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Cladosporium uredinicola SPEG. AND *Alternaria infectoria* E. G. SIMMONS, AS PROMISING BIOCONTROL AGENTS FOR *Bemisia argentifolii* BELLOWS&PERRING AND *Aphis gossypii* GLOV. ON TOMATOES

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ABSTRACT

Two *entomopathogenic* fungi, namely *Cladosporium uredinicola* and *Alternaria infectoria* were isolated from naturally infected nymphs and adults of both *Bemisia argentifolii* and *Aphis gossypii* infesting tomato plants. *C. uredinicola* was the most dominant species found with a rate of 35.8% and 35% on both *B.argentifolii* and *A.gossypii*, respectively. *A. infectoria* ranked second and was represented by 8.6% on *B. argentifolii* and 12% on *A.gossypii*. Laboratory bioassay studies indicated that *C. uredinicola* caused a higher mortality than *A. infectoria* for the two insect species at conidial concentration of 10 x 10^6 spores/ml, 4 days after treatment. The efficacy of both fungi as biocontrol agents against *B. argentifolii* and *A.gossypii* under semi-field conditions was also evaluated at fungal concentrations of 10 x 10^6 and 5 x 10^6 spore/ml.

Keywords: Biocontrol, tomatoes, Entomopathogenic fungi, *C. uredinicola*, *A. infectoria*, *B. argentifolii*, *A. gossypii*

INTRODUCTION

Whiteflies and aphids are serious insect pests of a wide range of crops in Egypt. The host range of *Bemisia argentifolii*. and *Aphis gossypii* includes many significant agricultural crops such as cotton and tomatoes. The damage occurs in a variety of ways. Feeding of whiteflies suck important nutrients, causing defoliation, stunting and poor plant yields. These insect pests also induce several plant physiological disorders, such as tomato irregular ripening and transmission of serious virus diseases (Taher, 1994; Toscano and Henneberry, 1994 and Conti, 1994). Reliance on chemical control of whiteflies and aphids leads to insect resistance, a dramatic increase in insect populations, high residue levels and adverse effects on the environment. Alternative methods of control, including the use of cultural methods, resistant varieties and biological control, could prevent aphid and Whitefly infestations and / or disease transmission and enhance activities of natural enemies.

The microbial control of insect pests is one of the most interesting means of insect control. It is well known that insects can be killed by fungi, either individually or in epizootics (Weiser, 1982). Some entomopathogenic fungi such as *Aschersonia aleyrodis, Verticillium lecanii* and *Beauvaria bassiana* were recorded as effective biological control agents against aphids and whiteflies (Hall, 1982; Fransen *et al.*, 1987; Fransen, 1990; Hsiao *et al.*, 1992). Three entomopathogenic fungi species belonging to genus *Cladosporium* have been recently recorded as promising biological control candidates for controlling whiteflies (*Bemisia spp*) in Mansoura region, Egypt (Abdel-Baky *et al*., 1998).

The aim of the present investigation is to, identify and study the seasonal incidence and to estimate the pathogenicity of different entomopathogenic fungi associated with *A. gossypii* and *B.argentifolii* on tomato plants in Mansoura region.

MATERIAL AND METHODS

Fungal Isolation, Identification and Incidence:

Insect cadavers showing natural external growth of fungi were collected and maintained in Petri-dishes containing potato dextrose agar (PDA) media. The inoculated Petri-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. These methods are the same as previously described by Shabana and Ragab (1997). To study the seasonal incidence of the isolated and identified entomopathogenic fungi, field samples of both *Bemisia argentifolii* and *A. gossypii* were collected from tomato plants during the period from September 1st, 2002 until March 16th, 2003.

