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Combining Effect of *Beauveria bassiana* (Bals.) and *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) on Sweetpotato Whitefly, *Bemisia tabaci* Gennadius (Aleyrodidae; Hemiptera)

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Abstract: Combined effects between the entomopathogenic fungi, *Beauveria bassiana* (Bals.) and whitefly parasitoid, *Eretmocerus mundus* Mercet on *Bemisia tabaci* (Genn.) were investigated under laboratory conditions. The competitive interactions among them were also evaluated either alone or in combination, in respect of the positive and negative effects. The deleterious effects on the parasitoid were extremely low, particularly when the parasitized nymphs exposed to the fungus later. In direct contact bioassay, fungus caused 5.1-15.3% mortality in post-releasing trial and from 8.9-22.1% in pre-releasing trial. Three to five days after treatment, *B. tabaci* nymphs were rejected as a host by *E. mundus* females due to the fungal infection. In infected nymphs, the majority of *E. mundus* females were not laid and no parasitism was detected. The control efficiency of the two natural enemies of *B. tabaci*, when used separately or in combination, varied according to the tested biological agent. *E. mundus* alone reduced pest populations by 19.4 and 51.1% in pre- and post-releasing trials, respectively. *B. bassiana* caused 38.1% in pre-releasing trial and 29.4% in post-releasing trial. Meanwhile, the interaction between fungus and the parasitoid in combination reduced the pest population by 51.2 and 72.3% in pre- and post-releasing trials, respectively.

Key words: Biocontrol agent, entomopathogenic fungi, *Bemisia tabaci*, whitefly natural enemies, interactions

INTRODUCTION

The protected environment in controlling whiteflies appears to be an ideal for successful application of biological control agent. This can be gained by combining the effect of pest natural enemies to enhance their efficiency and hence, to provide environmentally safer methods than using chemicals (Ehler, 2000). In this respect, Cory and Hoover (2006) showed that both entomopathogenic fungi and insect parasitoids and/or predators of arthropod natural enemies can contribute in suppressing pest populations when used jointly or independently. To encourage the role of whitefly natural enemies in pest control, they should be tested through interaction with other natural enemies in the agro-ecosystem.

Role of this fungi as virulence agent in suppressing whitefly populations was studied by Al-Deghairi (2008). The suitability of *B. bassiana*, as part of whitefly control measures in IPM programs, is critically important. Therefore, the fungus, *B. bassiana* and the whitefly parasitoid, *E. mundus*, have the potential to complement or interfere with each others, based on the environmental conditions and other biological factors (Kim *et al.*, 2005). This entomopathogenic fungi should be compatible with other naturally occurring biological control agents, such as, the hymenopteran

parasitoids, *Eretmocerus mundus*, in order to maximize the efficiency of the biological pest control. However, most experiments have focused on the effect of a particular biological agent and availability of data on susceptibility of parasitoids to the entomopathogenic fungi, *B. bassiana*, in particular, are insufficient. In addition, there is no information available regarding the non-target effects of *B. bassiana* on this parasitoid.

Since, new biotypes of *Bemisia tabaci* 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. Moreover, 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 in IPM programs (Roy and Pell, 2000). Thus, in order to use insect natural enemies more effectively in IPM program, 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). Obviously, Roy and Pell (2000) concluded that the synergistic interactions among natural enemies 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. This study deals with laboratory trials to investigate the susceptibility of interaction between *E. mundus* and *B. bassiana*, the widely used natural enemies for whitefly control. Thus the goal of this study focuses on the interactions between the entomopathogenic fungi, *B. bassiana* and the internal whitefly parasitoid, *E. mundus*. The capability of *B. bassiana* and/or *E. mundus* alone and in combination in controlling *B. tabaci* populations is also determined.

MATERIALS AND METHODS

Maintenance of Experimental Organisms

The sweetpotato whitefly was reared on the kidney bean, *Phaseolus vulgaris* L., under laboratory conditions $25\pm 2.2^{\circ}\text{C}$ and $70\pm 5\%$ R.H., College of Agriculture and Veterinary Medicine, Qassim University, KSA. The pest culture was originally initiated from individuals collected from squash plants, free of insecticides, in Qassim region. About hundred plastic pots cultivated with *P. vulgaris* were maintained until formation of two fully expanded leaves. Ten pairs of *B. tabaci* adults were introduced to the kidney bean leaves, confined under screen cages for 48 h in order to lay their eggs and then removed. The eggs were counted on each leaf and left till hatching and transformed to the 2nd instar nymphs.

