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Isolation of fungi from tomato rhizosphere and evaluation of the effect of some fungicides and biological agents on the production of cellulase enzymes by Nectria haematococca and Pythium ultimum var. ultimum

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Forty-five species and two species varieties belonging to twenty-six genera of fungi were isolated from 30 soil samples from the rhizosphere of tomato plants. The fungi most frequently isolated were *Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Gibberella fujikuroi, Nectria haematococca* and *Rhizopus stolonifer*.

Ridomil and Vitavax-captan (10, 50 and 100 ppm) had no significant effects on the activity of C_1 and C_x enzymes of *Nectria haematococca*. C_x enzyme activity was slightly increased at 10 and 50 ppm, but slightly decreased at 100 ppm. Vitavax-captan (10, 50 and 100 ppm) significantly decreased C_1 enzyme activities of *N. haematococca*. C_x enzyme activity was slightly increased at 10 and 50 ppm, but at 100 ppm it showed a slightly inhibitory effect. Ridomil caused a slight increase in the activity of C_x and C_1 enzymes by *Pythium ultimum* var. *ultimum* at low and moderate doses but the highest dose of Ridomil caused a slight reduction. Vitavax-captan slightly increased the activity of C_x and C_1 enzymes in *P. ultimum* var. *ultimum*.

Normal and sterilised filtrates of *Myrothecium verrucaria, Penicillium oxalicum* and *Trichoderma harzianum* induced a small decrease in C_1 enzyme activity of *Nectria haematococca*. The sterilised filtrates of the three fungi tested caused greater inhibition compared to the normal filtrate. The production of C_x enzyme was slightly increased with normal and sterilised filtrates of *Penicillium oxalicum* and *Trichoderma harzianum*, but was significantly increased by both types of filtrates of *Myrothecium verrucaria*. The two types of filtrate of all fungi tested did not significantly affect the activity of C_1 and C_x enzymes by *Pythium ultimum* var. *ultimum*.

Production of extracellular protein by *Nectria haematococca* was not significantly affected by any dose of the tested fungicides. It was slightly increased by the two types of filtrate of the three tested fungi but significantly increased by the normal filtrate of *Myrothecium verrucaria*. The normal filtrate of all the fungi tested enhanced extracellular protein production to a greater extent than the sterilised filtrate. Extracellular proteins of *Pythium ultimum* var. *ultimum* were slightly increased by all doses of Vitavax-captan and low doses only of Ridomil, also two types of filtrate of all tested fungi caused a slightly increasing effect.

Key words: Biological control, root-rot, fungicides, *Nectria haematococca, Pythium ultimum* var. *ultimum*.

Gherbawy Y. A. M. H. and Abdelzaher H. M. A. (1999): Izolace hub z rhizosféry rajčat a hodnocení účinku některých fungicidů a biologických agens na produkci celulázových enzymů druhů Nectria haematococca a Pythium ultimum var. ultimum. – Czech Mycol. 51: 157–170

Z rhizosféry rajčat bylo izolováno 45 druhů hub, z nichž se nejčastěji vyskytovaly druhy *Aspergilus flavus, A. fumigatus, A. niger, A. terreus, Gibberella fujikuroi, Nectria haematococca*

a *Rhizopus stolonifer*. U druhů *Nectria haematococca* a *Pythium ultimum* var. *ultimum* byl hodnocen účinek fungicidů Ridomilu a Vitavaxu – captanu na aktivitu celulázových enzymů; u druhů *Nectria haematococca* nebyl zjištěn žádný významný účinek těchto fungicidů na produkci C_1 a C_x enzymů. Nižší dávky (10 a 50 ppm) spíše zvyšovaly aktivitu enzymů, vyšší dávky (100 ppm) měly spíš inhibiční efekt. U druhu *Pythium ultimum* var. *ultimum* pouze Vitavax způsoboval slabé zvýšení aktivity C_1 a C_x enzymů. Studiem účinku filtrátů hub *Myrothecium verrucaria, Penicillium oxalicum* a *Trichoderma harzianum* bylo zjištěno, že tyto způsobily zvýšení aktivity C_x enzymu a snížení aktivity C_1 enzymu druhu *Nectria haematococca*, ale neměly žádný významný účinek na aktivitu celulázových enzymů druhu *Pythium ultimum* var. *ultimum*.

