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EFFECT OF *TRICHODERMA* SPP. AND FILTRATES ON SOME CHEMICAL COMPONENTS OF ONION PLANTS INFECTED WITH *SCLEROTIUM CEPIVORUM* AND *FUSARIUM OXYSPORUM* F. SP. *CEPAE*

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Abstract

Culture filtrates of *Trichoderma* spp. inhibited the linear growth of both *Sclerotium cepivorum* and *Fusarium oxysporum* f. sp. cepae. Also, the culture filtrates of *T.harzianum* altered the mycelial permeability of S. cepivorum more than *F. oxysporum* f. sp. *cepae.*

Amount of total free amino acids increased in onion plants infected with *S. cepivorum* or *F. oxysporum* f. sp *cepae* and also when plants were treated with *Trichoderma* spp. in the presence of one of the two pathogens. The same trend was observed with respect to amount of free phenols, while the same treatments reduced the amount of total carbohydrates and total phenols.

INTRODUCTION

Onion (*Allium cepae* L.) is attacked by several soil-borne fungi, under Egyptian conditions, such as *Fusarium oxysporum* f. sp *cepae* and *Sclerotium cepivorum* which cause basal rot and white rot diseases, respectively (Hanna 1990).

It was reported that some *Trichoderma* spp. produced antibiotics against soil fungi that cause diseases such as *T. harzianum* and *T. longibrachiatum* (Abd El-Moity

and Shatla 1981 and Michail *et al* . 1988).

Sterilized culture filtrates of *T. harzianum* and / or *T. longibrachiatum* affected mycelial growth, mycelial dry weight, sporulation, spore germination and mycelial cell wall permeability of several soil-borne fungi (Gaumann 1958, Pellizzari *et al.* 1970, Abd El-Moity and Shatla 1981, Emam 1982 and Michail *et al.* 1988).

Sirry *et al.* (1979) showed that bulbs of Giza 6 cultivars infected with *S. cepivorum* contained lower amounts of total and reducing sugars, total and conjugated phenols, while amino acids contents were greater than that in healthy ones.

Zahra (1984) reported that onion bulbs infected with *F. oxysporum* f. *sp. cepae* contained lower amounts of total, reducing, non-reducing sugars and total amino acids, but showed an increase in free and conjugated phenols compared with healthy ones. Also, the same trend was observed by Ramadan (1986) in case of onion bulbs infected with *F. oxysporum* f. *sp. cepae.*

Thus, the present investigation was carried out to study the effect of *T. harzianum* and its filtrate on growth, permeability of mycelial cells of two pathogenic fungi of onion. The effect of soil application of *Trichoderma* spp. on chemical components of onion bulbs infected with white rot and basal rot pathogen was also investigated.

MATERIALS AND METHODS

1. Efffect of sterilized culture filtrates of *Trichoderma* spp. on the linear growth of *S.cepivorum* and *F.oxysporum* f. sp. *cepae.*

Two *Trichoderma* spp. namely, *T. harzianum* and *T. longibrachiatum* were used to study the effect of their sterile culture filtrates on the growth of *S. cepivorum* and *F. oxysporum* f. sp. *cepae*, previously isolated from onion plants (Hanna 1990). Culture filtrates of both *T. harzianum* or *T. longibrachiatum* were obtained according to Abd El-Moity (1976) and were centrifuged for 15 minutes at 3000 rpm. and sterilized by autoclaving at 121°C for 20 minutes or using sintered glass filter G5. Sterilized culture filtrates were added to each Petri dish containing Gliotoxin fermentation medium at the rate of 10, 20 and 30% of the medium before

solidification. Filtrate free from Gliotoxin fermentation medium was used as control. Equal discs (4 mm in diameter) of each of *S. cepivorum* or *F. oxysporum* f. sp. *cepae* were transferred to the plates under aseptic conditions after solidification of the medium in five replicated series. The plates were incubated at 20°C for *S. cepivorum* and 25°C for *F. oxysporum* f. sp. *cepae*. Growth was recorded when mycelium covered the surface of the medium in the control.

