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INTERACTIONS AMONG THE ENTOMOPATHOGENIC FUNGUS, *Cladosporium uridenicolla* SPEG., AND TWO WHITEFLIES BENEFICIAL INSECTS, *Eretmocerus mundus* MERCERT AND *Coccienlla undecimpunctata* L.: FROM AN IPM PROSPECTIVE.

Abdel- Baky, N.F.
Economic Entomology Department, Faculty of Agriculture; Mansoura University (Email: nafabdel@mans.edu.eg)

ABSTRACT

Laboratory studies were initiated, for the first time, to evaluate the competitive interactions among the entomopathogenic fungus, *Cladosporium uridenicolla* and the two whiteflies natural enemies, the internal parasitoid, *Eretmocerus mundus* and the coccinellid predator, *Coccinella undecimpunctata*. The positive and negative effects of these combinations in regulating the population of the silverleaf whitefly, *Bemisia argentifolii*, were extensively studied.

The negative effects on the parasitoid were extremely low. No direct contact or moralities occurred among the parasitoid population when parasitizing the nymphs. Higher percentages of emerging parasitoid adults were also obtained. Meanwhile, when *Er. mundus* adults were exposed directly to the fungal suspension, the mortality percentage reached 5-7% only. Three to five days after treatment, *B. argentifolii* nymphs were rejected as a host by *Er. mundus* adult due to the fungal growth. The majority of *Er. mundus* females also laid no eggs and no parasitism was detected.

The susceptibility of *C. undecimpunctata* developmental stages to *C. uridenicolla* varied according to spore concentrations and the treated life stages. Eggs were more tolerant to the fungal infection, and hatchability reached 98.5%. The first instar larvae of *C. undecimpunctata* were more sensitive to infection by *C. uridenicolla*, followed by the 2nd instar and pupal.

Intra-guild predations were detected among the fungus and the predator, because of the predator feeding on the infected SLWF nymphs. Their feeding rates on the nymphs without mycelium varied between 38.65 and 56.5% for the larval instars and adult stage. Meanwhile, when the mycosis appeared on the SLWF nymphs, their feeding rates decreased sharply. Consequently, the pathogen could be considered as the "intra-guild predator" which was directly able to infect the coccinellid predator in the guild. In addition, *C. undecimpunctata* adults and larvae, that fed on the infected nymphs by the fungus, became also as intra-guild predators of the pathogen. Therefore, the use of both the fungus and the predator in combination had a deleterious effect on the biological control of whiteflies and could minimize the control efficiency.

The control efficiency of the three natural enemies of *B. argentifolii*, when used separately or in combination, varied according to the tested biological agent. Using *Er. mundus* alone reduced the pest populations by 38.1%, whereas, *C. uridenicola* shared by 19.4% in the pest population reduction. The interaction between *C. uridenicola* and *Er. mundus* raised the control efficiency to 57.8%. The use of both *Er. mundus* and *C. undecimpunctata* in combination reduced the pest population by 38.7%. The three biological control agents combined together reduced the pest population by 58.0%.

Combination between both *C. uridenicola* and *Er. mundus* caused a synergistic relationship (additive). The use of the pathogen in combinations with *Er.*

mundus improved suppression of *B. argentifolii* nymphs. Conversely, antagonism relationship (non-additive) among the pathogen in combinations with the coccinellid predator was detected. Therefore, the combination of C. uridenicola and whitefly parasitoids require further studies in other systems. The results suggest that the fungus and the parasitoid or the fungus and the predator may be used together for IPM program of the whiteflies, which may lead to viable control options. The fungus spore application should coincide with the later developmental stage of the parasitoid or the predator developmental stages to pass the sensitive stages in order to conserve these beneficial insects within the system.

Keywords: Entomopathogenic fungi, Cladosporium *uridenicolla*, *Eretmocerus mundus*, *Coccinella undecimpunctata*, *Bemisia argentifolii*, whitefly natural enemies, interactions, intra-guild predation, Biological control interactions.

INTRODUCTION

Microbial control agents offer alternatives to chemical pest control and are more selective than chemical insecticides (Fuxa, 1987). Further, bio-control agents can be integrated with other control agents to provide environmentally safer methods than chemicals. They may also secure protection after establishment within the host population (Fuxa, 1987; Goettel and Hajek, 2001).

Entomopathogenic fungi are considered less expensive with respect to deleterious side effects such as human health hazards and destruction of non-target organisms (Ahmed and Leather, 1994; Goettel and Hajek, 2001). Mycoinsecticides are very important and play a vital role in the natural regulation of many insect pests under natural conditions (Moore and Prior, 1993). To enhance their role in pest control should be tested through interaction with other natural enemies in the agro-ecosystem. Therefore, Poppy (1997) pointed out that both insect fungal diseases and arthropod natural enemies may contribute to the suppression of the insect pest populations either as individual species or as species complexes. The suitability of entomopathogenic fungi, as part of insect control measures in IPM programs, is critically important. So, mycoinsecticides and other insect natural enemies have the potential to complement or interfere with one another, based on the environmental conditions and other biological factors (Kim *et al.*, 2005). Since insect natural enemies have developed to be employed in multi-trophic relations, it is important to assess their interactions within natural enemies complexes if they are used in combination for IPM (Roy and Pell, 2000). Thus, in order to use insect natural enemies more effective in IPM program components, they should act in harmony with minimal antagonistic interaction between groups and other interventions (Lacey *et al.*, 1997). Multiple species of natural enemies can interact either synergistically/additively or antagonistically (Ferguson and Stilling, 1996; Roy and Pell, 2000). Clearly, Roy and Pell (2000) emphasized that the synergistic interactions may lead to higher mortality than the combined individual mortalities of the pest populations. Meanwhile, the additive mortality occurs if the biological agents do not interplay and, consequently, the total mortality levels by various agents are generally equal.

Evidently, the use of biological control technology has the potential to cause negative side effects to non-target organisms (Goettel and Hajek, 2001). The introduction or mass application of exotic fungal pathogen of pests, may affect some non-target species both in the local environment into which the agent was introduced and in the areas where they may spread (Hajek and Goettel, 2000). The risk may be slightly higher with exotic agents that are introduced to control native pests; however, no data has ever been presented that documents this concern (Carruthers and Poprawski, 1994).

