopsis obscurans* (Abd-El-Kareem et al. 2019a), and plant-parasitic nematodes (Abd-Elgawad 2019) are in progress. Field application and commercialization of such biocontrol agents are being investigated in Egypt and worldwide (e.g., Ziedan et al. 2011 and Ziedan et al., 2015; El-Mougy and Abdel-Kader 2014; Abd-Elgawad and Vagelas 2015; Abd-Elgawad 2017; Abd-Elgawad and Askary 2018). Yet, little attention is given to black root rot of strawberry which ranks high among strawberry diseases (El-Shemy et al. 2013). The aim of this study is to document the effects of some local *Trichoderma* spp. as safe biocontrol agents against strawberry black root rot under field conditions in Egypt. Also, some of the species herein have not been tested against the disease yet.

Materials and methods

Pathogens

Local pathogenic isolates of *F. solani*, *R. solani*, and *Pythium* sp., the causal agents of black root rot disease of strawberry plants, in addition to four antagonistic species of *Trichoderma*, i.e., *T. harzianum*, *T. viride*, *T. virinis*, and *T. koningii* were identified and provided by the Plant Pathology Department, National Research Centre, Giza, Egypt (Elshahawy et al. 2017; Abd-El-Kareem et al. 2019b).

Plant material and inoculum preparation

Strawberry seedlings (cv. Festival) were obtained from the Vegetable Crops Research Department, Agricultural Research Centre, Giza, Egypt. Inocula of antagonistic fungi were prepared by culturing each isolate on 50 ml potato dextrose broth (PDB) medium in 250-ml Erlenmeyer flasks for 15 days at 25 ± 2 °C. Inoculum of each fungus was prepared from its growing upper solid layers which were washed and blended in sterilized water. Colony-forming units (cfu) were adjusted to 10^6 cfu/ml using hemocytometer slide (Harman et al. 2004). A few drops of the emulsifier Tween 20 (Sigma Co.) and sticker were added.

Laboratory test

Trichoderma harzianum, *T. viride*, *T. virinis*, and *T. koningii* were tested for their inhibitory effect against strawberry black root rot fungi, i.e., *R. solani*, *F. solani*, and *Pythium* sp. in vitro. Each of the obtained fungal antagonist and the pathogenic fungus was grown on potato dextrose agar (PDA) medium for 7 days at 25 ± 2 °C. Disk of individual antagonistic fungi and disk of each pathogenic fungi were placed on opposite sides of Petri plates containing the PDA medium (John et al. 2010). Inoculated plates were incubated for 7 days at 25 ± 2 °C. Five plates for each particular treatment were used as replicates. The plates were then examined and growth area of plates inoculated only with the pathogenic fungus was measured. The reduction percent in growth area of the pathogenic fungi was calculated using the formula as follows: reduction% = $[(C - T)/C] \times 100$, where C is the average growth of pathogenic fungi in control treatment (cm^2) and T is the average growth of pathogenic fungi in presence of antagonistic fungus (cm^2).

Experimental design and treatments

Trichoderma harzianum, *T. viride*, *T. virinis*, and *T. koningii* were applied alone or in combination to study their effect on strawberry black root rot disease under field conditions. Actamyl 3 g/L (fungicide) and an untreated control were considered for comparison with the biocontrol efficacy as well as strawberry yield and enzyme activities. Experiments were carried out at fields previously known to be highly infested with strawberry

black root fungi at El-Qalioubia governorate, Egypt. So, naturally infested plots (4 × 8 m), each comprised of 8 rows (32 holes/row and one seedling was sown in each hole), in a randomized complete block design with three replicates (plots) for each treatment were established. Strawberry seedlings were planted in loamy clay, well-drained soil to a depth of 10 cm. Irrigation and nutrition and other agricultural practices were carried out as recommended (El-Shemy et al. 2013). Before planting, strawberry seedlings were dipped in spore suspension (10^6 cfu/ml) of each *Trichoderma* species for 5 min as single and combined treatments. The rhizosphere of each plant also received 80 ml of spore suspension (10^6 cfu/ml) for each species alone or in combination every 15 days, i.e., six times for a total of 3 months during which black root rot disease of strawberry usually grows and spreads rapidly under natural conditions.

