

Full Length Research Paper

Cellulose decomposing fungi and cellulase activity as affected by amistar and moncut fungicides

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Spray application of the two fungicides; Amistar (25% Azoxystrobin) and Moncut (25% Flutolanil) on faba bean plants in the field exhibited an inhibitive effect on the total and individual counts of cellulose decomposing fungi associated with roots and shoots of plants. The inhibitive effect of these fungicides depended mainly on the fungicide concentration. The inhibitive effect was increased with the increase in fungicide concentration at different periods of treatment. Forty four fungal isolates representing 35 species and 2 varieties belonging to 19 genera were screened for their abilities to produce exo- β -1,4 glucanase and endo- β -1,4 glucanase enzymes. All fungal isolates tested had the ability to produce cellulase enzymes, but with variable degrees. For exo- β -1, 4 glucanase, six isolates (represent 13.7% of total isolates) showed high cellulase activity. However, twenty one isolates (47.7% of total isolates) were found to be moderate in their cellulase activity. The remaining isolates (17 isolates, represent 38.6% of total isolates) were low producers of cellulase. For endo- β -1, 4 glucanase enzyme, five isolates (represent 11.4% of total isolates) showed high cellulase activity and twenty one isolates (47.7% of total isolates) had moderate ability to produce cellulase. The remaining isolates (18 isolates, represent 40.9% of total isolates) were low producers of cellulase. When these fungicides (at 100-800 ppm) were incorporated individually into the liquid culture medium specified for growth and an extracellular cellulase production was exerted an inhibitive effect on both mycelial growth and cellulase production of *Aspergillus flavus* var. *columnaris*, *A. fumigatus*, *A. ochraceous*, *Mucor hiemalis* and *Trichoderma harzianum*.

Key words: Fungicides, faba bean, cellulose decomposing fungi, cellulase enzymes.

INTRODUCTION

Broad bean (*Vicia faba* L.) is one of the most important winter crops in many parts of the world. It's production is concentrated in nine major regions; Northern Europe, Mediterranean, the Nile Valley, Ethiopia, Central Asia, East Asia, Oceania, Latin America and North America. Sometimes it is grown for green manure, but more generally for stock feed. Cultivated faba bean is used as human and animal food in many countries of the world. It can be used as a vegetable, green or dried, fresh or canned (Bond et al., 1985). Faba bean is attacked by several fungi causing many diseases including chocolate

spot (*Botrytis fabae* and *B. cinerea*), rust (*Uromyces viciae fabae*), black root rot (*Thielaviopsis basicola*), stem rots (*Sclerotinia trifoliorum*, *S. sclerotiorum*), root rots and damping-off (*Pythium* spp. and *Rhizoctonia* spp.), downy mildew (*Pernospora viciae*), leaf and pod spots or blight (*Ascochyta fabae*), foot rots (*Fusarium* spp.) (Van Emden et al., 1988). Recently, *Alternaria tenuissima* was found to be the pathogen of broad bean causing new leaf spot disease in Japan (Rahman et al., 2002).

Cellulose is the major component of plant biomass and the most abundant organic polymer on the earth. It is a linear homo-polysaccharide consisting of anhydrous glucose units that are linked by β -1,4-glycosidic bonds. Cellulases are capable of hydrolyzing β -1,4-glycosidic bonds in cellulose and have been broadly divided into

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

Fungicides	Amistar	Moncut
Chemical name	Methyl (α E)-2-[6-(2 cyanophenoxy)-4-pyrimidinyl]oxy]- α (methoxymethylene) benzeneacetate.	N-[3-(1-methylethoxy) phenyl]-2-(trifluoromethyl) benzamide
Active ingredient	Azoxystrobin 25% SC	Flutolanil 25%
Manufacturer	Syngenta Agro, Switzerland	Nihon Nohyako, Japan

Table 2. Doses of fungicides used for treatment of faba bean plants in the field.

Doses fungicides	Low	Recommended	High	Unit
Amistar	0.215	0.43	0.86	ml
Moncut	0.535	1.071	2.142	g

three types: endoglucanase (endo-1,4-D-glucanase, EG), cellobiohydrolase (exo-1,4-D-glucanase, CBH) and glucosidase (1,4-D-glucosidase, BG). EG acts in random fashion, cleaving linked bonds within the cellulose molecule. CBH removes cellobiose units from the nonreducing ends of the cellulose chain and BG degrades cellobiose and cellooligosaccharides to glucose (Saha, 2004). In nature there are many microorganisms, fungal and bacterial, produce enzymes that are capable of catalyzing the hydrolysis of cellulose. These fungi are members of *Aspergillus*, *Chaetomium*, *Mucor*, *Penicillium*, *Trichoderma* and Dematiaceous hyphomycetes as reported by several workers (Nelly, 1991; Abdel-Hafez et al., 1995; Moharram et al., 2004; El-Said et al., 2005, 2006; Vasil'chenko et al., 2005; El-Said and Saleem, 2008).

Several investigations were conducted on the effect of different fungicides on fungi in different places of the world (El-Ghaouth et al., 2001; Culbreath et al., 2002; Buck and Williams, 2003; Fravel et al., 2005; Meyer et al., 2006; Das et al., 2007; Groth, 2008; Sahile et al., 2008) as well as on mycelial growth and extracellular enzymes production by different fungal species (Abdel-Kader et al., 1989; Hassan, 1993; Saleem, 1995; Choi et al., 1997; Tsuji et al., 1997; Omar and Abd-Alla, 2000; Moharram et al., 2004; Fravel et al., 2005). This article was conducted to determine the selective effect of Amistar and Moncut fungicides on cellulose decomposing fungi associated with both roots and leaves of faba bean plant. In addition to the effect of these fungicides on cellulase enzyme production by the most active isolates of fungi.

MATERIALS AND METHODS

Pesticides used in the present investigation

Two fungicides commonly used in Egypt were employed in the

present investigation namely Amistar and Moncut. The fungicides are recently used in Egypt for controlling several fungal diseases of fruit trees, vegetables and economic crops. Their chemical names, active ingredients and their manufacturers are shown in Table 1.

Effect of fungicides on the mycobiota of faba bean plants

Treatment of plants by fungicides

Plants of 3 weeks age were sprayed with three doses of fungicides (low, medium 'recommended field dose' and high doses) as shown in Table 2. Untreated plants served as control.

Determination of rhizosphere, rhizoplane, phyllosphere and phylloplane fungi of faba bean

Plants treated and untreated with fungicides were removed from the soil and transferred to the mycological laboratory for assaying their fungal contents of rhizosphere, rhizoplane (root surface), phyllosphere and phylloplane (leaf surface) at intervals of 1, 2, 4, 6, 8 and 10 weeks of treatment.

Determination of rhizosphere soil fungi

Plants were randomly chosen and pulled from the soil. Soil particles adhered to roots treated and untreated with fungicides were collected. Rhizosphere soil fungi were determined using the dilution plate method (Johnson and Curl, 1972). Cultivated plates were incubated at 28°C for 7 days. The developing fungi were identified and counted. The numbers of fungi were calculated per mg of dry soil.

Determination of root surface (rhizoplane) fungi

Roots were subjected to series of washing by sterile distilled water. They were thoroughly dried between sterile filter papers, cut into equal segments (1 cm each) which were then palced on the surface of cellulose-Czapek's agar medium (4 plates). Plates were incubated at 28°C for 7 days and the developing fungi were identified, counted and calculated/20 root segments.

