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Three *Cladosporium* spp. As Promising Biological Control Candidates for Controlling Whiteflies (*Bemisia* spp.) In Egypt

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Abstract

Three species of entomopathogenic fungi, *Cladosporium uredinicola*, *C. cladosporioides* and *C. chlorocephalum* were found attacking whiteflies (*Bemisia* spp.) in Mansoura region, Dakahlia Governorate, Egypt. Their prevalence on *Bemisia* infesting seven plant hosts varied from 10.0 to 28.0% according to the plant host. The morphology of these three fungi and their infection symptoms is discussed. Attempts were made to initiate artificial infections in different stages of a laboratory culture of *Bemisia* spp. Results indicated that eggs were infected at a lower percentage (14 to 28%), whereas the hatching rate of eggs recorded a higher percentage (56%). Pathogenicity effect on nymphs and adults were very high (88%) and varied according to the spore concentrations and periods after infection. Laboratory studies revealed that *C. uredinicola* gave the highest infection percentages, followed by *C. cladosporioides* and *C. chlorocephalum*. Light regime studies showed that the three species of the fungus were more aggressive on *Bemisia* under a diurnal light regime than under continuous darkness.

Key words: Whitefly, *Bemisia tabaci*, *Bemisia argentifolii*, Entomopathogenic fungi, *Cladosporium uredinicola*, *Cladosporium cladosporioides*, *Cladosporium chlorocephalum*, Insect pathogens

Introduction

Silverleaf symptoms as a result of *Bemisia argentifolii* feeding observed in Egypt at different localities (Delta, Giza, El-Faiyum and Aswan) during a survey carried out from April 1991 until May 1993 on three host plants, lantana, tomato and squash (Lacey *et al*., 1993).

Bemisia spp. has become increasingly important as a key pest in temperate regions world-wide (Sanderson, 1987; Broadbent *et al*., 1989; Bellows *et al*., 1994; Abdel-Baky 1995; Fishpool *et al*., 1995; Kodeir, 1997). This insect pest causes severe damage due to direct feeding on the plants, contamination of the crop with sticky honeydew and transmission over 60 different plant viruses (Brunt, 1986; Byrne *et al*., 1990; Brown, 1991; Byrne and Bellows, 1991; Bedford *et al*., 1994). Both immature and adult stages are difficult to control with pesticides because of their preferred habitat on the under surface of leaves and their wide host range (Azab *et al*., 1969; Cock, 1986). The adults have a rapid reproduction rate (Butler *et al*., 1986; Byrne and Bellows, 1991) and this insect has developed cross resistance to several insecticides (Butter and Vir, 1989; Toscano *et al*., 1995).

In recent year, large scale control operations against *Bemisia* spp. have become necessary in different parts of the world. These campaigns are based on the use of chemical pesticides such as organophosphates and pyrethroids. Chemical controls provide only short-term solutions and raise concerns over pest resistance, human safety and environmental contamination. Chemical insecticide problems have provided the impetus to search for and develop alternative pest management options that are more sustainable and environmentally sound.

The use of suitable microbial agents instead of chemical insecticides has been demonstrated to be promising approach against *Bemisia* spp. (Hulden, 1986; Fransen *et al*., 1987; Helyer, 1993; Smith, 1993, Traboulsi, 1994). Apart from reduced environmental and human health hazards, these agents can have the potential of further spread within the pest population, causing an epizootic (Carruthers *et al*., 1993).