The parasitoid was collected from different plant hosts free of insecticides and its colony was initiated on *P. vulgaris* plants infested by whitefly nymphs and kept under laboratory conditions at $25\pm 2.2^{\circ}\text{C}$; $70\pm 5\%$ R.H. under 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 whitefly nymphs. The fungus was isolated from naturally infected whiteflies according to Abdel-Baky (2000) and Al-Deghairi (2008) and kept in slant Agar media at 5°C . The fungal spores were harvested from two weeks old cultures on autoclaved PDA media at $28\pm 2^{\circ}\text{C}$ by rinsing with sterilized distilled water.

Combined Effects of *B. bassiana* and *E. mundus* on *B. tabaci*

In order to assess the combining effect between the entomopathogenic fungus, *B. bassiana* and *E. mundus*, two trials were designed, included: whitefly nymphs treated with fungal suspension pre-releasing the parasitoid and whitefly nymphs treated with fungal suspension post-releasing the parasitoid. In each trial, thirty kidney bean pots infested by constant numbers of whitefly nymphs

(40 nymphs leaf⁻¹) were used. Three fungal concentrations were applied (Al-Deghairi, 2008) to assess the positive and negative impact on the parasitoid development and parasitism percentage. The trials were performed as follows:

Pre-Releasing Trial

Sixty kidney bean pots infested by whitefly nymphs were used in this trial, divided to two groups and treated with three fungal concentrations of 2×10^6 , 4×10^6 and 6×10^6 spores mL⁻¹. Twenty pots were used to each fungal concentration. A ratio of 1 parasitoid: 5 whitefly nymphs was released at two intervals. One group of the parasitoid were released immediately after treated by the fungal concentration, the other were released three days after the treatment. The parasitoid oviposition process behavior, symptoms of parasitism and parasitoid mortality by the fungus were studied carefully. Emergence of parasitoid adults was also recorded.

Post-Releasing Trial

Sixty plastic pots planted with kidney beans were also used in this trial. A constant number of the parasitoid (1 parasitoid: 5 WF nymphs) was introduced to kidney beans plants infested with whitefly 2nd instar under screen cages. The parasitoids were left for 24 h to oviposit their eggs and then removed. The pots were also divided to two groups; one group was treated, three days later, by fungal suspension at concentrations of 2×10^6 , 4×10^6 and 6×10^6 spores mL⁻¹. The other group, was treated by the fungal suspensions after five days from removing the parasitoid adults. Two days later after introducing the fungus, kidney bean leaves infested by WF nymphs were investigated by hand lens and examination was continued until parasitoid adult emergence. The mortalities of WF nymphs and emerging parasitoid adults by the fungus were observed under each fungal concentration. The obtained data were compared with the check treatment (treated by distilled water only).

Statistical Analysis

The mortalities number and values in all tests were subjected to ANOVA analysis. All statistical analysis were performed using CoStat Software program (1990). The percentages of mortality due to fungal activities were calculated according to Abbott (1925). The interaction between the two 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.

RESULTS

***Beauveria bassiana* on *Bemisia tabaci* Nymphs**

Beauveria bassiana caused different mortality rates among nymphs of *B. tabaci*. These mortality percentages were significantly different ($p < 0.05$) based on the fungal concentrations (Fig. 1). Infection levels were generally greater when higher spore concentrations of the fungus was used. All tested conidial concentrations were pathogenic and highly virulent among *B. tabaci* nymphs (Fig. 1). The average mortality percentages among *B. tabaci* nymphs were 18.75, 24.6 and 46.5 with 2×10^6 , 4×10^6 and 6×10^6 , respectively. This means that mortality among *B. tabaci* nymphs increased with the increase of the fungal concentrations and thus, sufficient control could be achieved when higher conidial concentrations were applied. The statistical analysis also revealed that nymphal mortality varied significantly among fungal concentrations and insect life stages (Fig. 1).