Disease assessment

The percentages of disease incidence were calculated 100 days after transplanting as follows:

$$\text{Disease incidence} = (\text{number of infected plants}/\text{total number of plants}) \times 100$$

Disease severity (DS) was recorded at the end of experiment according to original scale developed to evaluate a particular disease (Morocko 2006) as follows: 0 = plant well developed, no disease symptoms; 1 = no visible symptoms on above ground parts, 25% of roots discolored; 2 = plant slightly stunted, black necrosis on petiole bases, 26–50% of roots discolored; 3 = plant stunted, black necrosis on petiole bases, yellowing and death of outer leaves, 51–75% of roots discolored; 4 = plant severely stunted, outer leaves collapsed, younger leaves bluish green and wilting, > 75% of roots discolored; and 5 = plant dead.

$$\text{Disease severity}\% = \frac{\Sigma\ (\text{Disease grade} \times \text{number of plants in each grade})}{\text{Total number of plants} \times \text{highest disease grade}} \times 100$$

Effects on plant growth and yield

Fresh and dray weight/plant were measured after 90 days of planting. Accumulated strawberry yield (tons/feddan) for each treatment was determined.

Determination of enzyme activities

Extraction of enzymes

Plant leaves (g) were homogenized with 0.1 M sodium phosphate buffer (pH 7.1) at the rate of 1/3 w/v (Goldschmidt et al. 1968). The homogenate was centrifuged at 3000 rpm for 15 min. The supernatant was used to determine activities of the following enzymes.

Peroxidase assay

Peroxidase activity was measured by incubating 0.1 ml of enzyme extract with 4 ml of guaicol solution for 1 min at 25 °C and absorbance at 470 nm. The guaicol solution consisted of 3 ml of 0.05 M potassium phosphate, pH 7, 0.5 ml of 2% guaicol, and 0.5 ml of 0.3% H_2O_2 (Abeles et al. 1971). Peroxidase activity was expressed as the increase in absorbance at 470 nm/gram fresh weight/1 min using spectrophotometer (Spectronic 20-D).

Chitinase assay

The substrate colloidal chitin was prepared from chitin powder according to the method described by Ried and Ogryd-Ziak (1981). Determination of chitinase activity was carried out according to the method of Monreal and Reese (1969). One milliliter of 1% colloidal chitin in 0.05 M citrate phosphate buffer (pH 6.6) in test tubes and 1 ml of enzyme extract were added and mixed by shaking. Tubes were kept in a water bath at 37 °C for 60 min, then cooled and centrifuged before assaying. Reducing sugar was determined in 1 ml of the supernatant by dinitrosalicylic acid. The reaction was stopped by heating the tubes for 5 min at 100 °C. The tubes were cooled and 3 ml of distilled water was added before assay. Optical density was determined at 540 nm using spectrophotometer (Spectronic 20-D). Chitinase activity was expressed as mM *N*-acetylglucoseamine equivalent released/gram fresh weight tissue/60 min.

Statistical analysis

Tukey test for multiple comparisons among means was utilized (Neler et al. 1985).

Results

Laboratory test

The results in Table 1 indicated that all tested species of *Trichoderma* significantly reduced growth area of the three pathogenic fungi. The highest reduction in growth area was obtained with *T. harzianum*, *T. viride*, and *T. koningii* which reduced the growth area by more than 90.6% for the tested fungi. Meanwhile, *T. virinis* was less effective.

Field experiment

Effect on strawberry disease incidence and severity

The results in Table 2 revealed that the four species of *Trichoderma* significantly reduced disease incidence and severity. The highest reduction was obtained with their combined mixture which reduced the disease incidence and severity by 83.3 and 88.5% respectively. Individual treatment of *T. harzianum*, *T. viride*, and *T. koningii* resulted in reducing the disease incidence and severity by more than 70.8 and 74% respectively. Meanwhile, *T. virinis* was less effective.

Table 1 Effect of four *Trichoderma* spp. on growth area of strawberry black root rot disease

Treatment	*F. solani*		*R. solani*		*Pythium* sp.	
	Growth area (cm^2)	Reduction, %	Growth area (cm^2)	Reduction, %	Growth area (cm^2)	Reduction, %
Trichoderma harzianum	5.0c	92.1	5.3c	91.7	4.2c	93.4
T. viride	5.3c	91.7	4.5c	92.9	6.2c	90.3
T. virinis	8.0b	87.4	8.5b	86.6	10.0b	84.3
T. koningii	5.0c	92.1	4.2c	93.4	6.0c	90.6
Control	63.6a	0.0	63.6a	0.0	63.6a	0.0

Figures in a column with the same letter are not significantly ($P \leq 0.05$) different using Tukey test

Effect on some vegetative characters

The results in Table 3 showed that all tested species of *Trichoderma* significantly increased fresh and dry weight of strawberry plants. The highest increase was obtained with the mixture (*T. harzianum* + *T. viride* + *T.* virinis + *T. koningii*) which increased the fresh and dry weight by 83.3 and 176.9%, respectively. Individual treatment of *T. harzianum*, *T. viride*, and *T. koningii* increased fresh and weight by more than 105 and 68%, respectively. Meanwhile, *T. virinis* was less effective.

