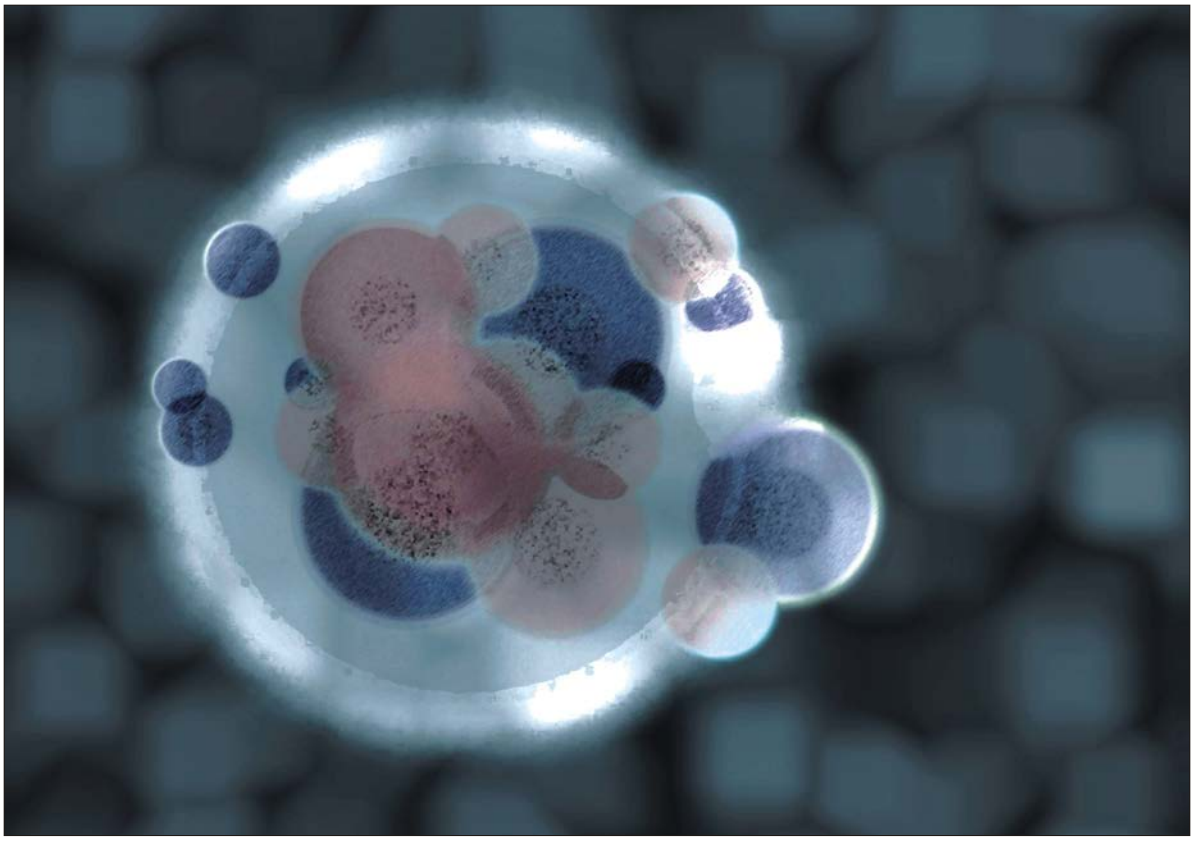

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In vitro Studies on the Toxicity of Culture Filtrates of Different Fungi on the Growth of *Fusarium oxysporum* f.sp. *vasinfectum*

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Abstract: The effects of culture filtrates of different fungi on the *in vitro* growth of *Fusarium oxysporum* f.sp. *vasinfectum* and the impact of these filtrates on wilt incidence were investigated on cotton seedlings cv. Barakat. The culture filtrates of the tested fungi were significantly inhibitory to the growth of *Fusarium oxysporum* f.sp. *vasinfectum*. Reduction in mycelial weight of the fungus was directly correlated with concentration of the fungal filtrate. *Arthrobotrys oligospora* culture filtrate at different concentrations was the most inhibitory as compared to the other fungi. The toxicity was largely influenced by the type of liquid culture medium and dilution used. *Rhizopus nigricans* culture filtrate had significant stimulatory effects on the growth of *Fusarium oxysporum* f.sp. *vasinfectum*. The culture filtrates of the tested fungi were also reduced the wilt incidence in cotton plants with, 75% reduction obtained by *Arthrobotrys oligospora* culture filtrate. Spore germination of *Fusarium oxysporum* f.sp. *vasinfectum* was also affected differentially by the use of the different fungal filtrates. Culture filtrates of fungi could be used as source of biological fungicides to control wilt disease in Gezira area of the Sudan.