Laboratory bioassay studies:

The most dominant fungi associated with the tested insects were used to evaluate their pathogenic effect on *B. argentifolii.* and *A. gossypii* under laboratory conditions. Hundred individuals from each insect species were chosen after surface-sterilization in a 1% sodium hydrochloride solution for 30 seconds and washed in distilled water (Abdel-Baky *et al*., 1998 & Abdel-Baky and Abdel-Salam 2003). Each Petri-dish containing 25 individuals was considered as one replicate. The insects were placed in a dark color blotter moistened with the fungal suspension with one concentration ($10x10^6$ spores/ml.) (Abdel-Baky *et al*., 1998). The data were collected daily and continued for seven days post-treatment, and subjected to variance analysis by SAS program (1988).

Field Evaluation:

Because both *C. uredinicola* and *A. infectoria* showed higher incidence and greater mortality rates under field and laboratory conditions, these fungi were selected for field evaluation against *B. argentifolii.* and *A. gossypii.*

For preparation of the fungal concentrations required for field application, the fungi were cultivated on PDA media at 28 °C. to obtain newly and fresh growth of the spores.

After intensive growth of both fungi, a little amount of sterile water was added to the harvest spores and mycelium. One ml of each fungal suspension was taken on glass slide to count the spores by means of light microscope. Then the counted spores were added to sterile water (one Liter) to prepare two concentrations 10×10^6 and 5×10^6 spore/ml.

Tomato seedlings were transplanted in the field and received the normal agronomic practices. The plants were covered with muslin cloth (1 x 1

m^2) to prevent any external infection. One month later, an artificial infestation by both *B. argentifolii* and *A. gossypii* were fulfilled under separated cages.

The cages were investigated at three days intervals to count the pests and continued until the pests reached their maximum numbers. The cages were divided into three groups. Five cages were treated with the higher concentration (10 x 10^6 spore/ml), five were treated with the low concentration (5 x 10^6 spore/ml) and the other five cages were treated with water only as a control.

Before treatments, the insects were counted directly (zero time). Meanwhile, samples from the plants of each group were taken after one day, two days, three days, five days, eight days, ten days and 15 days from treatment. The dead insects were investigated, counted and recorded.

Three leaves from each plant under the cages, were selected and transferred to the laboratory in plastic bags and investigated under the binocular. The insects which showed external growth of fungal mycelium were considered as dead by fungal spores. These tests were practiced with each fungus alone with the two concentrations, as well as, with the water treatment (control) .Mortality percentages were determined, recorded and subjected to statistical analysis.

RESULTS AND DISCUSSION

Fungal Isolation, Identification and Incidence:

Three entomopathogenic fungi, namely *Cladosporium uredinicola, Alternaria infectoria* and *Epicoccoum* spp. were found naturally attacking *B. argentifolii* and *A .gossypii* on tomato plants in Mansoura region. *C. uredinicola* was the most dominant species prevailing with a rate of 35.8% and 35% on both *B.argentifolii* and *A.gossypii*, respectively. *A. infectoria* ranked second and was represented by 8.6% on *B. argentifolii* and 12% on *A.gossypii. Epicoccoum* sp. came in the last place and was represented by a very low percentage (Figs. 1 & 2). The significance of *Cladosporium* spp. as effective biological control agents against whiteflies, aphids, and scale insects in the world have been reviewed (Pan *et al.*, 1989; Humber, 1991; Thumar and Kapadia, 1994; Baoyu *et al.*, 1997; Abdel-baky *et al.*, 1998; Abdel-baky and Abdel-Salam, 2003). Shabana and Ragab (1997) also recorded *A. infectoria* as an entomopathogenic fungus on the fig wax scale insect, *Ceroplastes rusci*, in Egypt for the first time. However, the present investigation is the first report of the occurrence of *A. infectoria* as a promising bio-control agent on *B. argentifolii* and *A. gossypii.*

Regarding the seasonal incidence, the three-entomopathogenic fungi appeared with the 1st sampling in the 1st week of September 2002. On *B. argentifolii, C. uredinicola* occurred with higher percentages from September until the end of December, then decreased during January 2003, disappeared in February and appeared again with lower percentage in March (Fig 3). The same trend was detected with *A. infectoria*, but its percentage fluctuated from high to low between months. With regard to the fungus *Epicoccum* sp., it appeared sometimes and its percentages ranged from 0.0 to 2% during the period of study (Fig. 3).