Under field conditions, *Cladosporium* species are epizootics and occur naturally with higher incidence (10.0-28.0%). Therefore, *Cladosporium* species are considered as bio-control agents against whiteflies and other sucking insects in Egypt (Abdel-Baky *et al.*, 1998). Accordingly, *C. uredinicola* is a native bio-control agent, the most dominant one and more virulent *in vivo* and under semi-field conditions (Abdel-Baky, 2000; Abdel-Baky and Abdel-Salam, 2003; Ragab and Abdel-Baky, 2004). Additionally, the fungus becomes established and provides long-term control in nature, as would be expected in classical control. Thus, the fungus is a valuable natural bio-control agent in regulating the populations of whiteflies in Egypt (Abdel-Baky *et al.*, 1998).

Eretmocerus mundus Mercet and *Coccinella undecimpunctata* L. are the most abundant indigenous beneficial insects against whiteflies under field conditions in Egypt (Abdel-Baky and Ragab, 2005; Saleh, 2005). They also form the key elements of biological control in IPM (Ardeh *et al.*, 2005).

Since *Bemisia argentifolii* and other whiteflies have very high reproductive rates and are very difficult to control with a single biological control agent, various species of natural enemies may be introduced simultaneously in greenhouses. These biological control agents may act synergistically, additively or antagonistically (Roy and Pell, 2000).

Therefore, the present study was carried out to characterize (**i**) the negative and positive side effects, and (**ii**) control efficiency, if *C. uridenicola* is integrated with the ecto-endparasitoid, *Er. mundus* and/or the coccinellid predator, *C. undecimpunctata*. This means that the goal will focus on the interactions between the fungal and two whiteflies natural enemies. The capability of the entomopathogenic fungus and *C. undecimpunctata*, and/or *Er. mundus* alone and in combination to control *B. argentifolii* populations is also determined.

MATERIAL AND METHODS

I: Experimental Organisms.

A- *Bemisia argentifolii* Bellows & Perring (Hemiptera: Aleyrodidae): The silverleaf whitefly (SLWF), *B. argentifolii*, was reared on the kidney bean, *Phaseolus vulgaris* L., under laboratory conditions 25±2.2°C an 70±5% R.H. SLWF culture was originally initiated from individuals collected from squash plants, untreated with insecticides, in Dakahelia governorate. About hundred pots cultivated with *P. vilgarius* were prepared until formation of two fully expanded leaves. Thus, ten pairs of *B. argentifolii* adults were introduced to

the kidney bean leaves, confined under screen cages for 48 hours in order to lay their eggs, and then removed. The eggs were counted on each leaf and were left until hatching and formation of the 2nd instar nymphs.

B- *Ertemocerus mundus* Mercet (Hymenoptera: Aphilinidae): The parasitoid was collected from different plant hosts free of insecticides and its colony was initiated on *P. velgarius* plants infested with SLWF nymphs. Different generations were reared under laboratory conditions of 25±2.2°C; 70±5% R.H. and normal photoperiod. To obtain adult parasitoids, the parasitoid pupae were isolated for adult emergence using small tubes. The adults were fed on honey solution and were placed on newly plants infested with SLWF nymphs.

C- *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae): Adults of the eleven spots lady beetles, *C. undecimpunctata*, were also collected from plant hosts infested by SLWF free insecticides and reared under laboratory conditions for one generation. Since *C. undecimpunctata* is a common predator preyed on wide host range of insect pests, individuals of the predator were used in this evaluation. The predator colony was reared on whiteflies and aphids until emergence the adults of the 2nd generation which used in these experiments.

D- *Cladosporium uridenicola* Spegazzini: The fungus was isolated from naturally infected whiteflies according to Abdel-Baky *et al.*, (1998) and kept in slant Agar media at 5 °C. The fungal spores were harvested from two weeks old cultures on autoclaved PDA media at 28±2 °C by rinsing with sterilized distilled water.

II. Interaction studies among three *B. argentifolii* natural enemies.

A- *Cladosporium uridenicolla* and *Ertemocrus mundus*.

In order to assess the interaction between the entomopathogenic fungus, *C. uridenicolla*, and *Er. mundus*, three trials were designed. The interactions were evaluated at three stages of parasitism, namely, **i**) SLWF nymphs parasitized by *Er. mundus* larvae, **ii**) SLWF nymphs treated with the fungal suspension then the parasitoid was introduced, and **iii**) SLWF nymphs and *Er. mundus* adults treated together directly after introducing the adults parasitoid by fungal suspension. Three fungal concentrations were used to assess the positive and negative impact on the parasitoid development and parasitism percentage. The trials were preformed as follows:

1. **Parasitized SLWF nymphs treated with *C. uridenicola*.**

A constant number of the parasitoid (1 parasitoid: 5 SLWF nymphs) was introduced to kidney beans infested with SLWF 2nd instar under screen cages. The parasitoid was left for 72 hours to lay its eggs and then removed. Five days later, after removing the parasitoid and signs of parasitism was noticed, the plant leaves, infested with SLWF nymphs, were treated by fungal suspension at concentrations of 4×10^{10}, 6×10^{10} and 10×10^{10} spores/ ml. Two days later, the plant leaves were investigated by hand lens and examination was continued until parasitoid adult emergence. The mortalities of SLWF nymphs and parasitoid adults by the fungus were recorded under each concentration. Five pots were treated with each fungal concentration and replicated three times.

2. **Non-parasitized SLWF nymphs treated with *C. uridenicola*, with the parasitoid adults introduced immediately after treatment.**

Forty five pots of kidney beans infested with 2nd instar nymphs of SLWF were used in this trial. Constant numbers of SLWF 2nd instar/plant leaf (40 nymphs/leaf) were counted and treated by the fungal suspension at concentrations of 4 X 10^{10}, 6 X 10^{10} and 10 X 10^{10} spores/ ml. Accordingly, the kidney bean pots were divided to three groups, each consisting of five pots per each fungal suspension and replicated three times. In the first group, *E. mundus* adults were introduced two days later after the fungal treatment by ratio of 1 parasitoid: 5 SLWF nymphs. The parasitoid oviposition process behavior, symptoms of parasitism and parasitoid mortality by the fungus were studied carefully. Emergence of parasitoid adults was also recorded.

3. **Both of non-parasitoid SLWF nymphs and parasitoid adults treated together with *C. uridenicola*.**

After introducing a constant number of the parasitoid (1 parasitoid: 5 SLWF nymphs) to kidney bean leaves infested by SLWF 2nd instar nymphs under screen cages, both parasitoid and SLWF nymphs were treated together by the fungal suspensions. The parasitoid adults and SLWF nymphs mortality were examined under the three fungal. Moreover, symptoms of parasitism, parasitism percentage, and parasitoid oviposition process were also recoded. Each fungal concentration consisted of five pots and thee replicates.

