

## ***Lecanicillium muscarium* as microbial insecticide against whitefly and its interaction with other natural enemies**

S.X. Ren\*, S. Ali, Z. Huang and J.H. Wu

Engineering Research Center of Biological Control, Ministry of Education, College of Natural Resource and Environment, South China Agricultural University, Wushan Road, Guangzhou City, 510642, P.R. China.

\* Corresponding author: email: [renxscn@yahoo.com.cn](mailto:renxscn@yahoo.com.cn)

*Bemisia tabaci* (Gennadius) has become a serious world-wide pest since the early 80s. Since it first recorded in china during 1949, the serious outbreaks of the pest in china during 1990s is thought to have been originated from the invasion of the new Bbiotype on ornamentals crops. Each year, farmers worldwide spend more than \$1 billion to control this pest, primarily by using insecticides. As a result, natural enemies are being sacrificed and many populations of whitefly have become resistant to conventional insecticides. Alternative control measures being investigated for whitefly include the use of different biological control agents (entomopathogenic fungi, predators parasitoids). Isolates of *Metarhizium anisopliae*, *Isaria fumosorosea*, and *Beauveria bassiana* (Balsamo) Vuillemin can infect whitefly under screen house or field conditions. Many species of predatory beetles and parasitoids have been used extensively as a model system in studies of population and behavioral ecology, partially due to their potential use in biological pest control. The biological control agents may act synergistically, additively or antagonistically. Synergetic interactions between pathogens and insect natural enemies can enhance control efficacy, whereas antagonistic interactions would reduce total control efficacy. Previous experience with whitefly demonstrates that integration of biocontrol agents may substantially contribute to sustainable management of damage caused by *B.tabaci* in both greenhouse and field cropping environments. Although many reports are available on short term detrimental effects of entomopathogenic fungi on different natural enemies, little is known about the indirect effects. The main objectives of this work are to review the pathogenic ability of *Lecanicillium muscarium* against whitefly and their impacts on the different life history parameters of other natural survival in relation to determining the role of natural mortality factors, including natural enemies and their efficient ratio on whitefly population dynamics.

**Keywords** Microbial control; compatibility; whitefly; agricultural microbiology; biological control

### **1. Background**

Whitefly (*Bemisia tabaci*) is one of the important crop pests attacking a wide range of crops and causing considerable losses either by direct feeding on crops or as a vector of various viral diseases. Whitefly is nearly cosmopolitan as a pest of greenhouse plants and occurs out door in warmer climates. *Bemisia tabaci* has a widespread distribution throughout the tropical regions of all continents. *Traileurodes abutiloneus* seems to be restricted to North America occurring in Mexico and in most states of the United States [1]. In southeastern United States, *B. tabaci* biotype B was first detected in 1986 from Poinsettia, but soon became a major pest of vegetables in south and central parts of Florida. Tomato was the crop most impacted, first from irregular ripening induced by nymphal feeding [2]. Since it was first recorded from china, several local outbreaks of *B. tabaci* were recorded in 1953 in Taiwan and in 1972 in Yunnan, and at present *B. tabaci* has spread from the southern to northern parts of china [3]. *B. tabaci* was considered as a non-severe pest until mid 1990s when sporadic outbreak occurred affecting a wide range of host plants [4]. The outbreak of the pest in china during 1990s was thought to be originated from the invasion of the new B biotype from ornamentals crops [5]. This B biotype can attack 176 plant species and is considered as a severe pest of vegetables, field crops ornamental plant and fruits [4].

Whitefly management includes the four cornerstones of integrated pest management resistance, biological control, chemical control and cultural practices. Extensive use of insecticides has led to the development of resistance in whiteflies [6], and the negative impact of pesticides on natural enemies of the whitefly have forced researchers to develop alternative means of control [7,8]. Microbial control is another approach being used these days for biological control of insect pests. The use of pathogens in biological control can be integrated with other natural enemies and the immediate use of a microbial control agent can protect the crop when parasitoids and predators are unable to maintain the pest population below the damage threshold. Entomopathogenic fungi are widely distributed throughout the fungal kingdom. Some insect-pathogenic fungi have restricted host ranges, while others have a wide host range, with individual isolates being more specific [9]. Entomopathogenic fungi of the genus *Aschersonia* are specific for whitefly and scale insects [10]. *Beauveria bassiana* and *paecilomyces fumosoroseus* have also shown strong potential for microbial control of nymphal whiteflies infesting cucurbit crops [11, 12, 13]. *Lecanicillium muscarium* Zare and Gams (previously known as *verticillium lecanii*) is a well-known pathogen of arthropods. The host range of this species is quite broad and includes homopteran insects as well as other arthropod orders. Wang *et al* [14] studied virulence of six strains of *L. muscarium* against sweet potato whitefly. Their results indicated that strain V16063, V3450 and Vp28 were virulent against *B. tabaci* having  $LC_{50}$  values of  $2.57 \times 10^5$ ,  $6.03 \times 10^5$  and  $6.05 \times 10^5$  conidia/ml respectively.