Determination of phyllosphere fungi

Known weights of treated and untreated leaves of faba bean were placed individually in sterile conical flasks containing 100 ml sterile distilled water. Flasks were shaken for 10 min. Ten ml of the suspension were transferred into flask (250 ml) containing 90 ml sterile distilled water. After five minutes of shaking, one ml of the final dilution was transferred to a sterile Petri-dish followed by addition of ~ 15 ml of liquid cellulose-Czapek's agar medium. Four replicates were prepared for each treatment and control. Plates were incubated at 28°C for 7 days and the developing fungi were identified, counted and calculated per mg of fresh weight of leaves.

Determination of leaf surface (phylloplane) fungi

Leaf samples from fungicides-treated and untreated faba bean plants were subjected to a series of washing with sterile distilled water. They were thoroughly dried between sterile filter papers, cut into equal segments (1 cm² each). Five segments were placed on the surface of cellulose-Czapek's agar medium in each plate. Four plates were prepared for each treatment and control. Plates were incubated at 28°C for 7 days, and the developing fungi were identified, counted and calculated/20 leaf segments.

Medium used for isolation of cellulose decomposing fungi

Cellulose-Czapek's agar medium (g/liter): Sodium nitrate, 3.0; Potassium dihydrogen phosphate, 1.0; Magnesium sulphate, 0.5; Potassium chloride, 0.5; Ferrous sulphate, 0.01; Cellulose powder, 20.0; Agar 15.0) was used for isolation of cellulose decomposing fungi. Rose bengal (0.1 mg/ml) and Chloramphenicol (0.5 mg/ml) were used as bacteriostatic agents (Smith and Dawson, 1944; Al-Doory, 1980).

Cellulase enzymes

Screening of fungal isolates for production of extracellular cellulase enzymes

Forty four fungal isolates (recovered from different parts of faba bean plants) representing 35 species and 2 varieties related to 19 genera were screened for their abilities to produce exo- and endo- β -1,4-glucanase enzymes on the solid media. Exo- β -1,4-glucanase (C₁-cellulase) and endo- β -1, 4-glucanase (C_x cellulase) were estimated according to the methods described by Eggins and Pugh (1962), Dingle et al. (1953) and Prasan and Verma (1979).

Effect of fungicides on mycelial growth and cellulase production by some fungi

Cultivation and culture conditions

Five fungal isolates namely, *Aspergillus flavus* var. *columnaris*, *A. fumigatus*, *A. ochraceus*, *M. hiemalis* and *T. harzianum* were used to study the effect of fungicides on mycelial growth and cellulase production since these fungi were found to be the highest activity of cellulase enzyme. Different doses of the two fungicides used ranging from 100 to 800 ppm were incorporated into 50 ml aliquots liquid medium. Cultures were inoculated with 2 agar mycelial discs (10 mm diameter) on which fungal species were allowed to grow. Modified Czapek's solution containing cellulose as a substrate was used for production of cellulase enzyme. After 7 days of incubation

at 28°C, cultures were filtered and the harvested mycelium was oven dried and weighed. Aliquots of 1 ml of the crude filtrate were assayed for enzymatic activity.

Determination of cellulase activity

Cellulase activity was assayed spectrophotometrically according to the method described by Nelson (1944) and modified by Naguib (1964), where 1 ml of 1% cellulose solution in acetate buffer (pH= 6) was used for exo- β -1, 4-glucanase enzyme. The activity was calculated as μ g reducing sugars per 1 ml of culture medium. Similar reaction mixtures using heated inactive enzyme solution were also used as control and water with reagents was used as a blank. A standard curve was plotted using aqueous solution of D-glucose with concentration from 10-120 μ g/ml.

Statistical analysis

Statistical analysis of data was carried out by one way analysis of variance and the means were separated by Tukey's honest significant difference test using Biostat 2007 statistical analysis program (Copyright © 2001-2009 Analystsoft).

RESULTS AND DISCUSSION

Effect of fungicides on cellulose decomposing fungi isolated from root surface of faba bean plant

Amistar and Moncut fungicides exhibited an inhibitive effect on the total counts of cellulose decomposing fungi isolated from rhizosphere and rhizoplane of faba bean plant by all doses used after all periods of treatment. The inhibitive effect was increased with the increasing of fungicide concentration after different period of treatment (Tables 3 to 6). The selective effect of different fungicides on rhizosphere and rhizoplane fungi was recorded by several workers (Abdel-Kader et al., 1983; Moubasher et al., 1984, 1991; Nan et al., 1991; Nan, 1995; Benignia and Bompeix, 2004; Moharram et al., 2004). Tridemorph and Benomyl fungicides exerted a toxic effect on the total count of root surface fungi of *Vigna sinensis* by the three doses used after all periods of treatment (Abdel-Kader et al., 1983; Moubasher et al., 1984). Nan et al. (1991) reported that, the total populations of fungi in the roots of red clover plants cultivated in pots treated with prochloraz fungicide (at concentration 240 mg/Kg dry soil) were lower than those from untreated controls. Nan (1995) reported that seed treatments with thiophanate-methyl fungicide or sulphuric acid copper+zinc complex reducing fungal infection of roots and reduced the total percentages of root segments yielding fungal colonies and frequencies of *Fusarium oxysporum* and *F. solani* and other species of *Fusarium*. Recently, Moharram et al. (2004) studied the effect of Kocide and Ridomil plus fungicides on cellulose decomposing fungi isolated from roots of tomato plant. They showed that, these fungicides had an inhibitive effect on cellulose decomposing fungi of tomato by all doses used after different periods of

Table 3. Count (per mg of dry soil) of rhizosphere fungi after different periods of faba bean treatment with various doses of Amistar on cellulose-Czapek's agar at 28°C.

