

VIRULENCE AND ENZYMATIC ACTIVITIES OF SOME ENTOMOPATHOGENIC FUNGI AGAINST WHITEFLIES AND APHIDS.

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ABSTRACT

Selection of *Cladosporium* spp., *Verticillium lecanii*, and *Epicoccum* sp. as bio-control agents against whiteflies and aphids was conducted on the bases of their pathogenicity to insect pests, safety to plant hosts and/or their abilities to produce extracellular enzymes to degrade the insect integument. *In vitro*, the tested fungi were active against the two-selected insects, in spite of the superiority of *Cladosporium* spp. to the others. In addition, the tested fungi caused higher mortality percentages among the whitefly populations than aphids. Among the five examined strains of each fungus, the isolates COMW3, COMW4 of *Cladosporium*; CfMW3, CfMW4 of *V. lecanii* and GbDW3 and GbDW4 of *Epicoccum* were very highly virulence as bio-control agents against the tested insect pests.

Cladosporium spp., *Verticillium lecanii*, and *Epicoccum* sp. produced several extracellular cuticle-degrading proteases, which are chymoelastase, being a determinant of pathogenicity. Multiple extracellular enzymes such as Lipase, Proteinase and Chitinase were detected in the culture media of *Cladosporium* spp., *V. lecanii*, and *Epicoccum* sp. High chitinase and proteinase activities were present in all fungal isolates grown on chitin and gelatin media. Meanwhile, lipase was produced only with the isolates COMW3, COMW4 of *Cladosporium*; CfMW3, CfMW4 of *V. lecanii* and GbDW3 and GbDW4 of *Epicoccum*. No cellulose hydrolysis was detected in cellulose media. Therefore, the two isolates of each fungal species were capable to produce insect cuticle degrading enzymes. The implications of entomopathogens producing multiple forms of enzymes are discussed in terms of pathogenicity and future research prospects.

The study of correlation between isolate virulence and enzyme activity for the three candidates fungal pathogens was fulfilled to elucidate the mechanisms by which the fungal isolates differed from one to another within the fungal species.

Keywords: *Cladosporium*; *Verticillium lecanii*; *Epicoccum*; entomopathogens; multiple enzyme producing; insect population regulation.

INTRODUCTION

The chemical control of whiteflies does not always show satisfactory results because wax secreted by the cuticle hampers insecticides penetration, and rapid development of population resistance to insecticides lowers its control efficacy (Osborne and Landa, 1992). Natural enemies

including insect myco-pathogenic, therefore, have drawn much attention as alternative means to bio-control of whiteflies, aphids and other insects (Lacey *et al.*, 1996; Abdel-Baky *et al.*, 1998 and Abdel-Baky, 2000). Greenhouse conditions with controlled temperature and high relative humidity, especially, offer an excellent environment for conidial germination and to increase entomopathogenic fungi ability to cause epizootics (Hall, 1982; Chandler *et al.*, 1993). Therefore, potential means of reducing the use of pesticide and their undesirable effects on human health and the environment are required (Hajek and Goettel, 2000).

In Egypt, Abdel-Baky *et al.*, (1998) and Abdel-Baky and Abdel-Salam (2003) recorded three species of *Cladosporium*, namely *C. uredinicola*, *C. cladosporioides* and *C. chlorocephalum*, *Verticillium lecanii* and *Epicoccum* sp. beside two other species, which naturally infected whiteflies, *Aphis gossypii* and *Empoasca* sp. under field conditions. They also showed that *Cladosporium* naturally occurred at high percentage of 10.0 to 28.0%. Moreover, they reported that *C. uredinicola* was the most dominant and more virulent in the laboratory tests. In addition, *C. uredinicola* proved to be the suitable bio-control agent towards whiteflies and aphids under semi-field conditions (Ragab and Abdel-Baky, 2004).

Generally, a myco-insecticide is composed of spores that settle on the insect cuticle, germinate, penetrate and lead to subsequent death of the insect host within few days (Moore and Prior, 1993; Arcas *et al.*, 1999). This is also a relatively long time. The main barrier to entomopathogenic fungi penetration is the cuticle chemical composition of an insect (Zackaruk, 1970). The insect cuticle (integument) is a composite material consisting of a thin lipid-protein rich epicuticle covering the bulky procuticle (Andersen, 1979). The procuticle is composed of the exo- and endocuticle that are composed mainly of chitin and protein, while the exocuticle is generally melanized (Andersen, 2002). The initial infection stage of most entomopathogenic fungi involves direct penetration of the insect cuticle. This penetration may occur through mechanical pressure in combination with enzymatic degradation (Charnley, 1984; Hassan *et al.*, 1989). For penetration through the insect cuticle, Deuteromycete fungi produce chitinase, proteinase, and lipase, commonly referred to as cuticle-degrading enzymes (Moraes *et al.*, 2003).