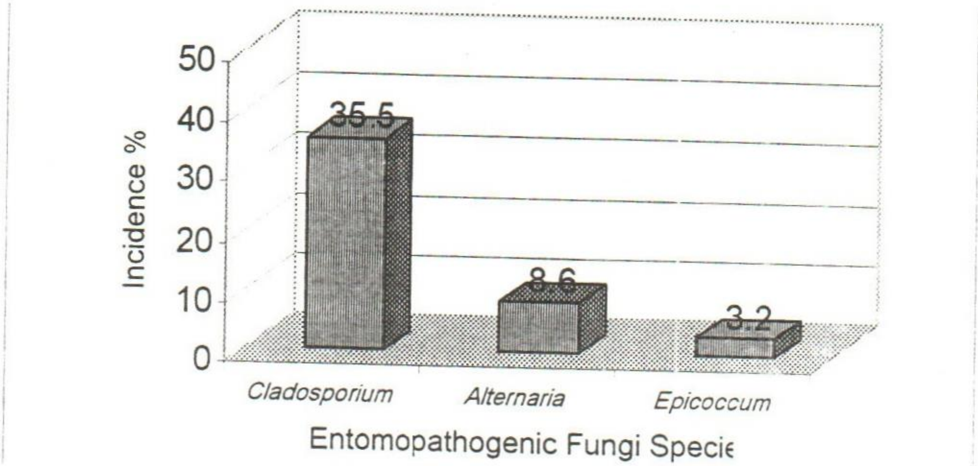

Figure (1) Incidence percentage of the three-entomopathogenic fungi isolated from *Bemisia* spp.

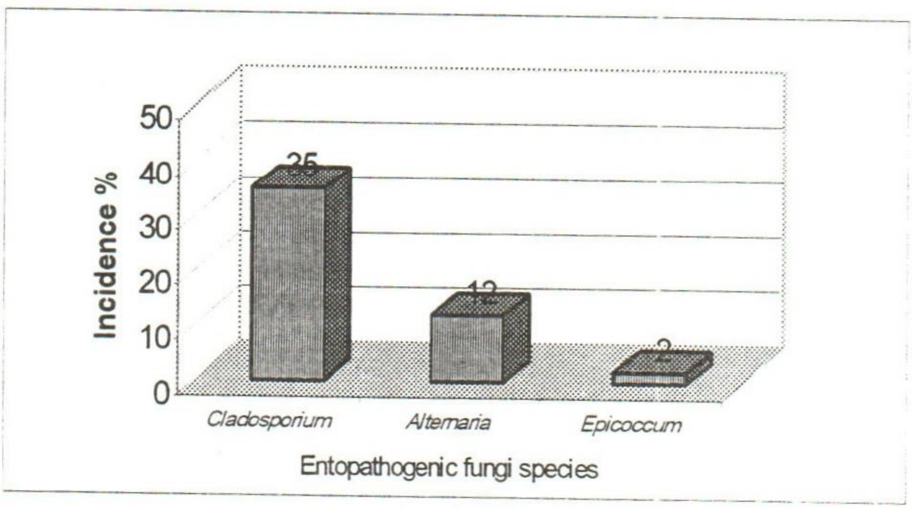

Figure (2) Incidence percentage of the three-entomopathogenic fungi isolated from *Aphis gossypii*.

On cotton aphid, the incidence of *C. uredinicola* and *A. infectoria* began with 1st week of September and continued until the 2nd half of December 2002. Both species disappeared completely during January and February 2003, then appeared again with low percentages in March (Fig 4). The natural occurrence of *C. uredinicola* as a bio-control agent against whiteflies and aphids in Egypt, was previously studied by Abdel-baky et *al.*, (1998). They found that *C. uredinicola* naturally attacked most of homopterous insects that existed in the Egyptian entomofauna. The prevalence varied according to the insect species and the plant host.

