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EVALUATION OF THE EFFICIENCY OF *PAECILOMYCES LILACINUS* AND *TRICHODERMA HARZIANUM* AS BIOLOGICAL CONTROL AGENTS AGAINST *ALTERNARIA SOLANI* CAUSING EARLY BLIGHT DISEASE OF EGGPLANT

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A B S T R A C T

Alternaria solani responsible for early blight of eggplant is a worldwide spread notably in Iraq. The use of biological control agents represents the best alternative and eco-friendly approach. Two soil-borne fungal (*Paecilomyces lilacinus* and *Trichoderma harzianum*) were tested in laboratory conditions using the dual confrontation technique at different concentrations of conidia filtrate (10, 20 and 30% conidia/mL) and *in vivo* as potential bio-agents against *A. solani*. *T. harzianum* possessed the highest antifungal potency against *A. solani* with an inhibition rate above 50% at a concentration of 30% conidia/mL. In greenhouse experiments, both *P. lilacinus* and *T. harzianum* treated preventively on eggplant leaves inoculated with *A. solani* lowered significantly the disease severity index (31.61% and 28.08%, respectively). Obtained results revealed an augmentation of the peroxidase activity (4.84 units/g/mL/min for *P. lilacinus*; 3.88 units/g/mL/min for *T. harzianum*) in comparison with positive and negative controls. The effect of these two antagonists is limited not only to the protection of eggplant plants but also to the improvement of their growth by increasing the fresh (27.01% for *P. lilacinus* and 34.45% for *T. harzianum*) and dry weights (3.31% for *P. lilacinus* and 5.90% for *T. harzianum*) of the above-ground portion. Based on our results, we can conclude that *T. harzianum* could be recommended for biological control use.

Keywords: *Alternaria solani*, antagonistic fungi, biocontrol activity, *in vitro* antagonism, *Solanum melongena*.

INTRODUCTION

Early blight is one of the serious foliar diseases of eggplants all over the world. The disease is caused by the species of *Alternaria* and the most destructive species is *A. solani* (El-Debaiky, 2018). Disease symptoms are characterized by formation of dark brown to black lesions with concentric rings and necrosis of leaf tissue between the veins (Iram *et al.,* 2018; Chohan *et al.,* 2019). The high level of humidity and frequency of precipitation following by the hot and dry weather greatly reduces the yield. In Iraq, the amount of eggplant infection has been estimated more than 60% (Salih & Abdul Ridha, 2019; Jindo *et al.,* 2021).

There are some methods to control this disease (chemical control, biological treatment, resistant varieties, crop rotation, etc.) which are able to inhibit spore germination and block appressorium formation (Rhouma *et al.,* 2016; El-Debaiky, 2018; Rhouma *et al.,* 2018). Increasing concerns for the public health and environment pollution have caused growers to search for an alternative strategy to control this disease (Esh *et al.,* 2011; Rhouma *et al.,* 2020). In addition, about $32-$45 million is spent annually of fungicides worldwide to control *Alternaria* sp. (El-Tanany *et al.,* 2018).

Therefore, the development of new alternative control methods is strongly required. Recently, new environmentally friendly tools (biological control) were tested to manage this disease worldwide (Chohan *et al.,* 2019; Salih & Abdul Ridha, 2019). Many species of antagonistic fungi (*Trichoderma* spp., *Paecilomyces* spp.,

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Penicillium spp., *Aspergillus* spp., etc.) presented high efficacy against *A. solani* under *in vitro* condition and able to reduce disease severity and induce plant resistance under greenhouse and field conditions (Esh *et al.,* 2011; Fontenelle *et al.,* 2011; Rhouma *et al.,* 2018). *Trichoderma* spp. has been shown to be the most effective biological control agents (BCA's) against many plant pathogens (El-Tanany *et al.,* 2018; Matrood *et al.,* 2020).

