academicJournals

Vol. 13(46), pp. 4330-4337, 12 November, 2014
DOI: 10.5897/AJB2014.13868
Article Number: 6017F0D48571
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Effect of equation pro and kema zed fungicides on cellulase and pectinase enzymes produced by some phytopathogenic fungi of broad bean

A. Saleem[1,2*], A. H. M. El-Said[1], T. A. Maghraby[1] and M. A. Hussein[1]

[1]Botany Department, Faculty of Science, South Valley University, 83523 Qena, Egypt.
[2]Biology Department, Faculty of Science, Taibah University, 30002 Almadinah Almonawarah, Saudi Arabia.

Received 17 April, 2014; Accepted 16 May, 2014

Two fungicides (equation pro and kema zed) were added to the medium in five doses (50,100, 200, 300 and 400 ppm active ingredient) to investigate the chemical control of cellulase and pectinase enzymes produced by some plant pathogens of broad bean. *Alternaria alternata*, *Alternaria citri*, *Alternaria* and *Cochliobolus spicifer* were isolated from diseased leaves of broad bean. The incorporation of these fungicides into the media for cellulase and pectinase enzymes exhibited an inhibitive effect on both cellulase and pectinase enzymes by all doses used except in few cases; the production of exo- and endo-β-1, 4-glucanase enzymes were slightly increased compared to the control at 50 and 100 ppm doses. The inhibitive effect of equation pro on endo- and exo-β-1,4-glucanase production ranged between 5.9 to 70.4% by *A. alternata*, *A. citri* and *C. spicifer*. However, kema zed exhibited an inhibitive effect on cellulases which ranged between 1.9 to 51.9%. On the other hand, the inhibitive effect of Equation pro on pectinase enzyme ranged between 20.1 to 75.6% by *A. citri* and *A. raphani* while kema zed exhibited an inhibitive effect ranged between 13.5 to 62.8%. The inhibitive effect of these fungicides on the mycelial growth of tested fungi was nearly similar to those for the enzyme production.

Key words: Fungicides, cellulase and pectinase enzymes, broad bean fungi.

INTRODUCTION

All enzymes consist of protein having catalytic properties and a non-protein part called a prosthetic group or coenzyme. Pesticides can interact either with the protein part of the enzyme molecule and completely inactivate it, or with the coenzyme and form stable compounds or unstable complexes. In both cases, the pesticides are classified as enzyme inhibitors (Gruzdyev et al., 1983).

The effect of fungicide was quite variable with different enzymes and fungal species. The biosynthesis of various enzymes was found to be controlled by a number of fungicides (Omar and Abd-Alla, 2000; Moharram et al., 2004; 2011; Gopinath et al., 2006; Dornez et al., 2008;

*Corresponding author. E-mail: asaleem@hotmail.com.

Özer et al., 2010; Saleem et al., 2012). There are several reports indicating the varying effect of fungicides on both mycelial growth, cellulase and pectinase production according to different factors including the kinds and doses of fungicides and the fungal species tested (Arinze and Yubedee, 2000; Moharram et al., 2004; Rathod and Chavan, 2010; Saleem et al., 2012). Gopinath et al. (2006) studied the effect of Propiconazole, Difenoconazole and Carbendazim fungicides on growth and hydrolytic enzymes production by *Colletotrichum capsici*. Three fungicides inhibited mycelial growth (radial growth and mycelial biomass) of *C*. *capsici* compared to controls. Among the fungicides, Propiconazole exhibited the highest level of inhibition followed by Difenoconazole and Carbendazim. The incorporation of fungicides into the growth medium, significantly reduced production of polygalacturonase, polygalacturonase trans-eliminase, pectin trans-eliminase and cellulases by *C*. *capsici*. The highest degree of inhibition was observed in Propiconazole, followed by Difenoconazole and Carbendazim.

Recently, Özer et al. (2010) evaluated the influence of nine fungicides on mycelial growth and poly-galacturonase activity of *Botrytis cinerea*. All fungicides except Triadimenol and Tebuconazole inhibited mycelial growth of fungal isolates. Cyprodinil + Fludioxonil, Myclobutanil and Imazalil inhibited polygalacturonase activity more than 50%. Fenhexamid had a lower inhibitory effect (<50%) on polygalacturonase activity. Procymidone and Pyrimethanil induced both PG activity and isoenzyme banding profile of isolates sensitive to these fungicides.

Hydrolytic enzymes play an important role in the pathogenicity of plants by facilitating fungal penetration through the host cell wall (Wanjiru et al., 2002). The major components of plant cell walls are cellulose, hemicellulose and lignin, with cellulose being the most abundant component (Han et al., 2003). Cellulose consists mainly of long polymers of β 1-4, linked glucose units and forms a crystalline structure (Shallom and Shoham, 2003). Cellulase enzymes complex is a multi-domain protein that consists of three major enzymes components which are endo-β-(1-4)-D-glucanase, exo-β-(1-4)-D-glucanase and β-glucosidase that works synergically in complex cellulose degradation (Duff et al., 1987). Generally, fungi produce three major types of cellulolytic enzymes: endoglucanase, exoglucanase and cellobiohydrolase (Klyosov, 1990). These enzymes are extracellular and inductive in nature (Enari, 1983). The ability to produce cellulase are widespread among fungi and has become the subject of extensive investigation (El-Said, 2001; Moharram et al., 2004; El-Said et al., 2005; Narasimha et al., 2006; Shanmugam et al., 2008; Abu-Bakar et al., 2010; Gautam et al., 2010; Sherief et al., 2010; Sarkar et al., 2011). Pectins are high molecular weight polysaccharides found in higher plants. They form the primary cell wall and the main components of the middle lamella (Alkorta et al., 1998). The hydrolysis of pectin backbone is obtained by the synergistic action of several enzymes (Gummadi and Panda, 2003). Among the enzymes secreted, polygalacturanase and pectinase are responsible for cell maceration and death of plant tissue (Fernando et al., 2001).