The development of a biocontrol system could play a crucial role in the integrated approach of *B. tabaci* management. Experiences with other species of whiteflies and evaluation of their population dynamics strongly suggested that biological control might reduce damage caused by *B. tabaci* in both greenhouse and field crops [15]. Suppression of whitefly populations can be achieved by exploiting the characteristics of each biological control agent when two or more agents are applied concurrently. However, experimental evidence indicates that biological control can be reduced or even disrupted by interactions between natural enemies. The development of effective biological control strategies in which parasitoids, predators and pathogens are integrated, requires knowledge of their interactions. The aim of the work presented in this review is to evaluate different biocontrol agents for their ability to integrate. This study investigates the outcome of interactions among three natural enemies of *B. tabaci* including fungi: *L. muscarium*, predator: *S. japonicum* and parasitoid: *Eretmocerus* sp.

## 2. Pathogenicity of *L. muscarium* against *Bemisia tabaci*

*L. muscarium* is a very common fungal pathogen attacking different insect species. It was capable of infecting a wide range of insect hosts from board geographical and climatic locations. Like all microorganisms, entomopathogenic fungi have specific biological characteristics that influence their activity in the environment [16]. To select fungal pathogen for controlling whiteflies it is necessary to study simple basic characteristics that are required to kill the target insects in both field and greenhouse conditions. Mor *et al* [17] compared 35 strains of *V. lecanii* from different hosts of geographical location against *B. tabaci* and found that the virulence on larvae of *B. tabaci* within these isolates ranged from 0 to 83%. Further study of these strains of *V. lecanii* against *B. argentifolii* (biotype B of *B. tabaci*) classified the 35 *V. lecanii* strains for degree of the virulence. Pathogenicity of *L. muscarium* involves adhesion of spores to the insect cuticle, germination, penetration and internal colonization culminating in host death [18]. In order to develop a laboratory bioassay system and to find out the effective conidial concentration for efficient assessment of fungal biocontrol agent against *B. tabaci*, four strains of *L. muscarium* derived from same geographical location and originated from the same host pest (*Trialeurodes* sp) were compared for their pathogenicity against third instar of *B. tabaci* to search for highly ovicidal isolate for further study for microbial control of whitefly pests.

The mortality caused by different isolates of *L. muscarium* on the third instar is presented in Table-1. *B. tabaci* mortality was significantly different between the different isolates varying from 87% to 56.19%, however the four strains were pathogenic against third instar at different concentrations. The isolate V20 was more virulent than other strains, whereas V26 isolate displayed similar level of virulence but statistically differed from V20. The lowest mortality was observed for V17 and V07. These results revealed that strains of *L. muscarium* derived from the same pest and same geographical location have different virulence against third instar of *B. tabaci*.

**Table 1** Cumulative mortality of *B. tabaci* caused by different concentrations of *Lecanicillium muscarium* isolates after 8 days.

Isolates	<i>L. muscarium</i> concentrations (conidia/ml)				
	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>6</sup>	10 <sup>7</sup>	10 <sup>8</sup>
V20	60.66±5.11 cA	62.63b±1.15cA	64.64±3.73 b cA	72.00±9.23 abA	87±1.73 aA
V26	42.00±1.15 bA	49.92±1.44 bA	51.34±9.11 bAB	56.54±11.75abA	78.57±6.96 aAB
V07	28.28±3.72 bB	34.59±7.24 bB	38.79±11.36 bB	51.93±9.40 abA	74.30±1.66 aB
V17	23.84±1.48 cB	32.11B±3.38bC	35.15±1.81abcB	42.06±12.01abA	56.19±1.04 aC

\*Mean in the same row with same small letters are not significantly different from each other (DMRT, P<0.05). Mean in the same column with same capital letters are not significantly different from each other (DMRT, P<0.05)

Modified from Fatiha et al[19]

The insect mortality started to increase 6 days after the inoculation and cumulative mortality was highest after 8 days. The LC<sub>50</sub> values were 1.07x10<sup>6</sup>, 1.19 x10<sup>7</sup>, 1.30x10<sup>8</sup> and 5.08 x10<sup>8</sup> for V20, V26, V07and V17 respectively (Table 2). LC<sub>90</sub> Values for V20 and V17 were 3.67 x10<sup>9</sup> and 7.69x10<sup>10</sup> conidial/ml respectively where as LC<sub>90</sub> value for V26 was 1.41x10<sup>10</sup> conidial/ml.