INTRODUCTION

Root rots are an enormously diverse group of diseases caused mainly by soil-borne pathogens. Some *Fusarium* and *Pythium* species that are not vascular pathogens produce toxins and enzymes particularly those degrading cell walls (Endo and Colt 1974; Wood and Jellis 1984). In the root-rot of many plants cellulases play an important part in pathogenesis (Mehrotra 1980).

Direct penetration of susceptible hosts by phytopathogenic fungi has been regarded as a mechanical process. However, it has been stated (Strobel and Mathre 1970; Agrios 1978) that extracellular enzymes produced by pathogenic fungi may help in the softening and disintegration of the host's cell wall to facilitate the penetration of the pathogen. Such enzymes might play an effective role in resistance or susceptibility to the disease initiation (Ferraris and Matta 1977; Misaghi 1982; Yehia et al. 1992).

The effect of fungicides on certain microbial processes such as decomposition of cellulose has been reviewed by several authors (Abdel-Kader et al. 1989; Ismail et al. 1989; El-Zayat et al. 1991; Hemida et al. 1993). Two of the most important fungicides that are used in Egypt for controlling serious tomato pathogens are Vitavax-captan and Ridomil.

As far as we know, little research has been done to evaluate the influence of microbial biocontrollers on the production and activity of cellulase enzymes by pathogenic fungi. Therefore , this work was conducted to evaluate the effect of some fungicides (Vitavax-captan and Ridomil) and biocontrol agent (*Myrothecium verrucaria, Penicillium oxalicum* and *Trichoderma harzianum*) on the production of cellulase enzymes by the causal agents of tomato root-rot (*Pythium ultimum* var. *ultimum* and *Nectria haematococca*) at Qena Governorate (Egypt), as an initial step in understanding this chemical and biological control system mechanism.

Materials and methods

I – Soil mycoflora

1 – Estimation of rhizosphere fungi

This part was carried out to make isolation for some fungi that are known as biological agents and grow saprophytically on tomato field soil as well.

Samples of rhizosphere soil were collected from the root system of 30 healthy tomato plants grown at the Farm of South Valley University, Qena, Egypt during March, 1997. From these soil samples, fungi were isolated on potato-dextrose agar (PDA) by the dilution plate method as described by Abdel-Hafez et al. (1990a). There were five replicate plates for each sample and the developing fungi were identified by reference to standard texts. Average total count (ATC; calculated per g dry soil in all samples), number of cases of isolation (NCI; out of 30 cases), occurrence remarks (OR) and total count percentage (TC %) of various fungal genera and species recovered from 20 rhizosphere soil samples were calculated.

2 – Isolation of *Pythium* species from tomato plant rhizosphere

Two methods were employed for isolation of *Pythium* spp. from tomato rhizospheric soil samples as follows:

1 – Particles of soil were placed in Petri-dishes containing VP3 medium (Ali-Shtayeh 1986) for selective isolation of *Pythium* species. The emerging hyphal tips were transferred to water agar medium for further purification from bacterial contamination (Abdelzaher et al 1994).

2 – Autoclaved segments of *Zea mays* leaf blades and autoclaved cucumber seed were used as baits. Five grams of rhizosphere soil were placed in a Petri dish. Ten ml of sterilised distilled water (Willoughby 1956, with modifications and Abdelzaher et al. 1995) were added to enable the baits to float on the surface. After five days of incubation at 25 °C the baits were removed, washed thoroughly with sterilised distilled water and blotted dry with sterile filter paper. Four baits were then placed at the edge a of Petri dish containing VP3 medium for selective isolation of *Pythium* spp. The plates were incubated at 20 °C for three days or until colonies appeared. The emerging hyphal tips were transferred to water agar medium for further purification from bacterial contamination (Abdelzaher et al. 1994).

Identification of *Pythium* species isolated in this investigation

Principally the key of Plaats–Niterink (1981) and Dick (1990) were principally used for identification, and descriptions by Waterhouse (1967) and Middleton (1943) were consulted for comparison or confirmation of the identifications!

II – Pathological study

Fusarium solani (anamorph of *Nectria haematococca*) was found to be the most prevalent species among the other *Fusarium* species in soil samples. *Pythium ultimum* var. *ultimum* was the only *Pythium* species from the tested soil samples. Therefore they were subjected to further experimentation.