The involvement of pectic enzymes in the degradation of pectic constituents of cell walls and middle lamella of plant tissues has been reported for diverse types of diseases (Ramos et al., 2010). Enzymes that attack pectic substances in the plant cell wall play a major role in pathogenicity (Cole et al., 1998). The role of pectin degrading enzymes in causing cell wall degradation is so important that it determines the virulence of many pathogens (Rogers et al., 2000). This investigation was carried out to study the effect of two fungicides commonly used in Egypt against phytopathogenic fungi of broad bean specially cell wall degrading enzymes which contribute in the first step of pathogenicity by penetrating of cell wall.

MATERIALS AND METHODS

Isolation of fungi

Fungi were isolated from 50 infected leaves samples of broad bean (*Vicia faba* L.) collected from different fields in Qena, Upper Egypt.

Effect of fungicides on cellulase enzymes

Equation pro and Kema zed fungicides were investigated for their effect on cellulaes and pectinase enzymes by the most active fungi for enzyme production. The chemical names, structural formula, active ingredient, manufacturers and agricultural uses of fungicides are shown in Table 1. Fungal isolates including *A. citri* and *Cochliobolus spicifer* (for exo-β-1,4-glucanase, C_1) and *A. alternata* and *A. citri* (for endo-β-1,4-glucanase, C_x) were cultured on 50 ml of the liquid medium containing cellulose or Carboxy methyl cellulose (CMC) as substrates. pH was adjusted and 6.50 ml of media were dispensed into each 250 ml Erlenmeyer conical flasks which were autoclaved for 15 min at 1.5 atm. Different doses of fungicides (50, 100, 200, 300 and 400 ppm, active ingredient) were individually added to the sterilized liquid medium under aseptic conditions. Media without fungicide served as control. Each flask was inoculated with two agar mycelial discs (10 mm diameter) of the tested fungal isolates obtained from a 7 days old culture. Inoculated flasks were incubated for 6 days at 30°C. Cultures were filtered and the filtrates were centrifuged at 4°C for 15 min at 15000 rpm. The clear supernatants were assayed for cellulase activity. Mycelial dry weights of the tested fungi were also determined.

Assay for cellulase activity (C_1 and C_x enzymes)

The method described by Nelson (1944) and modified by Naguib (1964) was employed as follows: 1 ml of each 1% cellulose powder for C_1-cellulase or CMC for C_X-cellulase was added separately to 1 ml of acetate buffer (pH = 6) and 1 ml of each culture filtrate. The

Table 1. Fungicides used their chemical names, active ingredients, manufacturers and agricultural uses.

Trade name	Equation Pro 52.5% WG	Kema zed 50%
Chemical name	Famoxadone: 3-anilino-5-methyl-5-(4 phenoxyphenyl)-2,4-oxazolidinedione. Cymoxanil: 2-2(2-cyano-2-methoxy iminoacetyl)-3-ethylurea.	Methylbenzimidazol-2-ylcarbamate
Active ingredient	Famoxadone 22.5% and Cymoxanil 30%	Carbendazim 50%
Manufacturer	Dobon Dinemores, France	Rotam Agrochemical, Hong Kong
Agricultural use	Systemic fungicide used to control many plant diseases including early and late blight of different vegetables such as potatoes and tomatoes as well as the powdery mildews of vitis.	Agricultural systemic fungicide used to control several plant diseases caused by pathogenic fungi on several types of fruits and vegetables including apple scab and powdery mildews of vitis.

mixtures were incubated for 30 min at 28°C. Similar reaction mixtures using distilled water with reagents were used as a blank. 3 ml of Nelson's solution were added and the reaction mixtures were shaken and placed in a boiling water bath for 15 min. After cooling, 3 ml of the arsenomolybdate solution was added, mixed thoroughly and then diluted to l0 ml with distilled water. The whole mixture was centrifuged to remove any turbidity. The amount of reducing sugars produced was estimated by determining the optical density (absorption spectrum) at 700 nm wave length with a spectrophotometer (Spectronic ® GeneSys ™ 2 PC). A standard curve was plotted using aqueous solutions of D-glucose with concentrations from 10 to 100 µg/ml.

Effect of fungicides on pectinase enzyme

Alternaria citri and *A. raphani* which were the most active pectinase producing isolates were cultured on liquid medium containing pectin as substrate. 50 ml of the liquid medium were dispensed into each 250 ml Erlenmeyer conical flasks which were autoclaved for 15 min at 1.5 atm. Different doses of fungicide (50, 100, 200, 300 and 400 ppm active ingradient) were individually added to the sterilized liquid medium under aseptic conditions. Media without fungicide served as control. Each flask was inoculated with two agar mycelial discs (10 mm diameter) of the tested fungal isolates obtained from seven days old cultures. Inoculated flasks were incubated for 6 days at 30°C. Cultures were filtered and the filtrates were centrifuged at 4°C for 15 min at 15000 rpm. The clear supernatants were assayed for pectinase activity. Mycelial dry weights of the tested fungi were also determined.