Effect on strawberry yield

The results in Table 4 indicate that all tested species of *Trichoderma* significantly increase strawberry yield. The highest increase was obtained with mixture of (*T. harzianum* + *T. viride* + *T.* virinis + *T. koningii*) which increased the yield by 117.1%. Individual treatment of *T. harzianum*, *T. viride*, and *T. koningii* resulted in increasing yield by 71.1, 57.1, and 64.3% respectively. Meanwhile, *T. virinis* was less effective.

Effect on enzyme activities

The results in Table 5 revealed that all tested *Trichoderma* species significantly increased activities of strawberry enzymes. The highest increase was obtained with their mixture which enhanced the peroxidase and chitinase activity by 150 and 160.9%, respectively. Individual treatment of *T. harzianum*, *T. viride*, and *T. koningii* increased peroxidase and chitinase activity by more than 100 and 117.4%, respectively. Meanwhile, *T. virinis* was less effective.

Discussion

Black root rot is a complex disease caused several fungi (Fang et al. 2012; Hutton et al. 2013; Juber et al. 2014). This complex disease is characterized by feeder rootlet killing, deterioration and blackening of the main root system, and a decline in vigor and productivity of the plant stand causing damage to the host and considerable reduction in the yield (Abdet-Sattar et al. 2008; Ceja-Torres et al. 2014). Using *T. harzianum* and *T. viride* as seedling dressing for strawberry suppression of root rot and improving yield components has long been tried (Sullivan 2004). Results in the present study indicated that all tested species of *Trichoderma* significantly reduced growth area of the pathogenic tested fungi. A considerable reduction in growth area was obtained with *T. harzianum*, *T. viride*, and *T. koningii*. Field experiment results revealed that all tested species of *Trichoderma* significantly reduced disease incidence and severity. The highest reduction was obtained with their mixture. In this respect, *Trichoderma* species have been known to evolve numerous mechanisms for enhancing plant and root growth (Harman and Bjorkman 1998). Thus, the colonization of the root system by rhizosphere competent strains of *Trichoderma* may result in increasing development of roots and/or aerial systems and crop yields. Other activities like the induction of plant systemic resistance and antagonistic effects on plant pathogenic nematodes have also been proposed (Sharon et al. 2001; Roatti et al. 2013; Harel et al. 2014). It is probable that during the plant-*Trichoderma* interactions, the

Table 2 Effect of four *Trichoderma* spp. on black root rot disease of strawberry plants under field conditions

Treatment	Black root rot disease			
	Disease incidence	Reduction, %	Disease severity	Reduction, %
Trichoderma harzianum	14.0c	70.8	10.0d	80.8
T. viride	12.0c	75.0	10.0d	80.8
T. virinis	21.0b	56.3	20.4b	60.8
T. koningii	14.0c	70.8	13.5c	74.0
T. mixture (T. h. + T. v. + T. v. + T. k.)	8.0d	83.3	6.0e	88.5
Actamyl 3 g/l (fungicide)	12.0c	75.0	13.0c	75.0
Control	48.0a	0.0	52.0a	0.0

Figures in a column with the same letter are not significantly ($P \leq 0.05$) different

Table 3 Effect of four *Trichoderma* spp. on some vegetative characters of strawberry plants under field conditions

Treatment	Weight (g)/plant			
	Fresh	Increase, %	Dry	Increase, %
Trichoderma harzianum	205.0b	105.0	32.0b	68.0
T. viride	200.0b	66.7	30.0b	130.8
T. virinis	180.0c	50.0	26.0c	100.0
T. koningii	202.0b	102.0	32.0	68.0
T. mixture (T. h. + T. v. + T. v. + T. k.)	220.0a	83.3	36.0a	176.9
Actamyl 3 g/l (fungicide)	160.0d	33.3	24.0d	84.6
Control	120.0e	0.0	13.0e	0.0

Figures in a column with the same letter are not significantly ($P \leq 0.05$) different

Table 5 Effect of four *Trichoderma* spp. on enzyme activities of strawberry plants under field conditions

Treatment	Enzyme activities			
	Peroxidase		Chitinase	
	Activity	Increase,%	Activity	Increase, %
Trichoderma harzianum	2.2b	120.0	5.0b	117.4
T. viride	2.1b	110.0	5.1b	121.7
T. virinis	1.5c	50.0	4.6c	100.0
T. koningii	2.0b	100.0	5.0b	117.4
T. mixture (T. h. + T. v. + T. v. + T. k.)	2.5a	150.0	6.0a	160.9
Actamyl 3 g/l (fungicide)	1.5c	50.0	4.8c	108.7
Control	1.0d	0.0	2.3d	0.0