The enzymatic activity of certain entomopathogenic fungi, such as, *Beauveria bassiana* and *Metrahizium anisopliae* at the cuticle surface of insects has been reported by Sosa-Gomez *et al.* (1997). Conidia germination was shown to require an exogenous carbon source (Lecuona *et al.*, 1997). Among various carbon sources, chitin and low levels of certain fatty acids were also shown to be efficiently utilized for that purpose, indicating that insect myco-pathogenic might use nutrients present on and in the insect integument (Woods and Grula, 1984; St. Leger, *et al.*, 1986 a & b). In addition, *M. anisopliae* produces several chitinolytic enzymes, which act after the pathogen's proteases which have significantly digested the cuticle protein and unmasked the chitin component of the cuticle (St. Leger, *et al.*, 1991; Reineke and Zebitz, 1996).

Therefore, the present study was conducted to select suitable fungal strains to our agro-ecosystem based upon the enzymes production

as well as highly virulent to the whiteflies and aphids. For this purpose, various isolates of *Cladosporium* spp., *Verticillium lecanii* and *Epicoccum* spp. collected from Egyptian fauna were compared with regard to their biological characters including their enzymatic activities. Screening of fungal isolates, which are highly virulent to a target pest, and suitable to indigenous greenhouse environment, are prerequisites to development of a fungal bio-control agent.

MATERIAL AND METHODS

ISOLATED FUNGI:

Three fungal species were isolated from naturally infected whiteflies and aphids species (Abd Allah, 2004), and selected to conduct this study. The selected fungi were *Cladosporium* spp., *Verticillium lecanii* (Zimm) and *Epicoccum* sp., all belonging to class Deturomycetes.

Key insect pests:

Whiteflies and aphids were the key pests used to carry out this study. *Bemisia argentifolii* (Bellows & Perring), *Trialeurodes ricini* (Misra), *Aphis gossypii* (Glov.), *A. cracivora* Koch. and *Myzus persicae* were the most common whiteflies and aphids chosen for laboratory studies.

Fungal Growth Media:

Four different media were used for fungal cultivation and growth and to evaluate their enzymatic activities. These media were Potato Dextrose Agar (PDA), Chitin Agar (CA), Gelatin Agar (GA) and Oil Agar (OA). The composition of the media was as reported by Merck (1994).

I. Bioassay test:

A: Insect preparation:

Two hundred individuals of each insect species were chosen after surface-sterilization in a 1% sodium hydrochlorite solution for 30 seconds, then rinsing three times with sterilized water and drying on paper tissue (Abdel-Baky, 2000).

B: Fungal spores suspension preparation:

Fungal inocula of *Cladosporium* spp., *V. lecanii* (Zimm) and *Epicoccum* sp. were prepared by transferring seven days old culture grown on slope PDA medium. The fungal growth was scrapped off and then 10 ml sterile water were added. In order to have a homogeneous suspension of both spores and mycelium, the tubes were shaken for one minute using Vortex Mixture (VM-300). The suspension was added to 90 ml sterile water in Erlenmeyer flask (250 ml). To perform the laboratory bioassay test (*in vitro*), one ml of the obtained spores suspension was prepared using a hemocytometer in the concentration of approximately 6×10^6 spores/ml and three different dilutions of spores suspension as 5×10^4 , 5×10^5 , and 5×10^6 spores/ml. were also prepared.

C: Laboratory Bioassay Test:

Ten individuals of each of the tested insects were placed on blotter papers moistened by fungal spores suspension in Petri-Dishes. In case of

whiteflies, dark color blotter papers were used in order to have a good contrast with the color of these insects. A piece of plant leaf was added after sterilization in each Petri-dish as a food source. This experiment was performed in 10 replicates (Abdel-Baky, 2000).

II. Enzymatic Activities of the tested fungi:

In this study, three enzyme types were measured since the insect cuticle composite material consisting of chitin, lipids, and protein according to (Berd, 1973) as follows:

A. Chitin Hydrolysis:

The fungal isolates were tested for chitin hydrolysis by inoculating Petri-dishes (9 cm diameter) containing chitin agar medium and incubated at 27°C. The results were taken seven days later by measuring the fungal growth diameter expressed in centimeters.

B. Gelatin Hydrolysis:

Gelatin hydrolysis was done by inoculating Petri-dishes (9 cm diameter) containing gelatin agar medium with fungal isolates and incubated at 27°C. The results were taken seven days later by measuring the clear zone in centimeter.

