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ANTIFUNGAL ACTIVITY OF *TAGETES ERECTA* EXTRACT AND *TRICHODERMA HARZIANUM* ON THE PATHOGENIC FUNGUS *FUSARIUM VERTICILLOIDES*

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Abstract

Plant extracts of *Tagetes erecta* and culture filtrate of *Trichoderma harzianum* was used to check their antifungal efficacy against *Fusarium verticilloides* mycelial growth. The results of this study revealed that the plant extracts and culture filtrate of *T. harzianum* significantly inhibited the mycelial growth of the pathogen. The effect was increased with the increasing of the concentration of extract and the period of treatment. The percentages of inhibition ranged between 20.93% – 65.65% in the case of plant extract of *T. erecta* and ranged between 13.43% - 50.81% in the case of culture filtrate of *T. harzianum.*

***Keywords*:** *Fusarium verticilloides*, Biocontrol, *Tagetes erecta, Trichoderma harzianum.*

Introduction

Plant extracts are generally assumed more acceptable and less hazardous than synthetic products and can be used as alternative antifungal treatment (Jobling, 2000; Guerrero-Rodríguez *et al*., 2007). Aqueous plant extracts from garlic, creosote bush, and clove inhibited the growth of *Fusarium oxysporum f. sp. lycopersici, Rhizoctonia solani,* and *Verticillium dahliae* (López-Benítez *et al*., 2005). *Tagetes erecta* L. is an annual ornamental plant. It has been shown that extracts of the marigold flower have antibacterial activity. In addition, in our previous study we demonstrated that the extracts prepared from the marigold root had inhibitory effects on a variety of common plant pathogens (Chen *et al*., 2003). A study by Céspedes *et al.* (2006) reported that chloroform/methanol extracts of *Tagetes lucida* inhibited 89% of the colony radial growth of *F. moniliforme*. *Trichoderma* spp. are effective biocontrol agents against different pathogens and some isolates are also known for their ability to induce systemic resistance in plants (Harman *et al*., 2004). The *Trichoderma harzianum* expresses inhibition against a broad spectrum of soil-borne pathogens including *F. oxysporum* f. sp. *cubense*, *F. oxysporum* f. sp. *niveum*, *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *cucumerinum* and *Rhizoctonia solani* (Huang *et al*., 2011). Study was conducted under greenhouse conditions revealed that the application of *T. harzianum* and chitosan (1 g/l) as root dipping treatment combined with chitosan (0.5 g/l) as foliar spray has reduced Fusarium crown and root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* incidence and severity by 66.6 and 47.6%, respectively (El-Mohamedy *et al*., 2014). The mechanisms of action of *Trichoderma* spp. include competition for space and nutrients, antibiosis, antagonism, inhibition of pathogen enzymes and plant growth enhancement (Abd-El-Khair *et al*., 2010; Howell, 2003). The objective of the present study is to assess the effect of *Tagetes erecta* extract and *Trichoderma harzianum* filtrate on the pathogenic fungus *Fusarium verticilloides.*

Material and Methods

Location of study: The study was conducted in the laboratory of Agriculture College – university of Almuthana. For the management of *F. verticilloides, Trichoderma harzianum* isolate and plant extracts of *Tagetes erecta* were used under in vitro conditions.

Source of *Trichoderma harzianum* isolate: Isolate of *T. harzianum* was obtained from the Ministry of science and Technology - Directorate of Agriculture Research - Department of Biotechnology.

Source of *Tagetes erecta* plants: *Tagetes erecta* plants were obtained from the native nurseries. The plant material (leaves, flowers and roots) were used in this study.

Effect of plant extracts on mycelial growth of *F. verticilloides*: The antifungal ability of methanol plant extracts was assessed by poisoned food technique described by (Nene and Thapliyal, 1993). Fresh samples of plant material of *Tagetes* plants were washed with tap water followed by distilled water. The samples were disinfected by using sodium hypochlorite (5%). The samples were then dried in an oven at 70 ºC for 2 days. After drying samples were ground to make powder and 10g of powder was dissolved in 100 ml of methanol. The samples were placed for 48 hrs in methanol which was then filtered by passing them through double layer filter papers. The filtrate is consider a stock solution(S) and different concentrations were prepared which were (S , S/2 , S/4).The PDA medium was poisoned by adding 10 ml of each concentration into 100 ml of medium. The medium was poured into sterilized Petri dishes and after solidification; these were inoculated with 5 mm block of pathogenic culture in the center of plates. The plates were incubated at 25 ºC and each treatment was replicated three times. However, control was retained by mixing the medium only with 10 ml of distilled water. After 24 hrs the growth of the pathogen in each Petri plate was detected and colony diameter was measured (Vincent, 1947).