2 . Effect of *T.harzianum* filtrates on the permeability of the mycelial growth of *S. cepivorum* and *F. oxysporum* f. sp. *cepae*:

Conical flasks (125 ml), each containing 50 ml Gliotoxin fermentation liquid medium, was used. Culture filtrate of *T. harzianum* previously sterilized by sintered glass G5 was added to the medium at the rate of 30% (V/v) (Abd-El-Moity 1976). Equal discs (4mm in diameter) of each of *S.cepivorum* or *F. oxysporum* f. sp. *cepae* were inoculated in each conical flask and then incubated at 20°C and 25 °C, respectively, for 7 days. After the incubation period of the two fungi, conduction of the solution was measured periodically at one hour interval during the experiment using a conductivity bridge according to Elnaghy and Heitefuss (1976). One ml chloroform was added to each flask and total leakage was measured. Results were expressed as u mhos/g fresh weight.

3. Effect of *Trichoderma* spp. application on chemical components of onion bulbs diseased with white rot and basal rot disease:

Extraction:

Two-gram samples of onion bulbs "improved Giza 6 cultivar" from each particular treatment of field experiment previously prepared by Abou-Zaid *et al.* (1991) were cut into small portions and immediately stored in brown bottles containing 30 ml of 95% ethanol. Bottles were kept in darkness at room temperature for four months. Ethanolic extracts were dried by air current using electric fan, then quantitiatvaly transferred to 5 ml 50% isopropanol and stored in vials at 1°C (Awad 1980).

Determination:

All isopropanol alcoholic extracts were used for determination of carbohydrates, total free amino acids and phenols.

1. Determination of carbohydrates:

Total and reducing carbohydrates were determined colourimetrically using picric acid method as described by Thomas and Dutcher (1924). The carbohydrates contents were determined as mg glucose/100g fresh matter. The difference between the total soluble and reducing carbohydrates is the value of non-reducing carbohydrates.

2. Determination of total free amino acids :

Quantitative determination of total free amino acids in sample extract was made according to Rosen (1957). The amount of total amino acids was calculated as glycine.

3. Determination of phenolic compounds:

Free, conjugated and total phenols were determined using the colourimetric method of folin Ciocalteau phenol reagent according to Snell and Snell (1953).

The difference between the total and free phenols is the value of conjugated phenols. The concentration of phenols was calculated as mg catechol.

RESULTS AND DISCUSSION

Culture filtrates of *T. harzianum* and *T. longibrachiatum*, sterilized either by filtration through sintered glass G5 or by autoclaving, inhibited the growth of *S. cepivorum* and *F. oxysporum* f. sp. *cepae* (Table 1). It is also clear that, increasing the concentration of culture filtrate from 10 to 30% resulted in reducing the mycelial growth of the two pathogenic fungi. Also, differences were significant between the two methods used (Table 1). These results are similar to those reported by Abd El-Moity and Shatla (1981) indicating that filtrates of *T. harzianum* sterilized by various methods showed different effects on mycelial growth of *S. cepivorum*.

The permeability of *S. cepivorum* and *F. oxysporum* f. sp. *cepae* mycelium was tested and data were tabulated in Tables (2 and 3).

Leakage of electrolytes from *S. cepivorum* and *F. oxysporum* f. sp. *cepae*,

Table 1. Effect of sterilized culture filtrates of *Trichoderma* spp. on the linear growth (cm) of *S. cepivorum* and *F. oxysporum* f. sp. *cepae*.