Laboratory bioassay studies:

All fungus species tested were pathogenic to the experimental insect at conidial concentrations (10 x 10^6 spores/ml). All fungus species differed significantly in virulence to *B. argentifolii* and *A. gossypii* nymphs (Table 1). After four days, *C. uredinicola* caused the higher mortality for the two insect species than *Alternaria*. The sensitivity of SLWF to *Cladosporium* infection was high as the mortality reached 64.2%, more than aphids (45.6%). Meanwhile, *A. infectoria* caused 33.5% and 27.8% mortality of *B. argentifolii* and *A. gossypii* population, respectively (Table 1).Similar results were reported by Abdel-baky and Abdel-Salam, (2003). They found that fungal species were significantly different in virulence to the whiteflies and the cotton aphid at conidial concentration of 10 x 10^6 spores/ml in laboratory bioassay studies.

Table (1): Efficacy of two fungal species isolated on two homopterous insects as a laboratory bioassay study.

Insect species	*C. uredinicola*	*A. infectoria*
B. argentifolii	64.2%	33.5%
A. gossypii	45.6%	27.8%

Field Evaluation:

A-Effect of two entomopathogenic fungi against *B. argentifolii*:

Table (2) represents the efficiency of two candidates of the entomopathogenic fungi, namely *C. uredinicola* and *A. infectoria* against *B. argentifolii* under semi-field conditions. Regarding *C. uredinicola*, the higher concentration (10 x 10^6 spore/ml) gave higher mortality rates, which reached 57.14%, 15 days after treatment. Meanwhile, the low concentration (5 x 10^6 spore/ml) showed 46.23 % after the same period of treatment. The data also revealed that the mortality percentages increased gradually with the longer time after treatment. The fungus caused higher infection rates under the semi-field condition compared with the water treatment (Table 2).

As for the efficiency of *A. infectoria*, the fungus proved satisfactory results against *B. argentifolii* compared with *C. uredinicola*. Fifteen days after treatment, *A. infectoriae* induced 37.40% and 26.49% of mortalities with (10 x 10^6 spore/ml) and (5 x 10^6 spore/ml), respectively (Table 2). It can be noted that the infection by *A. infectoriae* raised with elongation of time. In addition, the fungus created higher mortality percentages under the semi-field condition compared with the water treatment (Table 2).