Eretmocerus mundus* on *Bemisia tabaci

Releasing *E. mundus* against varied pest densities affected the control levels of *B. tabaci* (Fig. 5). Desirable control levels were achieved when higher number of parasitoids are released specially when

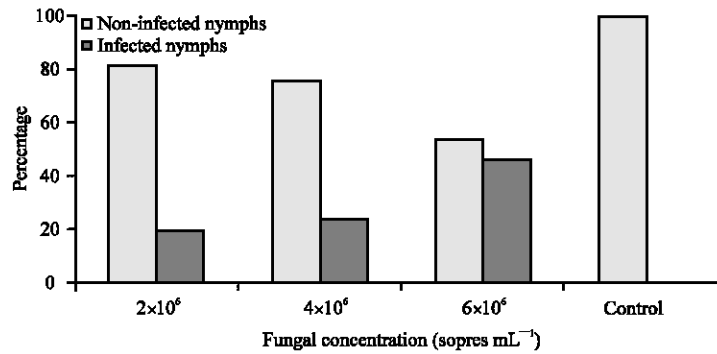


Fig. 1: Efficiency of *Beauveria bassiana* against *Bemisia tabaci* nymphs

Table 1: Summarization of the interacting between *Beauveria bassiana* and *Eretmocerus mundus* in controlling *Bemisia tabaci*

Statistical variables	Adult Whitefly emergence		Nymphs mortality by fungus only		Parasitized nymphs with fungal growth	
	Pre-releasing	Post-releasing	Pre-releasing	Post-releasing	Pre-releasing	Post-releasing
Mean±SE	57.2±2.4a	39.1±2.2b	91.80±4.5a	58.9±3.1b	26.9 ±1.7a	14.6±1.2b
df	28		28		28	
F (calculated)	119.81		122.05		44.09	
LSD at (0.05%)	3.38		6.12		3.81	
Statistical variables	Parasitized nymphs without fungal growth		Parasitoid emergence			
	Pre-releasing	Post-releasing	Pre-releasing	Post-releasing		
Mean±SE	87.4±3.9a	24.7±1.3b	83.4±2.6a	18.07±1.1b		
df	28		28			
F (calculated)	400.63		460.5			
LSD at (0.05%)	6.48		6.23			

*The numbers followed by the same letter(s) within a widest column are not significantly different at 5% level

low number of the pest is present. About 43.57% mortalities among *B. tabaci* population were achieved when releasing the parasitoids before the fungal treatments, while mortality reached 12.08% among whitefly nymphs that were treated by the fungus before introducing the parasitoids (Fig. 5).

Combined Effect of *B. bassiana* and *E. mundus* on *B. tabaci* Nymphs

Table 1 show the potential combining effect of two natural enemies of sweetpotato whitefly, *B. tabaci*, assessing five factors in two trials. The first factor was the emerged number of whitefly adults, which was high in pre-releasing trial compared with post-releasing trial. At least 57.2±2.4 WF adults, of 200 individuals, were emerged in pre-releasing trial compared to 39.1±2.2 adults in post-releasing trial (Table 1). The 2nd factor was the number of infected WF nymphs by *B. bassiana* alone, which gave 91.80±4.5 and 58.9±1.7 nymphs of 200 individuals, for pre- and post-releasing trials, respectively. The 3rd and 4th factors were related to the effect of *E. mundus*, which gave 87.4±3.9 and 24.7±1.3 of parasitized WF nymphs in pre- and post- releasing trials, respectively. Consequently, the number of emerged parasitoid was 83.4±2.6 and 18.07±1.1 in pre-releasing and post-releasing trial, respectively (Table 1). The last factor was dealing with the negative interaction between the two biological agent. This factor assessed weather the fungus affected the parasitization or the population of the parasitoid. In pre-releasing trial, the fungus destroyed 26.9±1.7 parasitized WF nymphs and destroyed 14.6±1.2 nymphs in post-releasing trial (Table 1).

**Negative Impact of the Interaction Among the Two Biological Control Agents
Indirect Contact**

Beauveria bassiana could destroy the parasitoid in its embryonic phase as it penetrates the pest cuticle either mechanically or biologically, therefore, the fungus could affect the emerged numbers of the parasitoid adults. In this respect, Fig. 2 shows the percentages of emerged and non-emerged parasitoids adults under three fungal concentrations at two time intervals of treatments. In pre-releasing trial, the percentages of non-emerged parasitoid adults were 1.3, 4.9 and 8.6%, with 13.57% for check treatment, with concentrations set at 2×10^6 , 4×10^6 and 6×10^6 spores mL^{-1} , respectively. Meanwhile, in post-releasing trial, the low spore concentrations 2×10^6 (spores mL^{-1}) caused 17.5% mortalities of the parasitoid in the embryonic phase, followed by moderate concentrations 4×10^6 (spores mL^{-1}) which caused 28.86%. On the other hand, higher fungal concentrations 6×10^6 (spores mL^{-1}) resulted to 34.89% mortalities in parasitoid at the embryonic developmental phase. Parasitoids mortality in the embryonic developmental phase was differed significantly based on the time of fungal treatments and fungal concentrations (Fig. 2).