Due to the importance of early blight disease on the eggplant caused by *A. solani* and the need for more essential information to manage this disease and to decrease the economic and environmental damage of the employ fungicides and given more researches on the integrated management of the early blight. The objectives of this investigation were to examine *Paecilomyces lilacinus* and *Trichoderma harzianum* for their ability to lower the growth of *Alternaria solani* in laboratory and greenhouse conditions.

MATERIAL AND METHODS

One pathogen (*Alternaria solani*) and two fungal antagonistic (*Paecilomyces lilacinus* and *Trichoderma harzianum*) were used in this study (*in vitro* and *in vivo* conditions). The fungal species used in the present research were obtained from the Laboratory of Plant Protection, College of Agriculture (Basra, Iraq), and they were isolated from leaves (pathogen) and rhizosphere (fungal antagonistic) collected from experimental field cultivated by eggplant plants in Basra, Iraq during January-December 2019.

Evaluation of antifungal activity of *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Alternaria solani* in laboratory condition: The effect of *in vitro* antagonism of *P. lilacinus* and *T. harzianum* on the growth of radial mycelium of *A. solani* was determined by the dual confrontation method. *P. lilacinus* and *T. harzianum* were grown on potato dextrose agar (PDA) medium and conidia filtrate were prepared according to the method described by Rhouma *et al.* (2018). Conidia were collected by adding 10 mL of sterilized distilled water and 0.02% Tween-80 to Petri dishes containing *P. lilacinus* and *T. harzianum* (7 days old). The conidial suspension was filtered and agitated for 5 min using a horizontal shaker (100% conidia/mL). The concentration of conidial suspension was adjusted at 10, 20 and 30% conidia per mL. Conidial viability was evaluated for each cells batch and only lots estimated to be >95% viable were considered in these experiments (Rhouma *et al.,* 2018).

A volume of 100 µL with indicated conidia filtrate concentrations of each antagonistic fungus was transferred aseptically into Petri dishes containing PDA. One disc plug (0.5 cm) of pathogen (4 days old) was placed in the center of PDA medium containing different concentrations of conidial suspension of each antagonistic fungus. The control was performed by placing one disc plug of pathogen in Petri dishes containing only PDA medium (PDA without conidia filtrate of antagonistic fungus). Ten Petri dishes per replication (5 replications) were carried out for each individual treatment and then incubated at 25 for 5 days (Matrood & Rhouma, 2021).

The mycelial growth inhibition of *A. solani* (I) was assessed by the formula of Rhouma *et al.* (2018): $I\ (\%) = ((D_o-D_n)/D_o) \times 100$. D_n represents the diameter of pathogen radial growth in the presence of the indicated conidia filtrate concentrations of antagonist, whereas; D_o represents the diameter of pathogen radial growth in the control treatment.

Evaluation of antifungal activity of *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Alternaria solani* in greenhouse condition: The experiment was carried out in the greenhouse as a randomized complete block design (three blocks each of 20 pots). Eggplant seedlings (cv. Barcelona) were placed in a pot containing peat and vermiculite (1:1); at the rate of one seedling in each pot. Before treatment, eggplant leaves were sterilized for two minutes by soaking in 3% solution of NaOCl and then washed with sterilized distilled water 3 times. The assay was achieved preventively by spraying eggplant leaves (10 mL) with 30% of conidia per mL of each antagonistic fungus. 10 mL (10^6 cfu\mL) of *A. solani* were applied 7 days after. Two controls were performed; positive (pathogen only) and negative (sterilized distilled water) controls. Immediately after inoculation plants were enclosed in plastic bags for 24 h to optimise conditions suitable for infection (Rhouma *et al.,* 2018).

