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Cladosporium spp. An Entomopathogenic Fungus for Controlling Whiteflies and Aphids in Egypt

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Abstract: Five species of entomopathogenic fungi were tested against whiteflies and aphids. *Cladosporium* spp. showed a high incidence on the two insect species tested (81% of the total isolated species). However, *C. uridenicola* was the predominant species isolated from both insect species during the two successive seasons of 1998 and 1999. Natural infection by *Cladosporium* ranged from 18.19-44.38% and 16.4- 45.27% in 1998 and 1999, respectively, according to insect species and the host plant. Statistical analysis revealed a high correlation (R =0.8759 ± 0.093) between the total investigated and total infected of each insect during the two successive years of study. The natural infection of *Cladosporium* to whitefly life stages was higher on whitefly nymphs (87.8%) than adults (8.08%) and eggs (4.15%) in 1998 and 1999. The results showed that the seasonal distribution of *Cladosporium* occurred all year around, but rates were higher during the period from June until November and that coincide with the high population of whiteflies and aphids. The weather had a significant effect on insects infection and had no effect on the others. The laboratory tests showed a high pathogenicity of the candidate on *A. gossypii, A. craccivora* and *B. agrentifollii* than other reported fungal.

Key words: Entomopathogenic fungi, *Cladosporium* spp., whiteflies, *Bemisia* species complex, *Bemisia argentifolii, Trialeurodes ricini,* Aphididae, *Aphis gossypii, A. craccivora, A. durantae,* epizootic, naturally occurrence, weather factors

Introduction

Whiteflies and aphids are globally important pests because of their direct damage and their role in transmission of plant viruses (Abdel-Baky 1995; Brown, 1998). Beside the appearance of the new whitefly biotypes, silverleaf whitefly (*Bemisia argentifolii* Bellows and Perring) in the Egyptian entomofauna, which attack different plant hosts with high population levels (Abdel-Baky and Abdel-Salam, 2000; Abdel-Baky *et al*., 2000). The castor oil whitefly (*Trialeurodes ricini* Misra) is responsible for the tomato yellow virus in Egypt (Idriss *et al*., 1997). These lead farmers to apply toxic insecticides frequently.

Chemical control has been confounded by the development of insecticide resistance in both whiteflies and aphids population. The development of high levels of resistance to most chemical insecticides (Dittrich *et al*., 1990; Kerns and Gaylor, 1992) has forced researchers to look for an alternative means of control (Lacey *et al*., 1996).

Microbial control agents offer alternatives to chemical pest control and more selective than chemical insecticides. Further, bio-control agents can be integrated with other control methods, they are environmentally more safer than chemically longer and may provide protection after establishment within the host population (Fuxa, 1987).

A number of fungal species have been recognized as promising biocontrol agents for insect pests (Gottel *et al*., 1990; Ferron *et al*., 1991). The insect fungi have had many successes because their characteristics (good epizootic, but slow action and over dependence on suitable environmental factors) make them useful after establishment. Many have relatively wide host ranges among insects. Another advantage is the fact that they do not have to be ingested by the insect host but can invade the host upon contact with the cuticle (Boucias *et al*., 1988).

Among the best fungal pathogens attacking whiteflies known, *Paecilomyces fumosoroseus, Beauveria bassiana, Verticillum lecanii* and *Aschersonia* spp. (Lacey *et al*., 1996). Meanwhile, *Pandora neoaphidis, Conidiobolus* sp., *V. lecanii, B. bassiana, Entomophothora* sp. and *Neozygotes fresenii are* known as aphid entomopathogenic fungi (Hatting *et al*., 1999). In spite of this, some authors consider *Cladosporium* spp. as a new promising biological control agent against homopterous insect. De Carvalho *et al*. (1972) reported that *Cladosporium herbarum* was associated as a controlling candidate against cashew whitefly, *Aleurodicus coccis* and also effective as biocontrol agent against three species of whiteflies namely, *Bemisia* sp., *Aleurothrixus* sp. and *Dialeurodes* sp. on various plant hosts in Venezuela (Rojas *et al*., 1998). Moreover, *C. aphids* recorded as bio-control agent against Alerurochiton *aceris* in Finland (Hulden, 1986) and *Chionaspis salicis* (L.) (Coccoidea: Diaspididdae). In china, *C. cladosporioides* caused 20-57% natural mortality of *Hemiberlesia pitysophila* under field conditions and 39% in the laboratory tests (Pan *et al*., 1989). Whereas, Thumar and Kapadia (1994) mentioned that *Cladosporium* spp. was able to infect *Aleurolobus barodensis* nymphs at all year times in India. Han *et al.* (1997) noticed that the entomopathogenic fungi, *Cladosporium* spp. naturally occurred and caused epizootic infection to the population of *Aleurocanthus spiniferus* in China.