Figures in a column with the same letter are not significantly ($P \leq 0.05$) different

fungus participates actively in protecting and improving its ecological niche. Such dual roles make *Trichoderma* strains appealing alternatives to soil fumigation technologies such as methyl bromide. Strains of *Trichoderma* may also be aggressive bio-degraders and act as competitors to fungal pathogens in their saprophytic phases, especially when nutrients are a limiting factor (Simon and Sivasithamparam 1989; Mansoori et al. 2013; Angelopoulou et al. 2014). Such strains have been reported as promoting activities of nonpathogenic bacteria and mycorrhizal fungi (Calvet et al. 1993). The ability of *Trichoderma* strains to synthesize substances inducing systemic acquired resistance (SAR)-like responses in plants was shown (Enkerli et al. 1999; Harel et al. 2014). The combined systemic biofungicides and plant growth promoters have great market potential if the molecular basis of the activities can further be identified. The strong biodegradation and substrate colonization performances of *Trichoderma* strains is the result of an amazing metabolic versatility and a high secretory potential which leads to the production of a complex set of hydrolytic enzymes. Similarly, the myco-parasitic process is based on the secretion of a rich cocktail of cell wall degrading enzymes able to hydrolize the cell wall of various fungi, i.e., chitinases, β-1,3-glucanases, and proteases (Benítez et al. 1998). Some lytic enzymes can be involved in both antagonistic and saprophytic processes providing an evolutionary advantage to strains with both biodegrading and antagonistic potential, for the efficient colonization of different ecological niches in soil (Tziros et al. 2007). In the present study, all tested species of *Trichoderma* significantly increased strawberry enzymes. The highest increase was obtained with their mixture which may be due to complementary effects.

Table 4 Effect of four *Trichoderma* spp. on strawberry yield under field conditions

Treatment	Strawberry yield	
	Ton/feddan	Increase, %
Trichoderma harzianum	12.0b	71.4
T. viride	11.0b	57.1
T. virinis	10.2c	45.7
T. koningii	11.5b	64.3
T. mixture (T. h. + T. v. + T. v. + T. k.)	15.2a	117.1
Actamyl 3 g/l (fungicide)	10.5c	50.0
Control	7.0d	0.0

Figures in a column with the same letter are not significantly ($P \leq 0.05$) different

These results are in harmony with those obtained by others (Cherif et al. 2007; Prlak and Kose 2009; Saksirirat et al. 2009). The increase of yield also might be due to the protected healthy root system that absorb and supply adequate amount of raw nutrient and/or the syntheses of these raw nutrient materials effectively in presence of high amount of chlorophyll and protein, leading to more fruit yield (Shoresh and Harman 2008 and Akladious and Abbas 2012). Also, our results agreed with others (Cherif et al. 2007; Latha et al. 2009; Prlak and Kose 2009; Saksirirat et al. 2009) concerning the enhanced activities of chitinase and peroxidase in treated strawberry plants compared to untreated ones. Likewise, some other results recorded that the efficacy of *Trichoderma* species on soil borne fungal diseases is even higher than fungicides and maintain longer (e.g., Ha 2010; Heydari and Pessarakli 2010)

Conclusion

Despite the continuous progress in cultivation and production of strawberry in Egypt in recent years due to its economic and social benefits, great yield losses by many pests and diseases are frequently associated with its plants. Black root rot disease of strawberry, caused by the fungi *Fusarium solani*, *Rhizoctonia solani*, and *Pythium* sp., ranks high among current serious diseases of strawberry. Chemical fungicides may be used to control the disease, but the current search aspires to use safe control measures

with a clear aim at the avoidance of human health hazards and environmental pollutions caused by chemicals. So, biocontrol agents were tried herein as safe alternatives. *Trichoderma harzianum*, *T. viride*, and *T. koningii* considerably reduced the growth area for all such pathogenic fungi. These four species significantly reduced black root rot disease incidence and severity under field conditions. The highest reduction was attained by mixture of the four species. Individual treatment of *T. harzianum*, *T. viride*, and *T. koningii* reduced both disease incidence and severity more than 70.8 and 74%, respectively. Moreover, this mixture considerably increased the strawberry fresh and dry weight as well as the yield. Also, *Trichoderma* species tested herein significantly increased two plant defense-related enzymes against pathogens in strawberry plants, i.e., peroxidase and chitinase activity. Thus, integrated pest management programs in ways that make these biocontrol agents complementary or superior to chemical fungicides should further be examined.

Abbreviations

cfu: Colony-forming units; PDA: Potato dextrose agar; PDB: Potato dextrose broth

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Authors' contributions

All authors participated in the development and implementation of the research plan and subsequently written it. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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