Assay for pectinase activity

Pectinase activity was assayed spectrophotometrically at 235 nm wavelength according to the method described by Sherwood (1966). The reaction mixture contained 3 ml of 0.1% pectin in 0.05 M Tris-HCl buffer (pH 8) and 0.5 ml culture filtrate. The mixture was incubated at 30°C for 3 h, and then 3 ml of 0.01 N HCl and 1 ml of the reaction mixture were mixed properly. The optical density was measured at 235 nm. One unit of pectinase activity was defined as the amount of enzyme causing an increase in the absorbance of 0.01 in 30 min.

Statistical analysis

Statistical analysis of the data was carried out by one way analysis of variance and the means were separated by Turkey's honest significant difference test using Biostat 2008 statistical analysis program (Copyright © 2001–2009 Analystsoft).

RESULTS

Effect of fungicides on cellulase enzymes

Five doses (50, 100, 200, 300 and 400 ppm, active ingredient) from each of the two fungicides (equation pro and Kema zed) were used to study the effect of fungicides on cellulase production by *A. citri* and *C. spicifer* for exo-β-1,4-glucanase (C_1) and *A. alternata* and *A. citri* for endo-β-1,4-glucanase (C_x) enzyme. Exo-β-1,4-glucanase produced by *A. citri* and *C. spicifer* were significantly inhibited by all doses of the two fungicides used; except in 50 ppm of Kema zed and 50 and 100 ppm of *C. spicifer*; the enzyme production was slightly increased compared to the control. Also, equation pro had more inhibitory effect than Kema zed fungicide on both fungi (Table 2, Figure 1). Mycelial growth of *A. citri* and *C. spicifer* were significantly inhibited by the all doses of the two fungicides and inhibition effect increased with increasing fungicides concentration, except in few cases where the mycelial growth of fungi were promoted by 50 or 100 ppm of Kema zed (Table 2 and Figure 1). Generally, the inhibitive effect of fungicides depends on the type and the dose of fungicides. The inhibitive effect was increased with increasing fungicide concentration (Table 2 and Figure 2). Endo-β-1,4-glucanase produced by *A. alternata* and *A. citri* was significantly inhibited by all doses of the two fungicides, except in 50 ppm dose of kema zed the enzyme production was slightly increased compared to the control. The inhibitive effect of the fungicides was increased by the increasing dose (Table 3 and Figure 3). Mycelial growth of *A. alternata* and *A. citri* were inhibited by all doses of fungicides, except in some cases at 50 ppm dose where the fungal growth were stimulated compared to the control. Generally, the inhibitive effect of the fungicides was increased by increasing the fungicide concentration. The inhibitive

Table 2. Effect of fungicides on growth and exo-β-1,4-glucanase production by *Alternaria citri* and *Cochliobolus spicifer* after 6 days of incubation at 30°C.

Fungicide	Equation Pro				Kema Zed			
	A. citri		*C. spicifer*		*A. citri*		*C. spicifer*	
Dose (ppm)	**E.P**	**D. wt**	**E.P**	**D. wt**	**E.P**	**D. wt**	**E.P**	**D. wt**
0 (control)	5.3	246	5.4	113	5.3	246	5.4	113
50	4.0*	120*	4.9	92*	5.5	284*	6.1*	125*
100	3.1*	109*	3.4*	49*	5.2	253	5.6	109
200	2.6*	85*	2.5*	35*	4.3*	217*	4.5*	93*
300	2.4*	65*	2.2*	26*	3.7*	177*	3.6*	63*
400	1.7*	60*	1.6*	24*	2.9*	137*	2.6*	52*
Inhibition percentage	24.5-67.9	51.2-75.6	9.2-70.4	18.6-78.8	1.9-45.3	11.8-44.3	16.7-51.9	3.5-54.0

E.P = Enzyme production (µg/ml) D. wt = Dry weight (mg/50 ml). Asterisked values mean significant difference from the control.

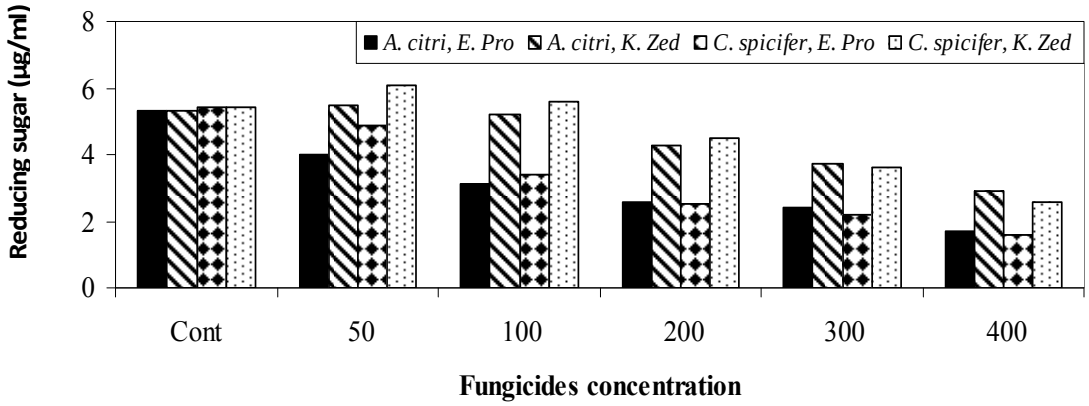

Figure 1. Effect of fungicides on exo-β-1,4-glucanase production by *Alternaria citri* and *Cochliobolus spicifer*.