The previous trials were compared with the check treatment (control) in which parasitized SLWF nymphs were treated only with sterile water.

B- *Cladosporium uridenicolla* and *Coccinella undecimpunctata*.

Different life stages (eggs, larval instars, pupae and adults) of *C. undecimpunctat* were used in this experiment. The trials were carried out as follows:

In case of *C. undecimpunctata* adults and larvae, three tests were conducted for each one according to the predator treatment by the fungal suspensions as follows:

1- The first was preformed by introducing SLWF nymphs daily, directly after treatment by three fungal concentrations, to feed *C. undecimpunctata* larvae and adults. In this trial, the coccinellid insects were free of fungal treatment. One newly emerged adult or larva was inserted in Petri-dish (9 cm diameter) and replicated 10 times. The same procedure was also followed with each larval instar (1st to 4th). This work was continued until the death of coccinellid larvae and adults or completed its life cycle. The behavior of individuals that emerged from these insects was observed. The prey consumption rates were also calculated to each stage.

2- The second was done by incubating the treated SLWF nymphs by the fungal spores at 25 °C for five days, until observing the mycoses signs. Then, *C. undecimpunctata* larvae and adults were fed daily on these nymphs. In this trial, the coccinellid insects were free of fungal treatment. One larva of each instar or adult was inserted in a Petri-dish and replicated 10 times. The trial was continued until the death of coccinellid larvae and adults or completed its life cycle. The behavior of individuals

that emerged from these insects was followed and the prey consumption rates were calculated for each stage.

3- The third one was preformed by treating both the coccinellid insects and SLWF nymphs inside a Petri-dish by the fungal spores. This means that the predator insects were exposed directly to the fungal suspension. Each stage was replicated 10 times for each fungal concentration. The trial was continued until the death of coccinellid larvae and adults or completed its life cycle. The behavior of individuals that emerged from these insects was observed and the prey consumption rates were calculated for each stage.

Coccinellia undecimpunctata eggs were placed in two groups, the newly deposited eggs and 3-days old eggs. Each treatment involved 10 egg masses (at least 15 eggs of each one) and replicated three times. The eggs of each group were treated by three fungal concentrations mentioned before. The treated eggs were incubated at 25 °C until hatching. Hatching percentage, egg viability (recoded as number of 1st instar larvae) and eggs mortality due to mycosis were recorded.

III. Statistical Analysis.

The mortalities number and values in all tests were subjected to ANOVA analysis. All statistical analyses were preformed using CoStat Software program (1990). The percentages of mortality due to fungal activities were calculated according to Abbott formula (1925). The interaction between the three bio-control agents and contribution of each biological agent if used alone or in combination to regulate the pest population were calculated by MINITAB program (1998).

RESULTS

I. Interactions between entomopathogenic fungus *C. uridenicolla* and endoparasitoid *Er. mundus*.

A- Parasitized SLWF nymphs treated with *C. uridenicola*.

The spores of *C. uridenicola* had great effect on non- parasitized SLWF nymphs (Fig. 1). The infection percentages of the SLWF nymphs were 20.47, 15.22, 8.95 and zero % with respect to higher, intermediate, lower spore concentrations and check treatment. Meanwhile, the fungal impact on the parasitized nymphs was slight and varied according to spore concentrations (Fig. 1). *Er. mundus* adults emerging from the treated parasitized nymphs reached 74.11% with the higher spore concentration, 80.14% with intermediate concentration of the fungus and 92.5% with lower spore concentration and 96.4 with the check treatment (Fig. 2). The data also revealed that there was no mortality among these adults due to the direct contact with spores and mycelium on the SLWF cadavers. In addition, the results showed no negative effects on the parasitized whiteflies nymphs treated by the fungal spores under field or greenhouses conditions. Moreover, the parasitoid efficiency increased with the intermediate and lower spore concentrations. Mortality percentages of the SLWF nymphs and the

percentages of the emerged parasitoid adults varied significantly according to spore concentrations (P≥0.05).

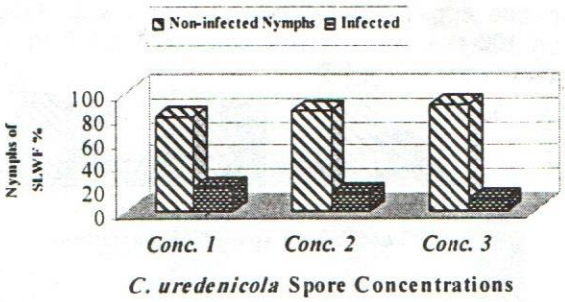


Fig (1): Infection percentage of the parasitized *B. argentifolii* nymphs by the entomopathogenic fungus, *C. uridenicola*, under three spore concentrations.

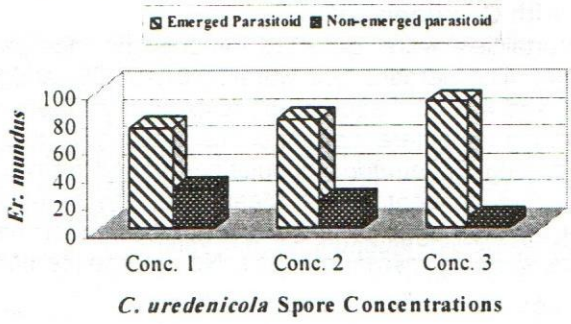


Fig (2): Percentages of emerged *Er. mundus* adults from parasitized *B. argentifolii* nymphs treated by three spore concentrations of the entomopathogenic fungus, *C. uridenicola*.

B- Non-parasitized SLWF nymphs treated with *C. uridenicola*, and the parasitoid adults introduced immediately after treatment.

C. uridenicola induced higher mortalities among the non-parasitized SLWF nymphs (Fig. 3). The higher spore concentration (10 X 10^{10} spores/ ml) caused 73.24% of the non-parasitized SLWF nymphs. Diminishing the spore concentration to 6 X 10^{10} and 4 X 10^{10} spores/ ml reduced the mortality percentages to 55.08 and 63.42% of the SLWF nymphs in comparison with higher concentration. The mortality percentage was affected greatly by the tested spore concentrations (P≥0.05).

Despite the direct contact of Er. *mundus* adults with the fungal spores, the mortality percentage among the parasitoid population did not rise over 5% under the higher spore concentration. No eggs were laid by *Er. mundus* females on the infected SLWF nymphs by *C. uredinicola* in comparison with healthy nymphs of the check treatment. This means that females could detect the infected nymphs and showed a rejection behavior to these hosts. Consequently, there were no emerged parasitoid adults.