**Table 2** LC<sub>50</sub> and LC<sub>90</sub> (conidia/ml) values for different *Lecanicillium muscarium* isolates

Isolates	LC <sub>50</sub>	LC <sub>90</sub>	Slope ± SE
V20	1.07×10 <sup>6</sup> (1.75×10 <sup>6</sup> -6.38×10 <sup>5</sup> )	3.67 ×10 <sup>9</sup> (4.78 ×10 <sup>9</sup> - 2.8×10 <sup>9</sup> )	0.23 ± 4.38
V26	1.19×10 <sup>7</sup> (1.64×10 <sup>7</sup> -8.58×10 <sup>6</sup> )	1.41 ×10 <sup>10</sup> (2.05×10 <sup>10</sup> - 9.57×10 <sup>9</sup> )	0.30 ± 5.64
V07	1.30×10 <sup>8</sup> (1.50×10 <sup>8</sup> -1.14×10 <sup>8</sup> )	1.33 ×10 <sup>10</sup> (1.69 ×10 <sup>10</sup> -1.04 ×10 <sup>10</sup> )	0.38 ±7.44
V17	5.08×10 <sup>8</sup> (5.84×10 <sup>8</sup> -4.41×10 <sup>8</sup> )	7.69 ×10 <sup>10</sup> (1.04 ×10 <sup>11</sup> -5.66 ×10 <sup>10</sup> )	0.23 ± 4.92

Modified from Fatiha et al [19]

### 3. The effect of *L. muscarium* on biological characteristics and life table of *Serangium japonicum* (Coleoptera: Coccinellidae)

Studies regarding biological control of whitefly, a serious pest in all tropical and subtropical regions of the world [19] have indicated that the coccinellid predator belonging to tribe *Serangium* (Coleoptera: Coccinellidae) are consistently performing as the best predator in the field as well as under laboratory conditions [21,22]. *S. japonicum* larvae can consume 25 to 50 whitefly eggs or nymphs in 24 h depending on the larval stage [23]. Previous experience with whitefly demonstrates that integration of biocontrol agents may substantially contribute to sustainable management of damage caused by *B. tabaci* in both greenhouse and field cropping environments [15]. Lethal and sub-lethal effects of entomopathogens on the biology of insects in general and on predators in particular are too complex to be observed. In cases of entomopathogens, the lethal and sub-lethal effects of the pathogen on beneficial insects (predators and parasitoids) with regard to fecundity, longevity and survivorship among others, are worth evaluating. The impacts of *L. Muscarium* isoalte V20 on the survival and reproduction of *S. japonicum* were evaluated in relation to determining the role of natural mortality factors, including natural enemies and their efficient ratio on whitefly population dynamics.

#### 3.1 Influence of *L. muscarium* on the development and survival of *Serangium japonicum*

The developmental period for all immature stages (eggs, 1st, 2nd, 3rd, 4th instar nymphs and pupae up to emergence) at the concentrations of 1×10<sup>6</sup>, 1×10<sup>7</sup> and 1×10<sup>8</sup> conidia/mL was significantly different when compared with the control colony respectively (Table 1). The shortest development period for different life stages was observed in the control colony, and the development period was longest for the colony treated with conidial concentrations of 1×10<sup>8</sup> spore/mL. The pre-imaginal developmental time was shortest for first, second and third instars, and longest for eggs, fourth instar larvae and pupae at different concentrations (1×10<sup>4</sup>, 1×10<sup>5</sup>, 1×10<sup>6</sup>, 1×10<sup>7</sup>, 1×10<sup>8</sup> conidia/mL) as shown in Table 3

**Table 3** Developmental period (Mean ± SEM) for different life stages of *Serangium japonicum* treated with different concentrations of *L. muscarium* (days).