effect of equation pro on endo- and exo-β-1,4-glucanase production ranged between 5.9 to 70.4% by *A. alternata*, *A. citri* and *C. spicifer*. However, kema zed exhibited an inhibitive effect on cellulases which ranged between 1.9 to 51.9% (Tables 2, 3 and Figures 1 to 4).

Effect of fungicides on pectinase enzyme

Five doses (50, 100, 200, 300 and 400 ppm, active ingredient) from two fungicides (equation pro and Kema zed) were used to study the effect of various concentrations of fungicides, in culture medium, on mycelial growth and pectinase production by *A. citri* and *A. raphani*.

Pectinase produced by *A. citri* and *A. raphani* was significantly inhibited by all doses of the fungicides. Generally, the inhibition effect of these fungicides was increased with the increase of fungicides concentration (Table 4 and Figure 5). Mycelial growth of *A. citri* and *A. raphani* were significantly inhibited by all doses of the two fungicides. Generally, the inhibitive effect of these fungicides on mycelial growth of the tested fungi depends on the type of fungicide and doses used. The inhibitory effect of these fungicides was increased with the increase of fungicide concentration. The inhibitive effect of Equation pro on pectinase enzyme ranged between 20.1 to 75.6% by A. citri and A. raphani while kema zed exhibited an inhibitive effect ranged between 13.5 to 62.8%. The inhibitive effect of these fungicides on the mycelial growth of tested fungi was nearly similar to those for the enzyme production (Table 4 and Figures 5 and 6).

DISCUSSION

In most cases, the equation pro and Kema zed fungicides showed an inhibitive effect on the mycelial growth, cellulase and pectinase enzumes, but with variable degrees which depend on the dose and the type of fungicide. Abdel-Kader et al. (1989) studied the effect of Euparen fungicide on mycelial growth and endo-1, 4 ß-D-

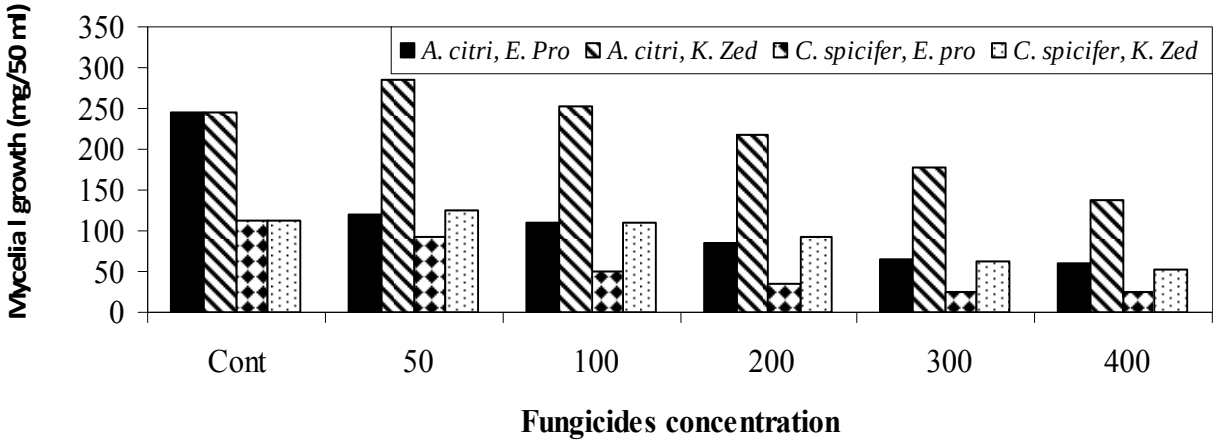

Figure 2. Effect of fungicides on mycelial growth of *Alternaria citri* and *Cochliobolus spicifer*.

Table 3. Effect of fungicides on growth and endo-β-1,4-glucanase production by *Alternaria alternata* and *A. citri* after 6 days of incubation at 30°C.

Fungicide	Equation Pro				Kema Zed			
	A. alternata		*A. citri*		*A. alternata*		*A. citri*	
Dose (ppm)	E.P	D. wt	E.P	D. wt	E.P	D. wt	E.P	D. wt
0 (control)	5.1	165	5.4	139	5.1	165	5.4	139
50	4.8	179*	4.6*	85*	5.0	185*	5.8	141
100	3.7*	145*	4.1*	75*	4.6	151*	4.9*	124
200	3.0*	112*	3.5*	63*	3.8*	132*	4.0*	99*
300	2.5*	70*	2.9*	45*	3.3*	126*	3.6*	94*
400	2.2*	58*	2.6*	41*	3*	119*	3.3*	82*
Inhibition percentage	5.9-56.9	12.1-64.8	14.8-51.8	38.8-70.5	2.0-41.2	8.5-27.9	9.2-38.9	10.8-41.0