Assessments were performed 3 weeks after inoculation. Observed areas symptoms were scored for disease index (DI) using a scale from 0 to 4; 0 = no spots; 1 = number of spots covering leaf about 1-3 spots, with yellowing of 1-25% of the leaf area; 2 = number of spots covering leaf about 4-6 spots, with yellowing of 26-50% of the leaf area; 3 = number of spots covering leaf about 7-9 spots, with yellowing of

51-75% of the leaf area; 4 = spots covering the totality of leaf, with yellowing of 75-100% of the leaf area. The disease severity index (DSI) were calculated for each block by the formula of McKinney: DSI (%) = (Σvn)/(20 × V)×100. v is the numeric value of DI scale, n is the number of plants assigned to the disease index scale, and V is the numeric value of the highest disease index scale (Matrood *et al.,* 2021).

After determining the fresh weight of the above ground portions, samples were dried in an oven at 60°C for 48 h for dry weight determination.

Peroxidase (POX) activity was evaluated according to the method of Velazhahan and Vidhyasekaran (1994). 3 ml of reaction mixture composed of 0.5 mL guaiacol, 1 ml phosphate buffer, 0.5 ml H_2O_2, 0.1 mL enzyme extract, and 0.9 ml water and the absorbance was examined at 470 nm.

STATISTICAL ANALYSIS

Statistical analysis was checked through the mean values of replicates using SPSS version 20.0 statistical software (SPSS, SAS Institute, USA) followed by one-way ANOVA. Homogeneity of variances and normality were performed by applying Least Significant Difference (LSD) Test. Differences between treatments were determined by LSD Test. All statistical tests were performed with a significance level of 1% ($P \leq 0.01$).

RESULTS AND DISCUSSION

Evaluation of antifungal activity of *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Alternaria solani* in laboratory condition: The data in Table 1 showed a significant decrease in the radial mycelial growth of the pathogen after *in vitro* interaction between *A. solani* (pathogen) and *P. lilacinus* and *T. harzianum* (antagonists) at different concentrations of conidia filtrate ($P < 0.01$). Laboratory examination demonstrated that the two antagonistic have an antifungal activity at 30% conidia/mL which varied from 46.74% (*P. lilacinus*) to 50.69% (*T. harzianum*) (Table 1).

Fontenelle *et al.* (2011) and El-Debaiky (2018) indicated that the existence of several BCA's (e.g.; *Trichoderma* spp. and *Paecilomyces* spp.) against *A. solani* is able to demonstrate higher inhibition of conidial production of up to 90% under laboratory condition. In the same sense, Chohan *et al.* (2015) and Olivia-Devi *et al.* (2017) observed that the mycelial growth of *A. solani* (above 90%) was dramatically lowered in the presence of *Trichoderma* species (*T. harzianum*, *T. hamatum* and *T. viride*) when used at higher concentration. Furthermore, Varma *et al.* (2008) pointed out the toxicity of *Trichoderma* spp. against *A. solani*. The possible mechanisms projected to clarify the antagonism were the competition and the antibiosis (progression, penetration, sporulation and colonization) (Benitez *et al.,* 2004; Rhouma *et al.,* 2018).

Table 1. Effect of direct confrontation of *Paecilomyces lilacinus* and *Trichoderma harzianum* on mycelial growth inhibition of *Alternaria solani*.

Fungal antagonists	Concentrations of conidia filtrate (% of conidia/ml)			Mean	$L.S.D_{0.01}$
	10	20	30		
Paecilomyces lilacinus	13.11	31.86	46.74	30.57	<0.01
Trichoderma harzianum	20.45	26.96	50.69	32.70	<0.01
Mean	16.78	29.41	48.72	Nd	Nd
$L.S.D_{0.01}$	Concentrations: 3.72 Interactions: 4.80				

Data are the average of five replicates (with ten Petri dishes per replicate).
Nd: not determined.

Evaluation of antifungal activity of *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Alternaria solani* in greenhouse condition: Statistical analysis revealed a significant differences between the treatments for all parameters ($P < 0.01$) (Table 2).

The antagonist application reduced the DSI and the values were ranged between 28.08% (*T. harzianum*) and 31.61% (*P. lilacinus*) (Table 2).