Effect of Culture Filtrate of *T. harzianum* on *F. verticilloides*: *Trichoderma harzianum* were cultured in conical flask containing Potato Dextrose Broth (PDB) for 20 days on rotary shaker at 25°C. The culture filtrate of *Trichoderma harzianum* was harvested after 20 days of incubation. To collect the filtrate, the liquid cultures were filtered through 2 layers of Whatman No.1 filter paper to remove hyphal fragments and finally filtered using a 0.22 um-sized membrane filter. Thus the samples were ready for further use. The filtrate of *Trichoderma* was mixed with PDA

separately to have different concentrations 12.5%, 25% and 50%. Supplemented, PDA mixtures were poured in sterilized petriplates. Then, 10 days old culture of 5 mm agar disc of *F. verticilloides* was placed in the center of the petriplates. The experiment was replicated 3 times and mycelial growth was measured after 7 days of incubation. For control, mycelium growth was counted in fresh PDA media without fungal filtrate. Observation of percent inhibition of mycelium growth (PIMG) was recorded by using the formula calculated by the formula: Inhibition % = (r1 - r2 / r1) × 100 Where r1 was the radial growth of pathogen in control, r2 was the radial growth of pathogen in treatment (Ghildiyal and Pandey, 2008).

Results and Discussion

Effect of plant extracts on mycelial growth of *F. verticilloides*: Plant extracts of *Tagetes erecta* were used to check their antifungal efficacy against *F. verticilloides.* Mycelial growth diameter was measured after 3, 5 and 7 days. The results of this study (Table 1) revealed that the plant extracts significantly inhibited the mycelial growth of the pathogen, and the effect was increased with the increasing of the concentration of extract and the period of treatment. After 7 days of treatment the radial growth of *F. verticilloides* was 28.3mm, 38.1mm and 46.1mm at the concentrations S , S/2 and S/4 respectively as compared with the control 82.4mm. The lowest percentage of inhibition of mycelial growth was recorded at the concentration S/4 after 3days which was 20.93% and the highest percentage of inhibition of mycelial growth was recorded at the concentration S after 7days which was 65.65%.

Table 1 : Antifungal activity of *Tagetes erecta* extract against *F. verticilloides*

Treatments	Concentrations	Mycelial growth of *F. verticilloides* (mm) after					
		3 days	%Inhibition	5 days	%Inhibition	7 days	%Inhibition
T. erecta extract	s	11.6	55.03	20.8	58.89	28.3	65.65
	s/2	14.2	44.96	26.3	48.02	38.1	53.76
	s/4	20.4	20.93	34.2	32.41	46.1	44.05
Control	0	25.8	-	50.6	-	82.4	-
$LSD_{0.01}$		0.49	0.072	0.62	0.042	0.71	0.128

Each value is a mean of three replicates

The effect of the extract of *T. erecta* on the growth of the mycelium of *F. verticilloides* may be due to the presence of active compounds that have antifungal effects like thiophenes which affect the growth of the pathogen and several studies were confirmed the effect of this compound against pathogens (Mares *et al*., 2002; Romagnoli *et al*., 1998). Another study shows that the essential oil from leaves and thiophene rich extracts from marigold roots have significantly good antifungal activity against a number of soil borne and foliar plant pathogens (Saha *et al*., 2012). Similar findings correlate with the findings of Martínez *et al.* (2014) who reported that marigold is effective against the *F. oxysporum*, because it restricts the disease symptoms 88.5% caused by the pathogen. The compounds viz., sesquiterpenes, saponins and flavonoids have certain anti-fungal properties, which is cent percent in marigold. Martínez (2012) reported that flavonoids are possessing antifungal activity against pathogens such as *Penicillium* sp. and *Rhizopus* sp. Phenolic compounds are active against the pathogen cell membranes, resulting in leakage of cytoplasmic. Similar study was conducted by Wavare *et al.* (2017) to evaluate the effectiveness of extracts of Marigold sp. (*Tagetes erecta* L.) against *Fusarium oxysporum* f. sp. *ciceri* and revealed that the floral water extract proved highly effective to reduce incidence of Fusarium wilt (69.31%) under greenhouse conditions. Kumar *et al.* (2019) investigated the antifungal activities of essential oil of *T. minuta* aerial parts against two plant pathogenic fungi *Aspergillus niger* and *Fusarium solani* and showed that the essential oil exhibited 100% inhibition of *A. niger* at 1% concentration level while 100% inhibition of *F. solani* was observed at 0.12% concentration level. Recent study about antifungal activities of the flowers' essential oil of *Tagetes minuta*, (Z)-tagetone and thiotagetone against *Candida lipolityca, Candida parapsilosis, Trichosporon asahii* revealed that the essential oil exhibited high activities with a minimum inhibitory concentration (MIC) of 46.75 µg/mL for *C. lipolityca*, 54.63 µg/mL for *C. parapsilosis* and 28.33 µg/ mL for *T. asahii*, while the compound (Z)- tagetone presented MIC values of 57.29 µM, 72.92 µM and 57.29 µM, respectively (de Oliveira *et al*., 2019).