Regarding the role of *Cladosporium* spp. against aphids, there are number of reports emphasize the ability of such fungus in reducing the population size of these pests (Lagowska, 1995; Vallejo *et al*., 1996; Han *et al*., 1997). In Egypt, Abdel-Baky *et al*. (1998) recorded three species of *Cladosporium* (*C. uredinicola, C. cladosporioides* and *C. chlorocephalum*) which infect *Bemisia* spp., *Aphis gossypii* and *Empoasca* sp. They reported that the fungus was occurred naturally at high percentage of incidence (10.0-28.0%)

under field conditions. They also reported that *C. uredinicola* was the most dominant one and more virulent in the laboratory tests. The aim of the present investigation is to study the pathogencity and epizootic of *Cladosporium* spp. and its seasonal occurrence on certain homopterous insects.

Materials and Methods

Survey sites, plant hosts and insects: The survey was carried out at three regions of Dakahlia Governorate (Mansoura, Talka and Aga). The pests involved in the survey were *Bemisia* spp., silverleaf whitefly (SLWF), *B. argentifolii,* castor oil whitefly (CQWF), *T. ricini,* cotton aphids, *Aphis gossypii* (Glov.) legume aphids, *A. craccivora* Koch. and duranta aphid, A. *durantae* Theobald. SLWF was surveyed on the three plant hosts (squash, cotton and Mexican fire plant, *Euphorbia prunifolia* Jacq.) while both *Bemisia* spp. and castor oil whitefly were surveyed only on castor oil plants, as this host was the only specific host for this pest. Cotton aphid (CA) was surveyed on the cotton, cucumber and eggplant. Meanwhile, the legume aphid (LA) was surveyed on cowpea and duranta aphid (DA) was surveyed on duranta plants.

Sampling Protocols

Whiteflies:

The silverleaf whitefly (SLWF): Twenty-five plants were chosen at random presenting plants of corner and center of the field. The sample was prepared by selecting five leaves from each plant (two leaves from each upper and lower third and one from the middle third of the main stem). Leaves from each group were removed and put in a plastic bag and transferred to the laboratory for examination. Eggs, small nymphs and large nymphs of SLWF were investigated under stereomicroscope and counted as a total/leaf. The infected individuals (cadavers) were also counted. This protocol was applied on cotton, squash and Mexican fire plant.

Castor oil whitefly (COWF): Castor oil plants were normally found on the edge of water canals, roads and around the edge of fields in Egypt. Five plants presenting five locations in Mansoura and Talka regions were chosen while the previous program, which was used with SLWF was applied. Since *Bemisia* spp. and COWF are more preferable to castor oil plants (Abdel-Baky *et al*., 2000), therefore, the differentiated between these two species was done on the base of pupal case shape and the methods of eggs laid on this host.

Aphids: Because of the newly formed parts of plants are more favorable to the aphid colonies for feeding and development, three leaves from the upper third of the main stem were chosen randomly, removed and put in a plastic bag and transferred to laboratory for investigation. The experimental samples were 75 leaves representing 25 plants of each crop. The same technique used with whiteflies was applied.

Fungal Isolation and Identification: The following two procedures were used in fungi isolation:

1- Insect cadavers showing natural external growth of fungi were collected and maintained in ptri-dishes contain potato dextrose agar (PDA) media. The inoculated ptri-dishes were kept in an incubator at 27 ± 2 °C and 75 ± 5% R. H. until further growth of the fungi. Spores of pure cultures were inspected under a compound microscope.
2- Predicted insects to be infected due to their abnormal movement were surface-sterilized in a 1% sodium hydrochlorite solution for 30 second and washed in distilled water. Then the insects were cultivated in ptri dishes (25 insects/dish) on PDA media and kept in an incubator under the same regime of temperature and R.H.

Identification of isolated fungi was done primarily in Plant Pathology Department, Fac, Agric., Mansoura University. Confermintaion of Fungus identification was based on the external symptoms and the morphology of the fungi and then habit characters were used in consultation with Waterhouse and Bradey (1992) and Humber (1997) and Commonwealth Mycology Institute, Kew, Surrey, England (Ellis, 1971, 1976) to confirm the preliminary identification..