Pathogenicity test (root-rot test)

The pathogenicity of *Nectria haematococca* and *Pythium ultimum* var. *ultimum* were tested on tomato seeds (cultivar Maramande). *Nectria haematococca* was grown for 14 days and *Pythium ultimum* var. *ultimum* for 10 days in 500 ml Erlenmeyer flasks containing a sterile mixture of cornmeal and sand (30:70, v / v) to which 50 ml of distilled water was added. Soil (loamy clay) was sterilised at 20 psi for 2 hours and then aerated for at least 2 weeks at room temperature before 100 ml of inoculum was incorporated into 900 g of sterilised soil. Control soils were inoculated with the sand-cornmeal mixture free from the fungus. The seeds were surface sterilised using 0.1 % mercuric chloride for 2 minutes, rinsed several times with sterilised water and then planted in infested soils in 100 g plastic pots (three seeds per pot). Non infested controls were included in all tests. The pathogenicity of each *Nectria* and *Pythium* isolate on tomato roots was based on a root-rot index (RRI) from 0 to 4, where 0 = healthy roots, 1 = 1 – 10 %, 2 = 11 – 25 %, 3 = 26 – 50 % and 4 = more than 50 % necrosis of the root system. The soils were watered to saturation every 2 – 3 days. The experiments were carried out in a illuminated growth cabinet (Precision, U. S. A.) at 30 °C and 5,000 Lux.

III – Enzymatical studies

1 – Fungicides

The two different fungicides used in this work were Vitavax-captan 75 % wp (Vitavax; 5,6-dihydro-2-methyl-1,4-Oxathiin-3-carboxanilido Captan; N –[Trichloromethylthio]-4-cyclohexene-1,2-dicarboximide) produced by Uniroyal International Division of Uniroyal Inc. Amity Road Bethany, Connecticut 06525 U. S. A., and Ridomil MZ 72 WP (8 % methyl D,L-N-[2,6 dimethyl phenyl]-N-[2 methoxy-acetyl]-alaninate) produced by Novartis Limited, Basle, Switzerland.

2 – Biological agents

Myrothecium verrucaria, Penicillium oxalicum and *Trichoderma harzianum* were isolated from rhizosphere samples and maintained on potato dextrose agar

(PDA) plates. Aliquots of 50 ml CMC-based liquid media (g/L of the following $NaNO_3$ 2.0, $KHPO_4$ 1.0, $MgSO_4$. $7H_2O$ 0.5, KCl 0.5 and carboxymethylcellulose CMC 10.0) were dispensed into 250 ml conical flasks. Each flask was inoculated with an agar mycelial disc (10-mm diameter) of the tested mould obtained from 7 days old cultures grown on a solid basal media. Cultures in flasks were incubated at 28 °C for two weeks and filtered. The culture filtrate was sterilised with Zites's bacterial filter. Some of the sterilised filtrate was autoclaved for 15 minutes at 120 °C, while the rest was considered normal filtrate.

Effect of fungicides and biological agents on endo-and exo-1,4 β-D-glucanase production.

Aliquots of 30 ml CMC-based liquid medium were dispensed into 100 ml conical flasks. After cooling, the tested fungicides (10,50 and 100 ppm, active ingredient/kg) and biocontrol agents (fresh and autoclaved) were added to the sterilised liquid medium. Flasks without fungicides and biocontrol agents were used as control. They were all inoculated with 1 ml of spore suspension of *Nectria haematococca* or *Pythium ultimum* var. *ultimum* and incubated at 28 °C without shaking.

Mycelial dry weight

After two weeks mycelia were collected by filtration and placed in an oven at 70 °C until constant weight. The dry weight of each treatment was calculated and is expressed as mg/30 ml media.

Enzymatic activity

Cellulase (C_x-cellulase) activity was measured by incubating a mixture of 1 ml of the culture filtrate and 0.5 ml of 0.5 % CMC in 50 mM citrate buffer (pH 5.2) at 37 °C for 30 minutes (Mandels et al. 1976).

C_1-cellulase activity was also measured in terms of filter paper activity by incubating a mixture containing 50 mg strip (10×10 cm) of Whatman No. 1 filter paper, 1 ml of 50 mM citrate buffer (pH 5.2) and 1 ml of the culture filtrate at 37 °C for 30 minutes (Chandrashekar and Kaveriappa 1988). The reaction was terminated by boiling in a water bath for 5 minutes and reducing sugars were determined photometrically according to Nelson (1944). Results were calculated using a glucose standard curve.