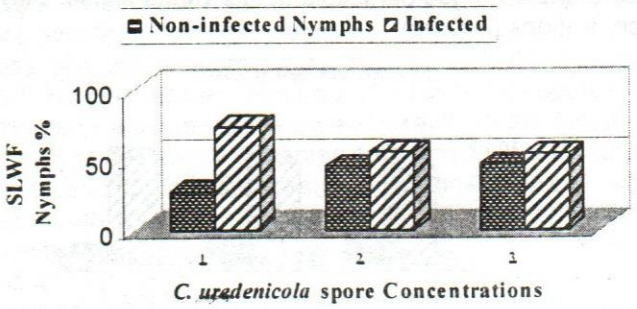


Fig (3): Mortality percentage of the non-parasitized *B. argentifolii* nymphs treated by three spore concentrations of the entomopathogenic fungus, *C. uridenicola*.

C- Non-parasitized SLWF nymphs and parasitoid adults treated together with *C. uridenicola*.

Higher mortalities were detected among the non-parasitized SLWF nymphs (Fig. 4). The percentages were 56.67, 55.29, and 54.55% at 10 X 10^{10}, 6 X 10^{10} and 4 X 10^{10} spores/ ml, respectively. There are no statistical differences among the different spore concentrations ($P \geq 0.05$). The mortality percentages among *Er. mundus* populations varied from 5 to 7% with the three tested spore concentrations. Meanwhile, the non-infected parasitoid adults did not lay their eggs or laid a few number under the infected SLWF nymphs before showing mycelium signs. No emergence of parasitoid adults occurred.

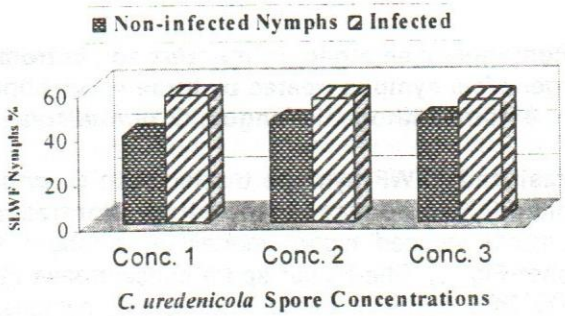


Fig (4): Mortality percentages of the non-parasitized *B. argentifolii* nymphs treated by three spore concentrations of the entomopathogenic fungus, *C. uridenicola*.

In conclusion, the negative side effects of the entomopathogenic fungus, *C. uridenicolla*, on the parasitoid *Er. mundus* population was very limited. There was no morality among the parasitoid population (indirect contact) when the parasitized SLWF were treated by the fungus (Table 1). Higher percentages of the parasitoid adults emerged from the treated SLWF

nymphs. Meanwhile, when the parasitoid adults were treated by the fungal suspension (direct contact), they rejected the infected SLWF nymphs as a host. The majority of females parasitoids laid no eggs, and in few cases these adults did not prefer the infected nymphs. As a result, no parasitism took place (Table 1).

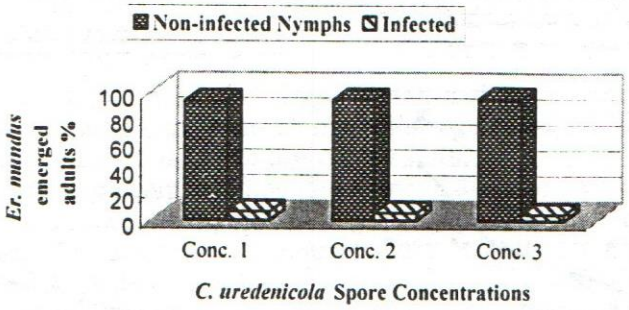


Fig (5): Percentages of infected and non-infected *Er. mundus* adults treated by three spore concentrations of the entomopathogenic fungus, *C. uridenicola*.

Table (1): mortality percentage and average number of total parasitoid female progeny treated by three spore concentrations of *C. uridenicola*.

Exposed procedures of the parasitoid to fungal spore suspensions	Mortality % of *Er. mundus*			Average of female progeny		
	$10 X10^{10}$ spores/ ml	$6 X10^{10}$ spores/ ml	$4 X 10^{10}$ spores/ ml	$10X10^{10}$ spores/ ml	$6 X 10^{10}$ spores/ ml	$4 X 10^{10}$ spores/ ml
Treated the parasitized SLWF nymphs	0.0 a	0.0 a	0.0a	0.0 *a*	0.0 *a*	0.0 *a*
Treated SLWF nymphs then introduced the parasitoid adults	5.45 a	4.11 ab	3.25 b	0.95 *b*	1.05 *ab*	1.20 *a*
Treated both of SLWF nymphs and parasitoid adults	6.95 a	5.85 ab	4.75 b	1.39 *c*	1.55 *b*	1.95 *a*

[a] the numbers followed by the same letter within a row are not significantly different at 1% level (Duncan Multiple Rang Test, Duncan, 1955)

II- Interactions between entomopathogenic fungus *C. uridenicolla* and coccinellid predator, *C. undecimpunctata*.

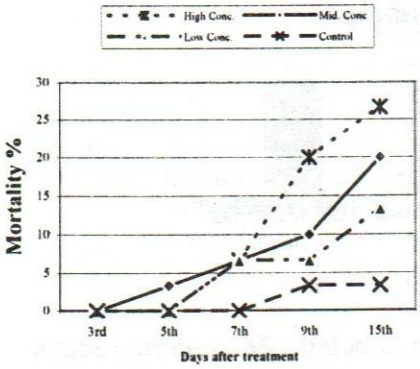
Positive and negative effects of interactions among *C. uridenicolla* and coccinellid predator, *C. undecimpunctata* were appeared as follows:

A- Mortality percentages among *C. undecimpunctata* adults.

1- When SLWF nymphs were treated by *C. uridenicolla* spore suspension, and introduced immediately to *C. undecimpunctata* adults.