Treatments (conidia/mL)	0.03% Tween-80	1×10 <sup>4</sup>	1×10 <sup>5</sup>	1×10 <sup>6</sup>	1×10 <sup>7</sup>	1×10 <sup>8</sup>
Egg	17.93 c (±0.08)	18.59 b (±0.10)	18.71 b (±0.11)	18.87ab (±0.10)	19.09 a (±0.12)	19.15 a (±0.11)
1st instar	13.83 c (±0.07)	13.93 c (±0.03)	14.01 c (±0.07)	14.28 b (±0.12)	14.28 b (±0.06)	14.62 a (±0.06)
2nd instar	11.94 d (±0.08)	12.14 cd (±0.05)	12.33bc (±0.04)	12.43 b (±0.09)	12.48 b (±0.07)	12.71 a (±0.08)
3rd instar	10.19b (±0.03)	10.30 b (±0.03)	10.35 b (±0.04)	10.86 a (±0.07)	10.96 a (±0.03)	10.96 a (±0.21)
4th instar	8.70c (±0.05)	8.82 bc (±0.04)	8.88 b (±0.04)	9.17 a (±0.05)	9.21 a (±0.05)	9.24 a (±0.04)
Pupa	4.53 c (±0.04)	4.58 c (±0.03)	4.72b (±0.04)	4.91 a (±0.70)	4.94 a (±0.03)	4.99 a (±0.03)

Means compared by one-way ANOVA, number within same row followed by different letters are significantly different (LSD Test, *P* < 0.05). Modified from Fatiha et al[24]

The percent survival of each stage (eggs, 1st, 2nd, 3rd, 4th instar nymphs, and pupae) treated with different concentrations up to emergence was not significantly different from the control colony ( $P > 0.05$ ). The survival decreased just slightly with the increasing concentrations from  $1 \times 10^4$  to  $1 \times 10^8$  conidia/mL when compared with the control. *Verticillium lecanii* was found to have no adverse effect on survival of *S. japonicum* larvae (Table 4).

**Table 4** Survival ratio (Mean  $\pm$  SEM) for different life stages of *Serangium japonicum* treated with different concentrations of *L. muscarium* (days).

Treatments (conidia/mL)	0.03% Tween-80	$1 \times 10^4$	$1 \times 10^5$	$1 \times 10^6$	$1 \times 10^7$	$1 \times 10^8$
Egg	0.75 a ( $\pm 0.028$ )	0.70 a ( $\pm 0.05$ )	0.71 a ( $\pm 0.016$ )	0.71 a ( $\pm 0.016$ )	0.71 a ( $\pm 0.033$ )	0.65 a ( $\pm 0.05$ )
1st instar	0.8a ( $\pm 0.028$ )	0.81 a ( $\pm 0.016$ )	0.80 a ( $\pm 0.028$ )	0.75a ( $\pm 0.028$ )	0.76 a ( $\pm 0.016$ )	0.73a ( $\pm 0.016$ )
2nd instar	0.81 a ( $\pm 0.033$ )	0.86 a ( $\pm 0.044$ )	0.83 a ( $\pm 0.016$ )	0.80 a ( $\pm 0.013$ )	0.83 a ( $\pm 0.044$ )	0.78 a ( $\pm 0.033$ )
3rd instar	0.86 a ( $\pm 0.016$ )	0.90 a ( $\pm 0.028$ )	0.86 a ( $\pm 0.016$ )	0.86 a ( $\pm 0.044$ )	0.83 a ( $\pm 0.016$ )	0.88 a ( $\pm 0.033$ )
4th instar	0.95 a ( $\pm 0.05$ )	0.98 a ( $\pm 0.016$ )	0.93 a ( $\pm 0.044$ )	0.93 a ( $\pm 0.044$ )	0.88a ( $\pm 0.072$ )	0.90 a ( $\pm 0.05$ )
Pupa	1.00a ( $\pm 0.00$ )	0.95 a ( $\pm 0.028$ )	0.96 a ( $\pm 0.033$ )	0.95 a ( $\pm 0.028$ )	0.93 a ( $\pm 0.044$ )	0.95 a ( $\pm 0.028$ )

Means compared by one-way ANOVA, number within same row followed by different letters are significantly different (LSD Test,  $P < 0.05$ ). Modified from Fatiha et al [24]