Bioassay: Each fungus associated with the insects tested was used to evaluate its pathogenic role on *B. argentifolii, A. gossypii* and *A. craccivora* under laboratory condition. Two hundred individuals from each insect species were chosen after surface-sterilized in a 1% sodium hydrochlorite solution for 30 s and washed in distilled water. Each prtidish contained 25 individuals was considered as one replicate. The insects placed in a dark color blotter moist with the fungal suspension with one concentration (10×10^6 spores/ml.) (Abdel-Baky *et al.*, 1998). A piece of plant leaf was added to each prti-dish after sterilization to be a source of food. Vandenberg (1996) and Abdel-Baky *et al.* (1998) techniques for preparation of tf.e fungal incula were followed. Data collected daily and continued for 7 days.

Statistical Analysis: Analysis of variance, correlation coefficient and stepwise regression models were used for Data analysis (CoStat Software, 1990). Percentages of *Cladosporium* infected homopterous insects were calculated by dividing the total number of infected insect species with *Cladosporium* (summed over all samples) by the total recorded numbers of each species sampled then multiplying by 100.

Results

Entomopathagenic fungi species: Five species of fungal pathogens were isolated as native bio-control agents in Dakahlia Governortae from whiteflies and aphids. The fungal names and their incidence percentages are presented in Fig. 1. Data showed that *Cladosporium* spp. was the most dominant one, which formed 80.87% of the total isolated species under both filed, and laboratory conditions. *Fusarium* sp. ranked the 2nd place with 7.6% followed by *Verticilium lecanii* with 5.7% of the total fungi recorded. Meanwhile, incidence of *Trichocithium roseum* and *Epicocum* sp. was lower which listed 3.9 and 2.1% of the total, respectively (Fig. 1). Moreover, *C. uredinicola* formalized over 90% of *Cladosporium* spp. that found attacking whiteflies and aphids (Fig. 2).

Incidence of *Cladosporium* spp. on some homopterous insects under field condition: *Cladosporium* spp. were

Table 1: Percentages of *Cladosporiurn* spp. incidence on certain homopterous insects at Dakahlia Governorate, Egypt

Insect species	Host plant	1998			1999		
		Total investigated insects	Total infected insects	Infection %	Total investigated insects	Total infected insects	Infection %
Bemisia spp.	Castor oil	6301	2476	30.30 b	12670	5736	45.27 a
B. argentifolii	Squash	12351	3611	29.24 d	10911	2576	23.61 d
B. argentifolii	Mexican fire	4531	857	18.19 g	3567	714	20.02 e
B. argentifolii	Cotton	7050	1500	21.28 f	7550	1500	19.87 e
T. ricini	Caster	16784	7449	44.38 a	15856	64397	42.24 b
A. gossypii	Cucumber	6857	2153	31.40 c	14863	3936	26.48 c
.4. *gossypii*	Egg plant	2553	643	21.99 f	5134	998	19.44 e
A. gossypii	Cotton	4070	780	19.16 g	4940	970	16.41 f
A. craccivora	Cowpea	13442	1932	14.37 h	10327	2447	23.70 d
A, durantae	Duranta	2839	688	24.23 e	4594	1290	28.08 c
L. S. D. 0.05 = 1.6863							

Means followed by the same letter in a column are not significantly differences (Duncan's Multiple Range Test)

Table 2: Stepwise regression analysis between the total investigated insects and total infected by *Cladosporium* spp. during 1998 and 1999

Insect species	Host plant	1998		1999	
		Regression Equation	R^2	Regression Equation	R^2
Bemisia app.	Castor oil	Y =- 43.24 + 0.247 X	0.9647	Y = 103.68 + 0. 281 X	0.9901
B. argentifolii	Squash	Y = 30.03 + 0.411 X	0.9478	Y = 05.541 4 + 0.438 X	0.8455
B. argentifolii	MeXican fire	Y = -5.359 + 0. 324 X	0.7807	Y = 11.52 + 0. 194 X	0.3911
8. argentifolii	Cotton	Y =- 35.89 + 0.537X	0.9478	Y =- 06.163 + 0.425X	0.9783
T. ricini	Caster	Y =. 19.73 + 0.484 X	0,9683	Y =.- 29.38 + 0,410 X	0.6645
A, gossypii	Cucumber	Y =- 54.50 + 0.371X	0.9794	Y =- 29.38 + 0,279 X	0.9827
A. gossypii	Egg plant	Y =- 61.62 + 0.358X	0.2626	Y = 08.391 + 0. 181X	0.8955
A. gossypii	Cotton	Y = 02.95 + 0.286 X	0.7919	Y =- 16.76 + 0. 393 X	0.7796
A. craccivora	Cowpea	Y = 25.49 + 0. 123X	0.9342	Y = 66.04 + 0.304 X	0.9191
A. durantae	Duranta	Y =- 2.104 + 0.259 X	0.7845	Y =- 17.09 + 0.1318 X	0.8757