Key words: Toxicity, culture filtrates, *Fusarium oxysporum* f.sp. *vasinfectum*, cotton, Sudan

INTRODUCTION

Recent information has clearly confirmed the existence of the true pathogenic wilt caused by the fungus *Fusarium oxysporum* f.sp. *vasinfectum* in the Gezira scheme of the Sudan (Yassin and Dafalla, 1982; Ulloa *et al*., 2006; Keristin *et al*., 2001). Severe infections and economic losses are observed and predominantly occurred in the susceptible cotton (*Gossypium barbadense* L.) cultivars. *Fusarium* wilt disease in Sudan has increased in proportion due to increasing and intensified cotton production in the area. At present, there is no any effective control measures to overcome the disease, despite that, several tactics has been practiced (Yassin and Dafalla, 1982). The most effective so far, to control the disease is the growing of resistant varieties. Consequently, the recent trend is to increase the area under medium staple Acala cottons e.g., Barac (67)B which are immune to vascular wilt at the expense of the long staple Barakat cultivar, is a safeguard against vascular wilt with a resultant improvement in yield potential. The culture filtrates of several soil-borne fungi, as biological option have been shown to affect nematodes (Liu and Chen, 2000; Mankau, 1969; Khan *et al*., 1984) and exhibit a range of specificities in their antagonistic activity towards them (Siddiqui and Mahmood, 1996; Adekunle and Akinsanmi, 2005). However, the effect of the fungal culture filtrates and its bioactivity on other fungi has been little studied (Nafe-Roth, 1972). In the present study, the effect was evaluated in culture filtrates of the different fungi on the growth of *Fusarium oxysporum* f. sp. *vasinfectum*, infectivity and wilt incidence and the conditions necessary to obtain the maximum fungicidal effect of the filtrate.

MATERIALS AND METHODS

Fungal isolates and filtrate preparation: Seven soil fungi namely *Alternaria alternata* (Nees), *Arthrobotrys oligospora* (Fresenius), *Aspergillus niger* (Link), *Cephalosporium acremonium* (Corda), *Cercospora beticola* (Fresenius), *Cladosporium cladosporoides* (Link) and *Rhizopus nigricans* (Kuhn) were isolated from groundnut and cotton infected plants or from trees infected with vascular wilt. Isolates of *Fusarium oxysporum* f.sp. *vasinfectum* were obtained from the roots of susceptible cotton cv. Barakat grown at the Gezira Research Station (GRS), Sudan. These fungi were cultured for 10-15 days in 150 mL of Czapek-Dox liquid medium

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at 25°C. The fungal culture of *A. oligospora* originated from Gezira area of Sudan obtained from Professor Al-Nur El-Amin of Gezira University. Filtrates were obtained by filtering through filter paper (Whatman No. 1) under aseptic conditions. Then clarified by centrifugation at 6000 rpm for 10 min and were taken as standard solutions S. Different dilutions i.e., S/2, S/4, S/8 and S/10 of each culture filtrate were prepared by adding required amount of sterile deionized distilled water.

***In vitro* assessment of culture filtrates on *Fusarium oxysporum* f.sp. *vasinfectum*:** To determine the effect of culture filtrates of the tested fungi on the growth of *Fusarium oxysporum* f.sp. *vasinfectum*, aliquots of 25 mL filtrates of different dilutions of each fungus was separately added in 175 mL Czapek-Dox liquid medium in conical flask. Flasks containing Czapek-Dox medium alone (200 mL) served as control. Flasks were then inoculated with *Fusarium oxysporum* f.sp. *vasinfectum* and incubated at 25°C. Each set of treatments was replicated 5 times. After 15 days the fungal mats were harvested through Whatman filter paper No. 2 (which removes mycelium), then gently pressed between the folds of blotting paper to remove the excess amount of water and weighed. Conidia were recovered from inoculated flasks and the influence on spore germination of *Fusarium oxysporum* f.sp. *vasinfectum* was determined according to the method described by Domsch *et al.* (1980).

Bioactivity of fungal culture filtrates as influenced by type of media: As a general rule, the bioactivity of the culture filtrates and production of toxic metabolites is greatly influenced by the culture medium (Smith and Moss, 1985). Therefore, the activity of the culture filtrates of different fungi was tested after culturing fungi on Czapek-Dox, Corn meal and Malt liquid media. Media were autoclaved at 115°C for 20 min before inoculation. The three types of media were then inoculated with different fungi. The media thus inoculated were kept in laboratory temperature (22-27°C).