C. Lipid Hydrolysis:

Lipid hydrolysis was determined by inoculating Petri-dishes (9 cm diameter) containing oil agar medium at 27°C. The result was taken 7 days later by adding copper sulfate solution to Petri-dishes. The bluish green agar color appeared around the fungal growth indicating positive results.

III. Statistical Analysis:

The obtained Data were subjected to analysis of variance (ANOVA) by SAS Software program (SAS, 1990). In addition, the results were compared at 0.05 and 0.01% probability levels using Least Significant Difference (LSD).

RESULTS

Bioassay test:

Laboratory experiments on the two selected isolates of *Cladosporium* spp., *Verticillium lecanii* and *Epicoccum* sp. against whiteflies life stages and aphids showed a favorable control of *Cladosporium* spp. towards the two pests, followed by *V. lecanii* and *Epicoccum* sp. with the three spores concentrations (Table 1).

Table (1): Effect of the three fungal species on the mortality percentages of whiteflies and aphids, *in vitro*.

Fungal species and isolates	Whiteflies Mortality %			Aphids Mortality %		
	Conc.1	Conc.2	Conc.3	Conc.1	Conc.2	Conc.3
<i>Cladosporium</i> sp.	COMW3	89.0	93.5	71.5	58.0	45.0
	COMW4	84.0	84.0	61.0	50.0	39.0
<i>Verticillium lecanii</i>	CFMW3	71.5	73.5	60.0	30.0	33.0
	CFMW4	70.5	69.5	60.5	43.0	36.0
<i>Epicoccum</i> sp.	GBDW3	62.0	57.0	55.0	43.0	41.0
	GBDW4	65.5	58.0	53.5	44.0	40.0

The bioassay results were secured five days later. The fungal isolates were also more effective against whiteflies than aphids. The mortality percentages were higher than 80% with *Cladosporium* spp., 70 with *V. lecanii* and 50% in case of *Epicoccum* sp. isolates. The mortality percentages varied from 30 to 58% in aphid populations (Table 1).

II. The Extracellular Enzymatic Activities of the Selected Fungi:

One of the most important parameters used in the fungal selection is the production of extracellular enzymatic capabilities to degrade insect cuticle. The examined extracellular enzymes such as lipase, cellulase, proteinase, and chitinase proved that these fungi were highly virulent against the target pests beside their safety towards its host plants. The isolates, CoMW1, CoMW2, CoMW3, CoMW4 and CoMW5 (*Cladosporium*); CfMW1, CfMW2, CfMW3 and CfMW4 (*Verticillium*) and GbDW1, GbDW2, GbDW3, GbDW4 and GbDW5 of *Epicoccum* were detected during their growth for fungal characteristics (Table 2).

Table (2): Assessment of some enzymatic activities of the tested fungal isolates.

Tested fungal isolates	Enzymatic Activities			
	Lipase	Cellulase	Proteinase	Chitinase
<i>Cladosporium</i> spp.				
CoMW1	-	-	+	+
CoMW2	-	-	+	+
CoMW3	+	-	++	++
CoMW4	+	-	++	++
CoMW5	-	-	+	+
<i>Verticillium lecanii</i>				
CfMW1	-	-	+	+
CfMW2	-	-	+	+
CfMW3	+	-	++	++
CfMW4	+	-	++	++
<i>Epicoccum</i> sp.				
GbDW1	-	-	+	+
GbDW2	-	-	+	+
GbDW3	+	-	++	++
GbDW4	+	-	++	++
GbDW5	-	-	+	+

Where's: Co: castor oil, M: Mansoura University, W: whitefly, D: Dekerns; Cf: cauliflower, Gb: Green bean, (1, 2, 3, 4, 5): number of isolates

The enzymes activities of each isolate were measured for the three fungal species (Table: 2). All these isolates showed negative results regarding cellulase production. This is quite well for plant cell safety being out of degradation capabilities. In addition, all isolates exhibited negative reaction for lipase except isolates CoMW3, CoMW4, CfMW3, CfMW4 GbDW3, and GbDW4 of the tested fungal. This will be convenient for the wax layer hydrolysis in insect cuticle. In case of proteinase and chitinase, all the tested fungal isolates showed high potentialities which were satisfactory for degradation of the insect body wall. The qualitative values of both proteinase and chitinase are listed in Table (3).