Direct Contact

Parasitoids may succeed in avoiding fungal infection during the embryonic phase, however, direct contact with the fungus which covers the body of whitefly nymphs during its emergence from the pupal case or during its search for non-infected hosts may contribute to its mortality. Mortality percentages in *E. mundus* populations in pre-releasing trial were 8.9, 14.8 and 22.1% for the three fungal concentrations used (2×10^6 , 4×10^6 and 6×10^6 spores mL^{-1}), respectively (Fig. 3). In the post-releasing trial, mortality percentages were 5.1, 9.2 and 15.3% for the three fungal concentrations used (2×10^6 , 4×10^6 and 6×10^6 spores mL^{-1}).

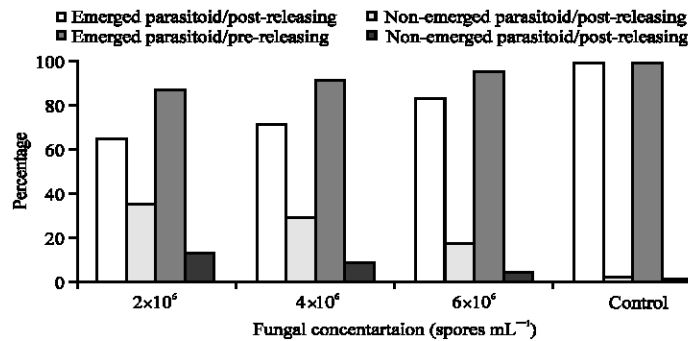


Fig. 2: Negative impact of *Beauveria bassiana* on the whitefly parasitoid, *Eretmocerus mundus* under three fungal spore concentrations

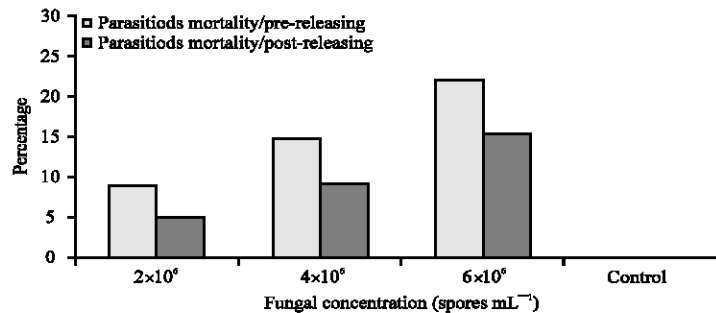


Fig. 3: Mortality percentages among *Eretmocerus mundus* population treated by *Beauveria bassiana*

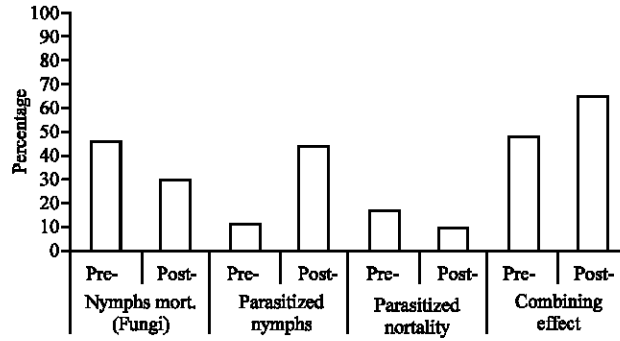


Fig. 4: Percentages of the fungal growth and the oviposited eggs/female parasitoid in pre- and post releasing treatments

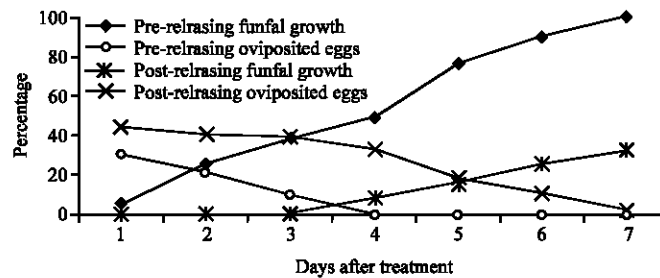


Fig. 5: An Average impact of the interactions among *Beauverava bassina* and *Eretmocerus mundus* in controlling *Bemisia tabaci*