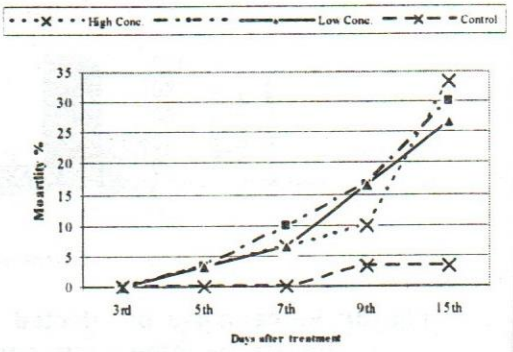
The predator mortality was lower, appeared late seven days after treatment, and reached its maximum after 15 days. All the three fungal concentrations caused mortality among *C. septemunctata* adult populations,

but the mortality percentages differed significantly (P≥0.05) according to the spore concentrations (Fig 6). Higher mortality rates (26.67%) occurred when the spore concentration 10 X 10^{10} was used. Median and low spore concentrations induced 20 and 13.33% mortality percentages among the predator populations in comparison with the check treatment (3.33%) (Fig. 6).



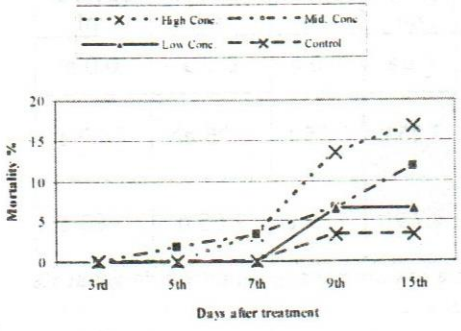
A

Fig. (6): Mortality percentages of *C. undecimpunctata* adults treated by three *C. uridenicolla* spore concentrations



B

Fig.(7):Mortality percentages of *C. undecimpunctata* adults treated by three *C. uridenicolla* spore concentrations



C

Fig. (8): Mortality percentages of *C. undecimpunctata* adults treated by three *C. uridenicolla* spore concentrations

Figures Legend:

A: Nymphs of *B. argentifolii* treated by C. *uridenicolla*, and then introduced immediately to feed *C. undecimpunctata* adults (Fig. 6).

B: Both of *B. argentifolii* nymphs and *C. undecimpunctata* adults treated together by C. *uridenicolla* suspension (Fig. 7).

C: Nymphs of *B. argentifolii* treated by C. *uridenicolla*, and left until appearance of infection signs then introduced to feed *C. undecimpunctata* adults (Fig. 8).

2- When both SLWF nymphs and *C. undecimpunctata* adults were treated together by *C. uridenicolla* spore suspension.

Mortality was observed among the predator population on the 5th day after treatment (Fig. 7). It increased gradually and reached its maximum within 15 days. The mortality percentages significantly varied in accordance with the spore concentrations (P≥0.01). In this treatment, higher mortality rates occurred when compared with the other two predator trials. C. uridenicolla spore concentrations caused 33.33, 30.00 and 26.67% with higher, median and lower spore concentrations (Fig. 7). The check treatment showed 3.33% among its populations.

3- When SLWF nymphs treated by C. *uridenicolla* spore suspension, and SLWF nymphs incubated till mycelium appeared on the cadaver and introduced to C. undecimpunctata adults.

Lowest mortality percentages appeared among the predator population in comparison with the two previous trials (Fig. 8). These percentages reached 16.67, 11.67 and 6.67% with spore concentrations of 10×10^{10}, 6×10^{10} and 4×10^{10} spores/ml, respectively. In the check treatment, mortality among the predator population recoded only 3.33% (Fig. 8). The mortality rates varied significantly according to the spore concentrations (P≥0.05).

B- Susceptibility of *C. undecimpunctata* developmental stages to *C. uridenicolla* treatment.

The susceptibility of *C. undecimpunctata* developmental stages to *C. uridenicolla* was studied under laboratory conditions (Table 2). The eggs were more tolerant to the fungal infection, which was expressed by hatchability. The later was higher with low spore concentration (98.5%), followed by 92 and 85% with median and high spore concentration (Table 2). The first instar larvae of *C. undecimpunctata* were more sensitive to infection by *C. uridenicolla*, which mortalities reaching 50, 40, and 33.33 with 10×10^{10}, 6×10^{10} and 4×10^{10} spores/ ml, respectively. The 2nd instar and pupal stage were in equal in their susceptibility the fungal infection under the three spore concentrations. Similar results were obtained regarding the susceptibility of 3rd, 4th instar larvae and the adult stage at all spore concentrations in comparison with the control (Table 2). Statistically, infection of *C. undecimpunctata* differed significantly according to spore concentrations, days after treatment and the predator developmental stages (P≥0.05).

Table (2): Susceptibility of *C. undecimpunctata* developmental stages to the entomopathogenic fungus, *C. uridenicola*.

Fungal Spore Concentrations	Egg Hatch-ability %	Mortality %					
		1st instar Larvae	2nd instar Larvae	3rd instar Larvae	4th instar Larvae	Pupae	Adults
10×10^{10} spores/ ml	85	50	26.67	10	6.67	26.67	6.67
6×10^{10} spores/ ml	92	40	23.33	6.67	6.67	23.33	3.33
4×10^{10} spores/ ml	94.5	33.33	16.67	6.67	3.33	18.33	3.33
Control (Water treatment)	98.5	6.67	3.33	3.33	0	0	0

C- Feeding rates of *C. undecimpunctata* life stages on treated SLWF nymphs by the fungus.

Feeding rates of *C. undecimpunctata* life stages varied on infected or non-infected nymphs, as well as, on the degree of mycelium growth and its appearance and covering the cadavers (Fig. 9). Feeding on infected SLWF nymphs without mycelium appearance, showed 45.5, 56.5, 42.5, 38.65 and 40.15%, in comparison with its feeding on healthy nymphs, for larval instars and adult stage. Meanwhile, when the mycosis appeared on the SLWF nymphs, the predator life stages fed on the nymphs but with minimum capacity in comparison with the infected nymphs without mycelium appearance and healthy nymphs (Fig. 9).

The predator feeding rates decreased to 10.2, 14.5, 18, 25.4 and 30.5% for larval instars and adult stage. Moreover, feeding rates differed significantly according to the prey healthy and degree of mycelium appearance on the cadavers.