Table 3: Correlation coefficient between the percentages of infected insects by *Cladosporium* spp. in 1998 and 1999

Variables	Correlation coefficient parameters			
	R ± S.E	Slope b ± S.E	Y Int (a) ± S.E	Sign. Sign
% of *Cladosporium* infection in 1998 and 1999.	0.8759 ± 0.093	0.8906 ± 0.094	2.851 ± 0.0049	***

Table 4: Efficacy of five fungal species isolated on three homopterous insects

Insect species	Spores concentration (10×10^{66})									
	Cladosporium spp.		*Fusarium* sp.		*Verticillium lecanii*		*Trichacithiu raseum*		*Epicacum* sp.	
	Mean ± SE	% of infection	Mean ± SE	% of infection	Mean ± SE	% of infection	Mean ± SE	% of infection	Mean ± SE	% of infection
A. passypii	18.75 ± 1.2	37.5	8.50 ± 0.71	13.0	9.0 ± 1.31	18.0	4.25 ± 0.55	8.0	2.25 ± 0.31	4.5
A. craccivora	19.0 ± 1.4	38.0	5.75 ± 0.75	11.5	6.75 ± 0.95	13.5	3.25 ± 7.41	6.5	1.75 ± 0.20	3.5
B. argentifolii	41.75 ± 2.6	83.5	10.75 ± 1.01	21.5	13.5 ± 1.20	27.0	3.75 ± 0.43	7.5	2.00 ± 0.15	4.0

found naturally attacking most of homopterous insects that exist in Egyptian entomofauna. The prevalence varied according to the insect species and the plant host type (Table 1). Regarding its suppression role on whiteflies, the fungus caused high infection rates, which reached 44.38% and 42.24% on castor whiteflies in 1998 and 1999, respectively. The infection percentages of *Cladosporium* against *Bemisia* spp. on castor oil plants were high and formed 39.30% in 1998 and 45,27 in 1999. Percentage of SLWF cadavers infected by *Cladosporium* spp. ranged from 18.19-39.24% in 1998 and 19.87-23.61% in 1999 (Table 1). The lowest rate of infection against SLWF was recorded on both cotton and Mexican fire plants in the two successive years.

A high prevalence of the disease caused by *Cladosporium* against cotton aphid occurred on cucumber plants (31.40% and 26.48%) in both years, respectively. Meanwhile, the occurrence of the fungus as a natural enemy of *A. gossypii* on cotton fields was lower 19.16% in 1998 and 16.41% in 1999. The effect of *Cladosporium* spp. on *A. durantae* was high 24.23% and 28.08% in both years, respectively, than its effect on *A. craccivora* (Table 1). The natural occurrence of *Cladosporium* spp. showed variation on whitefly life stages as it was 87.8, 8.05 and 4.15% for nymphs, adults and eggs, respectively (Fig. 3).

Table 2 shows the regression equations, which express the numerical relationship between the fungus *Cladosporium* and each of the tested insect pests in the two years of study. The statistical analysis revealed the presence of a high relationship among the fungus and its victims. The results, also, indicate that the fungus has a continuos effect from year to another as seen from Table 3.

Seasonal colonization of *Cladosporium* spp. under field condition: Natural occurrence of *Cladosporium* spp. was observed all year around with different infection rates. Figure 4 and 5 show the seasonal abundance and the yearly distribution of the fungus on the homopterous insects involved in this study. Levels of infection extend from June until the end of November in the two years of study

(Fig. 4, 5). The degree of fungus association with both *Bemisia* spp. and *T. ricini* on castor oil plants was relatively higher and continued all year around, then decreased to low levels from December until May of each year. The fungus caused an epizootic diseases to whiteflies from Sept. until end of November in both years (Fig. 4, 5). The same trend occurred with the SLWF on cotton plants and the fungus was effective against the pest from July until Sept. in 1998 and 1999. Moreover, *Cladosporium* incidence on aphid species was high and the infection rates were varied according to aphid species and its population size under field condition.
The fungus prevalence in the field was found associated with the favorable environmental conditions during the period from June until November {temperature average 21.19 (15.73 - 30.43) and R. H. 60.96 (33.80 - 85.67) in both years}.