Production of extracellular protein

Extracellular protein production was also assessed (Lowry et al. 1951).

RESULTS AND DISCUSSION

Rhizosphere fungi

Forty-five species and 2 species varieties belonging to 26 genera were isolated from the rhizosphere soil of tomato plants.

Aspergillus was the most common genus isolated (Table 1). It was recovered from all samples and amounted to 39.5 % of total fungi. Of the nine species isolated *Aspergillus flavus, A. fumigatus, A. niger* and *A. terreus* were the most prevalent. They were recovered from 50 – 80 % of the samples comprising 5.4 – 15.4 % of total fungi. The species of *Aspergillus* isolated during the study were previously encountered in various types of Egyptian soil as reported by Abdel-Hafez et al. (1990a,b,c, 1995) and Abdelzaher et al. (1997b).

Gibberella, Nectria and *Rhizopus* were also isolated with high frequencies of occurrence. They were recovered from 56.7 %, 60 % and 63.3 % of the samples comprising 3.9 %, 7.8 % and 9.9 % of total fungi, respectively. They were represented by *Gibberella fujikuroi, G. gordonia, G. intricans, Nectria haematococca* and *Rhizopus stolonifer*. Abdel-Hafez et al. (1995) isolated the three genera previously from sugarcane rhizosphere at Qena Governorate. They found the three genera constituting 16.7 %, 25 % and 41.7 % of the samples matching 1.3 %, 3.1 % and 0.9 % of total fungi. Abdelzaher et al. (1997b) also isolated the three previously genera from the rhizosphere of a maize plant in El – Minia Governorate (Egypt). They recovered these genera from 40 %, 55 % and 30 % of the samples matching 2.6 %, 7.8 % and 1.5 % of total fungi.

Cochliobolus spicifer, Curvularia ovoidea, Mucor hiemalis, Mycosphaerella tassiana and *Myrothecium verrucaria* were isolated in moderate frequency of occurrence. They represented 26.7 – 43.3 % of the samples matching 1.7 – 3.7 % of total fungi. Most of the above fungal species were previously isolated, but with different incidences, from the rhizosphere of several plants cultivated or growing in Egypt (Abu El-Souod et al. 1988; Abdel-Hafez et al. 1990 a,c, 1995; Abdelzaher et al. 1997a) or other parts of the world (Nagaraja 1990, 1991; Rajendra and Saxena 1991). The remaining genera and species were isolated rarely or in small quantities (Table 1).

Occurrence of *Pythium* species in rhizosphere soil of tomato

The results of *Pythium* isolation from the tomato rhizosphere indicated that *Pythium ultimum* var. *ultimum* was present in the tomato rhizosphere of the field studied. *P. ultimum* var. *ultimum* was isolated from Egypt as a causal agent of wheat damping – off (Abdelzaher et al. 1997b). It was previously mentioned from Egyptian soil (El-Helaly et al. 1972; Abdelzaher et al. 1997a).

Table 1 Average total count (ATC), number of cases of isolation (NCL), occurrence remarks (OR) and percentage of total count of various fungal genera and species recovered from 20 rhizosphere soil samples of tomato plants on PDA medium at 28 °C.