Several entomopathogenic fungi were isolated, described, established and their role as biocontrol agents against whiteflies recently explored. Scanty information is available regarding the use of *Cladosporium* spp., as biological control agents, specially on whiteflies (Fransen, 1990). The entomopathogenic fungi *Cladosporium herbarum* was reported by De Carvalho *et al*. (1972) as factor in controlling *Aleurodicus cocois* and *Cladosporium aphids* against *Aleurachiton aceris* (Hulden, 1986). The latter species was also found on *Chionaspis salicis* (L.) (Coccoidea, Diaspididae). In China, Pan *et al*. (1989) isolated *Cladosporium cladosporioides* and used it for controlling *Hemiberlesia pitysophila*. It caused 39% mortality in laboratory tests and 20-57 percent in field tests. The European Biological Control Laboratory (EBCL) of the USDA approved two fungal species, *Paecilomyces fumosoroseus* in Pakistan, India and Nepal and *Verticillium lecanii* in Spain as biological control agents against whiteflies (Lacey *et al*., 1993). In India, Thumar and Kapadia (1994) showed that nymphs of *Aleurolobus barodensis* (Maskell) were infected by *Cladosporium* sp., during most of the year. Gindin and Ben Zeev (1994) isolated *Conidobolus coronatus* and

Conidobolus spp. from *B. tabaci* in Isreal as promising biocontrol candidates for this insect pest.

Entomopathogein fungi are poised to become valuable tools in IPM programs due to a better understanding of the insect infection process and advances in mass production and formulation techniques that promote efficacy and conidial stability in storage and application (Moore and Prior, 1993). The goal of the present study was to provide preliminary information of the incidence of entomopathogenic fungi of the genus *Cladosporium* and their possible use as biological control agent against whiteflies in Egypt.

Materials and Methods

Field survey: Attempts were carried out during 1996 to survey the fungi associated with whiteflies on various plant hosts growing in Dakahlia Governorate, Egypt. The insects were observed on the following plant hosts; Squash (*Cucurbita peop* L.: Cucurbitaceae), Cabbage (*Brassica oleracea* var. *Capitata* L.: Cruciferae), Labanet El-Homara weed (*Euphorbia prunifolia* Jacq: Euphorbiceae), Lantana plants (*Lantana camara* L.: Verbeanceae), *Hibiscus* (*Hibiscus rosa-sinensis* L.: Malvaceae), Duranta (*Duranta plumeri* var. *variegate* L.: Verbenaceae) and cotton (*Gossypium barbadense* L.: Malvaceae). The associated insects were examined visually and by using the leaf disc method. The whitefly life stages infected with fungi were collected and transferred to the laboratory for isolation and identification.

Cultivation and isolation: Fifty naturally infected adults, nymphs and eggs of whiteflies were surface disinfected in 1 percent sodium hypochlorite for 1 minute then washed with sterilized water. The same number of adults, nymphs and eggs were left without sterilization as a check. Both groups were washed thoroughly with distilled water, dried on tissue paper and placed on potato dextrose agar (PDA) supplemented with streptomycin sulphate (3.7 mg/ml) and chloramphenicol (2.5 mg/ml). Plates were incubated for 3 days at $22 \pm 2^{\circ}C$ under 12 h alternating cycles of Near Ultra Violet (NUV) light and darkness. The plates were examined under a stereo binocular microscope and associated fungi were isolated.

Identification of the associated fungi: The single-spore isolation technique was employed to obtain fungal isolates in pure cultures. Pure cultures were incubated for 7 days at $22 \pm 2^{\circ}C$. Spores of the pure cultures were inspected under a compound microscope. Fungi were identified in consultation with the Commonwealth Mycology Institute, Kew, Surrey, England (Ellis, 1971, 1976).

Whitefly colonies: To obtain insects free from natural infection, whitefly adults (*Bemisia* spp.) were collected from cabbage and squash fields by aspirator and maintained in large screen cage ($125 \times 60 \times 50$ cm) on new squash plants for 48 h until the adults oviposited. The squash plants were transferred to another cage and kept until emergence of the nymphs. These insects were used for the bioassay studies.

Pathogenicity of *Cladosporium* spp. to the whitefly life stages: One hundred whitefly adults were selected and placed on dark color blotters moistened with the fungal spore suspension in petri-dishes. Each petri-dish contained 25 individuals and was considered as one replicate. A piece of squash leaf was introduced to the perti-dish after sterilization to be used as a source of food for the adults. Disk of squash leaves containing 25 nymphs or eggs were cut and placed in the petri-dish after dipping it in the fungal spore suspension. After incubation, the plates were examined under a stereo microscope to study the symptoms of the fungus on the adults, nymphs and eggs. The same number of different life sages of *Bemisia* were washed thoroughly in distilled water and used as checks using the same conditions as for the treated insects. Four replicates were run for each life stage, while all treatments were replicated twice during this study.