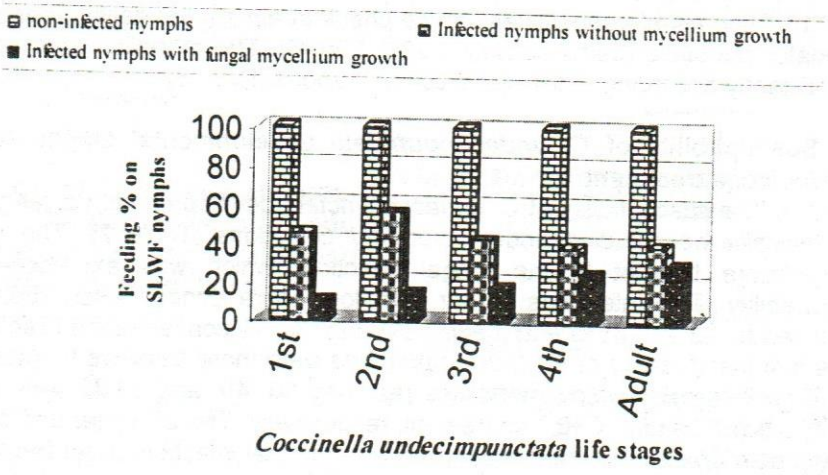


Fig (9). Feeding percentages of different *C. undecimpunctata* life stages on *B. argentifolii* nymphs treated by the entomopathogenic fungus, *C. uridenicola*.

III. Integration among the three biological agents in controlling *Bemisia argentifolii*.

The three natural enemies of *B. argentifolii*, applied alone and in combination, were able to suppress *B. argentifolii* population, thus delaying its increase by about one month (= one generation time) compared with the check treatment. In this respect, the regression analysis elucidated the sharing rates of each natural enemy when used alone or in combination with others in controlling the pest under semi-field conditions (Table 3).

Er. mundus alone decreased the pest populations by 38.1% (Table 3). Meanwhile, *C. uridenicola* was able to reduce the pest population by 19.4%. On the contrary, the combined effect of *C. uridenicola* and *Er. mundus* raised the control efficiency to 57.8%. Both *Er. mundus* and *C. undecimpunctata* combined together suppressed the pest population by

38.7% only. The combined effect of the three biological control agents reduced the pest population by 58.0% (Table 3).

Table (3). Regression analysis among the three SLWF, *B. argentifolii*, bio-control agents in the pest control if they used separated or in combinations.

Control effectiveness when integration the three bio-control	Regression Analysis				SLWF Bio-control agents		
	R^2 %	Adjusted R^2 %	C-p	S	*C. uridenicola*	*Er. munuds*	*C. undecimpunctata*
Control Efficiency %	38.1	34.7	7.6	3.8307		X	
	19.4	15.0	14.7	4.3712	X		
	57.9	53.0	2.0	3.2506	X	X	
	38.7	31.5	9.4	3.9240		X	X
	58.0	50.1	4.0	3.3480	X	X	X
Regression Equations							
C. uridenicola	Y= 11.5 + 0.257 X						
Er. munuds	Y= 11.5 + 0.257 X						
C. undecimpunctata	Y= 11.5 + 0.257 X						
Interact among the three bio-control factors	Y= 6.90 + 0.123 *Cladosporium* + 0.259 *Er. mundus* + 0.020 *C. undecimpunctata* Where Y is Control efficiency.						

DISCUSSION

Entomopathogenic fungi may contribute with other insect natural enemies to the control of insect pests either as individual species or as species complexes (Roy and Pell, 2000). With the increased use of parasitoids and predators in biological control programs, it is important to evaluate their interactions within complexes of natural enemies if they are used effectively in IPM (Pell and Roy, 2000; Lacey and Mesquita, 2002). Fungal pathogens have impact on the beneficial insects or non-target organisms either through direct infection of these organisms or through indirect effects, principally depletion of the target population (Goettel and Hajek, 2001). Direct infection of any beneficial insect or non-target organisms usually has undesirable effects in any biological control program. Moreover, Rosenheim *et al.* (1995) explained the indirect effects which a pathogen may interfere with the natural enemy complex by reducing the pest population or rendering the pest host unsuitable for other natural enemies.

When the entomopathogenic fungus, *Cladosporium uridenicola* and whiteflies parasitoid, *Eretmocerus mundus* and/or the coccinellid predator, *Coccinella undecimpunctata* are applied separately or in combination, it is important that they may be mutually compatible or interfere under field conditions. Due to its numerous hosts, *C. uridenicola* infected various species of homopterous insect (Abdel-Bakey *et al.*, 1998; Abdel-Baky, 2000). Thus, the fungus could potentially pose a threat to beneficial insects and non-target organisms. Since, interactions among these three biological control agents in

controlling *B. argentifolii* has not yet been studied, its necessary determine its role when acting with any other beneficial insect. Combined use of *C. uridenicola* and *Er. mundus*, and both of *C. uridenicola* and *C. undecimpunctata* may be more efficient than being separately used and may result in complete mortality of the pest.

Interaction among *C. uridenicola* and *Er. mundus* was positively in favor of the insect parasitoid (Figs, 1-5). Comparatively with the previous studies, most interactions between entomopathogenic fungi and the insect parasitoid were asymmetrically in favor of the pathogen (Hochberg and Lawton, 1990). Nevertheless, the relative timing of parasitism and fungal infection is often crucial to the final competitive outcome.

In the current study, a higher percentage of adult parasitoid emerged from the treated parasitized SLWF treated by *C. uridenicola* meaning that the parasitized host individuals were less susceptible to infect by the fungus than unparasitized ones. This may be due to the changes in hosts caused by the parasitoid or its progeny after parasitism (Vinson and Iwantsch, 1980 a and b). This is generally defined as host regulation. Changes in parasitized hosts may include morphological, biochemical and physiological or physical activities within the nymphal host, as well as, host cuticle melanization (Fransen and van Lenteren, 1993 and 1994). Additionally, the authors correlated the changes in host susceptibility with the emergence of the parasitoid larvae from the eggs inside the host in case of *Encarsia Formosa* and *Aschersonia alyerodis*.

In general, Fransen and van Lenteren (1993) summarized the factors that may influence the effective colonization of parasitized greenhouse whitefly by *E. formosa* after treatment by *A. alyerodis* as follows: **1**) increasing parasitized hosts survival when treated by entomopathogenic fungi after parasitization can induce a decrease in host susceptibility for infection; **2**) penetration of parasitized larvae by the fungus may be more or less difficult than unparasitized hosts because of indirect changes in the host cuticle; **3**)competition for food between the parasitoid larva and the fungus may be present; and **4**) after successful penetration of the parasitized host, subsequent colonization by the fungus may be hampered due to the defense mechanisms. This latter interpretation was in agreement with Vinson and Iwantsch (1980 a & b). The current study also shows that *Er. mundus* females were able to discriminate between infected nymphs by *C. uridenicola,* that have a mycelium growth, and non-infected nymphs. This may explain the rejection of the parasitoid adults to use the infected SLWF nymphs as a host, as no parasitism was detected (Fransen and van Lenteren, 1993).