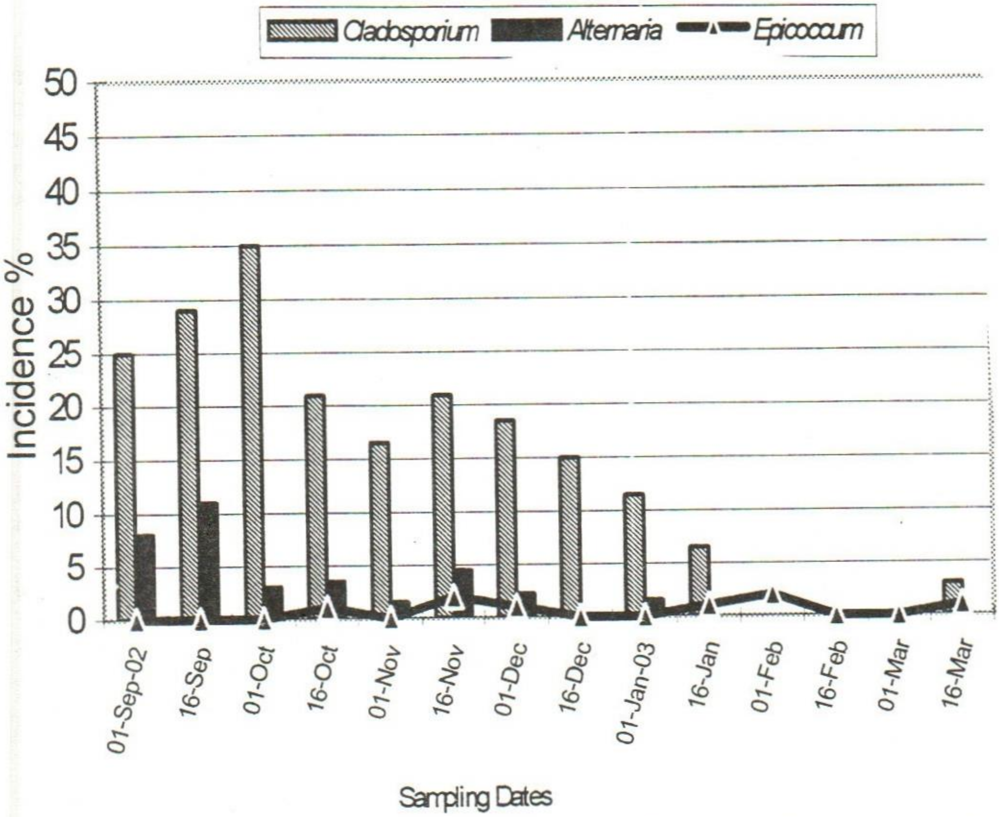

Figure (3) Seasonal incidence of the entomopathogenic fungi isolated

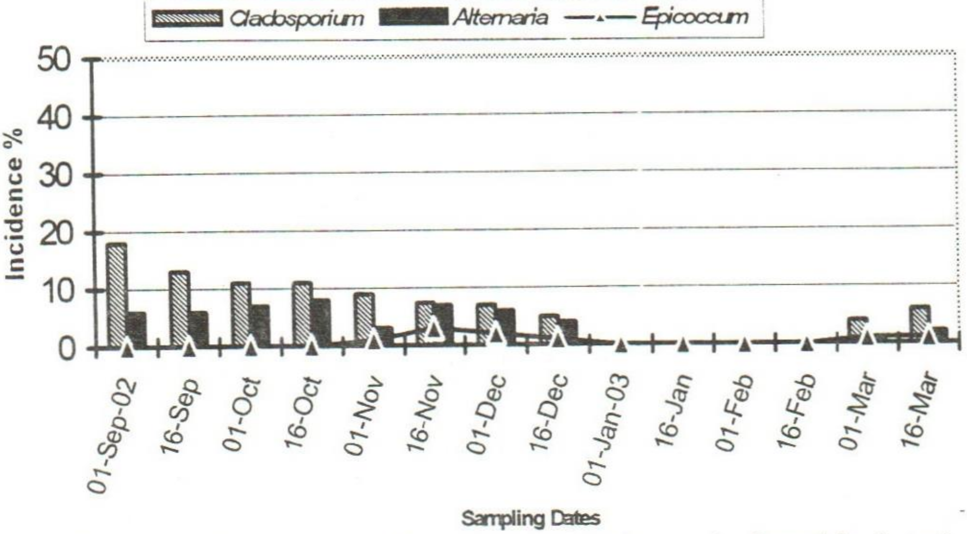

Figure (4) Seasonal incidence of the entomopathogenic fungi isolated from *Aphis gossypii*.

Table (2). Mortality Percentage of *Bemisia argentifolii* immature in tomato plants treated by two fungal species under semi-field condition.

Fungal species	Days After Treatment	Treatments		
		Control (Water Only)	Fungal Concentration (5×10^6)	Fungal Concentration (10×10^6)
Cladosporium uredinicola.	1	00.00 c	00.00 g	00.00 g
	2	00.00 c	03.64 f	08.30 f
	4	00.00 c	06.75 e	14.03 e
	7	03.64 b	14.03 d	26.49 d
	10	05.19 b	23.90 c	36.88 c
	12	07.79 a	31.69 b	49.35 b
	15	07.79 a	46.23 a	57.14 a
Alternaria infectoriae	1	00.00 c	00.00 f	00.00 g
	2	00.00 c	0.52 g	04.16 f
	4	00.00 c	5.71 e	08.83 e
	7	03.64 b	11.95 d	14.03 d
	10	05.19 b	17.66 c	20.26 c
	12	07.79 a	22.34 b	27.53 b
	15	07.79 a	26.49 a	37.40 a

[a] Values within a column followed with the same letter (s) are not significantly different according to LS means test (P=0.05).