Laboratory bioassay studies: All fungal species tested were pathogenic to the three insect species tested at conidial concentrations of 10 x 10^6 spores/ml. Fungal species were significantly differed in virulence to the SLWF, cotton aphid and legume aphid (Table 4). At 3 d, *Cladosporium* spp. caused the higher mortality in this short time for the insect species than all other fungal species. Sensitivity of SLWF to *Cladosporium* infection was high as the mortality reached 83.5% than both aphids *(A. gossypii* 37.5% and *A. craccivora* 38.0%). *Verticillium lecanii* was the other second fungus, which caused 37.0, 18.0 and 13.5% mortality in *B. argentifolii, A. gossypii* and 4. *craccivora* population, respectively. On the other hand, the efficacy of *Tricocithium roseum* and *Epicocum* sp. was low in comparison with the other species tested (Table 4).

Discussion

The success of the entompathogens in insect control are largely due to epizootiologcal or ecological factors, i.e. the speed of kill and economic injury level (EIL) of the pest, effects of the ecosystem and environment, timing and transmission and host resistance (Fuxa, 1997). Moreover, the efficiency of a natural enemy in bio-control is influenced by the host range, specificity of the fungal pathogen, or strain, the host's physiological state, nutrition, defense mechanisms, cuticle and epicuticular of the pest (McCoy *et al*., 1988; Boucias *et al*., 1988). The significance of *Cladosporiurn* spp. as a one of the effective biological control agents against whiteflies, aphids, and scale insects in the world have been reviewed (De Carvalho *et al*., 1972; Roberts and Humber, 1981; Hulden, 1986; Pan *et al*., 1989; Humber, 1991; Thumar and Kopadia, 1994; Han *et al*., 1997; Abdel-Baky *et al*., 1998). Farias and Filho (1987), found that *Cladosporium* spp. was the most important fungus isolated from nymphs of *Aleurothrixus aepium* in cassava plants. The fungus caused 82.2% mortality of the insect nymphs under field conditions. The percentage *Bemisia* spp. infected naturally by *Cladosporium* were varied from 10.0 - 28.0% according to the plant host and over 80% in the laboratory tests (Abdel-Baky *et al*., 1998). The virulence of *Cladosporium* varied on the base of insect species and type of plant host (Table 1). Hare and Andereadis (1983) explained the role of plant host on the infection rate of the fungus. They referred to significant differences in susceptibility of *Leptinotarsa decemlineata* to *Beauvera bassiana* when reared on four different Solanceae species, considering fungal prevalence in field and mortality in laboratory. This interpretation explains why the infection of the insect was varied within plant species? Whereas, plant morphology may also interfere with insect susceptibility to fungal infection (Hare and Andreadis, 1983; Boucias *et al*., 1984). The insect species, plant host, the quality of fungal inoculum and the climatic conditions during the survey could account for the differences in percentages of *Cladosporium* spp. observed between insects.
Data presented in Table 2 might throw some light on the infection relationships, which govern the fungus incidence. This consequence relationship of fungus infection under field condition might be possible to utilize in controlling the insect pests. The relationship between all infected insects in two years of study was very high (R $=0.8759 \pm 0.093$) and indicates that the degree of correlation between the two variables showed stronger relationship that is actually present under the field condition (Table 3).
Entomopathogenic fungi in nature cause a regular and tremendous mortality of the pests (Steinhaus, 1949). The seasonal distribution of *Cladosporium* sop, mostly tends to be high from June to November (Fig. 4, 5) and coincide with population of the insect. Furthermore, the increase in whiteflies or aphids population followed by an increasing in the infection level if climate conditions and ecological factors were favorable for the fungus (Carruthers and Soper, 1987; Abdel-Baky *et al*., 1998).
The high incidence of *Cladosporium* infection on the castor oil plants with *Bemisia* spp. and *T. ricini* in 1998 and 1999 (Table 1) may be due to one or more of the following reasons: 1) castor oil plants cultivated and grown on the edges of fields or/and on the border of water canals, whereas water evaporation's are higher enough to spore germination and widespread of fungal spores, 2) increase the fogging particularly in the end of the summer and fall and 3) whiteflies immature harbor on the lower surfaces of plant leaves while the nymphs prefer the lower leaves on the main stem of plant. All these ecological factors provide humidity around both insect and fungus, which increase the infection of *Cladosporium* and cause the epizootic. The results are in agreement with number of reports which shows the role of natural field epizootics followed periods of high rainfall or relative humidity and link disease outbreaks with ambient moisture (Hajek and St. Leger, 1994; Carruthers *et al*., 1997). Gottel *et al.* (1990) pointed out that fungi with wide host ranges are frequently and even more specific under field condition. Such specificity is thought to be due to the complex biotic and abiotic interactions the field. This is in agreement with the results obtained in this study (Table 4)
The perennial agroecosystem offer a permanence in vegetation that could potentially allow host and pathogen populations to persist in some degree balance (Franz, 1971). This could be lead to residual pathogen activity or widespread infection, called an epizootic, because the fungus can multiply and persist on its host insect (Ferron, 1981). The high epizootic potential of *Cladosporium* spp. which observed on both whiteflies and aphids populations can be attributed to many factors which, when considered in total, describe a pathogen well adapted to parasitism of a highly mobile, rapidly developing host which attains high population densities during favorable periods of spring,