In conclusion, the negative effects of *B. bassaina* on *E. mundus* population were very limited compared to chemical insecticides, if used in the appropriate time. Indirect mortalities among the parasitoids in pre-releasing trial were very low and ranged from 4.9 to 13.57% when parasitized WF nymphs were treated by the fungus (Table 1). Meanwhile, when the parasitoid adults were treated by the fungal suspension (direct contact), they rejected the infected WF nymphs as a host. The majority of female parasitoids laid no eggs and in few cases showed a non-preference to infected nymphs (Fig. 4).

Positive Interaction Effect Between the Two Biological Control Agents

The entomopathogenic fungi, *B. bassaina* and the internal whitefly parasitoid, *E. mundus*, alone and together, were able to suppress *B. tabaci* population. In this respect, the regression analysis showed that sharing rates of each one of the two natural enemy when used alone or together in controlling the pest under laboratory conditions (Table 2, Fig. 5).

E. mundus alone decreased the pest populations by 19.4% in pre-releasing trial and 51.1% in the post-releasing trial (Table 2). Meanwhile, *B. bassaina* was able to reduce the pest population by 38.1 and 29.4% in both pre- and -post releasing trials, respectively. On the contrary, the combined effect of *B. bassaina* and *E. mundus* raised the control efficiency to 51.2% in pre-releasing treatment and 72.3% in the post-releasing trial. This means that both biological control agents of whitefly interact positively and enhanced the pest control efficiency, if applied in correct way particularly after releasing the parasitoid after four to five days (Fig. 4, 5).

Table 2: Regression analysis of the interactions among *Beauveria bassaina* and *Eretmocerus mundus* in controlling *Bemisia tabaci* either applied separately or in combinations

Efficiency of two biological control agents	Pre-releasing the parasitoids				Post-releasing the parasitoids			
	Regression analysis		<i>B. tabaci</i> Bio-control agents		Regression analysis		<i>B. tabaci</i> Bio-control agents	
	R ² (%)	Adjusted (R ² %)	<i>B. bassaina</i>	<i>E. mundus</i>	R ² (%)	Adjusted (R ² %)	<i>B. bassaina</i>	<i>E. mundus</i>
Control efficiency (%)								
<i>E. mundus</i>	19.4	15.0		X	51.1	43.1		X
<i>B. bassaina</i>	38.1	34.7	X		29.4	24.0	X	
Two agents combined	51.2	43.10	X	X	72.3	64.7	X	X
Regression equations								
<i>B. bassaina</i>	Y = 11.5 + 0.257 X				Y = 14.45 + 0.561 X			
<i>E. mundus</i>	Y = 11.5 + 0.257 X				Y = 9.65 + 0.297 X			
Interaction between the two biological agent	Y = 11.45 + 0.443 <i>B. bassaina</i> + 0.259 <i>E. mundus</i> where, Y is control efficiency				Y = 15.76 + 0.623 <i>B. bassaina</i> + 0.852 <i>E. mundus</i> where, Y is control efficiency			

Accepted and Rejected Whitefly Nymphs Treated by *Beauveria bassaina* as a Host by *Eretmocerus mundus* Females

Figure 4 shows percentages of fungal growth and number of oviposited eggs/female parasitoids when *B. tabaci* nymphs treated by the fungus before or after releasing the parasitoids. In pre-releasing trials, fungal growth was very low one day after treatment, giving only 5%. Meanwhile, the growth of the fungus was accelerated in the 2nd day giving a 25%, then increased sharply which reach its completed development within the 6th day of treatment. Thus, the fungal mycelium covered the cadavers of whitefly nymphs destroying them and making them un-healthy. Consequently, female parasitoids rejected the nymphs and never oviposit their eggs except in the first two days before the fungal mycelium covered the nymphal body (Fig. 4).

No fungal growth was observed within 1st three days after releasing the parasitoids. However, the development of the fungal growth was very low in comparison with the pre- releasing treatment. Although, whitefly nymphs were accepted as a host by parasitoids' female giving very high oviposition percentages ranged from 44 to 32% after four days from treatment, the female parasitoids rejected the hosts and number of oviposited eggs decreased sharply towards the end of the trial when the fungus was at its maximum growth (Fig. 4).