### 3.2 Influence of *L. muscarium* on fecundity, pre ovipositional period and female longevity of *Serangium japonicum*

The fecundity of females showed significant differences among the treatments including  $1 \times 10^4$ ,  $1 \times 10^5$  and  $1 \times 10^6$  conidia/mL, when compared to the control ( $F = 1.66$ ,  $df = 5$ ,  $P = 0.0490$ ). The maximum number of eggs (569.70 eggs) was laid by the control beetles, whereas the lowest fecundity was observed in  $1 \times 10^8$  conidia/mL, having an average value of 510.18 eggs/female (Table 5)

The duration of the pre-oviposition period of *S. japonicum* showed no significant differences for different treatments when compared to the control ( $F = 0.82$ ,  $df = 5$ ,  $P = 0.5427$ ) (Table.3). The pre-oviposition period was almost 7 days in the control, and  $1 \times 10^4$  and  $1 \times 10^6$  conidia/mL concentrations (Table 5).

Longevity of adult females treated with different concentrations ( $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$ ,  $1 \times 10^7$ ,  $1 \times 10^8$  conidia/mL) did not vary significantly ( $F = 0.05$ ,  $df = 5$ ,  $P = 0.9985$ ) among the treatments and the control, with the shortest longevity in  $1 \times 10^6$  conidia/mL at 63.9 days compared to longest, 66.6 days in the control. *V. lecanii* showed no effect on the longevity of *S. japonicum* females (Table 5).

**Table 5** The fecundity, preoviposition and longevity (Mean  $\pm$  SEM) of *Serangium japonicum* treated with different concentrations of *L. muscarium*

Treatments (conidia/mL)	0.03% Tween-80	$1 \times 10^4$	$1 \times 10^5$	$1 \times 10^6$	$1 \times 10^7$	$1 \times 10^8$
Fecundity	569.70 a ( $\pm 8.01$ )	556.20 a ( $\pm 2.14$ )	552.20 a ( $\pm 9.07$ )	538.60 ab ( $\pm 7.35$ )	526.33 b ( $\pm 2.60$ )	510.18 b ( $\pm 4.62$ )
Preoviposition (days)	6.58 a ( $\pm 0.19$ )	6.54 a ( $\pm 0.18$ )	6.62 a ( $\pm 0.18$ )	6.50 a ( $\pm 0.18$ )	6.87 a ( $\pm 0.12$ )	6.75 a ( $\pm 0.16$ )
Longevity (days)	66.62 a ( $\pm 6.28$ )	67.74 a ( $\pm 5.87$ )	63.87 a ( $\pm 6.82$ )	63.87 a ( $\pm 7.14$ )	65.50 a ( $\pm 5.30$ )	64.62 a ( $\pm 5.49$ )

Means compared by one-way ANOVA, number within same row followed by different letters are significantly different (LSD Test,  $P < 0.05$ ). Modified from Fatiha et al[24]

### 3.3 Influence of *L. muscarium* on life table characteristics of *Serangium japonicum*

The value of the net reproduction rate observed for the control was significantly higher when compared to different treatments ( $F = 12.02$ ,  $df = 5$ ,  $P = 0.0044$ ). The net reproductive rate was highest in the control with a value of 102.10 and was lowest for  $1 \times 10^8$  conidia/mL having a value of 84.07. There were no significant difference observed for values

of  $r_0$  among the concentrations of  $1 \times 10^4$ ,  $1 \times 10^5$  and  $1 \times 10^6$  conidia/mL with values of 96.40, 93.53, 92.48 respectively (Table 4).

The values of  $r_m$  were not significantly different among the treatment ( $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$ ,  $1 \times 10^7$ ,  $1 \times 10^8$  conidia/mL) and the control ( $F = 1.61$ ,  $df = 5$ ,  $P = 0.2873$ ). Also, the mean generation time ( $T$ ) was not significantly different among the treatments when compared to the control ( $F = 0.17$ ,  $df = 5$ ,  $P = 0.9636$ ).