Table (3): Qualitative activities of chitinase and proteinase of the tested fungal isolates

Tested fungal isolates	Chitinase Growth diameter (G, cm)	Proteinase		
		Zone diameter (cm)		H/G
		G	H	
<i>Cladosporium</i> spp.				
CoMW1	1.0	2.3	2.8	1.2
CoMW2	1.7	1.8	2.1	1.2
CoMW3	2.5	1.3	2.4	1.9
CoMW4	2.9	1.0	2.5	2.5
CoMW5	0.8	2.0	2.0	1.0
<i>Verticillium lecanii</i>				
CfMW1	2.1	3.2	3.2	1.0
CfMW2	1.5	2.8	3.1	1.1
CfMW3	5.0	2.0	3.0	1.5
CfMW4	4.5	1.5	2.1	1.4
<i>Epicoccum</i> sp.				
GbDW1	2.8	1.5	1.5	1.0
GbDW2	1.7	2.3	2.3	1.0
GbDW3	4.4	1.5	1.7	1.1
GbDW4	4.8	1.2	1.5	1.3
GbDW5	3.3	1.8	1.8	1.0

Where's: Co: castor oil, M: Mansoura University, W: whitefly, D: Deken.s; Cf: cauliflower, Gb: Green bean, (1, 2, 3, 4, 5): number of isolates.

Regarding the chitinase activity, Fig. (1) indicates that the isolates CoMW3 and CoMW4 of *Cladosporium*; CfMW3 and CfMW4 of *V. Lecanii* and GbDW3 and GbDW4 of *Epicoccum* showed highly enzymatic activities against insect cuticle. Fungal growth diameters were 2.5, 2.9; 5, 4.5, 4.4, and 4.8 cm for isolates CoMW3, CoMW4, CfMW3, CfMW4 GbDW3, and GbDW4 on chitin water agar medium, respectively. The importance of this

enzyme is due to the conversion of chitin of insect body into its deacetylated form chitosan, glucose amine polymer. Proteinase activity expressed in gelatinase activities, the isolates belonging to genus *Cladosporium* showed appreciative values of gelatin hydrolysis (Fig.2). The isolates CoMW3 and CoMW4 were superior to the other tested isolates of the same genus, being 1.9 and 2.5 cm as H/G values, respectively.

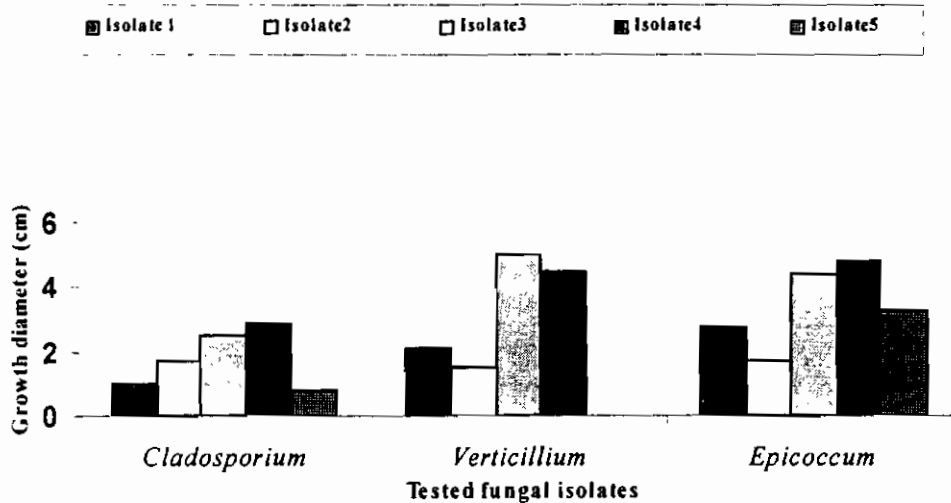


Fig. (1): Qualitative activity of chitinase produced by tested fungal isolates.

Similar results were obtained in case of the genus *Verticillium* isolates. The isolates CfMW3 and CfMW4 exhibited better activities than the other isolates tested in gelatin agar medium (Fig. 3). The values of proteinase, expressed as H/G of the isolates belonging to genus *Epicoccum* (Fig. 4) were 1.3 and 1.0 cm for GbDW3 and GbDW4 strains, respectively. Furthermore, the H/G% values for each fungus were plotted against the growth diameter or against the zone diameter for the *Cladosporium* (Figs. 5 A & B), *Verticillium* (Figs. C & D) and *Epicoccum* (Figs. E & F).

The H/G % values of the isolates CoMW3, CoMW4, CfMW3, CfMW4 GbDW3, and GbDW4 gave 190, 250; 150, 140 and 110, 130% for *Cladosporium*, *Verticillium* and *Epicoccum*, respectively. In addition, the linear regression of H/G % against the growth diameter was expressed as $y = -109.57x + 340.07$ ($r^2 = 0.8461$); $y = -28.006x + 191.51$ ($r^2 = 0.8155$) and $y = -22.254x + 144.94$ ($r^2 = 0.5040$) for *Cladosporium*, *Verticillium* and *Epicoccum*, respectively.