To prepare fungal inocula, Vandenberg (1996) technique was followed. Spores from the pure cultures were scraped from the surface of the plates with a sterile glass rod and suspended in 200 ml of sterile distilled water. The fungal spore suspension was then filtered through a tissue paper. Two concentrations of the conidial spore suspension, (4×10^6) and (10×10^6) per ml of each fungal species, were used. The treatments were incubated at $22 \pm 2^{\circ}C$ under 12 h light and 12 h dark. Effect of light on fungal growth: Petri-dishes containing PDA were inoculated with a conditional suspension from the tree fungal species. From these plates, 5 mm disks of the mycelia growth were taken and transferred to the center of petri-dishes (15 cm diameter) containing PDA media. The plates were incubated at $25 \pm 2^{\circ}C$ under either 12 h light followed by 12 h darkness or 24 h darkness for 10 days. The diameter of the colonies was used as a measurement of the growth response. The measurements were taken after 4, 6, 8 and 10 days of incubation.

Statistical analysis: Statistical analysis was carried out to determine the effect of fungal spore concentration on the different life stages of *Bemisia* spp. under laboratory and field conditions using one way analysis of variance (ANOVA), Correlation and regression analysis (CoStat Software, 1990).

Results

Identification, description and symptoms of *Cladosporium* species: The culture obtained revealed the presence of three species of *Cladosporium* attacking *Bemisia* sp. They were *C. uredinicola* (Speg.), *C. chlorocephalum* (Fresen.). This is the first report of these fungi in Egypt on *Bemisia* spp. The morphology, description and pathogenicity symptoms of three species were:

***Cladosporium uredinicola* (Speg.):** Colonies effuse, olivaceous, velvety. Conidiophores straight or flexuous,

occasionally branched, spetate, usually with groups of 2-3 scars at the apex and sometimes lower down, pale olive smooth or minutely verruculose, up to 300 μ long, 3-5 μ thick. Ramo-conidia 25-30 μ long. Conidia spherical, fusiform, ellipsoidal oblong, very pale olive, smooth 0-3 septate, 3-5–3 μ diam or 7-25 × 3-6 μ (Ellis, 1976).

***Cladosporium chlorocephalum* (Fresen.):** In culture colonies effuse, olive green, Conidiophores: stipe dark brown to black up to 680 μ long 14-24 thick. Conidia olive or pale brown smooth or verruculose, 0-septate. In young cultures 0-2 septate. Ramo-conidia measuring 8-34 × 4-6 μ and long branched chains of ellipsoidal and spherical conidia 4-8 μ 3.5 μ or 3-6 μ diameter (Ellis, 1971).

***Cladosporium cladosporioides* (Fresen.):** Colonies effuse, olive green or oliveaceous brown, velvety. Conidiophores sometimes up to 350 μ long but generally much shorter, 2-6 μ thick, pale to mid olivaceous brown, smooth or verruculose. Ramo-conidia 0-1 septate, up to 30 μ long. 2-5 μ long branched chains, mostly 0-septate, 3-7 × 2-4 μ pale of olivaceous brown, smooth but verrculose in some strains. It occurs as a secondary invader on many different plants and has been isolated from air soil and textiles (Ellis, 1971).

The examination of field-collected and artificially inoculated whiteflies elucidated the following symptoms. Filed-collected whiteflies showed different stages of the infection process. In primary stages of infection, a white mycelium was flattened (especially the abdomen) and had a lot of wrinkles from the dorsal view, this only occurred in the case of adults. In advanced cases of the infection process, the cadavers were completely covered with thick and dense dark green mycelium (olive color).