Weeks after treatment	1				2				4				6				8				10			
	Doses				Doses				Doses				Doses				Doses				Doses			
Genera and species	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H
Total count	3.7	2.1*	1.4*	0.7*	4.9	4.0	2.3*	1.6*	5.0	2.9*	1.6*	1.3*	4.7	3.2*	2.0*	1.2*	4.7*	2.5*	1.9*	1.2*	5.0	3.2*	2.1*	1.8*
<i>Alternaria alternata</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.2	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Aspergillus</i>	0.8	0.5	0.4*	0.1*	1.8	1.6	1.2*	0.8*	2.3	1.4*	0.7*	0.7*	1.3	1.0	0.8*	0.5*	1.1	0.5*	0.4*	0.3*	1.2	1.2	0.8	0.8
<i>A. flavus</i>	0.4	0.4	0.3	0.1	0.4	0.4	0.4	0.2	0.5	0.2	0.2	0.1*	0.3	0.2	0.2	0.1	0.4	0.2	0.2	0.1	0.4	0.3	0.2	0.2
<i>A. fumigatus</i>	0.2	0.0	0.1	0.0	0.4	0.3	0.3	0.2	0.8	0.6	0.3*	0.4*	0.8	0.5	0.3*	0.3*	0.2	0.1	0.0	0.0	0.2	0.2	0.1	0.1
<i>A. niger</i>	0.0	0.0	0.0	0.0	0.6	0.6	0.4	0.3	0.6	0.4	0.2*	0.2*	0.2	0.2	0.2	0.1	0.3	0.1	0.1	0.1	0.3	0.3	0.2	0.2
<i>A. ochraceous</i>	0.2	0.1	0.0	0.0	0.2	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.2	0.2	0.2
<i>A. terreus</i>	0.0	0.0	0.0	0.0	0.2	0.2	0.1	0.1	0.3	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.2	0.1	0.1	0.0	0.3	0.2	0.1	0.1
<i>Chaetomium globosum</i>	0.4	0.2	0.1	0.1	0.5	0.4	0.2	0.1*	0.2	0.1	0.1	0.0	0.4	0.2	0.1	0.2	0.3	0.2	0.1	0.0	0.4	0.3	0.3	0.2
<i>Emericella</i>	1.0	0.5*	0.2*	0.2*	1.4	1.3	0.6*	0.4*	1.4	1.2	0.7*	0.6*	1.2	0.8	0.5*	0.3*	1.6	0.9*	0.8*	0.3*	1.5	0.9*	0.6*	0.6*
<i>E. nidulans</i>	0.4	0.3	0.1	0.1	0.6	0.4	0.2*	0.2*	0.8	0.5	0.3*	0.3*	0.8	0.6	0.3*	0.2*	0.7	0.4	0.4	0.1*	0.8	0.4*	0.2*	0.2*
<i>E. nidulans</i> var. <i>lata</i>	0.6	0.2*	0.1*	0.1*	0.8	0.9	0.4*	0.2*	0.6	0.7	0.4	0.3	0.4	0.2	0.2	0.1	0.9	0.5*	0.4*	0.2*	0.7	0.5	0.4	0.4
<i>Fusarium oxysporum</i>	0.1	0.0	0.2	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.2	0.2	0.1	0.0	0.3	0.1	0.2	0.1	0.2	0.1	0.1	0.0
<i>Gibberella fujikuroi</i>	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.4	0.2	0.1	0.1	0.2	0.1	0.1	0.0
<i>Mucor hiemalis</i>	0.1	0.1	0.0	0.0	0.2	0.1	0.0	0.0	0.2	0.1	0.0	0.0	0.3	0.1	0.0	0.0	0.2	0.1	0.1	0.1	0.0	0.0	0.0	0.0
<i>Myrothecium roridum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.2	0.1	0.1
<i>Nectria haematococca</i>	0.5	0.4	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.1	0.0	0.0	0.4	0.3	0.2	0.0*	0.3	0.2	0.2	0.2	0.3	0.1	0.0	0.0
<i>Penicillium chrysogenum</i>	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.2	0.1	0.1	0.3	0.2	0.0	0.1	0.2	0.1	0.1	0.1
<i>Phoma hebarum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhizopus stolonifer</i>	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
<i>Stachybotrys chartarum</i>	0.7	0.4	0.3*	0.1*	0.4	0.3	0.0*	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.2	0.0*	0.0*

C = Control; L= Low dose; R = Recommended dose; H = High dose; Astrisked values mean significant difference from control.

treatment.

Aspergillus, *Chaetomium*, *Emericella*, *Fusarium*, *Gibberella* and *Nectria* were the most common genera of fungi isolated from root surface of faba bean plant on cellulose-Czapek's agar. The counts of these genera were decreased by all doses used after different periods of treatment;

except in some cases, the counts were increased or not affected at certain periods and doses. From the previous genera, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Chaetomium globosum*, *Emericella nidulans*, *E. nidulans* var. *lata*, *Fusarium oxysporum*, *Gibberella fujikuroi* and *Nectria haematococca* were the most common

species. The counts of these species were decreased by all doses used after all periods of treatment; except in some cases, the counts were not affected or increased at certain doses and periods (Tables 3 to 6). The selective effect of various fungicides on cellulose decomposing fungi was recorded by several workers (Abdel-Kader et

Table 4. Count (per mg of dry soil) of rhizosphere fungi after different periods of faba bean treatment with various doses of Moncut on cellulose-Czapek's agar at 28°C.

Weeks after treatment	1				2				4				6				8				10			
Doses	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H
Total count	3.7	2.5*	1.5*	1.4*	4.9	3.0*	2.1*	1.7*	5.0	3.0*	2.5*	2.1*	4.7	3.1*	2.1*	1.5*	4.7	2.6*	1.7*	1.4*	5.0	3.0*	2.4*	1.8*
<i>Alternaria alternata</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0
<i>Aspergillus</i>	0.8	0.6	0.6	0.4	1.8	1.3	1.0*	0.7*	2.3	1.5*	1.1*	0.7*	1.3	0.8	0.3*	0.4*	1.1	0.5*	0.3*	0.2*	1.2	0.6*	0.4*	0.4*
<i>A. flavus</i>	0.4	0.3	0.3	0.2	0.4	0.2	0.2	0.1	0.5	0.4	0.2	0.2	0.3	0.2	0.1	0.1	0.4	0.2	0.1	0.1	0.4	0.1	0.1	0.1
<i>A. fumigatus</i>	0.2	0.2	0.2	0.1	0.4	0.4	0.4	0.2	0.8	0.5	0.5	0.2*	0.8	0.4*	0.1*	0.1*	0.2	0.1	0.0	0.0	0.2	0.1	0.1	0.1
<i>A. niger</i>	0.0	0.0	0.0	0.0	0.6	0.4	0.2*	0.2*	0.6	0.3	0.1*	0.1*	0.2	0.1	0.1	0.1	0.3	0.1	0.1	0.0	0.3	0.2	0.1	0.1
<i>A. ochraceous</i>	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
<i>A. terreus</i>	0.0	0.0	0.0	0.0	0.2	0.2	0.1	0.1	0.3	0.3	0.3	0.2	0.0	0.1	0.0	0.1	0.2	0.1	0.0	0.0	0.3	0.2	0.1	0.1
<i>Chaetomium globosum</i>	0.4	0.2	0.1	0.1	0.5	0.0*	0.2	0.1*	0.2	0.1	0.0	0.0	0.4	0.2	0.1	0.0*	0.3	0.1	0.0	0.0	0.4	0.3	0.3	0.2
<i>Emericella</i>	1.0	0.8	0.5*	0.5*	1.4	0.8*	0.5*	0.4*	1.4	0.8*	0.6*	0.6*	1.2	0.9	0.6*	0.4*	1.6	1.0*	0.6*	0.6*	1.5	1.0*	0.6*	0.5*
<i>E. nidulans</i>	0.4	0.4	0.3	0.3	0.6	0.3	0.2*	0.2*	0.8	0.5	0.4*	0.4*	0.8	0.6	0.3*	0.3*	0.7	0.4	0.2*	0.2*	0.8	0.5	0.2*	0.2*
<i>E. nidulans var. lata</i>	0.6	0.4	0.2*	0.2*	0.8	0.5	0.3*	0.2*	0.6	0.3	0.2*	0.2*	0.4	0.3	0.3	0.1	0.9	0.6	0.4*	0.4*	0.7	0.5	0.4	0.3*
<i>Fusarium oxysporum</i>	0.1	0.1	0.0	0.2	0.2	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.2	0.1	0.1	0.0	0.3	0.1	0.1	0.1	0.2	0.2	0.2	0.1
<i>Gibberella fujikuroi</i>	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.1	0.1	0.1	0.4	0.2	0.1	0.1	0.2	0.1	0.1	0.1
<i>Mucor hiemalis</i>	0.1	0.1	0.0	0.0	0.2	0.1	0.0	0.0	0.2	0.1	0.1	0.1	0.3	0.2	0.1	0.0	0.2	0.1	0.1	0.1	0.0	0.0	0.0	0.0
<i>Myrothecium roridum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.4	0.2	0.2	0.1
<i>Nectria haematococca</i>	0.5	0.3	0.1*	0.1*	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.4	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.3	0.2	0.2	0.2
<i>Penicillium chrysogenum</i>	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.3	0.2	0.2	0.1	0.2	0.1	0.1	0.1
<i>Phoma herbarum</i>	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.2	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhizopus stolonifer</i>	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.2	0.2	0.2	0.1	0.3	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.2	0.1	0.1	0.1
<i>Stachybotrys chartarum</i>	0.7	0.3*	0.1*	0.1*	0.4	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.2	0.2	0.0*