The higher the diameter of the fungal colony and diameter of the clear zone, the more virulent of the fungus isolate on their insect host. In addition, the less growth zone diameter in combination with the wider diameter of clear zone indicated that the fungal isolates had potentialities in the tested extracellular enzymatic production. Consequently, this reflected higher attack on the insect pest.

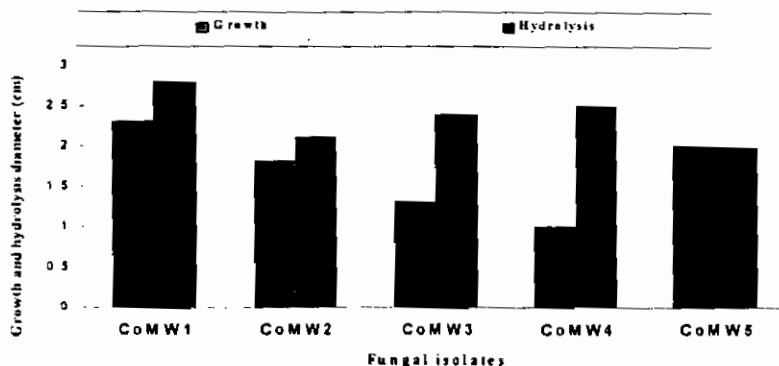


Fig. (2): Diameter of growth and hydrolysis zones of the selected *Cladosporium* isolates on gelatin agar medium.

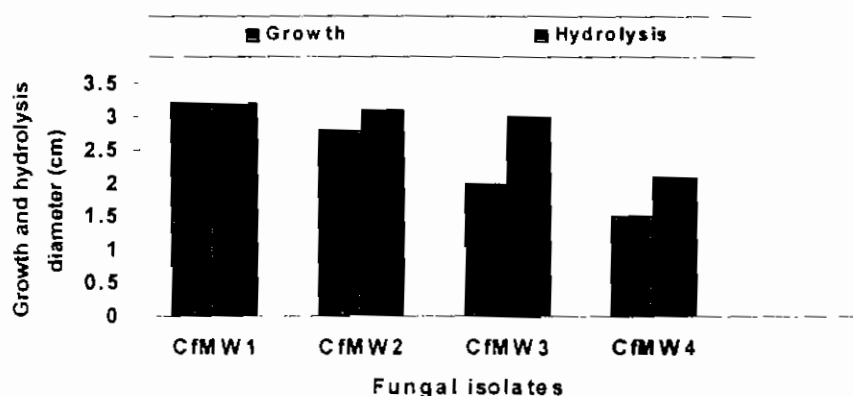


Fig. (3): Diameter of growth and hydrolysis zones of the selected *Verticillium* isolates on gelatin agar medium.