Genera & species	ATC	NCI & OR	TC %
Acremonium strictum	4400	5L	1.8
Alternaria	3200	7L	1.3
A. alternata	2600	5L	1.1
A. tenuissima	600	2R	0.3
Aspergillus	94200	30H	39.5
A. candidus	1800	3R	0.8
A. flavus	19800	21H	8.3
A. fumigatus	17600	15H	7.4
A. niger	36800	24H	15.4
A. ochraceus	1800	5L	0.8
A. sydowii	1200	4L	0.5
A. terreus	12800	17H	5.4
A. ustus	1400	4L	0.6
A. violaceus	1000	3R	0.4
Botryotrichum atrogriseum	600	2R	0.3
Chaetomium globosum	2400	4L	1.0
Cladosporium cladosporioides	800	2R	0.3
Cochliobolus	8400	12M	2.5
C. lunatus	3200	5L	1.3
C. spicifer	5200	9M	2.2
Cunninghamella echinulata	4200	7L	1.8
Curvularia ovoidea	8200	13M	3.4
Emericella	4600	6L	1.9
E. nidulans	2000	4L	0.8
E. nidulans var. *dentata*	1800	3R	0.8
E. nidulans var. *lata*	800	2R	0.3
Eurotium amstelodami	2000	2R	0.8
Fusarium oxysporum	7200	7L	3.0
Gibberella	9200	17H	3.9
G. fujikuroi	4400	11M	1.8
G. gordonia	1400	3R	0.6
G. intricans	3400	8M	1.4
Humicola grisea	2400	2R	1.0
Mucor	8800	8M	3.7
M. circinelloides	1200	2R	0.5
M. hiemalis	4000	8M	1.7
M. racemosus	3600	6L	1.5
Mycosphaerella tassiana	5400	10M	2.3
Myrothecium verrucaria	8800	8M	3.7
Nectria haematococca	18600	18H	7.8
Papulaspora immersa	1000	2R	0.4
Penicillium	11000	7L	4.6
P. aurantiogriseum	1200	4L	0.5
P. chrysogenum	4200	7L	1.8

Table 1 cont.

Genera & species	ATC	NCI & OR	TC %
P. funiculosum	2200	2R	0.9
P. oxalicum	1000	3R	0.4
P. puberulum	1200	4L	0.5
P. purpurogenum	1200	2R	0.5
Phoma glomerata	1400	3R	0.6
Rhizopus stolonifer	23600	19H	9.9
Setospheria rostrata	1600	4L	0.7
Stachybotrys chartarum	2000	4L	0.8
Trichoderma harzianum	1600	3R	0.7
Ulocladium chartarum	3200	7L	1.3
Gross total counts	238800		
Number of genera	26		
Number of species	45 + 2 Var.		

Occurrence remarks: H = high occurrence, 15 – 30 cases (out of 30); M = moderate occurrence, between 8 – 14 cases; L = low occurrence, 4 – 7 cases; R = rare occurrence, 1 – 3 cases.

Table 2 Root rot index (RRI) of tomatoes infected with *Nectria haematococca* and *Pythium ultimum* var. *ultimum* after 24 days of sowing in infested soil.

Fungi	No. of germinated seeds out of 30 seeds	No. of damped – off seedlings	No. of root rotted seedlings	RRI*
Nectria haematococca	30	5	25	4
Pythium ultimum var. *ultimum*	28	8	20	4

* Root-rot index results are attributed to tomato plants that escaped from the damping-off.

Pathogenicity test (root – rot test)

Nectria haematococca and *Pythium ultimum* var. *ultimum* isolated from the tomato rhizosphere were used in this study. Isolates of *Nectria haematococca* and *Pythium ultimum* var. *ultimum* were highly pathogenic to the root of tomato seedlings in greenhouse pathogenicity trials when soil was infested with the cormeal-sand inoculum (Table 2).

Khallil and Ammar (1994) reported that both normal and autoclaved culture filtrates of *Fusarium solani* caused upper yellowing and epinasty of tomato leaf cuttings (var. Marmande). The culture filtrate was less effective by either autoclaving or minimising concentration. Also, they reported that the total death due to *F. solani* application into the soil was 63.04 %. Such results make clear that the tested isolate of *F. solani* was highly pathogenic.

Pythium ultimum var. *ultimum* can become a severe parasite of many plants, it is a causal agent of damping-off and root-rots of many crops (Plaats–Niterink 1981).

Table 3 Effect of Ridomil and Vitavax-captan on the production of biomass, exo-(C_1) and endo-C_x β–1,4-glucanase and extracellular protein by *Nectria haematococca* and *Pythium ultimum* var. *ultimum*.