Sterilization by	Fungi	*S. cepivorum*				*F. oxysporum f. sp. cepae*			
		Conc. culture filtrates (%)				Conc. culture filtrates (%)			
		10	20	30	Aver.	10	20	30	Aver.
Filtration (sintered glass G5)	*T. harzianum No. 1*	9.00	9.00	8.40	8.80	9.00	7.76	7.08	7.94
	T. harzianum No. 2	9.00	8.00	7.30	8.10	8.42	7.20	6.86	7.49
	T. longibrachiatum	7.84	5.16	4.18	5.73	6.84	6.14	5.42	6.13
	Average	8.61	7.39	6.63	7.54	8.09	7.03	6.45	7.19
	Control filtrate free	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Autoclaved	*T. harzianum No. 1*	9.00	9.00	8.40	8.80	8.60	8.00	6.84	7.81
	T. harzianum No. 2	9.00	8.00	7.50	8.17	8.50	7.54	6.50	7.51
	T.longibrachiatum	8.72	5.52	4.32	6.19	7.64	6.64	5.90	6.72
	Average	8.91	7.51	6.74	7.72	8.25	7.39	6.41	7.35
	Control filtrate free	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
	L. S. D. at 5% to Fungi	0.062				0.124			
	Filtrate (conc.)	0.054				0.108			
	Fungi x Conc.	0.108				0.216			
	Sterilization	0.044				0.088			

Table 2. The effect of *Trichoderma harzianum* filtrate on permeability of *S. cepivorum*.

Time (h)	Conductivity (umhos)		Conductivity of culture filtrate (A)	Chloroform added (umhos)		Total conductivity of culture filtrate (B) after adding chloroform	% of total leakage $\frac{A \times 100}{B}$
	S. cepivorum + Trichoderma filtrate	*S. cepivorum*		*S. cepl + Trichod. filtrate*	*S. cep.*		
0	120	100	20	170	120	50	40.0
1	180	155	25	250	200	50	50.0
2	270	240	30	320	265	55	54.5
3	300	260	40	380	320	60	66.6
4	340	300	40	400	340	60	66.6
5	400	350	50	450	380	70	71.4
6	450	380	70	560	480	80	87.5
7	450	380	70	560	480	80	87.5
8	450	380	70	560	480	80	87.5

Table 3. The effect of *Trichoderma harzianum* filtrate on permeability of *Fusarium oxysporum* f. *sp. cepae.*

Time (h)	Conductivity (umhos)		Conductivity of culture filtrate (A)	Chloroform added (umhos)		Total conductibity of culture filtrate (B) after adding chloroform	% of total leakage $\frac{A \times 100}{B}$
	Fusarium +Trichoderma filtrate	*F. oxysporum*		*Fusar. + Trichod. filtrate*	*F. ox.*		
0	220	200	20	300	250	50	40.0
1	280	250	30	360	300	60	50.0
2	355	320	35	400	340	60	58.3
3	400	360	40	450	390	60	66.6
4	430	385	45	490	425	65	69.2
5	460	400	60	520	440	80	75.0
6	460	400	60	520	440	80	75.0
7	460	400	60	520	440	80	75.0
8	460	400	60	520	440	80	75.0

during incubation period at room temperature (25-27°C), showed that culture filtrates of *T. harzianum* increased cell permeability of *S. cepivorum* from 40.0 at zero time to 87.5 after 8 hours, while it was 75.5 for *F. oxysporum* f. sp. *cepae* at 8 hours. Changes in permeability might be due to the action of substances in culture filtrate of *T. harzianum*. Impairment of permeability would greatly influence the physiological function of the cells. These data are in accordance with those mentioned by other workers (Gaumann 1958, Elnaghy and Heitefuss 1976 and Emam 1982).

Changes in chemical components of onion bulbs associated with white rot and basal rot diseases of onion in presence or absence of *Trichoderma* were studied to determine total free amino acids, carbohydrates and phenol components. Results obtained showed an accumulation of amino acids in plant tissues infected with both *S. cepivorum* and *F. oxysporum* f. sp. *cepae* when compared with total amino acids in control treatment and inoculation with *Trichoderma* spp. only (Table 4). Invasion of the host tissues with the aforementioned pathogens caused degradation of reducing and non-reducing sugar contents, thus decreasing the amount of total sugar. On the other hand, the control treatment or treated with *Trichoderma* spp. showed high amount of total carbohydrates compared with diseased plants (Table 4). This finding supported the results obtained by Sirry *et al.* (1979), Zahra (1984) and Ramadan (1986).