In the laboratory, after the spores attached to the cuticle and germinated, the bodies of both adults and nymphs became enlarged, cylindrical and increased in size hollowed by emergence of the white mycelium. In some cases, a large sticky ball showed on cadavers in which the cuticle became very thin and hollowed out. When this ball emerged on the end of the abdomen, the ovaries showed inside it. After 7-10 days, the mycelium color changed to dark green (Fig. 1).

Incidence of the fungi under filed conditions: Data presented in Table 1 illustrate that the percentages of infected *Bemisia* species varied from one plant host ot another and from one time of the year to another. A high prevalence (25.47%, n = 11303) of the disease caused by the *Cladosporium* spp. was recorded on whiteflies on squash plants, while a lower prevalence occurred on whiteflies on cabbage plants (17.4%, n= 2584). The percentages of infected whiteflies ranged from 22.5% (n = 284) on cotton plants to 17.5% (n = 1027) on Hibisucs. The data indicated that the peak of naturally infected whiteflies began during second half or September and tended to increased until the first of November. The statistical analysis revealed that a highly significant difference was observed between the infected numbers of *Bemisia* spp. on squash plants and other host plants tested. No significant differences were found among the diseased insects on cabbage, Euphorbia, Lantana, Hisbiscus, Duranta and Cotton (L.S.D 0.5 = 46.73264).

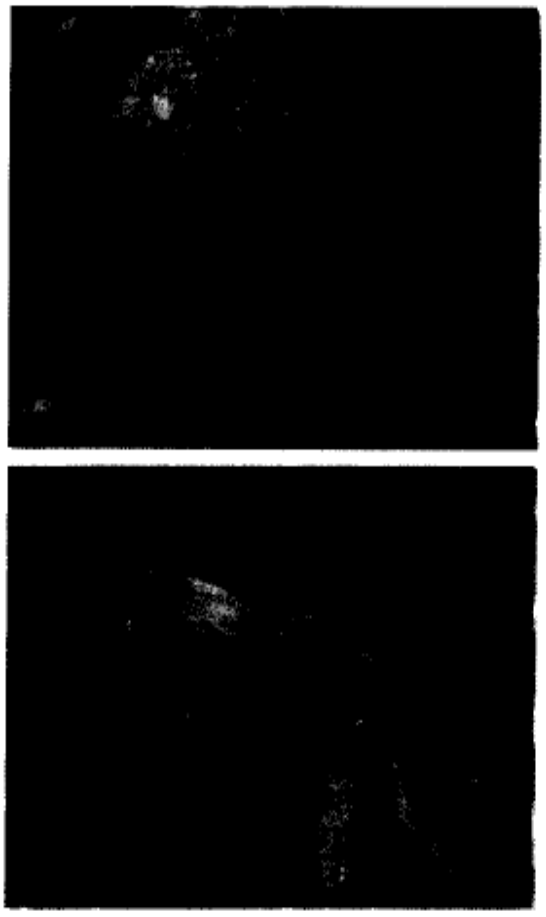

Fig. 1: Infection symptoms of *Cladosporium* spp. on *Bemisia* spp. (A: nymph; B: adult)

The result in Table 2 indicate that Relative Humidity (R.H.) is a key factor affecting the rate of infections. Also, "R" values showed a prefect linear association between R.H. and he infection of *Bemisia* spp. The regression analyses fit the model equation: $Y = 15526.82 + 683.382\ X_1 + 655.712\ X_2 + 1010.33\ X_3 + 1182.84\ X_4$ (Where X_1, X_2, X_3 and X_4 are maximum temperature, minimum temperature, maximum RH. and minimum RH., successively) and the R^2 value was highly significant ($r^2 = 0.8778$).