C = Control; L= Low dose; R = Recommended dose; H = High dose; Astrisked values mean significant difference from control.

al., 1983; Moubasher et al., 1984, 1987; Das et al., 1995). Moharram et al. (2004) studied the effect of Kocide and Ridomil plus fungicides on cellulose decomposing fungi isolated from roots of tomato plant. They showed that, these fungicides had an inhibitive effect on cellulose decomposing fungi by all doses used after different periods of treatment. The most common fungi were *Alternaria alternata*, *Aspergillus flavus*, *A. fumigates*, *A. niger*, *A. terreus*, *Cochliobolus spicifer*, *Emericella nidulans*, *E. nidulans var. lata*,

Mycosphaerella tassiana, *Penicillium chrysogenum* and *P. oxalicum*. Their counts were reduced or completely eliminated by all doses used after different periods of treatment. Fravel et al. (2005) studied the effect of azoxystrobin, chlorothalonil, mancozeb, mefenoxam and thiram fungicides on *Fusarium* wilt caused by *Fusarium oxysporum*. Azoxystrobin and chlorothalonil were most toxic to the fungus and reduced growth rate and final colony size at 10 ppm or greater concentrations.

Effect of fungicides on cellulose decomposing fungi isolated from leaf surface of faba bean plant

The two fungicides tested exhibited a depressive effect on the total counts of cellulose decomposing fungi isolated from phyllosphere and phylloplane fungi of faba bean plant by all doses used after all periods of treatment. Generally, the depressive effect was increased with the increasing of fungicide concentration (Tables 7 to

Table 5. Count (per 20 root segments) of rhizoplane fungi after different periods of faba bean treatment with various doses of Amistar on cellulose-Czapek's agar at 28°C.

Weeks after treatment	1				2				4				6				8				10			
	Doses				Doses				Doses				Doses				Doses				Doses			
Genera and species	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H
Total Count	25	23	19	12*	28	25	21*	18*	41	28*	24*	19*	42	33	27*	26*	38	34	24*	19*	44	40	30*	29*
<i>Acremonium strictum</i>	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1	0	2	1	1	0	2	1	1	0
<i>Alternaria alternata</i>	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0
<i>Aspergillus</i>	0	0	0	0	0	0	0	0	4	2	1	1	9	5*	3*	3*	10	8	3*	1*	12	12	6*	5*
<i>A. flavus</i>	0	0	0	0	0	0	0	0	2	1	1	1	3	2	1	1	4	2	1	1	6	6	2*	2*
<i>A. fumigatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	4	2	2	2	4	4	2	0*	4	4	2	2
<i>A. niger</i>	0	0	0	0	0	0	0	0	2	1	0	0	2	1	0	0	2	2	0	0	2	2	2	1
<i>Cochliobolus spicifer</i>	0	0	0	0	0	0	0	0	2	1	1	0	2	2	1	1	0	0	0	0	3	2	1	1
<i>Emericella nidulans var. lata</i>	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fusarium</i>	3	2	2	1	6	4	4	3	2	1	1	1	4	3	2	2	6	6	4	4	4	3	3	3
<i>F. oxysporum</i>	3	2	2	1	4	3	3	2	2	1	1	1	3	2	2	2	4	4	3	3	4	3	3	3
<i>F. semitectum</i>	0	0	0	0	2	1	1	1	0	0	0	0	1	1	0	0	2	2	1	1	0	0	0	0
<i>Gibberella</i>	2	1	1	1	2	2	1	1	5	3	3	3	2	2	2	2	4	4	1	1	4	4	3	3
<i>G. fujikuroi</i>	2	1	1	1	2	2	1	1	3	2	2	2	2	2	2	2	2	2	0	0	4	4	3	3
<i>G. pulicaris</i>	0	0	0	0	0	0	0	0	2	1	1	1	0	0	0	0	2	2	1	1	0	0	0	0
<i>Nectria haematococca</i>	20	20	16*	10*	16	15	14	14	20	16*	16*	12*	20	18	16*	16*	15	14	14	13	16	16	14	14
<i>Phoma herbarum</i>	0	0	0	0	4	3	2	0*	5	3	2	2	0	0	0	0	0	0	0	0	3	2	2	2
<i>Rhizopus stolonifer</i>	0	0	0	0	0	0	0	0	0	0	0	0	3	2	2	2	0	0	0	0	0	0	0	0

C = Control; L = Low dose; R = Recommended dose; H = High dose; Astrisked values mean significant difference from control.

10). In this respect, Abdel-Kader et al. (1983) reported that, the fungicide Tridemorph was almost regularly inhibited the total counts of phyllosphere and phylloplane fungi of *Vigna sinensis* by three doses after 10 days and till the end of the experiment. Moubasher et al. (1984) reported that, Benomyl was almost regularly toxic to the total counts of phyllosphere and phylloplane fungi and the toxicity persisted till the end of the experiment. Recently, Moharram et al. (2004) studied the effect of Kocide and Ridomil plus fungicides on the phyllosphere and phylloplane

fungi of tomato plant. They reported that, the two fungicides had an inhibitive effect on cellulose decomposing fungi of tomato by all doses used after different periods of treatment.

A. alternata, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Emericella nidulans var. lata*, *P. chrysogenum* and *Rhizopus stolonifer* were the most common fungi isolated on cellulose-Czapek's agar. The counts of these fungi were reduced by all doses of fungicides after different periods of treatment; except in some cases, the counts of these fungi was not affected or

increased at certain periods and treatments (Tables 7 to 10). Annapurna and Rao (1982) studied the effect of foliar application of Captan, Dithane, Carbaryl and Atrataf on leaf extract and phylloplane counts of maize fungi. Generally the microbial counts in treated samples were less than the control.

In Egypt, the phyllosphere and phylloplane fungi of *Vigna sinensis* were markedly reduced as a result of treatment with Tridemorph and Benomyl fungicides (Abdel-Kader et al., 1983; Moubasher et al. 1984). Moharram et al. (2004) investigated

Table 6. Count (per 20 root segments) of rhizoplane fungi after different periods of faba bean treatment with various doses of Moncut on cellulose-Czapek's agar at 28°C.