Greenhouse assessment of fungal culture filtrates on wilt incidence: Effect of culture filtrates obtained from different fungi on *Fusarium* wilt incidence was studied under greenhouse conditions. Seeds of susceptible cotton cultivar cv. Barakat were sown in 500 mL pots containing steam-sterilized topsoil. Each pot that contained one seedling was inoculated at 7 days after planting with 50-80 thousands chlamydospores/cc of *Fusarium oxysporum* f.sp. *vasinfectum*as described by Yassin and Dafalla (1982). After fungus inoculation, 5 mL of 10-day-old culture filtrates of different fungi and Czapek-Dox control treatments were applied. The treatments were added to the base of the seedlings around were *Fusarium oxysporum* f.sp. *vasinfectum* inoculum had been placed. The experiment was arranged in a randomized complete block design with 5 replicates. Percent reduction in wilt incidence was recorded after 75 days of inoculation.

Statistical analysis: Data obtained from repeated experiments were pooled and the means were used in analysis for significant differences using appropriate statistical procedure (SAS Institute Inc., Cary, NC, U.S.A).

RESULTS

Results presented in Table 1 indicated that the fungal filtrates of the different tested fungi significantly ($p = 0.05$) suppressed the growth of *Fusarium oxysporum* f.sp. *vasinfectum*. Percent reduction in mycelial weight of *Fusarium oxysporum* f.sp. *vasinfectum* was directly correlated to the concentration of the fungal filtrate. The tested culture filtrates were differed in their influence on *Fusarium oxysporum* f.sp. *vasinfectum* depending on the type of culture and dilution used (Table 1 and 3).The maximum reduction in mycelial weight (83.52%) was observed in the S concentration of *Arthrobotrys oligospora* followed by *Cercospora beticola* (80.77%), *Cladosporium cladosporoide*s (79.04%) and *Alternaria alternata* (75.28%). The minimum reduction, on the other hand, was brought about by the culture filtrate obtained from *Rhizopus nigricans* (59.57). However, the reduction in mycelial weight is decreased when the culture dilution was increased with no significant differences between dilutions of each treatment (Table 1). This was clearly detected in the ½ concentration and 1/4 of the culture filtrate of the fungus *Arthrobotrys oligospora* (82.31%) and also in dilution 1/4 and 1/8 of the *Alternaria alternata* culture filtrate (67.31%). Even in 1/10 concentration the reduction in mycelial weight of *Fusarium oxysporum* f.sp. *vasinfectum* was significant in all of the tested fungal cultures. Subsequently, the culture filtrate of the fungus *Rhizopus nigricans* showed stimulatory effects in the 1/10 concentration, with 6.04% increase in mycelial weight of *Fusarium oxysporum* f.sp. *vasinfectum* over controls (Table 1). However, different concentrations of the culture filtrates of *Fusarium oxysporum* f.sp. *vasinfectum* did not significantly affect the growth of the fungus itself.

Results obtained from greenhouse experiment showed that the culture filtrates of the tested fungi were also reduced the wilt incidence in cotton plants with 75% reduction obtained by *Arthrobotrys oligospora* culture

Table 1: Influence of the different dilutions of fungal filtrates on the growth of *Fusarium oxysporum* f.sp. *vasinfectum*

	Mycelial fresh weight of *Fusarium oxysporum* f.sp. *vasinfectum* in different dilutions of fungal filtrates							
Fungal species (filtrates)	0 (control)	S(standard)	S/2	S/4	S/8	S/10	DSD (at 1%)	LSD (at 5%)
Alternaria alternata (Nees)	-	4.50 (-75.28)	5.16 (-71.65)	5.95 (-67.31)	5.95 (-67.31)	6.56 (-63.96)	1.333	0.652
Arthrobotrys oligospora (Fresenius)	-	3.00 (-83.52)	3.22 (-82.31)	3.22 (-82.31)	4.00 (-78.03)	5.00 (-72.53)	1.100	1.010
Aspergillus niger (Link)	-	5.42 (-70.22)	5.98 (-67.15)	6.00 (-67.04)	6.43 (-64.68)	9.00 (-50.55)	1.999	1.300
Cephalosporium acremonium (Corda)	-	6.00 (-67.04)	6.12 (-66.38)	6.50 (-64.29)	6.97 (-61.71)	7.40 (-59.35)	1.033	1.011
Cercospora beticola (Fresenius)	-	3.50 (-80.77)	4.00 (-78.03)	5.00 (-72.53)	6.11 (-66.43)	8.41 (-53.80)	2.001	1.751
Cladosporium cladosporoides (Link)	-	3.75 (-79.04)	4.00 (-78.03)	4.60 (-74.73)	5.13 (-71.82)	7.11 (-60.94)	1.231	0.541
Rhizopus nigricans (Kuhn)	-	7.36 (-59.57)	8.50 (-53.30)	11.31 (-37.86)	15.71 (-13.69)	19.30 (+6.04)	2.939	1.812
Czapek-Dox medium (control)	18.20	-	-	-	-	-		
CD at 1%		1.810	1.980	2.000	1.995	2.416		
CD at 5%		1.125	1.333	1.400	1.500	1.567		