Successful development of the parasitoid and increasing its efficiency when combined with the fungal pathogen depend on the timing of fungal spore applications to the hosts of parasitoids. This may be useful to determine whether the insect hosts were first infected by the pathogen or were first parasitized by the parasitoid as in case of *Verticillium lecanii* infecting *Aphidius nigripes*, a parasitoid of potato aphid, *Macrosiphum euphorbiae* (Askary and Brodeur, 1999). Fransen and van Lenteren, (1994) also reported that the infection rates of *E. Formosa*, a greenhouse whitefly

parasitoid, by *A. aleyrodis* varied with timing of spore application after parasitism. They found that when fungal spore was applied on parasitized whitefly nymphs one to three days after oviposition in the WF nymphs, the parasitized nymphs were significantly reduced because of eggs laid by the parasitoid just after or at the time of spores application which may succumb by fungal infection. Meanwhile, when fungal spores were applied 4-10 days, they did not reduce parasitism in comparison with the control treatment.

Balstospores of *Paecilomyces fumosoroseus* sprayed on *Trialeurodes vaporarium* nymphs 4 days after releasing *E. formosa* did not inhibit parasitoid development (Kim *et al.*, 2005). Finally, parasitization at an early phase of fungal infection is, accordingly, detrimental to the parasitoid progeny survival. The ability of *Er. mundus*, in the current study, to discriminate between infected and non-infected SLWF nymphs at a later phase, gave the parasitoid the opportunity to successfully parasitize healthy WF nymphs, and thereby to cause host mortality complementary to the fungal treatment. This complies with the results of Rosa *et al.* (2000), who mentioned that the high virulence of *Metarhizium anisopliae* and *Beauveria bassiana* to the bethylid parasitoid *Prorops nasuta*, did not affect significantly the predatory or parasitic capacity of *P. nasuta*.

The present results also explain, for the first time, the potential impact of the entomopathogenic fungi, *C. uridenicola* on coccinellid predator, *C. undecimpunctata* when used in combination. Impact of entomopathogenic fungi on the coccinellid predatory species revealed, however, that the three species *B. bassiana*, *Paecilomyces farinosus* and *P. fumosoroseus* infected the predators during hibernation in Poland (Ceryniger and Hodak, 1996; Ceryniger, 2000). The different responses exhibited, in the current study, by the coccinellid predator, *C. undecimpunctata* to infected SLWF nymphs proved the complexities of the interactions among natural enemies in the agro-ecosystem.

C. uridenicola showed different pathogencity effects towards the predators based on spore concentrations and susceptibility of the developmental stages. Decreasing the fungal spore concentrations and indirect contact of the predator led to minimize the lethal effect of the fungus on the predator life stage (Figs, 6-9; Table 1). The 1st and 2nd instar larvae and pupae of the predator were more susceptible to the fungal treatment than other stages (Table 1). James *et al.* (1995) attributed this phenomenon to the heavy hairs on coccinellid larvae more than on its prey, which may facilitate conidial pick up. In another study, Pavlyushin, (1996) reported that *V. lecanii, B. bassiana* and *P. fumosoroseus* had an entomocidal effect on the larvae of *Chrysoperla carnea* and *Ch. sinica* as well as on *Cycloneda limbifer*. Larval mortality depended on the infection dosage. At a spore concentration of 5 and 25 million/ml, mortality of *Chrysoperla* spp. was 4% and at 100 million spores/ml the mortality reached 28%. The larvae of *C. limbifer* were more sensitive to *B. bassiana*. Similar results on the effects of *B. bassiana* and *P. fumosoroseus* against the coccinellid, *Serangium parcesetosum* Sicard, a predator of whiteflies, were observed in the laboratory (Poprawski, *et al.*, 1998). They reported that the predator had significantly lower survival when sprayed with

B. bassiana than with *P. fumosoroseus*. However, survival was not affected by the dosage rates for each pathogen.

Intra-guild predation is a dramatic expression on intervention among natural enemies, which could lead to antagonism and reduced host mortality and its dominance in the biological control agents (Polis and Holt, 1992; Rosenheim *et al.*, 1995). Generally, the previous studies on the interactions among entomopathogens and insect natural enemies consider the pathogen as the "intra-guild predator" which is directly able to infect the coccinellid predator in the guild (Goettel and Kajek; 2001). However, in case of the coccinellid predator that fed on infected and sporulating SLWF nymphs with the fungus can also be preyed upon. Accordingly, *C. undecimpunctata* adults and larvae become also as intra-guild predator of the pathogen. This is in harmony with Pell *et al.* (1997) and Roy *et al.* (1998) who found, in non-choice laboratory studies, that *C. sepetmpunctata* adults and the carabid, *Pterostichus madidus* consumed aphids at a late stage of *Erynia neoaphids* infection. Meanwhile, the 4th instar larvae of *C. sepetmpunctata* partially consumed sporulating infected aphids. Conversely, Roy *et al.* (2001) referred to the ability of Coccinilldae to vector conidia from a colony infected by *E. neoaphids* to uninfected one. Thus, these predators could help in fungal dispersal under field condition, which leads to fungal epizootic. They also reported that other common aphid predators such as larval stages of the syrphid, *Episyrphus balteatus* and the chrysopid, *C. carnea* never consumed infected aphids.

In conclusion, *C. undecimpunctata* adults and larvae may positively affect the biological control potential of *C. uridenicola*, while *Er. mundus* will negatively affect the biological control potential of this fungus (Table 2). The successful augmentation of these natural enemies may be impeded by the antagonism, however, careful management ensuring temporal separation of the interacting natural enemies could result in effective biological control (King and Bell, 1978). In addition, the use of an interaction among the biological control agents is required through understanding of the dynamic relationship between pathogens, parasitoids, predators and insect hosts. Moreover, the successful manipulation of natural enemies in IPM program is dependent upon such an understanding. Therefore, the present study indicates that the fungus *C. uridenicola* may be compatible with the action of the parasitoid or the predator under field conditions, provided that pathogen applications, and parasitoid or/and predator releasing are timed not to coincide.

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التفاعلات بين كل من الفطر الممرض للحشرات *Cladosporium uridenicolla* واثنين من الأعداء الحيوية للذباب الأبيض، الطفيل الداخلى *Eretmocerus mundus* ومفترس أبو العيد *Coccienlla undecimpunctata* : من منظور المكافحة المتكاملة للآفات.