Pathogenicity of *Cladosporium* spp. to different life stages of *Bemisia* spp: Pathogenicity in egg: Laboratory investigations showed that whitefly eggs were not easily infected by spores in the first two days following inoculation, but needed 5 to 10 days to allow conidial germination and penetration of the eggs. Table 3 shows

that the percentage of infected egg varied depending upon the spore concentration and the species of *Cladosporium*. A high infection percentage was found with *C. uredinicola*, which reached 25 and 28 percent, followed by *C. cladosporioides* (18 and 19%), while *C. chlorocephalum*, had a low percentage (14 and 15%), with concentrations of 4×10^6 ml and 10×10^6 ml of fungal spore, respectively. A high significant difference was found between *C. uredinicola* and *C. chlorocephalum* in attacking the eggs using a fungal spore concentration of 4×10^6/ml versus a concentration of 10×10^6 fungal apores/ml. There was no significant differences between the concentration used regarding mortality.

The results showed that the hatchability of *Bemisia* eggs varied according to the fungal species. As presented in the Table 3, a high percentage of hatching was observed with *C. chlorocephalum*, which reached 56 and 51 percent when 4×10^6 and 10×10^6 of fungal spores/ml were used, followed by *C. cladosporioides* (52 and 48%). *C. uredinicola* showed the lowest percentage of hatchability (38%) with 4×10^6 and 36 percent with 10×10^6 spores/ml. The data revealed that were highly significant difference among the hatching rates in eggs treated with the three species of *Cladosporium* compared with the check. Moreover, highly significant differences were observed among *C. uredinicola*, check and the other two species when 4×10^6 spores/ml was used.

Pathogenicity of *Calosorium* species to *Bemisia* numphs: Data presented in Table 4 shows that the after inoculating nymphs of *Bemisia* spp. with *Cladosporium* spp. spore suspensions (4×10^6 and 10×10^6 fungal spores/ml), symptoms were observed in 53, 38 and 47 percent of the nymphs after two days with *C. uredinicola*, *C. chlorocephalum* and *C. cladosporioides*, respectively. The infection percentage increased sharply and reached 70 percent (*C. uredinicola*), 52 percent (*C. chlorocephalum*) and 61 percent (*C. cladosporioides*) after five days.

With the second fungal spore concentration (10×10^6 spore/ml), only *C. uredinicola* caused a high infection rate (75%) two days after treatment (Table 4). The percentages of infection in the nymphs with the other three fungal species reached 87, 48 and 51 percent, respectively, five days after inoculation.

In general, the results showed that the high concentration of fungal spores gave a highly mortality rate. Data were confirmed by the statistical analysis, which revealed a significant difference in the pathogeniciy rates in the nymphs of *Bemisia* by *C. uredinicola* compared with the other two fungal species.

Pathogenicity of *Cladosporium* species to *Bemisia* adults: Table 5 shows that the infection percentage of *Bemisia* adults treated with two concentrations of conidial suspensions of *Cladosporium* spp. were higher in comparison with nymphs and eggs.

Two days after treatment the adults showed high infection percentages when *C. uredinicola* was used (60 and 70%), followed by *C. cladosporioides* (50 and 53%), then *C. chlorocephalum*, which gave the lowest percentage (36 and 46%), with concentrations of 4×10^6 and 10×10^6 respectively. Similarly, the percentages of infected *Bemisia* adults increased to 68 and 88 percent with *C. uredinicoia* 60 to 65 percent with *C. cladosporioides* and 53 and 50 percent with *C. chlorocephalm* (4×10^6 and 10×10^6 spores/ml, respectively) five days after each treatment.

The differences in patholgenicity of the three fungal species were highly significant at the 1 percent level after two days. After five days, the differences were significantly only between *C. uredinicola* and the other two species when using 4×10^6 spores/ml.

Effect of light regimes of fungal growth: The growth rate of *Cladosporium* spp. under two light regimes are shown in Fig. 2. The data indicate that the development of *Cladosporium* spp. was faster under 12 h, alternating cycles of NUV light than under continuous darkness. The growth of *C. uredinicola* under both regimes was better than the other two species of *Cladosporium*. Its diameter reached 7.78 and 6.24 cm, on alternating or on continuous darkness, 10 days after plating on PDA, respectively. There was a significant difference between the development of these species under the two light regimes, as seen in Fig. 2.