Organism	Fungicides	Doses (ppm)	Dry weight (mg / 30 ml media)	Cellulase production (mg reducing sugars / ml crude enzyme / 30 min / 30 ml media)		Extracellular protein (mg egg albumin protein / 30 ml media)
				C_1	C_x	
Nectria haematococca	Control		62.1	3.3	3.6	0.48
	Ridomil	10	80.3*	1.2	7.6	0.43
		50	88.6*	1.8	7.0	0.28
		100	49.2*	1.2	0.6	0.7*
	Vitavax-captan	10	48.3*	0.6*	7.6	0.4
		50	45.5*	0.2*	4.2	0.63
		100	47.9*	0.8*	3.0	0.65
Pythium ultimum var. *ultimum*	Control		32.6	0.13	0.18	0.19
	Ridomil	10	50.2*	0.16	0.26	0.28
		50	37.7*	0.29	0.08	0.17
		100	18.4*	0.06	0.06	0.11
	Vitavax-captan	10	23.5*	0.19	1.36	0.20
		50	18.5*	0.56	1.14	0.25
		100	10.8*	0.63	1.18	0.30

* Significantly different from the control at 5 % level. Each value is the average of three replicates.

Enzymatic activities

1 – Fungicides

The mycelial dry weight of *Nectria haematococca* and *Pythium ultimum* v. *ultimum* were significantly increased by low and moderate doses of Ridomil but at high doses the effect became inhibitory. On the contrary the dry weight of both fungal species were significantly decreased by all doses of Vitavix-captan (Table 3 and 4). Abdalla and Manci (1979) reported that 50 to 300 ppm of the herbicide Stomp reduces the mycelial dry weight of *Pythium* using liquid media.

On the other hand, the mycelial dry weight of *Nectria haematococca* and *Pythium ultimum* var. *ultimum* was significantly increased by the untreated (normal) filtrate of *Myrothecium verrucaria, Penicillium oxalicum* and *Trichoderma harzianum*. The autoclaved filtrate of the previous biological agents caused a slight decrease in the dry weight of both tested fungi, but the aufiltrate of *Trichoderma. harzianum* caused a slight increase (Table 3 and 4).

Table 3 reveals that the Ridomil effect on C_1 cellulase of the culture filtrate of *Nectria haematococca* was slightly inhibitory. Its activity against CMC was slightly promoting at low and moderate doses, but it was non-significantly inhibited at

Table 4 Effect of *Myrothecium verrucaria*, *Penicillium oxalicum* and *Trichoderma harzianum* filtrate on the production of biomass, exo-(C_1) and endo-(C_x) β–1,4-glucanase and extracellular protein by *Nectria haematococca* and *Pythium ultimum* var. *ultimum*

Organism	Biocontrol agents	Filtrate type	Dry weight (mg / 30 ml media)	Cellulase production (mg reducing sugars / ml crude enzyme / 30 min / 30 ml media)		Extracellular protein (mg egg albumin protein / 30 ml media)
				C_1	C_x	
Nectria haematococca	Control		62.1	3.3	3.6	0.48
	Myrothecium verrucaria	Normal	78.3*	2.3	8.1*	0.7*
		Sterilised	60.5	1.7	5.9	0.63
	Penicillium oxalicum	Normal	66.2	2.0	5.8	0.65
		Sterilised	60.1	1.5	4.6	0.55
	Trichoderma harzianum	Normal	83.2*	2.1	5.4	0.68
		Sterilised	64.5	2.0	4.2	0.5
Pythium ultimum var. *ultimum*	Control		32.6	0.13	0.18	0.19
	Myrothecium verrucaria	Normal	38.2*	0.49	0.82	0.28
		Sterilised	32.0	0.45	0.16	0.25
	Penicillium oxalicum	Normal	35.4	0.78	3.2*	0.27
		Sterilised	30.3	0.17	0.40*	0.22
	Trichoderma harzianum	Normal	43.5*	0.09	0.28	0.20
		Sterilised	36.7	0.63	0.24	0.26

* Significantly different from the control at 5 % level. Each value is the average of three replicates. Normal = Untreated

high doses. This results indicate that *Nectria haematococca* was able to tolerate the lower concentrations of Ridomil.

Table 3 shows that the Ridomil effect on C_1 production by *Pythium ultimum* var. *ultimum* was slightly promoting at low and moderate doses but was slightly inhibited at high doses. In the case of C_x it was slightly promoting at low doses only and slightly decreasing at both moderate and high doses. Adaptation of fungi to low doses of toxicants has been demonstrated by many investigators (El–Khadem et al. 1979; Kataria and Dodan 1982; Sayed et al. 1990).

Vitavax-captan caused a significant inhibition of C_1 enzyme activity of *Nectria haematococca* at the different experimental doses. It was also slightly promoting at its low and moderate doses with C_x enzyme production, but its high doses had a slightly inhibitory effect.