Values are means of 5 replicates, in parenthesis are percent reduction (-) or stimulation (+) of *Fusarium oxysporum* f.sp. *vasinfectum* growth over control

Table 2: Percent reduction in wilt incidence of cotton (*Gossypium barbadense*) cv. Barakat at 75 days of inoculation with culture filtrates of different fungi

Treatments	(%) Reduction in wilt incidence (transformed into degrees)
Alternaria alternata	22.01
Arthrobotrys oligospora	75.14
Aspergillus niger	50.00
Cephalosporium acremonium	66.00
Cercospora beticola	44.00
Cladosporium cladosporoides	58.01
Rhizopus nigricans	36.00
Czapek-Dox medium (control)	00.00
CD at 5%	2.94

Each value is a mean of 5 replicates

Table 3: Influence of the culture medium on the bioactivity of *Arthrobotrys oligospora* culture filtrate on *Fusarium oxysporum* f.sp. *vasinfectum* (10 day-old culture filtrate)

	Mycelial weight of *Fusarium oxysporum* f.sp. *vasinfectuum*		
Culture filtrate dilution	Czapek-Dox	Corn-meal	Malt
0 (water control)	16.08[a]	20.62[a]	18.46[a]
S/2	13.00[b]	16.00[b]	14.52[b]
S/4	13.01[b]	16.00[b]	14.11[b]
S/8	12.95[b]	15.08[b]	14.00[b]
S/10	13.00[b]	15.00[b]	13.95[b]

Means followed by the same letter in columns are not significantly different ($p<0.05$) according to the Student[s] test, number of 5 replicates

Table 4: Influence of the different fungal culture filtrates on spore germination of *Fusarium oxysporum* f. sp. *vasinfectum*

Treatments	Spore germination (%)
Alternaria alternata	44.00
Arthrobotrys oligospora	30.66
Aspergillus niger	55.14
Cephalosporium acremonium	66.17
Cercospora beticola	28.00
Cladosporium cladosporoides	37.00
Rhizopus nigricans	70.11
Water (control)	90.37
CD at 5%	1.980
CD at 1%	2.730

Values are means of 5 replicates, spore germination calculated from 100 mixtures of micro and macro-conidia

filtrate (Table 2). Spore germination of *Fusarium oxysporum* f.sp. *vasinfectum* was also affected differentially by the use of the different fungal filtrates, with maximum suppression (72.00%) in *Cercospora beticola* followed by *Arthrobotrys oligospora* (69.34%) (Table 4). The influence of the culture medium on the bioactivity of *Arthrobotrys oligospora* against *Fusarium oxysporum* f.sp. *vasinfectum* was apparent, as the culture filtrate of Corn meal was less active compared to Malt and Czapek-Dox and the maximum activity was obtained from the later medium (Table 3).

DISCUSSION

The bioactivity of the tested culture filtrates of different fungi on the growth of *Fusarium oxysporum* f.sp. *vasinfectum* was very efficacious in inhibiting mycelial fresh weight *in vitro*. In the greenhouse experiment, filtrates of various fungal strains resulted in reduced wilt symptoms. The filtrates of these fungi also resulted in a variable reduction in spore germination of *Fusarium oxysporum* f.sp. *vasinfectum*. Also the influence of the culture medium on the bioactivity of culture filtrates was very important as the filtrate of Corn meal was less active as compared to Malt and Czapek-Dox media.

The toxicity of culture filtrates of different fungi on the growth of *Fusarium oxysporum* f.sp. *vasinfectum* might be attributed to the production of certain toxic metabolites and/or enzymes by them in the culture medium. Present findings are corroborated with those of Ghewande *et al*. (1984) who reported the efficacy of some fungal culture filtrates against other fungi. Other workers specified that several strains of *Arthrobotrys oligospora* had antimicrobial activity and linoleic acid was responsible for activity in cultures of different fungal species (Anke *et al*., 1995). Furthermore, some species of *Alternaria*, *Rhizopus*, *Aspergillus*, *Cephalosporium* and *Cercospora* are known to produce one or more mycotoxins or antibiotics (Daub and Ehrenshaft, 2000; Manzoni and Rollins, 2002; Das and Pal, 1974; Ghewande *et al*., 1984). For example, species of

Cephalosporium, *Alternaria*, *Cercospora* and *Cladosporium* are known to produce mycotoxins like cephalosporin, alternariol, beticolin and cladosporin, respectively (Jacyno *et al*., 1993; Lim *et al*., 2002).

The present study identifies some culture filtrates of fungi as potential source of biological fungicides which enables only the *Fusarium* wilt pathogen to be killed, without destroying all the soil micro fauna as current chemicals are doing and this specificity appears as an additional point in the fungicidal mycotoxins research.

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