Weeks after treatment	1				2				4				6				8				10			
	Doses				Doses				Doses				Doses				Doses				Doses			
Genera and species	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H
Total Count	25	21	19	14*	28	27	18*	11*	41	31*	25*	19*	42	37	30*	22*	38	33	24*	19*	44	38	32*	27*
<i>Acremonium strictum</i>	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	2	1	1	0	2	2	1	1
<i>Alternaria alternata</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
<i>Aspergillus</i>	0	0	0	0	0	0	0	0	4	2	1	1	9	12	7	3*	10	8	5*	3*	12	11	7*	6*
<i>A. flavus</i>	0	0	0	0	0	0	0	0	2	2	1	1	3	6	2	2	4	4	2	2	6	6	3	3
<i>A. fumigatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	0*	4	2	2	0*	4	3	3	2
<i>A. niger</i>	0	0	0	0	0	0	0	0	2	0	0	0	2	2	1	1	2	2	1	1	2	2	1	1
<i>Cochliobolus spicifer</i>	0	0	0	0	0	1	0	0	2	2	0	0	2	2	2	1	0	0	0	0	3	2	1	1
<i>Emericella nidulans var. lata</i>	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fusarium</i>	3	2	2	0	6	6	3	0*	2	2	1	1	4	2	2	1	6	6	2*	2*	4	4	4	3
<i>F. oxysporum</i>	3	2	2	0	4	4	2	0*	2	2	0	0	3	2	2	1	4	4	2	2	4	3	3	3
<i>F. poae</i>	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0
<i>F. semitectum</i>	0	0	0	0	2	1	1	0	0	0	0	0	1	0	0	0	2	2	0	0	0	0	0	0
<i>Gibberella</i>	2	1	1	1	2	2	1	1	5	4	4	1*	2	2	1	1	4	3	1	1	4	5	5	4
<i>G. fujikuroi</i>	2	1	1	1	2	2	1	1	3	2	2	0	2	2	1	1	2	2	1	1	4	4	4	3
<i>G. intricans</i>	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1
<i>G. pulicaris</i>	0	0	0	0	0	0	0	0	2	1	1	1	0	0	0	0	2	1	0	0	0	0	0	0
<i>Nectria haematococca</i>	20	18	16*	13*	16	16	14	10*	20	18	16*	14*	20	16*	16*	16*	15	14	14	13	16	12*	12*	10*
<i>Phoma herbarum</i>	0	0	0	0	4	2	0*	0*	5	3	3	2	0	0	0	0	0	0	0	0	3	2	2	2
<i>Rhizopus stolonifer</i>	0	0	0	0	0	0	0	0	0	0	0	0	3	2	2	0	0	0	0	0	0	0	0	0

C = Control; L= Low dose; R = Recommended dose; H = High dose; Astrisked values mean significant difference from control.

the effect of Kocide and Ridomil plus fungicides on the phyllosphere and phylloplane fungi of tomato plant. They found that, the two fungicides had an inhibitive effect on several cellulose decomposing fungi associated with tomato leaves and these were *A. alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Cochliobolus spicifer*, *Emericella nidulans*, *E. nidulans var. lata*, *Mycosphaerella tassiana*, *Nectria haematococca*, *P. chrysogenum* and *P. oxalicum*.

It is worth to mention that, several plant diseases caused by numerous pathogenic fungi were found to be controlled by various fungicides includes maize smut fungus *Sphacelotheca reiliana* (Ramirez and Medrano, 1991), downy mildew of onion caused by *Peronospora destructor* (O'Brien, 1992), wheat leaf rust caused by *Puccinia recondite* (Scott, 1995), oat leaf stripe pathogen *Pyrenophora avenae* (Brear et al., 1997), apple powdery mildew fungus

Podosphaera leucotricha (Reuveni et al., 1998), daylily rust caused by *Puccinia hemerocallidis* (Buck and Williams, 2003), tomato *Fusarium* wilt caused by *Fusarium oxysporum* (Fravel et al., 2005), sugar beet leaf spot caused by *Cercospora beticola* (Khan and Smith, 2005), *Rhizoctonia* foliar blight of soybean (Meyer et al., 2006), barely leaf rust caused by *Puccinia hordei* (Das et al., 2007), rice sheath blight caused by *Rhizoctonia solani* (Groth, 2008) and faba bean chocolate spot

Table 7. Count (per mg leaves) of phyllosphere fungi after different periods of faba bean treatment with various doses of Amistar on cellulose-Czapek's agar at 28°C.

Weeks after treatment	1				2				4				6				8				10			
	Doses				Doses				Doses				Doses				Doses				Doses			
Genera and species	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H
Total Count	4.0	2.3*	1.5*	0.6*	4.9	3.7*	2.8*	1.6*	5.7	4.3*	3.3*	2.9*	2.6	2.0	1.5*	1.2*	5.0	3.4*	2.4*	1.5*	5.9	5.3	4.0*	2.9*
<i>Alternaria alternata</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.7	0.6	0.6	0.8	0.6	0.6	0.5	0.9	0.7	0.4*	0.3*	0.0	0.0	0.0	0.0
<i>Aspergillus</i>	0.6	0.4	0.2*	0.1*	3.1	2.4	2.2*	1.1*	3.1	2.3	1.9*	1.5*	0.4	0.3	0.3	0.0	1.1	0.6*	0.3*	0.1*	1.9	1.7	1.5*	1.1*
<i>A. flavus</i>	0.3	0.1	0.0	0.0	0.7	0.5	0.3*	0.2*	1.4	1.0*	0.8*	0.7*	0.1	0.1	0.1	0.0	0.5	0.3	0.1*	0.1*	0.3	0.3	0.3	0.2
<i>A. fumigatus</i>	0.2	0.2	0.2	0.1	0.8	0.5	0.4*	0.1*	1.0	0.7	0.7	0.6*	0.2	0.1	0.1	0.0	0.3	0.2	0.1	0.0	0.5	0.5	0.4	0.4
<i>A. niger</i>	0.0	0.0	0.0	0.0	1.5	1.4	1.3	0.7*	0.4	0.3	0.3	0.1	0.1	0.1	0.1	0.0	0.2	0.1	0.1	0.0	0.6	0.5	0.5	0.3
<i>A. ochraceous</i>	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.2	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.4	0.3	0.2	0.2
<i>A. terreus</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0
<i>Curvularia launata</i>	0.3	0.1	0.1	0.0	0.2	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0
<i>Emericella</i>	0.2	0.1	0.1	0.0	0.6	0.4	0.1*	0.2*	0.2	0.1	0.0	0.1	0.3	0.2	0.1	0.1	0.2	0.2	0.1	0.0	1.3	1.1	0.8*	0.8*
<i>E. nidulans</i>	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.3	0.2	0.1	0.1	0.1	0.1	0.0	0.0	0.6	0.5	0.3	0.3
<i>E. nidulans var. lata</i>	0.2	0.1	0.1	0.0	0.5	0.3	0.1*	0.1*	0.2	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.7	0.6	0.5	0.5
<i>Fusarium oxysporum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.4	0.3	0.3	0.0	0.0	0.0	0.1	0.5	0.3	0.2	0.2	0.0	0.0	0.0	0.0
<i>Humicola grisea</i>	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.2	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.2	0.1
<i>Mucor racemosus</i>	0.5	0.3	0.3	0.2	0.0	0.0	0.0	0.0	0.3	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.2	0.1	0.1
<i>Mycosphaerella tassiana</i>	0.9	0.6	0.4*	0.1*	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.4	0.2	0.2	0.1	0.5	0.4	0.3	0.1*
<i>Myrothecium roridum</i>	0.6	0.3	0.3	0.1*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.2	0.2	0.1	0.0	0.0	0.0	0.0	0.6	0.5	0.4	0.2*
<i>Nectria haematococca</i>	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.5	0.3	0.3	0.2	0.4	0.4	0.2	0.2
<i>Penicillium chrysogenum</i>	0.2	0.1	0.0	0.0	0.3	0.2	0.2	0.1	0.1	0.1	0.0	0.0	0.4	0.4	0.3	0.3	0.8	0.6	0.6	0.5	0.3	0.3	0.2	0.1
<i>Scopulariopsis brevicaulis</i>	0.0	0.0	0.0	0.0	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.3	0.2	0.1	0.0	0.0	0.0	0.0
<i>Setosphaeria rostrata</i>	0.0	0.0	0.0	0.0	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.2	0.1
<i>Stachybotrys chartarum</i>	0.6	0.4	0.1*	0.1*	0.0	0.0	0.0	0.1	0.4	0.3	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.1	0.1

C = Control; L= Low dose; R = Recommended dose; H = High dose; Astrisked values mean significant difference from control.

caused by *Botrytis faba* (Sahile et al., 2008).