Fig. 2: Developmental rates of *Cladosporium* spp. Under two light regimes (A: *C. uredinicola*; B: *C. cladosporioides* and C: *C. Chlorocephalum*).

Table 1: Infection percentages of *Bemisia* spp. By *Cladosporim* spp. On certain plant host from the 2nd half of July till the 1st half of December 1996, in Mansoura region, Egypt

		Plant Hosts						
		Squash	Cabbage	Euphorbia	Lantana	Hibiscus	Duranta	Cotton
% of naturally infected *Bemisia* spp.								
July	2nd half	0.0	13.7	14.1	15.2	15.8	12.2	0.0
August	1st half	0.0	13.3	14.1	18.5	16.3	14.3	10.0
	2nd half	22.1	15.7	15.9	20.8	16.7	15.8	20.0
Sep.	1st half	23.1	16.9	17.1	20.5	17.7	21.4	26.7
	2nd half	26.0	18.5	17.4	20.7	18.5	22.5	23.4
October	1st half	26.2	18.1	18.6	19.3	21.4	23.8	0.0
	2nd half	27.3	25.2	24.3	23.6	24.5	32.7	0.0
Nov.	1st half	26.3	16.5	28.0	22.6	16.4	19.6	0.0
	2nd half	25.2	15.6	26.5	18.2	16.3	19.8	0.0
Dec.	1st half	0.0	12.7	0.0	17.2	10.9	15.8	0.0
Total investigated WF		11303.0	2584.0	1977.0	1644.0	1027.0	765.0	284.0
Mean % of infected WF		25.47	17.4	19.7	19.9	17.5	18.4	22.5
Mean infected WF/Plant ± SE		115.3a ±27.93	45.0b ±9.1	39.0 ±6.5	32.8 ±6.2	18.0 ±3.8	14.1 ±2.8	6.4 ±3.7

Mean followed by a different letter in a row are significantly different ($p < 0.05$)

Table 2: Correlation coefficient between infection of *Bemsia* spp. by *Cladosporium* spp. on seven host plants and certain weather factors during the period of study in Mansoura region, Egypt

Weather factors (x)	Corr. (R)	Slope (b)	Y Int. (A)
Maximum temperature	0.20487	0.83729	58.2858
Minimum temperature	0.07816	3.02589	180.4215
Average temperature	0.14994	5.03184	114.9942
Maximum R.H.	0.73432	-179.377	15153.88
Minimum R.H.	0.74453	-13.299	784.0207
Average R.H.	0.75097	-25.0762	1830.6095

Table 3: Effect of two spore concentrations prepared from three species of *Cladosporium* on infection and hatchability of *Bemisia* spp. eggs

Fungus species	4×10^6				10×10^6			
	% mortality	Mean ± SD	% hatching	Mean ± SD	% mortality	Mean ± SD	% hatching	Mean ± SD
C. uredinicola	25	6.25 ± 1.5a	38	9.50 ± 1.29	28	7.00 ± 1.41a	36	9.00 ± 2.16
C. chlorocephalum	14	3.50 ± 1.29b	56	14.00 ± 0.82	15	3.75 ± 0.96b	51	12.75 ± 0.96
C. cladosporioides	18	4.50 ± 1.29ab	52	12.75 ± 0.96b	19	4.75 ± 0.96ab	48	12.00 ± 0.82bc
Check (untreated)	0	0	88	22.00 ± 2.16	0	0	83	20.75 ± 1.50a

Mean followed by a different letter in a row are significantly different ($p < 0.05$)

Table 4: Effect of two spore concentrations prepared from three species of *Cladosporium* on infection and hatchability of *Bemisia* spp. eggs