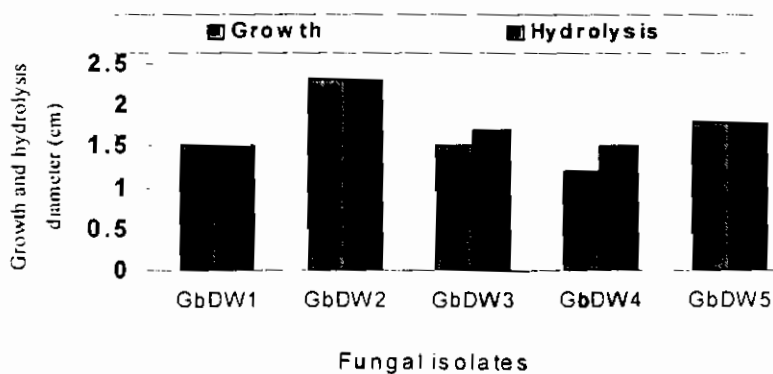
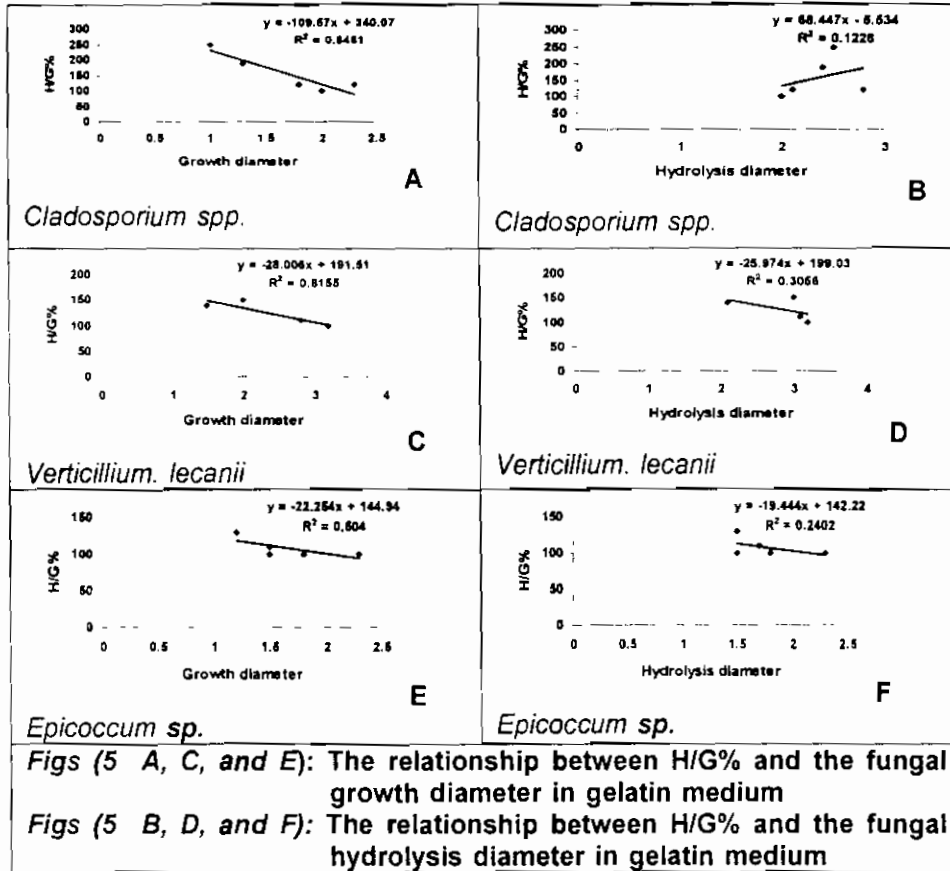
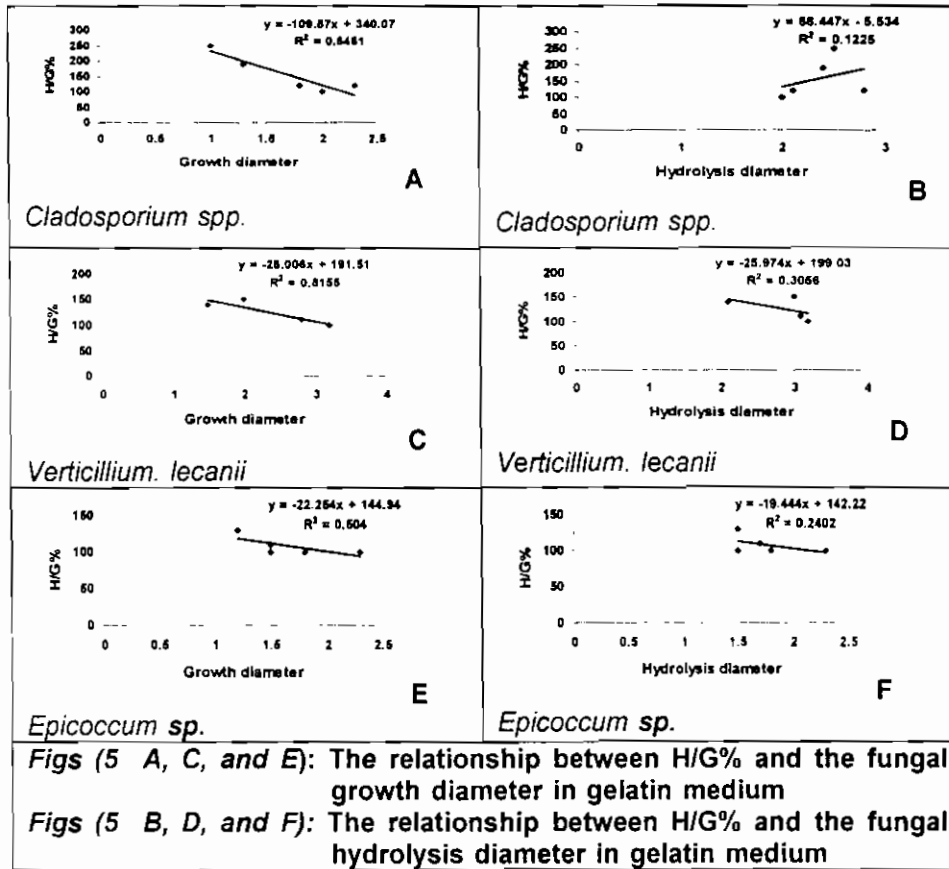


Fig. (4): Diameter of growth and hydrolysis zones of the selected *Epicoccum* isolates on gelatin agar medium.