Screening of fungi for cellulase production

Forty four fungal isolates represent 35 species

and 2 varieties including 19 genera were screened for their abilities to produce exo- β -1, 4 glucanase and endo- β -1,4 glucanase. All isolates were recovered from different parts of faba bean plant. All fungal isolates tested had the ability to produce cellulase enzymes, but with variable

degrees. For exo- β -1, 4 glucanase, Six isolates (represent 13.7% of total isolates) showed highcellulase activity. Twenty one isolates (47.7% of total isolates) were found to be moderate cellulase activity. The remaining isolates (17 isolates, 38.6% of total isolates) were low

Table 8. Count (per mg leaves) of phyllosphere fungi after different periods of faba bean treatment with various doses of Moncut on cellulose-Czapek's agar at 28°C.

Weeks after treatment	1				2				4				6				8				10							
Doses	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H				
Genera and species	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H
Total Count	4.0	2.3*	1.4*	1.0*	4.9	3.8*	2.8*	2.1*	5.7	4.8	3.4*	1.8*	2.6	2.2	1.5*	0.8*	5.0	3.1*	1.7*	1.8*	5.9	4.4*	3.7*	2.8*				
<i>Alternaria alternata</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.6	0.4	0.3*	0.8	0.7	0.5	0.3*	0.9	0.5*	0.2*	0.2*	0.0	0.0	0.0	0.0				
<i>Aspergillus</i>	0.6	0.3	0.2*	0.0*	3.1	2.6	2.1*	1.8*	3.1	2.6	1.9*	0.9*	0.4	0.3	0.3	0.0*	1.1	0.7*	0.2*	0.2*	1.9	1.5	1.2*	0.9*				
<i>A. flavus</i>	0.3	0.1	0.1	0.0	0.7	0.5	0.4	0.4	1.4	1.1	0.8*	0.5*	0.1	0.1	0.1	0.0	0.5	0.3	0.1*	0.1*	0.3	0.1	0.1	0.1				
<i>A. fumigatus</i>	0.2	0.1	0.1	0.0	0.8	0.7	0.7	0.5	1.0	0.9	0.6*	0.2*	0.2	0.1	0.1	0.0	0.3	0.2	0.1	0.0	0.5	0.5	0.4	0.3				
<i>A. niger</i>	0.0	0.0	0.0	0.0	1.5	1.3	1.0*	0.8*	0.4	0.3	0.3	0.1	0.1	0.1	0.1	0.0	0.2	0.1	0.0	0.1	0.6	0.4	0.4	0.2*				
<i>A. ochraceous</i>	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.2	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.4	0.4	0.3	0.3				
<i>A. terreus</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0				
<i>Curvularia launata</i>	0.3	0.1	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0				
<i>Emericella</i>	0.2	0.2	0.1	0.0	0.6	0.4	0.3	0.2*	0.2	0.2	0.1	0.2	0.3	0.2	0.2	0.1	0.2	0.1	0.0	0.0	1.3	0.9*	0.9*	0.7*				
<i>E. nidulans</i>	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.3	0.2	0.2	0.1	0.1	0.1	0.0	0.0	0.6	0.4	0.4	0.3				
<i>E. nidulans var. lata</i>	0.2	0.2	0.1	0.0	0.5	0.3	0.2	0.2	0.2	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.7	0.5	0.5	0.4				
<i>Fusarium oxysporum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.6	0.4	0.1*	0.0	0.0	0.0	0.0	0.5	0.3	0.2	0.2	0.0	0.0	0.0	0.0				
<i>Humicola grisea</i>	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.1	0.1				
<i>Mucor racemosus</i>	0.5	0.4	0.2	0.2	0.0	0.0	0.0	0.0	0.3	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.0				
<i>Mycosphaerella tassiana</i>	0.9	0.5*	0.3*	0.3*	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.4	0.3	0.2	0.2	0.5	0.3	0.3	0.2				
<i>Myrothecium roridum</i>	0.6	0.3	0.3	0.2*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.3	0.1	0.1	0.0	0.0	0.0	0.0	0.6	0.4	0.4	0.3				
<i>Nectria haematococca</i>	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.4	0.3	0.3	0.4	0.3	0.3	0.2				
<i>Penicillium chrysogenum</i>	0.2	0.1	0.0	0.0	0.3	0.3	0.2	0.1	0.1	0.1	0.0	0.0	0.4	0.4	0.3	0.3	0.8	0.5	0.4*	0.4*	0.3	0.2	0.2	0.1				
<i>Scopulariopsis brevicaulis</i>	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.2	0.2	0.2	0.0	0.0	0.0	0.0				
<i>Setosphaeria rostrata</i>	0.0	0.0	0.0	0.0	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.2	0.2				
<i>Stachybotrys chartarum</i>	0.6	0.3	0.3	0.2*	0.0	0.0	0.0	0.0	0.4	0.3	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.1	0.1				

C = Control; L= Low dose; R = Recommended dose; H = High dose; Astrisked values mean significant difference from control.

producers of cellulase. For endo- β -1, 4 glucanase enzyme, Five isolates (represent 11.4% of total isolates) showed high cellulase activity. However, twenty one isolates (47.7% of total isolates) had moderate ability to produce cellulase and the

remaining isolates (18 isolates, 40.9% of total isolates) were low producers of cellulase (Table 11). Moharram et al. (2004) screened seventy two fungal isolates recovered from tomato plant for their ability to produce cellulase enzymes. They

showed that all fungal isolates tested had the ability to produce cellulase enzymes, but with variable degrees. *Aspergillus flavus*, *Cunninghamella echinulata*, *Emericella nidulans var. lata*, *Fusarium oxysporum* and *Penicillium*

Table 9. Count (per 20 leaf segments) of phylloplane fungi after different periods of faba bean treatment with various doses of Amistar on cellulose-Czapek's agar at 28°C.