E.P = Enzyme production (µg/ml) D. wt = Dry weight (mg/50ml). Asterisked values mean significant difference from the control.

glucanase productions by 11 cellulose-decomposing fungi. The mycelial growth of *Trichoderma viride* was significantly increased by the medium dose and not affected by the other two doses. On the other hand, the mycelial growth and endo-1,4 ß-D-glucanase production of *A. alternata, Fusarium moniliforme*, *Myrothecium verrucaria* and *Thermoascus aurantiacus* were nil at the three doses (4.8, 23.8, 47.5 ppm) of Euparen while *Aspergillus niger* and *Penicillium chrysogenum* were not significantly affected by any dose. The other fungi includes, *A. flavus*, *Chaetomium globosum*, *Drechslera halodes* and *F. solani* which were significantly inhibited or completely eliminated at least by the higher doses. Omar and Abd-Alla (2000) investigated the effect of two fungicides (Afugan and Tilt) at 50 ppm on the activity of various enzymes including cellulase and protease by four nodule colonizing fungi namely *A. awanori*, *A. flavus*, *P. chrysogenum* and *T. koningii*. They found, that, the enzyme activity exhibited different responses to pesticide application. The effect of the two fungicides was essentially stimulatory. Moharram et al. (2004) reported that, the different doses of Kocide caused a regular significant decrease in mycelial growth and cellulase (C_1) production by *A. flavus*, *Cunninghamella echinulata*, *Emercilla* var. *lata*, *F. oxysporum* and *P. aurantiogriseum*. Lower doses of Ridomil plus (50 and 100 ppm) stimulated mycelial growth and cellulase (C_1) production in *A. flavus* and *F. oxysporum.* High doses of Ridomil reduced both fungal growth and enzyme production. Recently, Saleem et al. (2012) studied the effect of Amistar and Moncut fungicides on fungal growth and cellulase production by *A. flavus* var. *columnaris*, *A. fumigatus*, *A. ochraceous*, *Mucor hiemalis* and *T. harzianum*. The incorporation of fungicides in the culture medium for cellulase production exhibited an inhibitive effect on mycelial growth and cellulase production of all fungi by all doses used (100 to 800 ppm).

Mehta (1984) demonstrated that, the maximum reduc-

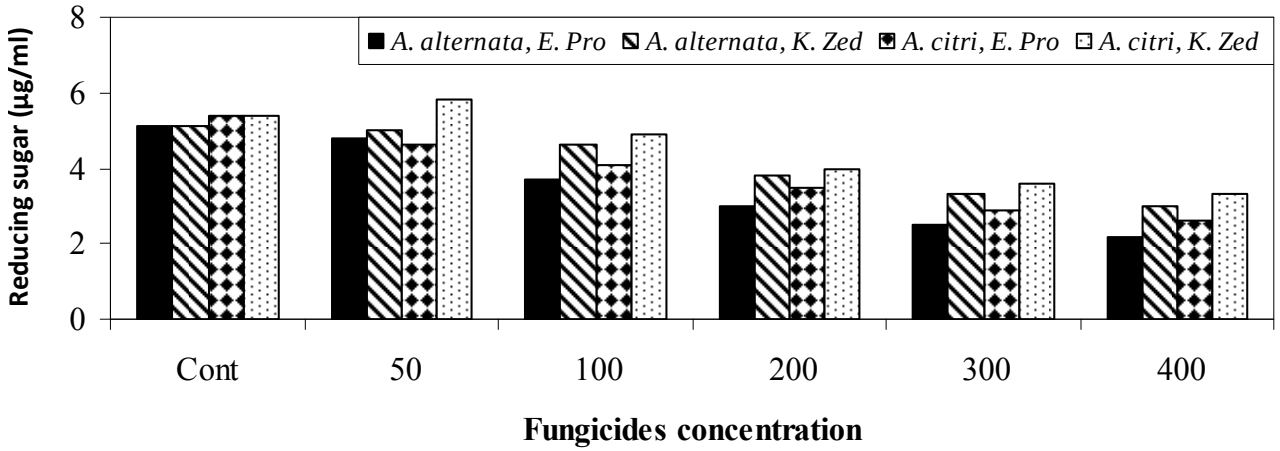

Figure 3. Effect of fungicides on endo-β-1,4-glucanase production by *Alternaria alternata* and *A. citri*.