DISCUSSION

Important criteria for development of a myco-insecticide for biological control can be easily produced on an artificial media, high spore yield and high virulence against target organisms (Meeks *et al.*, 2002). The present study indicates that the three entomopathogenic fungi isolated from naturally infected whiteflies and aphids were effective in the reduction of insect populations *in vitro*. However, the genus *Cladosporium* was the most effective (Table 2). This variation within the entomopathogenic fungi may be due to one or more factors that dominate during the course of study. Milner and Spore (1981) mentioned other factors, which influence the effectiveness of entomopathogenic fungi, mainly light, air movement, food availability, and insect physiological conditions. They also attributed the frequent lower effect of the fungi to some components of insect epicuticle, which inhibit the fungal germination and growth. Moreover, the germination and penetration of fungal spores into the insect cuticle constitute an important step in the fungus infection processes. In addition, the reduction in mortality rates amongst the fungal isolates (Table 1) may be attributed to antifungal compounds on the surface of the insect cuticle that hampered conidial



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germination on the insect cuticle against some fungal species and strains more than others (Smith & Grula, 1982; Chandler *et al.*, 1993; Meeks *et al.*, 2002). In another study, Smith & Grula, (1981) proved that fatty acids with a short carbon chain, such as valeric and caproic acids isolated from insect cuticle, inhibited the spore attachment and germination of *Beauveria bassiana*. Hall (1982) also observed unsatisfactory control by *V. lecanii* against *Trialeurodes vaporariorum* and *Aphis gossypii* under greenhouse conditions. According to Buckner *et al.*, (1999), the mortality of whitefly nymphs differed from other insects because they produced profuse amounts of cuticular lipids, especially long-chain wax esters. These lipids are produced in such a thick layer that could inhibit fungal spores from penetrating the cuticle layer (James, 2001). These combinations of factors may explain the variations of the efficacy of many entomopathogenic fungi more than others.

The insect pathogenic fungi produce a complement of extracellular enzymes that degrade the integuments of their hosts (St. Leger *et al.*, 1986 a & b; Paterson, *et al.*, 1994; Hsiao, 1998). Penetration through the host cuticle is the mode of entry for most myco-insecticides (Charnley, 1984). During the fungal infection, the first step prior of penetration is the adhesion of fungi to the host cuticle (Boucias and Pendland, 1991).

In the present investigation, no cellulase enzyme was detected with *Cladosporium*, *Verticillium* and *Epicoccum* (Table 3). This means that the fungi were safe to the plants and more specialized on insects than on plant hosts. This finding was in accordance with Ellis (1971) protocol, who mentioned that these species had no pathogenic effects on their plant hosts associated with whiteflies and aphids. Similar results were obtained by Meeks *et al.* (2000).

In addition, the fungi were able to grow and hydrolyze the lipids, chitin, and protein. The enzymes produce from these fungi may explain the effect of the fungi in controlling the insect pests by digesting and hydrolyzing the insect cuticle. This interpretation complies with the results of Fargues (1984) who speculated that the enzymes present on the conidial surface might assist in the consolidation of the infectious propagule to the cuticle surface.

Enzymes having esterase, proteinase, and/or lipase activity may be used to digest the epicuticle. Histological studies of pre-germinating conidia on insect cuticle suggest that modification of the epicuticle does occur before germ tube formation (David, 1976; Reineke and Zebitz; 1996). Elucidating the mechanisms regulating the secretion of these enzymes is essential to understand pathogen growth and development in its insect host.

Substantial knowledge of the physiology and biochemistry of the proteases and chitinases has been gained in the recent years (Paterson *et al.*, 1994). In *Metarhizium anisopliae* and other entomopathogens, chitinase is required for only a brief period during penetration of host cuticle and is tightly regulated by chitin degradation products (Napolitano and Juarez, 1997). Proteases have an additional role in providing nutrients, before and after the cuticle are penetrated. Consequently, regulation is looser, with production being triggered in response to limitation for nutrients such as carbon and nitrogen (St. Leger, *et al.*, 1986a). However, production is

enhanced when the pathogen is grown on insect cuticle. Recently, the role of insect cuticle lipids on activity of entomopathogenic fungi has been figured out. There are two possible scenarios, some of them might be used as poorly accessible sources of energy for spore germination and the others may have a specific antifungal effect (Lecuona, *et al.* 1997).