Weeks after treatment	1				2				4				6				8				10			
	Doses				Doses				Doses				Doses				Doses				Doses			
Genera and species	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H
Total count	40	29*	22*	20*	62	43*	37*	25*	65	50*	38*	29*	70	55*	45	36*	70	45*	36*	18*	71	54*	42*	42*
<i>Acremonium strictum</i>	0	0	0	0	0	0	0	0	4	2	1	1	0	0	0	0	8	4*	2*	2*	3	2	2	2
<i>Alternaria alternata</i>	0	0	0	0	10	7	5*	2*	14	11	11	8*	12	8*	7*	7*	6	2*	2*	0*	4	3	0*	0*
<i>Aspergillus</i>	36	27	22*	20*	34	28	25	18*	22	20	14*	12*	16	14	11*	8*	27	18*	18*	10*	40	34	28*	28*
<i>A. flavus</i>	10	9	8	8	10	9	7	7	8	7	7	4*	4	3	2	2	9	6	6	4*	10	9	9	9
<i>A. fumigatus</i>	10	8	6*	4*	4	3	3	2	4	4	2	2	8	8	6	4*	4	4	4	2	12	12	8*	8*
<i>A. niger</i>	8	6	4*	4*	10	9	9	5*	8	8	4*	4*	4	3	3	2	6	4	4	4	14	11	9*	9*
<i>A. ochraceous</i>	8	4*	4*	4*	6	4	4	2*	2	1	1	1	0	0	0	0	6	2*	2*	0*	4	2	2	2
<i>A. terreus</i>	0	0	0	0	4	3	2	2	0	0	0	1	0	0	0	0	2	2	2	0	0	0	0	0
<i>Chaetomium globosum</i>	0	0	0	0	0	0	0	0	4	2	2	0*	0	0	0	0	5	3	2	2	4	3	3	3
<i>Cochliobolus spicifer</i>	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	2	2	0	0	0	0	0	0
<i>Emericella nidulans</i> var. <i>lata</i>	4	2	0*	0*	2	1	1	0	4	3	2	0*	0	0	0	0	4	4	2	0*	9	4*	4*	4*
<i>Fusarium oxysporum</i>	0	0	0	0	0	0	0	0	4	3	2	2	6	4	4	2*	2	2	0	0	0	0	0	0
<i>Myrothecium roridum</i>	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	2	2
<i>Nectria haematococca</i>	0	0	0	0	4	3	2	2	6	4	2*	2*	8	6	6	6	4	2	2	0*	0	0	0	0
<i>Penicillium chrysogenum</i>	0	0	0	0	6	4	4	3	5	4	4	4	10	8	6*	4*	4	2	2	0*	4	3	1	1
<i>Rhizopus stolonifer</i>	0	0	0	0	4	0*	0*	0*	0	0	0	0	13	11	11	9*	8	6	6	4*	4	3	2	2
<i>Ulocladium botrytis</i>	0	0	0	0	0	0	0	0	2	1	0	0	3	2	0	0	0	0	0	0	0	0	0	0

C = Control; L= Low dose; R = Recommended dose; H = High dose; Astrisked values mean significant difference from control.

aurantiigriseum, *chrysogenum* were the most active producers of exo- β -1,4 glucanase. However, *Alternaria alternata*, *Aspergillus tamarii*, *Cunninghamella echinulata*, *Emericella nidulans* and *P. chrysogenum* were the most active fungal isolates for endo- β -1,4 glucanase. El-Said and Saleem (2008) screened 42 fungal isolates recovered from soil for their abilities to produce endo 1,4- β -glucanase enzyme (CMase) on solid medium. Ten isolates (23.80% of total isolates)

showed high cellulolytic activity and these were *A. alternata*, *Aspergillus flavus*, *A. fumigatus*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Mucor racemosus*, *Papulaspora immersa*, *Rhizopus stolonifer* and Sterile mycelia. Sixteen isolates (38.09%) were either moderate or low producers of cellulase enzyme. Several fungal species were reported as cellulase producers with variable degrees by several workers (Abdel-Hafez et al.,

1995; El- Said, 2001; El-Said et al., 2006; El-Said and Saleem, 2008).

Effect of fungicides on mycelial growth and cellulase production of some fungi

Since these fungi (*Aspergillus flavus* var. *columnaris*, *A. fumigatus*, *A. ochraceous*, *M. hiemalis* and *T. harzianum*) were found to be the

Table 10. Count (per 20 leaf segments) of phyloplane fungi after different periods of faba bean treatment with various doses of Moncut on cellulose-Czapek's agar at 28°C.

Weeks after treatment	1				2				4				6				8				10			
	Doses				Doses				Doses				Doses				Doses				Doses			
Genera and species	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H	C	L	R	H
Total count	40	37	23*	20*	62	42*	31*	21*	65	48*	35*	22*	70	57*	45*	27*	70	54*	46*	29*	71	45*	42*	36*
<i>Acremonium strictum</i>	0	0	0	0	0	0	0	0	4	2	2	0*	0	0	0	0	8	4*	2*	0*	3	2	2	2
<i>Alternaria alternata</i>	0	0	0	0	10	6*	2*	2*	14	12	12	9*	12	12	10	6*	6	4	4	2*	4	3	2	2
<i>Aspergillus</i>	36	35	23*	20*	34	28	21*	17*	22	19	15*	10*	16	13	10*	5*	27	25	19*	15*	40	27*	25*	23*
<i>A. flavus</i>	10	9	6*	6*	10	9	8	8	8	7	6	4*	4	3	3	2	9	9	7	7	10	10	8	8
<i>A. fumigatus</i>	10	8	6*	6*	4	2	2	0*	4	4	3	2	8	6	4*	3*	4	4	4	2	12	4*	4*	4*
<i>A. niger</i>	8	10	7	6	10	9	9	7	8	6	6	4*	4	4	3	0*	6	8	6	6	14	10*	10*	8*
<i>A. ochraceous</i>	8	8	4*	2*	6	4	2*	2*	2	2	0	0	0	0	0	0	6	2*	0*	0*	4	3	3	3
<i>A. terreus</i>	0	0	0	0	4	4	0*	0*	0	0	0	0	0	0	0	0	2	2	2	0	0	0	0	0
<i>Chaetomium globosum</i>	0	0	0	0	0	0	0	0	4	3	2	2	0	0	0	0	5	4	4	4	4	3	3	3
<i>Cochliobolus spicifer</i>	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	1	2	2	2	0	0	0	0	0
<i>Emericella nidulans var. lata</i>	4	2	0*	0*	2	1	1	0	4	2	0*	0*	0	0	0	0	4	2	2	2	9	4*	4*	0*
<i>Fusarium oxysporum</i>	0	0	0	0	0	0	0	0	4	2	2	0*	6	4	4	2*	2	2	2	0	0	0	0	0
<i>Myrothecium roridum</i>	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	1	1
<i>Nectria haematococca</i>	0	0	0	0	4	2	2	0*	6	4	0*	0*	8	6	4*	3*	4	3	3	2	0	0	0	0
<i>Penicillium chrysogenum</i>	0	0	0	0	6	4	4	2*	5	2	2	1*	10	8	6*	2*	4	4	4	2	4	2	2	2
<i>Rhizopus stolonifer</i>	0	0	0	0	4	1	1	0*	0	0	0	0	13	11	9*	8*	8	4*	4*	2*	4	3	3	3
<i>Ulocladium botrytis</i>	0	0	0	0	0	0	0	0	2	2	0	0	3	1	1	0	0	0	0	0	0	0	0	0

C = Control; L= Low dose; R = Recommended dose; H = High dose; Astrisked values mean significant difference from control.