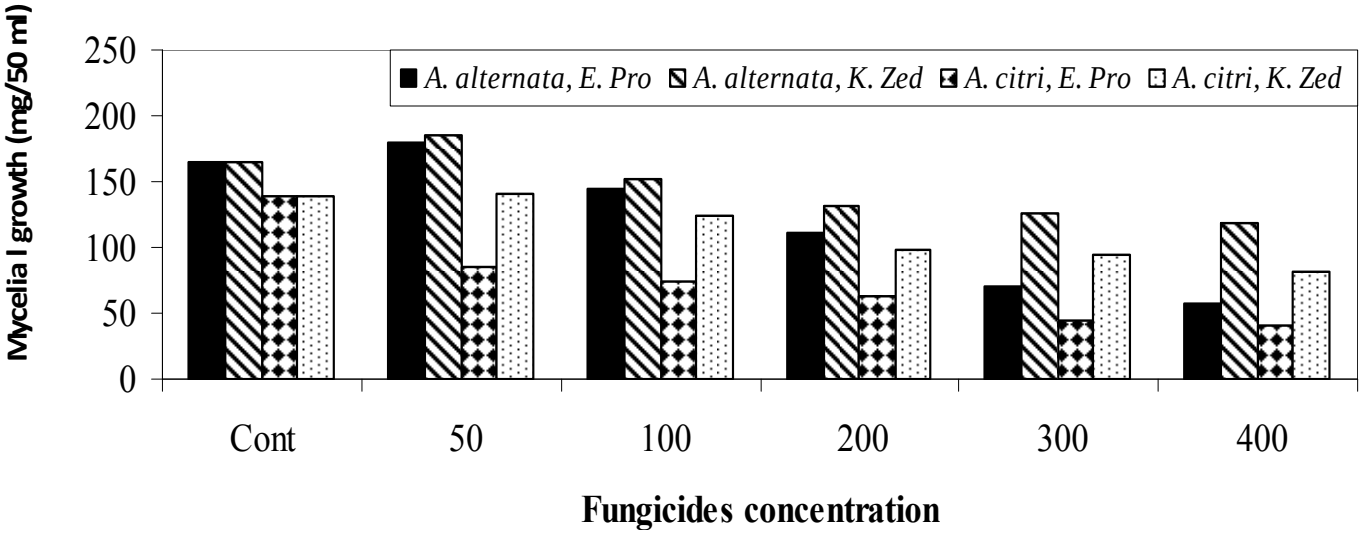

Figure 4. Effect of fungicides on mycelial growth of *Alternaria alternata* and *A. citri*.

Table 4. Effect of fungicides on growth and pectinase production by *Alternaria citri* and *A. raphani* after 6 days of incubation at 30°C.

Fungicide	Equation Pro				Kema Zed			
	A. citri		*A. raphani*		*A. citri*		*A. raphani*	
Dose (ppm)	**E.P**	**D. wt**	**E.P**	**D. wt**	**E.P**	**D. wt**	**E.P**	**D. wt**
0 (control)	2.58	114	2.36	118	2.58	114	2.36	118
50	2.06*	102*	1.79*	85*	2.14*	104*	2.04*	114
100	1.66*	78*	1.59*	58*	1.78*	81*	1.78*	94*
200	1.33*	62*	1.17*	48*	1.52*	65*	1.56*	65*
300	1.02*	51*	0.96*	41*	1.21*	56*	1.14*	63*
400	0.63*	40*	0.65*	34*	0.96*	50*	0.97*	57*
Inhibition percentage	20.1-75.6	10.5-64.9	24.1-72.4	28.0-71.2	17.1-62.8	8.8-56.1	13.5-58.9	3.4-51.7

E.P = Enzyme production (U/ml) D. wt = Dry weight (mg/50ml). Asterisked values mean significant difference from the control.

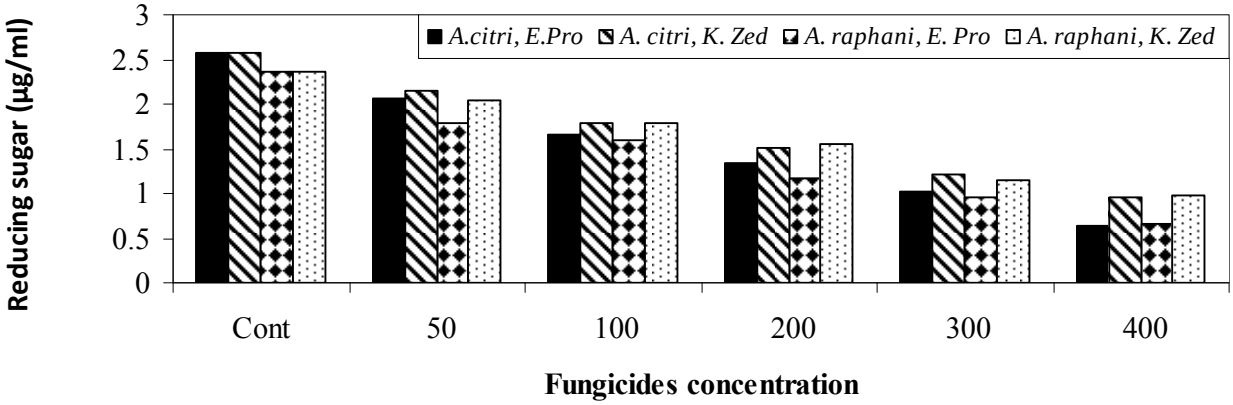

Figure 5. Effect of fungicides on pectinase production by *Alternaria citri* and *A. raphani*.