B-Effect of two entomopathogenic fungi against *Aphis gossypii*:

C. uredinicola and *A. infectoriae* showed different mortality rates against *Aphis gossypii* under semi-field conditions, but not the same higher levels compared with their effects against *B. argentifolii* (Tables 2 & 3). After 15 days, *C. uredinicola* caused 44.44% mortality rate with the concentration (10×10^6 spores/ml) and 36.36% with concentration of 5×10^6 spores/ml (Table 3).

On the contrary, *A. infectoriae* recorded moderate mortality percentages against *A. gossypii* compared with *C. uredinicola*. Fifteen days after treatment, the mortality percentages by *A. infectoriae* reached 30.30 and 23.23% with (10×10^6 spores/ml) and (5×10^6 spores/ml), respectively (Table 3). It may be obvious that the infection by *A. Infectoriae* rose with longer time after treatment. Moreover, the fungus caused higher mortality percentages under the semi-field condition compared with untreated control water (Table 3).

In conclusion, both fungi induced different mortality rates under semi-field condition, but *Cladosporium* spp. efficiency was greater than that of *A. infectoria* under the same conditions. Also, the results showed that the mortality rates increased gradually with longer time of exposure after treatment.

Table (3). Mortality Percentage of *Aphis gossypii* in tomato plants treated by two fungal species under semi-field condition.

Fungal species	Days After Treatment	Treatments		
		Control (Water Only)	Fungal Concentration (5×10^6)	Fungal Concentration (10×10^6)
Cladosporium uredinicola.	1	00.00 e	00.00 g	00.00 g
	2	00.00 e	04.04 f	06.06 f
	4	00.00 e	06.06 e	09.09 e
	7	04.04 c	15.15 d	18.18 d
	10	06.06 b	21.21 c	23.23 c
	12	09.09 a	28.28 b	31.31 b
	15	10.75 a	36.36 a	44.44 a
Alternaria infectoria	1	00.00 e	00.00 e	00.00 g
	2	00.00 e	00.00 e	00.00 g
	4	00.00 e	03.03 d	06.06 e
	7	04.04 c	06.06 c	11.11 d
	10	06.06 b	12.12 bc	22.22 c
	12	09.09 a	15.15 b	27.78 b
	15	10.75 a	23.23 a	30.30 a

[a] Values within a column followed with the same letter (s) are not significantly different according to LS means test (P=0.05).

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الفطران كلادوسبوريم يوريديناكولا و الترناريا انفكتوريا كعاملين واعدين للمكافحة البيولوجية لحشرتى الذبابة البيضاء ومنَ القطن على محصول الطماطم

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تم عزل الفطرين كلادوسبوريم يوريديناكولا و الترناريا انفكتوريا من الحوريات والحشرات الكاملة للذبابة البيضاء ومنَ القطن. وقد وجد أن النوع كلادوسبوريم يوريديناكولا هو الأكثر شيوعا عن النوع الأخر، حيث بلغت نسبة الحشرات المصابة بة طبيعيا فى الحقل ٣٥ % على كلا النوعين من الحشرات فى حين بلغت الأصابة بالفطر الترناريا انفكتوريا ٨% على الذبابة البيضاء و١٢% على منَ القطن.

كما تم ايضا دراسة اختبارات القدرة المرضية لكلا الفطرين على كلا النوعين من الحشرات معمليا وحقليا. وقد أسفرت النتائج المعملية على أن إستخدام الفطر كلادوسبوريم يوريديناكولا بتركيز ١٠X١٠ ٦/جرثومة/مل يعطى نسبا أعلى للموت على كلا النوعين من الحشرات عن الفطر الترناريا انفكتوريا وذلك بعد أربعة أيام من المعاملة.كما تم كذلك تقييم فعالية كلا النوعين من الفطريات ضد النوعين من الحشرات تحت ظروف نصف حقلية وذلك باستخدام تلك الفطريات بتركيزين مختلفين هما ٥X١٠ ٦/جرثومة/مل و ١٠X١٠ ٦/جرثومة/مل.