This study showed the correlation between isolate virulence and enzyme activity for the three candidates fungal pathogens to elucidate the mechanisms by which the fungal isolates differed from one to another within the fungal species. Further studies are needed to clarify the molecular basis of the mechanisms by which the fungal strains vary in the metabolism of hydrocarbons, the unique components of the insect cuticular barrier which enable them to survive against microbial, chemical and physical attack. The answer to these questions might help to improve the efficiency of microbial pest control strategies.

In conclusion, entomopathogenic fungi invade insects by a combination of mechanical pressure and enzymatic degradation of the cuticle. Therefore, St. leger *et al.*, (1986 b) suggested that proteinases play a major role in the cuticle degradation by *M. anisopliae*, as chitinolytic enzymes appearing after the enzymes of the proteolytic complex. This is going along with cuticular structure in which protein masks chitin. Moreover, Screen *et al.* (2001) reported that wild-type levels of chitinase are not limiting for cuticle penetration. Recently, Nahar *et al.* (2004) suggested that constitutive chitin deacetylase from *M. anisopliae*, in the absence of induced chitinase, converts cuticular chitin to chitosan and thus plays an important role in cuticle softening and pathogenesis.

In view of the need to new IPM tools, a possible alternative is insect pathogens that can secrete high levels of lipase, chitinase and proteinase enzymes. This might enable the use of these deacetylase enzymes in conjunction with myco-pesticide formulation in IPM to facilitate faster mortality. Indeed, the cost-effective production of these enzymes will be evaluated and calculated in further study.

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القدرة المرضية والنشاط الأنزيمي لبعض الفطريات الممرضة للذباب الأبيض واليمن.

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تختلف مواصفات إختيار المسبب المرضي للأفات الحشرية حسب نوع الحشرة ونوع المسبب المرضي، ولكن استخدمت ثلاثة من المعايير الهامة عند إختيار فطريات *Epicoccum* و *Cladosporium Verticillium lecanii* الأبيض واليمن. هذه المواصفات هي: القدرة المرضية للفطر - كونها آمنة للعائل النباتي - المقدرة على إفراز إنزيمات محللة لجدار جسم الحشرة.

ولقد أشارت الدراسة المعملية أن هذه الفطريات تميزت بفاعليتها في خفض تعداد حشرات الذباب الأبيض واليمن، حيث أظهرت هذه الفطريات في ارتفاع النسبة المؤوية لموت أفراد الذباب الأبيض واليمن، مع تفوق سيادة فطر *Cladosporium* عن الفطريين الآخرين. كما أشارت الدراسة على قدرة أنواع الفطريات الثلاثة في ارتفاع نسبة الموت لأفراد الذباب الأبيض عن نسبة الموت في حشرات اليمن المستخدمة في هذه الدراسة.

وقد تم عزل خمسة عزلات لكل من فطري *Epicoccum* و *Cladosporium* وأربعة من فطر *V. lecanii*، ولكن كانت العزلات COMW3, COMW4 لفطر *Cladosporium*، و CfmW3, CfmW4 لفطر *V. lecanii* و GbDW3 و GbDW4 لفطر *Epicoccum* من أكفا العزلات في قدرتها المرضية ونشاطها الأنزيمي مما يجعله أحد أهم العوامل البيولوجية المبشرة في مكافحة حشرات الذباب الأبيض واليمن. وقد أظهرت الدراسة أيضا أن لهذه الفطريات الثلاثة قدره عالية في إفراز بعض الأنزيمات المحللة لجدار جسد الحشرة (محللات كيميائية)، وهذه الإنزيمات تحدد القدرة المرضية للفطر المستخدم. ومن الإنزيمات التي أنتجتها الفطريات في بيئة النمو المستخدمة، إنزيمات الليبيز، البروتينيبيز و أنزيم الشيتينيبيز.

سجل ارتفاع في النشاط الأنزيمي لإنزيمات البروتينيبيز و الشيتينيبيز مع كل عزلات الفطريات التي نمت على بيئة البروتين والشيتين، ولكن أنزيم الليبيز أنتج فقط مع العزلات COMW3, COMW4 لفطر *Cladosporium*، و CfmW3, CfmW4 لفطر *V. lecanii* و GbDW3 و GbDW4 لفطر *Epicoccum*. ولم يستدل على إفراز إنزيم السليوليبيز المحلل لجدر الخلايا النباتية مع كل عزلات الفطريات المستخدمة في الدراسة.

وتوضح هذه الدراسة الارتباط بين القدرة المرضية والنشاط الأنزيمي لثلاثة من الفطريات المبشرة في مجال مكافحة البيولوجية لحشرات الذباب الأبيض واليمن، حيث تلقى الضوء على ميكانيكية عمل هذه الفطريات وكذلك توضيح سبب الاختلاف في القدرة المرضية بين عزلات الفطر الواحد.