most active cellulase producers, they employed to study the effect of fungicides on cellulase production. Cellulase production of *Aspergillus flavus* var. *columnaris*, *A. fumigatus*, *A. ochraceous*, *M. hiemalis* and *T. harzianum* was inhibited by all doses of fungicides. The degree of inhibition depended mainly on the fungicide concentration in the culture medium for enzyme production. Generally, the inhibitive effect of the fungicide was increased with the increasing of fungicide concentration. On the other hand, the effect of fungicides on the mycelial growth of *Aspergillus flavus* var. *columnaris*, *A. fumigatus*, *A.*

ochraceous, *M. hiemalis* and *T. harzianum* was similar to their effect on the enzyme production. The fungicides tested inhibited the mycelial growth of all tested fungi and the inhibitive effect was increased with the increasing of fungicide concentration in the culture medium (Tables 12 and 13). Peterauer et al. (1992) investigated the effect of Benomyl on growth and cellulase formation of *Trichoderma reesei*, *T. harzianum* and the Benomyl-resistant mutant *T. harzianum*. *T. reesei* produced the highest amount of cellulase and *T. harzianum* produced the lowest cellulase amounts. Growth of both strains was

equally inhibited by 2 µg/ml of Benomyl. Recently, Moharram et al. (2004) investigated the effect of two systemic fungicides Kocide and Ridomil plus on mycelial growth and cellulase production by different fungal species including *Alternaria alternata*, *Aspergillus flavus*, *A. tamarii*, *Cunninghamella echinulata*, *Emericella nidulans*, *E. nidulans* var. *lata*, *Fusarium oxysporum*, *Penicillium aurantiogriseum* and *P. chrysogenum*. They recorded reduction effect on mycelial growth and cellulase production by tested fungi particularly at high doses (200-400 ppm) of fungicides.

Table 11. Screening of fungal isolates for their abilities to produce cellulase enzymes.

Fungal isolates	Source	Cellulases	
		C ₁	C _x
<i>Acremonium strictum</i>	Phylloplane	24 M	21 M
<i>Alternaria alternata</i>	Phylloplane	26 M	26 M
<i>Aspergillus carneus</i>	Phyllosphere	20 L	24 M
<i>A. flavus</i>	Phyllosphere	19 L	20 L
<i>A. flavus</i>	Rhizosphere	20 L	21 M
<i>A. flavus</i> var. <i>columnaris</i>	Phyllosphere	33 H	30 H
<i>A. fumigatus</i>	Phylloplane	35 H	30 H
<i>A. fumigatus</i>	Phyllosphere	33 H	28 M
<i>A. fumigatus</i>	Rhizosphere	35 H	29 H
<i>A. niger</i>	Rhizosphere	19 L	22 M
<i>A. ochraceous</i>	Rhizoplane	21 M	21 M
<i>A. ochraceous</i>	Phylloplane	32 H	29 H
<i>A. sydowii</i>	Phyllosphere	21 M	22 M
<i>A. sydowii</i>	Rhizosphere	20 L	23 M
<i>A. tamaritii</i>	Phyllosphere	25 M	21 M
<i>A. terreus</i>	Phyllosphere	23 M	19 L
<i>A. terreus</i>	Rhizosphere	25 M	24 M
<i>A. ustus</i>	Rhizosphere	20 L	23 M
<i>A. versicolor</i>	Phylloplane	22 M	21 M
<i>Cladosporium cladosporioides</i>	Phyllosphere	18 L	17 L
<i>Chaetomium globosum</i>	Phylloplane	20 L	14 L
<i>Curvularia lunata</i>	Phyllosphere	24 M	19 L
<i>Emericella nidulans</i>	Phyllosphere	20 L	20 L
<i>E. nidulans</i> var. <i>lata</i>	Phyllosphere	22 M	25 M
<i>Fusarium oxysporum</i>	Rhizoplane	14 L	20 L
<i>F. poae</i>	Rhizoplane	23 M	16 L
<i>F. semitectum</i>	Rhizoplane	21 M	14 L
<i>Gibberella fujikuroi</i>	Rhizoplane	25 M	18 L
<i>G. intricans</i>	Rhizoplane	20 L	15 L
<i>G. pulicaris</i>	Rhizoplane	17 L	16 L
<i>Mucor hiemalis</i>	Rhizosphere	29 H	15 L
<i>M. racemosus</i>	Phyllosphere	20 L	16 L
<i>Mycosphaerella tassiana</i>	Phylloplane	26 M	22 M
<i>Myrothecium roridum</i>	Phylloplane	20 L	18 L
<i>Nectria haematococca</i>	Rhizoplane	20 L	15 L
<i>Penicillium brevicompactum</i>	Phylloplane	25 M	24 M
<i>P. chrysogenum</i>	Phylloplane	26 M	27 M
<i>P. chrysogenum</i>	Phyllosphere	24 M	28 M
<i>P. funiculosum</i>	Phyllosphere	24 M	22 M
<i>Phoma herbarum</i>	Rhizoplane	24 M	26 M
<i>Rhizopus stolonifer</i>	Rhizosphere	25 M	27 M
<i>Setosphaeria rostrata</i>	Phylloplane	18 L	16 L
<i>Stachybotrys chartarum</i>	Phyllosphere	16 L	17 L
<i>Trichoderma harzianum</i>	Phyllosphere	24 M	30 H

High activity > 28; Moderate activity = 21-28; Low activity < 21.

The inhibitive effect of different fungicides on mycelial growth and extracellular enzymes of several fungi was

recorded by many workers (Abdel-Kader et al., 1989; Saleem, 1995; Omar and Abd-Alla, 2000; Fravel et al.,

Table 12. Effect of Amistar on growth and cellulase production by some fungi after 7 days of incubation at 28°C.

Fungi dose (ppm)	<i>A. flavus</i> var. <i>columnaris</i>		<i>A. fumigatus</i>		<i>A. ochraceous</i>		<i>M. hiemalis</i>		<i>T. harzianum</i>	
	EP	D wt	EP	D wt	EP	D wt	EP	D wt	EP	D wt
0 (control)	4.28	192	4.36	210	5.42	150	3.48	149	5.06	183
100	4.08	159*	4.16	153*	4.60*	132*	3.06*	130	4.76	162*
200	3.66*	140*	3.82*	125*	3.44*	117*	2.80*	108*	4.40*	155*
400	3.36*	118*	3.56*	116*	3.28*	102*	2.54*	92*	4.14*	138*
600	3.06*	85*	3.24*	104*	3.00*	87*	2.08*	87*	3.84*	121*
800	2.46*	68*	3.06*	89*	2.76*	74*	1.54*	72*	3.72*	109*

E.P = Enzyme production (µg/ml) D wt = Dry weight (mg/100 ml) Asterisked values mean significant difference from the control.

Table 13. Effect of Moncut on growth and cellulase production by some fungi after 7 days of incubation at 28°C.

Fungi dose (ppm)	<i>A. flavus</i> var. <i>columnaris</i>		<i>A. fumigatus</i>		<i>A. ochraceous</i>		<i>M. hiemalis</i>		<i>T. harzianum</i>	
	EP	D wt	EP	D wt	EP	D wt	EP	D wt	EP	D wt
0 (control)	4.28	192	4.36	210	5.42	150	3.48	149	5.06	183
100	3.86	160*	4.22	189	5.18	143	2.94*	118*	4.82	171
200	3.68*	148*	4.02	165*	4.86*	133*	2.64*	109*	4.56*	157*
400	3.50*	125*	3.78*	137*	4.40*	118*	2.46*	96*	4.20*	143*
600	3.00*	88*	3.46*	116*	4.06*	103*	2.14*	82*	4.12*	127*
800	2.74*	60*	3.18*	103*	3.74*	88*	1.92*	67*	3.96*	104*

E.P = Enzyme production (µg/ml); D wt = Dry weight (mg/100 ml); Asterisked values mean significant difference from the control.

2005; Meyer et al., 2006).

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