DISCUSSION

Entomopathogenic fungi may interact with other insect natural enemies either as individual species or as species complexes (Roy and Pell, 2000). Consequently, a competition may occur as interactions between closely related species or among unrelated organisms (Hochberg and Lawton, 1990). Increasing demands for entomopathogenic fungi and parasitoids in biological control programs, made it important to evaluate their interactions to maximize their efficiency in IPM programs (Pell and Roy, 2000; Lacey and Mesquita, 2002). Fungal pathogens have impact on the beneficial insects or non-target organisms either through direct and/or indirect infection of these organisms (Ehler, 2000). 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 where a pathogen may interfere with the natural enemy complex by reducing the pest population or rendering the host pest unsuitable for other natural enemies. This completely in agreement with the current studies (Fig. 2, 3). *B. bassaina* caused direct and indirect mortalities among *E. mundus* populations and mortalities were differed based on the fungal concentrations.

When *Beauveria bassiana* and *Eretmocerus mundus* are applied separately or in together, it is important that they may be mutually compatible or interfere under field conditions. Due to its numerous hosts, *B. bassiana* infects various insect species (Al-Deghairi, 2008), thus, the fungus could potentially pose a threat to other beneficial insects and non-target organisms. Combined use of *B. bassiana* and *E. mundus* may be more efficient than being used separately resulting in complete mortality of the pest.

Interaction among *B. bassiana* and *E. mundus* was positively in favor of the insect pest control. Comparatively with the earlier 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 emerged parasitoid adults were recorded in post-releasing treatment meaning that the parasitized host individuals were less susceptible to infection by the fungus than unparasitized ones. This may be due to the changes in the host caused by the parasitoid or its progeny after parasitism (Vinson and Iwantsch, 1980). This is generally defined as host regulation. Changes in parasitized hosts may include morphological, biochemical and physiological or physical activities within the nymphal stage, as well as, host cuticle melanization (Fransen and Van Lenteren, 1993, 1994). Additionally, the researchers correlated the changes in host susceptibility with the emergence of the parasitoid larvae from the eggs inside the host as happen in *Encarsia formosa* and *Aschersonia alyerodis*.

In general, Fransen and Van Lenteren (1993) shown the factors that may influence the effective colonization of parasitized greenhouse whitefly by *E. formosa* after treatment by *A. alyerodis* as follows: increasing parasitized hosts survival in post-releasing trials can induce a decrease in host susceptibility for infection, fungal penetration of the parasitized larvae may be more difficult than unparasitized hosts because of indirect changes in the host cuticle competition between unrelated organisms may be present and after successful penetration of the parasitized host, the fungus may be hampered due to the defense mechanisms. This latter interpretation was in agreement with Vinson (1976) and Vinson and Iwantsch (1980). The current study also shows that *E. mundus* females were able to discriminate between infected nymphs by *B. bassiana*, that have a mycelium growth and non-infected nymphs. This may explain the rejection of the parasitoid adults to use the infected WF nymphs as a host.

Successful development of the parasitoid and increasing its efficiency when combined with the fungal pathogen depends on the timing of fungal spore applications to the hosts of the 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. alyerodis* varied with the timing of spore application after parasitism. They found that when fungal spores were applied on parasitized whitefly nymphs one to three days after oviposition, the parasitized nymphs were significantly reduced as a result of cumulative infection by fungus and then by parasitism. However, when fungal spores were applied 4-10 days after introducing parasitoids, parasitism wasn't reduced compared with the control. Finally, parasitization at an early phase of fungal infection is, accordingly, detrimental to the parasitoid progeny survival. In this study, *E. mundus* adults were able to discriminate between infected and non-infected WF 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*.

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 earlier studies on the interactions among entomopathogens and insect natural enemies consider the pathogen as the intra-guild predator which may directly be able to infect the other natural enemies in the guild (Ehler, 2000).

In conclusion, *E. mundus* negatively affected the biological control potentiality of this fungus (Table 1). 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 biological control agents is required through understanding of the dynamic relationship between pathogens, parasitoids and host insects. Moreover, the successful manipulation of natural enemies in IPM program is dependent upon such an understanding. Therefore, the present study indicates that the fungus *B. bassaina* may be compatible with the action of the parasitoid or the predator under field conditions, giving that pathogen applications and parasitoid or/and predator releasing are timed not to coincide.

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