Vitavax-captan showed a slightly promotive effect on the production of C_1 and C_x enzymes by *Pythium ultimum* var. *ultimum* (Table 3).

These results were basically similar to those obtained by Gupta and Prasad (1969). They reported that fungicides may inhibit or reduce the production and activity of various enzymes from several fungi. Sporulation of the fusaria was

reduced by 50 % by Vitavax at 50 ppm (Mathure et at. 1971). Lukens and Sisler (1958) and Owens and Novotny (1959) suggested that captan acts by inhibiting a number of enzymes in phosphorus metabolism, certain oxidases and dehydrogenases, carboxylase and coenzyme-A. Soil drenched with 25 ml of 0.2 % Vitavax suspension per 2000 g soil/pot significantly reduced the post-emergence damping-off of tomato seedlings caused by *Pythium aphandermatum* (Nene and Thapliyal 1979).

Hemida et al. (1993) found that Tilt induced a significant decreasing inhibitory effect on activities of C_x produced by *Fusarium solani*. On the other hand, they reported that the activity of C_x produced by the same fungus was significantly increased with doses of Primextra, but the insecticide Polytrin was the only pesticide to show a significant toxicity on the activity of C_1 produced by *F. solani*.

Production of extracellular proteins by *Nectria haematococca* was not significantly affected by the low or moderate doses of Ridomil, but the highest dose significantly increased their production. On the other hand, only the low doses of Vitavax-captan slightly decreased the production of extracellular protein, but the moderate and high doses slightly increased them (Table 3).

With regard to the production of extracellular protein by *Pythium ultimum* var. *ultimum* under the effect of Ridomil, it was found that production was inhibited at moderate and high doses but enhanced at low doses. On the other hand, Vitavax-captan showed a slightly promotive effect on the production of extracellular protein at all experimental doses (Table 3).

El-Abyad et al. (1988) reported that Prometryn at high doses (128 and 256 ppm) disturbed membrane permeability and increased the production of extracellular protein of two *Fusarium* spp. which cause wilt. Abdel-Basset et al. (1992) reported that the significant inhibitory effect of Selecron on the extracellular protein produced by *Fusarium solani* was confined to low doses after 12 and 16 days and to high doses after 8 – 20 days.

2 – Biological agents

Table 4 shows that C_1 cellulase of *Nectria haematococca* was inhibited by both types of filtrate (normal & sterilised) of all tested biological agents. The sterilized filtrate of all tested fungi showed strong inhibition in comparison to the normal filtrate. On the other hand the activity of the filtrate of tested fungi on C_x production was slightly promotive with normal and sterilised filtrates of *Penicillium oxalicum* and *Trichoderma harzianum*, but significantly promotive at both types of *Myrothecium verrucaria* filtrate.

Culture filtrates of *Penicillium oxalicum* induced a significant increase in C_x activity of *Pythium ultimum* var. *ultimum* (Table 4) but had no effect on C_1 activity. Culture filtrates of the other two fungi had no effect on C_x and C_1 activity.

Howell (1991), Ghisalberti and Sivasithamparan (1991), and Jensen and Wolffhechel (1995) reported that strains of *Trichoderma* and *Gliocladium* produce many different secondary metabolites. Some of these seem to play a key role in many interactions causing antibiosis and lysis of the pathogen. Bertagnolli et al. (1996) reported that a greater degree of inhibition of growth of the fungal pathogen *Rhizoctonia solani* was observed when culture filtrate from *Trichoderma harzianum* was added to the medium than when the culture filtrate from *Rhizoctonia solani* was added to *Trichoderma harzianum* cultures. Extracellular enzymes including β–1,3-glucanase, chitinase and cellulase (Cruz et al. 1993; Lorito et al. 1994; Harman et al. 1995) are effective in disrupting the mycelium of the pathogen.

The untreated filtrate of *Myrothecium verrucaria* had a significant effect on the production of extracellular proteins by *Nectria haematococca*, all other filtrates did not show a significant effect in comparison with control (Table 4).

The two types of filtrate of all tested fungi showed a not significant increasing effect on the production of extracellular protein by *Pythium ultimum* var. *ultimum* (Table 4).

Further studies concerning substances involved in the inhibition of cellulase enzyme activity by *Nectria haematococca* under the effect of the tested biological agents should be studied.

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