نجدى فاروق عبد الباقى
قسم الحشرات الإقتصادية – كلية الزراعة – جامعة المنصورة

أجريت دراسات معملية لتقييم التفاعل التنافسى بين الممرض الفطرى *Cladosporium uridenicolla* واثنين من الأعداء الحيوية للذباب الأبيض هما، طفيل *Eretmocerus mundus* و أبو العيد ١١ نقطة *Coccinella undecimpunctata*. تعتبر هذه الدراسة الأولى من نوعها لدراسة إمكانية

ا لتكامل بين هذه العناصر البيولوجية الثلاثة المستخدمة فى مجال المكافحة الحيوية للذباب الأبيض. وتهدف الدراسة إلى لتقييم التأثير السلبى والإيجابى للتفاعل بين الفطر وكل من الطفيل والمفترس.

وتشير النتائج إلى أنه عند إستخدام الفطر والطفيل معا، كان التأثير السلبى للتفاعل عديم القيمة. ففى حالة التعريض غير المباشر للطفيل (عند معاملة الحوريات المتطفل عليها) لم تسجل حالات موت بين أفراد الطفيليات الخارجة من طور العذراء، بل على العكس كانت نسبة خروج الحشرات الكاملة للطفيل عالية جدا مقارنة بالكنترول. أما فى حالة تعريض الطفيل مباشرة للفطر(معاملة الطفيل والحوريات معا) فإن نسبة الموت لم تتعدى ٥إلى٧%. وفى حالة معاملة حوريات الذباب الأبيض بالفطر وتركها لمدة ٣ إلى ٥ أيام، فإن الحشرات الكاملة للطفيل رفضت تلك الحوريات واستخدامها كعائل. وأيضا لم تسجل حالات وضع بيض للطفيل على الحوريات التى نما عليها الفطر، وفى بعض الحالات القليلة جدا وضع بيض بمعدل منخفض جدا، الأمر الذى لم يسجل معه أى حالات للتطفل.

و بالنسبة لمفترس أبو العيد، فقد كان حساسا جدا للمعاملة بالفطر مقارنة بالطفيل، واختلفت هذه الحساسية باختلاف أطواره المختلفة. حيث كان بيض المفترس أكثر مقاومة للإصابة بالفطر و بلغت النسبة المئوية للفقس ٩٨,٥، ٩٤,٥، ٩٢و ٨٥% مع الكنترول والتركيزات المنخفضة، المتوسطة والمرتفعة، على التوالى. أما الأطوار اليرقية، فكان العمر الأول أكثر حساسية للإصابة بالفطر، يلية العمر اليرقى الثانى ثم طور العذراء. بينما كان العمر اليرقى الثالث والرابع وطور الحشرة الكاملة أكثر تحملا للإصابة بالفطر عن باقى أطوار المفترس.

وقد اكتشفت ظاهرة الافتراس بين أطوار المفترس والفطر، حيث تغذت أطوار المفترس المختلفة فماعدا العذراء على حوريات الذباب الأبيض المصاب بالفطر. وفى حالة إصابة الحوريات بالفطر مع عدم ظهور أعراض المرض، بلغت نسبة التغذية ٤٥,٥، ٥٦,٥، ٤٢,٥، ٣٨,٦٥و ٤٠,١٥% للأعمار اليرقية الأولى، الثانية، الثالثة، الرابعة وطور الحشرة الكاملة على التوالى، مقارنة بالكنترول (التغذية على حوريات غير مصابة بالفطر). أما الحوريات التى ظهرعليها ميسليوم الفطر، فإن تغذية أطوار المفترس إنخفضت إلى ١٠,٢، ١٤,٥، ١٨، ٢٥,٤، و ٣٠,٥% للأعمار اليرقية الأولى، الثانية، الثالثة، الرابعة وطور الحشرة الكاملة على التوالى. ولهذا فإن الفطر يعتبر كمفترس حيث يصيب أطوار حشرة أبو العيد ١١نقطة مباشرة مما أدى إلى موت أفرادها، وعليه أيضا تعتبر حشرة أبو العيد مفترسة لتغذيتها على حوريات الذباب الأبيض المصابة بالفطر. وفى كلتا الحالتين فإن هذه الظاهرة تقلل من فاعلية المكافحة البيولوجية عند استخدام الفطر والمفترس معا.

أما فاعلية المكافحة عند استخدام العناصر الثلاثة فى مكافحة الذباب الأبيض، سواء كل عنصر على حده أو مندمجين معا، فيتوقف على طبيعية عنصر المكافحة البيولوجية المستخدم. فعند إستخدام الطفيل منفردا ساعد فى خفض تعداد الحشرة بنسبة ٣٨,١%، أما إستخدام الفطر منفردا فقد أدى إلى موت ١٩,٤% من تعداد الحشرة. أما تفاعل الطفيل مع الفطر(تطبيقيهما معا) أدى إلى زيادة فاعلية المكافحة البيولوجية للحشرة بنسبة ٥٧,٨%. على العكس، أستخدام الطفيل مع المفترس، لأن نسبة موت الحشرة لم تتعد ٣٨,٧%. فى حالة إستخدام العناصر الثلاثة معا، فإن نسبة الخفض فى عشيرة الحشرة لم تتعد ٥٨,٠%.

وعليه، فإن إستخدام الفطر والطفيل نتج عنه علاقة تعاونية مثمرة (زيادة فاعلية المكافحة). وبالتالى فإن تطبيق كل من الفطر والطفيل فى برامج مكافحة الحشرة فإنه من المحتمل تحسن فاعلية المكافحة لحوريات الذباب الأبيض. على العكس، فإن إستخدام الفطر والمفترس نتج عنه علاقة تضاد (لم يساعد على زيادة فاعلية المكافحة). وبناء علية، فإن الإستخدام المشترك للفطر والطفيل و المفترس ربما يستحق دراسة أخرى تحت ظروف نصف حقلية أو حقلية. وبالتالى فإن هذه الدراسة تقترح إمكانية إستخدام الفطر والطفيل معا، أو الفطر والمفترس معا فى برامج المكافحة المتكاملة للآفات للذباب الأبيض الأمر الذى يؤدى خيارات للمكافحة قابلة للتطبيق، بشرط رش جراثيم الفطر فى مواعيد محددة تتزامن مع الأطوار الأخيرة من حياة الطفيل، أو مع الأطوار الأقل حساسية من حياة المفترس وذلك لحفظ وصيانة هذه الأعداء الحيوية داخل النظام البيئى الزراعى.