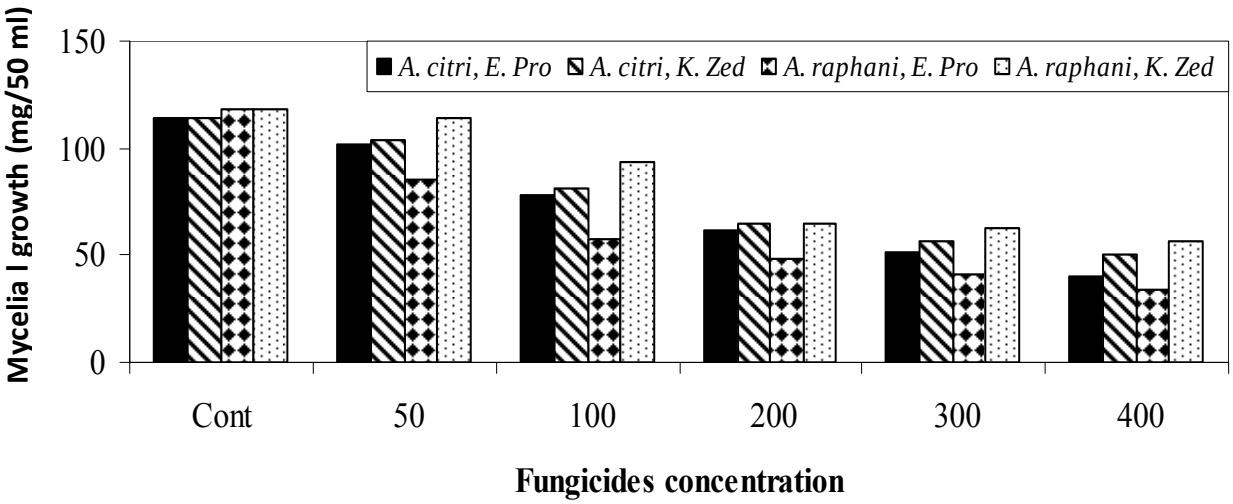

Figure 6. Effect of fungicides on mycelial growth of *Alternaria citri* and *A. raphani*.

tion of pectin methylgalacturonase enzyme synthesis by *A. tenus* was achieved by Brassicol. A high enzyme inhibition was exhibited by Thiram, Cuman, Sultaf and Blimix, respectively. Pectin methylgalacturonase production by *A. tenuis* was also greatly affected by Blitox, Sultaf, Thiram and Brassicol, respectively. Concerning mycelial growth, inhibition of growth was quite variable with different fungicides. Cuman, Blimik and Sultaf showed a stronger inhibition of mycelial growth than Brassicol and Cosan. Blimix was the most effective fungitoxic in nature, other fungicides showed a moderate toxic effect on *A. tenuis*. Gopinath et al. (2006) reported that Propiconazole, Difenoconazole and Carbendazim fungicides inhibited mycelial growth (radial growth and mycelial biomass) of *C. capsici* when compared to controls. Among the fungicides, Propiconazole exhibited the highest level of inhibition, even at lower concentrations. It was followed by Difenoconazole and Carbendazim in descending order. Incorporation of fungicides into the growth medium significantly reduced production of polygalacturonase, polygalacturonase trans-eliminase, pectin trans eliminase and cellulases by *C. capsici*. The highest degree of inhibition was observed in Propiconazole, followed by Difenoconazole and Carbendazim. Recently, Özer et al. (2010) reported that all studied nine fungicides except Triadimenol and Tebuconazole inhibited mycelial dry weight of *Botrytis cinerea* isolates <50%. Cyprodinil + Fludioxonil, Myclobutanil and Imazalil inhibited pectinase activity more than 50%. Fenhexamid had a lower inhibitory effect (<50%) on pectinase activity. Procymidone and Pyrimethanil induced both pectinase activity and isoenzyme banding profile of isolates sensitive to these fungicides.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Abdel-Kader MIA, Moubasher AH, Abdel-Mallek AY (1989). studies on the effect of the fungicide Euparen on cellulose-decomposing fungi in Egyptian soil. J. Basic Microbiol. 29:329-335.

Abu Bakar NK, Abd-Aziz S, Hassan MA, Ghazali FM (2010). Isolation and selection of appropriate cellulolytic mixed microbial cultures for cellulases production from oil palm empty fruit bunch. Biotechnology, 9 (1):73-78.

Alkorta I, Garbisu C, Llama MJ, Serra JL (1998). Industrial applications of pectic enzymes: A review. Process Biochem. 33:21-28.

Arinze AE, Yubedee AG (2000). Effect of fungicides on *Fusarium* grain rot and enzyme production in maize (*Zea mays* L.). Global J. Pure Appl. Sci. 6(4):629-634.

Cole L, Dewey FM,Hawes CR (1998). Immunocytochemical studies of the infection mechanisms of *Botrytis fabae*: II. Host cell wall breakdown. New Phytologist 139:611-622.

Dornez E, Gebruers K, Joye IJ, Ketelaere B, Lenartz J, Massaux C, Bodson B, Delcour JA, Courtin MC (2008). Effects of fungicide treatment, N-fertilisation and harvest date on arabinoxylan, endoxylanase activity and endoxylanase inhibitor levels in wheat kernels. J. of Cereal Sci. 47: 190-200.

Duff SJB, Cooper DG, Fuller OM (1987). Effect of media composition and growth conditions on production of cellulase and β-glucosidase by a mixed fungal fermentation. Enzyme Microb. Technol. 9:47-52.

El-Said AHM (2001). Phyllosphere and phylloplane of banana cultivated in Upper Egypt and their cellulolytic ability. Mycobiol. 29:210-217.

El-Said AHM, Abdel-Hafez SII, Saleem A (2005). Effect of Herbized and Touchdown herbicides on soil fungi and production of some extracellular enzymes. Acta Microbiol. et Immunol. Hung. 52(1):103-128.