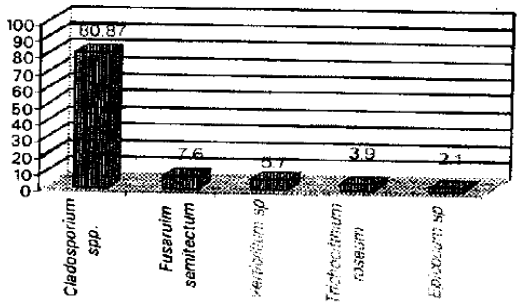

Fig. 1: Percentages of five-entomophatogenic fungi incidence isolated from whiteflies and aphids in Dakahlia Governorate, Egypt

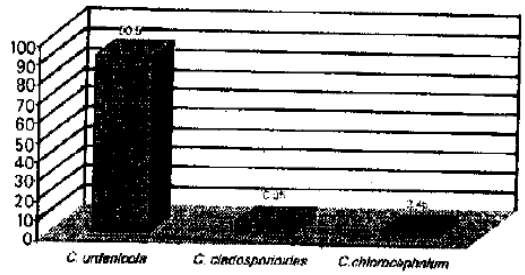

Fig. 2: Percentage of *Cladosporium* incidence under field condition

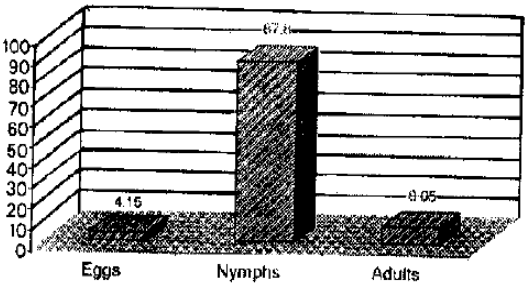

Fig. 3: Percentage of *Cladosporium* spp. infection against whitefly life stages under field condition

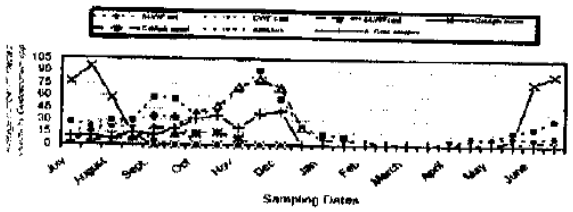

Fig. 4: Seasonal incidence of *Cladosporium* spp. on six homopterous species in 1998

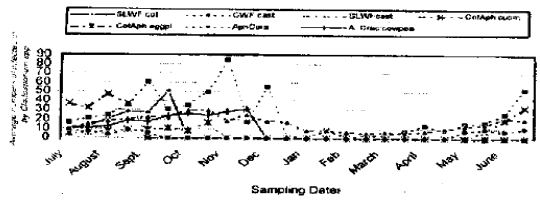

Fig. 5: Seasonal incidence of *Cladosporium* spp. on six homopterous species in 1999

summer and fall.

In conclusion, *Cladosporium* spp. appears to be one of the most effective native bio-control agents against homoptrous insects in Egypt. However, to achieve good control treatments, it must be incorporated into IPM program

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