Effect of *T. harzianum* culture filtrate on *F. verticilloides* growth: The results of this study had been presented in table (2). The results had been revealed that the culture filtrate of *T. harzianum* has great potential to inhibit the growth of *F. verticilloides*. The effect of culture filtrate was increased with the increasing of the concentration of culture filtrate. The radial growth of mycelium were 36.2 mm, 44.3 mm and 51.6 mm a the concentrations 50%, 25% and 12.5% respectively after 7days of treatment as compared with the control 73.6mm. The lowest percentage of inhibition of mycelial growth was recorded at the concentration 12.5% after 3days which was 13.43% and the highest percentage of inhibition of mycelial growth was recorded at the concentration 50% after 7days which was 50.81%.

Table 2 : Activity of *T. harzianum* filtrate on *F. verticilloides* growth

Treatments	Concentrations	Radial mycelial growth of *F. verticilloides* (mm) after					
		3 days	%Inhibition	5 days	%Inhibition	7 days	%Inhibition
T. harzianum culture filtrate	50%	11.2	44.27	26.4	45.67	36.2	50.81
	25%	15.6	22.38	30.6	37.03	44.3	39.80
	12.5%	17.4	13.43	36.3	25.30	51.6	29.89
Control	0%	20.1	-	48.6	-	73.6	-
$LSD_{0.01}$		0.68	0.072	2.19	0.176	3.54	2.93

The effect of culture filtrate of *T. harzianum* against *F. verticilloides* may be due to the presence of certain compounds such as N-phenylethylenediamine, phenol, pthalic acid, diallylamine and propanal in the filtrate of *Trichoderma* (Anita *et al.,* 2012). The result of this study is in agreement with many workers who have also reported the inhibitory effect of culture filtrate of *Trichoderma* spp. upon several plant pathogens. Mishra *et al*. (2011) reported that more than 50% growth inhibition was found at 10% cell free culture filtrate of *T. viride* against pathogens like *R. solani, S. rolfsii, M. phaseolina* and *C. capsici* while at 20% concentration 100 % mycelial growth inhibition was observed which suggest the inhibitory action of cell free culture filtrate of *Trichoderma* as found in the present experiment. Bokhari and Parveen, (2012) found that culture filtrates of *T. harzianum* caused reduction in the growth of *Fusarium solani* by 21.3. The results of this study are also confirmatory with previous findings made by different scientists. *Trichoderma* species have characteristic to grow rapidly and have ability to suppress the pathogen by competing them for food and habitat (Devi *et al*., 2012) and also by inhibit pathogen through mycoparasitism (Khirood and Jite, 2012). Study was conducted by Ferrigo *et al*. (2014) to evaluate the effect of *T. harzianum* against the plant pathogenic fungus *F. verticillioides* and they showed that seed biopriming with *T. harzianum* can be a promising and environmentally friendly way to control *F. verticillioides* kernel colonization and fumonisin accumulation. The result of this study is in agreement with the study of Gawade *et al*. 2012 who found the isolate of *Trichoderma* have good antagonistic effect on the mycelial growth of *F. moniliformae* and they showed thathe effect was increased with the increasing of the concentration of culture filtrate. *Allium Sativum, Euclyptus globulus* and *Lantana camara* and two bio-control agents (*Trichoderma harzanium* and *Trichoderma viridi*) were evaluated to check their antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici*. It was observed that all the plant extracts showed significant results. *S. aromaticum* was the most effective in control of *F. oxysporum* f. sp. *Lycopersici* followed by *A. Sativum, E. globulus and L. camara*. Both the bio-control agents inhibited the growth of fungus *T. harzianum* showed 42.60% growth inhibition and T. *viride* exhibited 36.69% growth inhibition. Application of plant extracts and bio-control agents are cost effective, easily available and ecofriendly for the management of fusarium wilt disease (Khan, et al. 2017). *Trichoderma virens* and *T. viride* significantly increased the amount/activity of secreted antifungal metabolites in response to volatile compounds (VCs) produced by 13 strains of *Fusarium oxysporum*, a soilborne fungus that infects diverse plants. This response suggests that both *Trichoderma* spp. recognize the presence of *F. oxysporum* by sensing pathogen VCs and prepare for attacking pathogens (Li, et al. 2018).

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