Application of *Trichoderma* spp. to soil containing either *S. cepivorum* or *F. oxysporum* f. sp. *cepae* resulted in onion bulbs containing total phenols lower than diseased plants, but was higher than control. Such difference might be due to accumulation of phenols in tissues nearby cells invaded by the pathogen. In this respect, Goodman *et al.* (1967), El-Shabrawy (1978) and Ramadan (1986) reported similar results.

Table 4. Effect of soil application with *Trichoderma* spp. on reducing, non-reducing and total carbohydrates level, and total free and bound phenol contents as well as total amino acids of onion bulbs in presence or absence of *S. cepivorum* and *F. oxysporum* f. sp. *cepae*.

Treatments Fungi soil infestaion	Reducing, Non-reducing and total carbohydrates in onion bulbs (mg/g fresh weight)			Total, Free and conjugated phenol contents in onion bulbs (mg/g fresh weight)			Total amino acids in onion bulbs (mg/g fresh weight)
	R	N. R.	T.	Free phenols	conugafeet phenols	Total phenols	
1- *Sclerotium cepivorum*	71.87	40.63	112.50	0.625	4.187	4.812	177.5
2- *Fusarium oxysporum* f. sp. *cepae*	112.50	65.62	178.12	0.687	4.688	5.375	202.5
3- *Trichoderma harzianum* No. 2	25.00	153.12	178.12	0.187	2.688	2.875	102.5
4- *Trichoderma longibrachiatum*	21.87	215.63	237.50	0.312	2.938	3.250	102.5
5- Uninfested (control)	32.81	192.19	225.00	0.312	1.688	2.000	102.5
6- *T. harzianum* 2 + *S. cepivorum*							
Diseased plants (S.c,)	21.87	18.75	40.62	1.375	1.562	2.937	202.0
Plant free from disease	16.37	152.38	168.75	0.250	2.587	2.937	65.0
7- *T. harzianum* 2 + *F. oxysporum*							
Diseased plants (F. ox.)	106.25	34.37	140.62	1.062	3.313	4.375	207.0
Plants free from disease	29.69	148.43	178.12	0.375	4.000	4.375	35.0
8- *T. longibrachiatum* + *S. cepivorum*							
Diseased plants (S. c.)	71.87	45.32	117.19	0.750	1.125	1.875	205.0
Plants free from disease	21.87	215.63	237.50	0.250	4.000	4.250	82.5
9- *T. longibrachiatum* + *F. oxysporum*							
Diseased plants (F. ox)	23.44	154.68	178.12	0.437	3.625	4.062	65.0
Plant free from disease	18.75	206.25	225.00	0.312	6.438	6.750	17.5

R = Reducing carbohydrates N. R. = Non-reducing carbohydrates T = Total carbohydrates

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تأثير الترايكودرما ورواشحه على المحتوى الكيميائى لنباتات البصل المحقونة بفطرى سكليروشيوم سيبفورم وفيوزايوم أوكسيسبورم سيبا

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كان لراشح التريكودرما تأثير مثبط لنمو كل من فطرى سكليروشيوم سيبفورم وفيوزاريوم اوكسسبورم سيبا . كما أنها أدت الى تغيير نفاذية ميسليوم الفطر سكليروشيوم سيبفورم عن ميسيليوم الفطر فيوزاريوم اوكسبسيورم سيبا. هذا وقد زادت الأحماض الأمينية الحرة والفينولات الحرة فى نبات البصل التى أعديت بفطر سكليروشيوم سيبفورم أو فيوزاريوم اوكسيسبورم سيبا وايضا عند معاملة النباتات بجنس الترايكودرما فى وجود أحد الفطرين بينما أدت المعاملة بنفس المعاملات السابقة إلى إنقاص كل من الكربوهيدرات والفينولات الكلية.