The efficiency of the two fungal antagonists on the development parameters was also studied. Both *P. lilacinus* and *T. harzianum* improved the weights of fresh (27.01% and 34.45%, respectively) and dry (3.31% and 5.90%, respectively) above ground (stem + leaves) of eggplant inoculated by *A. solani* (Table 2).

The peroxidase (POX) activity showed that *P. lilacinus* (4.84 units/g/mL/min) and *T. harzianum* (3.88 units/g/mL/min) exhibited a greater activity than controls (positive control = 1.95 units/g/mL/min; negative control = 1.68 units/g/mL/min) when plants were inoculated with *A. solani* (Table 2).

Table 2. Effect of preventive treatments of two fungal antagonists (*Paecilomyces lilacinus* and *Trichoderma harzianum*) on the disease severity index (DSI), fresh (FWA) and dry (DWA) weights of above ground (stem + leaves) portions and peroxidase (POX) activity in the eggplant leaves inoculated with *Alternaria solani* after 7 days of pathogen inoculation in experimental greenhouse.

Treatments	DSI (%)	FWA (g)	DWA (g)	POX activity (units/g/ml/min)
Paecilomyces lilacinus	31.61	27.01	3.31	4.84
Trichoderma harzianum	28.08	34.45	5.90	3.88
Positive control	66.86	18.73	2.97	1.95
Negative control	0	40.10	6.09	1.68
$L.S.D_{0.01}$	<0.01	<0.01	<0.01	<0.01

Data are the average of 20 eggplant plants per treatment and per replicate (3 replicate).
DSI: Disease severity index; FWA: Fresh weight of above ground (stem + leaves) portions; DWA: Dry weight of above ground (stem + leaves) portions; POX: Peroxidase.

Present results are in analogy with Selim (2015), Adhikari *et al.* (2017), Roy *et al.* (2019) and Viriyasuthee *et al.* (2019) showing the specific effect of *Paecilomyces* spp. and *T. harzianum* against *Alternaria* spp. under greenhouse and field conditions. These authors documented that plant treated preventively with those antagonistic depicted the lowest disease severity index.

Viriyasuthee *et al.* (2019) and Moreno-Gavíra *et al.* (2020) pointed out that the direct interaction between antagonists (*Paecilomyces* spp. and *Trichoderma* spp.) and *A. solani* increased the dry and the fresh weights of above ground portions, which supports our results. Hibar *et al.* (2005) and Rhouma *et al.* (2018) revealed that the use of BCA's as plant growth stimulators, because of its production of hormones of growth and improved the transfer of minerals nutrition.

Foliar treatment with BCA's enhanced the resistance of plants to many phytopathogens and improved the peroxidase activity (Deborah *et al.,* 2001). Alfiky and Weisskopf (2021) observed that foliar treatment of plants with *Paecilomyces* spp. and *Trichoderma* spp. against *Alternaria* spp. improved the plant resistance. Analog results were obtained with *T. harzianum* against *Meloidogyne javanica* (Sharon *et al.,* 2001). Magesh and Ahiladevi (2017), and Niño-Medina *et al.* (2017) revealed that foliar treatment with antagonistic fungi against *Alternaria* spp. increased the catalase and peroxidase activities and that this increase could be related to lignification.

CONCLUSION

Foliar treatment of eggplant with spore suspensions of *T. harzianum* lowered the severity of early blight disease caused by *A. solani* and promoted the fresh and dry weights of above ground (stem + leaves) portions. Based on our results, we can conclude that *T. harzianum* could be recommended for biological control use.

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COMPETING INTERESTS

The authors declare that they have no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study. All authors have approved the manuscript for submission.

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Abdulnabi A. A. Matrood	:	Planning and designing experiments, implementation of experiments, contributed in writing, editing and reviewing manuscript
Abdelhak Rhouma	:	Planning and designing experiments, implementation of experiments, contributed in writing, editing and reviewing manuscript