Fungus species	2 days				5 days			
	4×10^6		10×10^6		4×10^6		10×10^6	
	% infected Nymph	Mean ± SD	% infected Nymph	Mean ± SD	% infected Nymph	Mean ± SD	% infected Nymph	Mean ± SD
C. uredinicola	53	13.25 ± 0.96a	75	18.75 ± 2.22a	70	17.5 ± 1.29a	87	21.75 ± 1.26a
C. chlorocephalum	38	9.50 ± 1.29b	39	9.75 ± 1.71b	52	13.0 ± 0.82c	48	12.00 ± 1.41b
C. cladosporioides	47	11.75 ± 0.96ab	37	9.25 ± 2.75b	61	15 ± 0.82b	51	12.75 ± 1.50b

Mean followed by a different letter in a row are significantly different ($p < 0.05$)

Table 5: Infection rates of *Bemisia* adults exposed to three species of *Cladosporium* at two concentrations after two and five days

Fungus species	2 days				5 days			
	4×10^6		10×10^6		4×10^6		10×10^6	
	% infected adults	Mean ± SD	% infected adults	Mean ± SD	% infected adults	Mean ± SD	% infected adults	Mean ± SD
C. uredinicola	60	15.0 ± 0.82a	76	19 ± 0.81a	69	17.5 ± 1.71a	88	22 ± 1.83a
C. chlorocephalum	36	09.0 ± 0.82c	46	11.5 ± 1.29	53	13.25 ± 0.96b	50	12 ± 0.85c
C. cladosporioides	50	12.5 ± 1.29b	53	13.25 ± 2.75b	60	15 ± 0.82ab	65	16.25 ± 0.86b

Mean followed by a different letter in a row are significantly different ($p < 0.05$)

Discussion

The survey study on *Bemisia* spp. and their natural enemies during two successive seasons (1996 and 1997) noted the silverleaf symptoms on squash leaves as described by Suhuster *et al*. (1991). Since the silverleaf disorder is associated with the feeding habits of the new stain of *Bemisia* (*B. biotype* or *B. argentifolii*) (Perring *et al*., 1993), the authors suggest that the species of *Bemisia* found in Mansoura region in the new strain of whitefly (Strain B, *B. argentifolii*) and not *B. tabaci* (or possibly a mixture of the two). These findings are in agreement with the observation of Lacey *et al*. (1993) and the esterase electromorph analysis results of Brown *et al*. (1995) on Egyptian whitefly strains collected from tomato fields.

The impact of *Bemisia* spp. on filed crops is increasing to a dramatic level. It is currently recognized as one of the most significant pests of several plant hosts (Roditakis, 1990; Summers *et al*., 1995). Traditionally, control programs for this insect have depended totally on the regular application of insecticides (Byrne *et al*., 1990; Dittrich *et al*., 1990). These insecticides have provided only ephemeral suppression of *Bemisia* populations and their toxicity to the environment and non-target species has probably led to increases whitefly outbreaks (DeBach and Rose, 1977; Rose and Woolley, 1984). Because of these problems, the development of considerable resistance to insecticides in whitefly populations (Parbhaker *et al*., 1985; Dittrich *et al*., 1990; Toscano *et al*., 1995) and its preferred habitat on the underside of foliage, there is considerable pressure to search, rediscover its native natural enemies and establish them in outdoor crops. Consequently, the search for new biological control agents has intensified within the past five years (Lacey *et al*., 1993). Studies were carried out in Mansoura region to evaluate the role of *Bemisia* natural enemies (predators, parasitoids and pathogens).

Three species of entomopathogenic *Cladosporium*, *C. herbarum*, *C. aphids* and *C. cladosporioides* were recorded (De Carvalho *et al*. 1972; Hulden, 1986; Pen *et al*., 1989). This study recorded for the first time in Egypt the occurrence of these three species of *Cladosporium*, isolated only from *Bemisia* life stages as host specific entomopathogenic fungi attacking *Bemisia* spp.

The entomopathogenic fungi appear to offer the best prospects for parasitizing *Bemisia* spp., because of its environmental safety and potential to spread in fields during high humidity periods (Ekbom, 1979, 1981). Therefore, *Cladosporium* spp. are likely to be effective natural agents against this insect in Egypt.