Enari TM (1983). Microbial cellulases. In Fogarty WF (ed.) Microbial enzymes and biotechnology. Applied Sciences Publishers, London.
Fernando THPS, Jayasinghe CK, Wijesundera RLC (2001). Cell wall degrading enzyme secretion by *Colletotrichum acutatum*, the causative fungus of secondary leaf fall of *Hevea brasiliensis* (rubber). Mycol. Res., 105: 195-201.

Gautam SP, Bundela PS, Pandey AK, Jamaluddin L, Awasthi MK, Sarsaiya S (2010). Optimization of th medium for the production of cellulase by the *Trichoderma viride* using submerged fermentation. Int. J. Environ. Sci. 1 (4): 656-665.

Gopinath K, Radhakrishnan NV, Jayaraj J (2006). Effect of propiconazole and difenoconazole on the control of anthracnose of chilli fruits caused by *Colletotrichum capsici*. Crop Protect. 25:1024-1031.

Gruzdyev GS, Zinchenko VA, Kalinin VA, Stovtsov RI (1983). Chemical Protection of Plants. Edited by Gruzdyev, G.S. Puplished by Leib G., Russian.

Gummadi SN, Panda T (2003). Purification and biochemical properties of microbial pectinases- a review. Process Biochem. 38:987-996.

Han SO, Yukawa H, Inui M, Doi RH (2003). Regulation of expression of cellulosomal cellulase and hemicellulase genes in *Clostridium cellulovorans*. J. Bacteriol. 185:6067-6075.

Klyosov AA (1990). Trends in biochemistry and enzymology. Biochemistry 29:10577-10585.

Mehta P (1984). Inhibitory effect of some plant growth regulators, production of pectin methylgalacturonase by *Alternaria solani* and *A. tenuis.* Folia Microbiol. 29:43-50.

Moharram AH Abdel-Hafez SII, El-Said AHM, Saleem A (2004). Effect of two systemic fungicides on cellulose decomposing fungi of tomato plants and on some enzymatic activities. Acta Microb. et Immun. Hung. 51(4):403-430.

Moharram AM, El-Said AHM, Saleem A and Hamed A (2011). Effect of amistar and moncut fungicides on fungi of faba bean plant and on amylase activity. Afr. J. Microbiol. Res. 5 (26):4492-4507.

Naguib MI (1964). Effect of sevin on the carbohydrate and nitrogen metabolism during the germination of cotton seeds. Ind. J. Exp. Biol. 2:149-152.

Narasimha G, Sridevi A, Buddolla V Subhosh CM, Rajasekhar RB (2006). Nutrient effects on production of cellulolytic enzymes by *Aspergillus niger*. Afric. J. of Biotech. 5(5):472-476.

Nelson N (1944). A photometric adaptation of the somogyi methods for determination of glucose. J. Biol. Chem. 153:375-380.

Omar SA, Abd-Alla MH (2000). Physiological aspects of fungi isolated from root nodules of faba bean (*Vicia faba* L.). Microbiol. Res. 154:339-347.

Özer N, Köycü ND, Delen N (2010). Analysis of polygalacturonase enzyme production in isolates of *Botrytis cinerea* sensitive to different fungicides used in vineyards. J. Phytopathol. 158(11-12): 744-749.

Ramos AM, Gally M, Garcia MC, Levin L (2010). Pectinolytic enzyme production by *Colletotrichum truncatum*, causal agent of soybean anthracnose. Rev. Iberoam Micol. 27(4):186-190.

Rathod GM, Chavan AM (2010). Extra-cellular pectinase activity of post-harvest fungi from papaya fruits in presence of different influencing factors. J. Exp. Sci. 1(2):7-11.

Rogers LM, Kim YK, Guo W, Gonza lez Carranza L, Li D, Kolattukudy PE (2000). Requirement for either a host or pectin-induced pectate lyase for infection of *Pisum sativum* by *Nectria hematococca*. Proc. Natl. Acad. Sci. 97:9813-9818.

Saleem A, Moharram AH, El-Said AHM, Hamid A (2012). Cellulose decomposing fungi and cellulase activity as affected by amistar and moncut fungicides. Afr. J. Microbiol. Res. 6 (21): 4457-4470.

Sarkar S, Girisham S, Reddy SM (2011). Cellulase activity in fungal infected banana fruits-Effect of different synthetic media on cellulase production. J. Recent Advan. Appl. Sci. 26:12-17.

Shallom D and Shoham Y (2003). Microbial Hemicellulases. Curr. Opin. Microiol. 6:219-228.

Shanmugam P, Mani M, Narayanasamy M (2008). Biosynthesis of cellulolytic enzymes by *Tricothecium roseum* with citric acid mediated induction. Afr. J. Biotech. 7(21):3917-3921.

Sherief AA, El-Tanash AB, Atia N (2010). Cellulase production by *Aspergillus fumigatus* grown on mixed substrate of rice straw and wheat bran. Res. J. Microb. 5(3):199-211.

Sherwood RT (1966). Pectin lyase and polygalacturonase production by *Rhizoctonia solani* and other fungi. Phytopathology, 56: 279-286.

Wanjiru WM, Zhensheng K, Buchenauer H (2002). Importance of cell wall degrading enzymes produced by *Fusarium graminearum* during infection of wheat heads. Eur. J. Plant Pathol. 108(8):803-810.