The abundance of *Cladosporium* spp. found its incidence and establishment under field condition on different life stages of *Bemisia* spp. on various plant hosts showed it was a promising candidate for biological control of whiteflies in Egypt. Natural epizootics of *Cladosporium* spp. occurred only at the end of summer and during the fall. This may be attributed to the optimum temperatures, high relative humidity and the amount of precipitation that occurred during this time of year (Table 2), which is in agreement with the findings of Fawcett (1944); Ponomarenko, *et al*. (1975) Lacey *et al*. (1993). High humidity is a vital requirement for spore germination, establishment of infection, sporulation and consequently, the capacity to produce and epizootic (Ekbom, 1979, 1981). The dispersal of entomopathogenic fungi among crop hosts is due to the movement of whitefly adults that are carrying the spores (Carruthers *et al*., 1993). This may explain the epizootics of *Cladosporium* spp. in Mansoura region.

Numerous epizootics recorded on squash, followed by cabbage, Euphorbia, Lantana, Hibiscus, Duranta and Cotton (Table 1), may be related to the heavy infestation and feeding preference of *Bemisia* for these hosts (Hoffman and Frodsham, 1993; De Quattro, 1997). This finding is in agreement with the results of Spasova *et al*. (1980) in Bulgaria with respect to *Aschersonia placena* on the larva of *Trialeurodes vaporariorum*. Our field survey studies showed that the percentages of infected *Bemisia* different from 17.43 to 25.47 percent (Table 1), showing that the *Cladosporium* spp. became naturally epizootic in the fields against this insect. *Cladosporium* spp. were observed during 1997 associated with *Bemisia* nymphs on poinsettia plants (*Euphorbia pulcherrima* Willd.), cotton aphids, *Aphis gossypii* Glover and the cotton leafhopper, *Emposasca lybica* De Berg.

The ability of *Cladosporium* species to infect *Bemisia* eggs was low. This may be due to the egg chorion invasion form fungal spores which need time to adapt, geminate and penetrate the egg shell. Fransen *et al*. (1987) reported that eggs of *T. vaporariorum* were not infected by *Ascheronia* species. The rates of infection for *Bemisia* life stages were higher under laboratory conditions (Table 4 and 5) even though the infection percentages were low under field conditions. Manipulation of climate factors and use of certain additives in formulation could increase the infection rate of *Bemisia* eggs on field plants (Fransen, 1994).

With nymphs and adults, the situation was different Results showed over 50 percent pathogenicity of *Cladosporium* spp. at both inoculums concentration. Nymphs (2nd to 4th instar) and adults were susceptible to the three species of *Cladosporium*. These results are in agreement with the results of Hussey (1958), Ekbom (1979), Hall (1982) and Masuda and Maeda (1999), when *V. lecanii* was used against the greenhouse whitefly. The infection process of *Cladosporium* spp. occurred within two days after inoculation and revealed some of the characteristics associated with these fungi, such as fast germination and high sporulation rate. These data agree with the results of Jackson *et al*. (1985) on *V. lecanii*. Light intensity and light duration are factors responsible for the sporulation of many fungi (Leach, 1965, 1971; Trione and Leach, 1969). Our result reveal that the development of *Cladosporium* spp. was dependent on the diurnal light regime; this means that these species could sporulate and yielded more spores when grown under 12 h alternating cycles than under continuous darkness. In this respect, our results are in agreement with the findings of Misaghi *et al*. (1978) and Cotty *et al*. (1983), who found that a diurnal light regime is required for *Alternaria* sp. to sporulate. The results presented here show strong evidence that *Cladosporium* spp. are effective entomopathogenic fungi against *Bemisia* spp. These fungi were highly virulent on whitefly life stages caused natural

epizootics on the insect under field conditions, are easy to produce in the laboratory and can be formulated to be used as an entomopathogenic biocontrol agent against whiteflies.

More information on the *Cladosporium* spp. mode of action, prevalence in the fields and greenhouses and integration with other beneficial insects needs to be obtained in future experiments.

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