**Table 5** Life table parameters (Mean  $\pm$  SEM) of *Serangium japonicum* treated with different concentrations of *L. muscarium*  
Means compared by one-way ANOVA, number within same row followed by different letters are significantly different

Treatments (conidia/mL)	0.03% Tween-80	$1 \times 10^4$	$1 \times 10^5$	$1 \times 10^6$	$1 \times 10^7$	$1 \times 10^8$
$r_0$ (Progeny/female)	102.10 a ( $\pm 2.73$ )	96.40 ab ( $\pm 1.96$ )	93.53 b ( $\pm 0.74$ )	92.48 b ( $\pm 2.43$ )	84.37 c ( $\pm 1.11$ )	84.07 c ( $\pm 2.32$ )
$r_m$ (Progeny/female)	0.0998 a ( $\pm 0.0013$ )	0.0996 a ( $\pm 0.1241$ )	0.0986 a ( $\pm 0.0005$ )	0.0983 a ( $\pm 0.0008$ )	0.0965 a ( $\pm 0.0018$ )	0.0969 a ( $\pm 0.0015$ )
$T$ (days)	46.32 a ( $\pm 0.32$ )	45.84 a ( $\pm 0.16$ )	45.99 a ( $\pm 0.34$ )	46.00 a ( $\pm 1.22$ )	45.92 a ( $\pm 0.85$ )	45.74 a ( $\pm 0.60$ )
$\lambda$ (days)	1.105 a ( $\pm 0.001$ )	1.104 a ( $\pm 0.000$ )	1.103 a ( $\pm 0.006$ )	1.103 a ( $\pm 0.009$ )	1.101 a ( $\pm 0.001$ )	1.101 a ( $\pm 0.002$ )

(LSD Test,  $P < 0.05$ ). Modified from Fatiha et al[24]

#### 4. Effect of *Lecanicillium muscarium* on *Eretmocerus sp. nr. furuhashii* (Hymenoptera: Aphelinidae)

Parasitoids have been used extensively as a model system in studies of population and behavioral ecology, partially due to their potential use in biological pest control (Godfray and Shimada 1999). *Eretmocerus sp. nr. furuhashii* (Hymenoptera: Aphelinidae), a parasitoid of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), has a widespread distribution worldwide [25]. This species is recognized as one of the most important natural enemies of *B. tabaci* having generated a lot of interest in countries where *B. tabaci* is a problem. They oviposit under the host and develop in a vital capsule within the host [26]. Adult parasitoids preferentially oviposit within 3rd and 4<sup>th</sup> instar nymph of whitefly and the greatest rate of development occurs when the 3<sup>rd</sup> instar of whitefly are parasitized [26, 27, 28]. To date, most of the studies on whitefly parasitoids show their ability to establish and maintain a high level of parasitism but generally, secondary effects of entomopathogenic fungi on endoparasitic insects are little known [29]. The effect of different concentrations of *L. muscarium* on different development stages (pupa and adult) of *E. sp. nr. Furuhashii* was studied to observe the presence of any positive interaction among biocontrol agents with minimum risk hazards.

##### 4.1 Effect of *L. muscarium* on young larvae and pupae of *Eretmocerus sp. nr. Furuhashii*

The percentage emergence of *E. sp. nr. furuhashii* against different conidial concentrations after 6 and 12 days of parasitoid oviposition is shown in table-1. The emergence of parasitoids from the whitefly nymphs treated with *L. muscarium* spores and control after 6 days of *E. sp. nr. furuhashii* parasitization showed significant differences among different treatments and the control ( $df=5$ ,  $F=14.51$ ,  $P=0.0001$ ). The results indicated that the number of parasitized larvae surviving decreased with increasing concentrations of *L. muscarium*. Successful parasitoid emergence was greatest (67.77%) in the control and lowest (28.88%) at a conidial concentration of  $1 \times 10^8$  conidia/ml.

Immatures of *E. sp. nr. furuhashii* 12 days postoviposition were affected by fungal application and no significant differences were detected in the mean number of parasitoids successfully emerging in the control and at  $1 \times 10^4$ ,  $1 \times 10^5$  and  $1 \times 10^6$ ,  $1 \times 10^7$  and  $1 \times 10^8$  conidia/ml ( $df=5$ ,  $F=0.4361$ ,  $P=1.40$ ). Maximum percentage emergence of adult parasitoid in control was 75.55% (Table 6).

**Table 6** Percent emergence of *Eretmocerus* sp. nr. *fulvipes* adults following application of increased spore concentrations of *L. muscarium* after 6 and 12 days of oviposition at  $25 \pm 2$  °C

Treatments (conidia/ml)	6 days			12days		
	Parasitoid Host	Emerged Adults	Emergence %	Parasitoid Host	Emerged Adults	Emergence %
0.03%Tween-80	90	61	67.77 ± 2.93 a	90	68	75.55±2.93a
1×10 <sup>4</sup>	90	55	61.11± 4.84a	90	62	70.00 ±3.84a
1×10 <sup>5</sup>	90	44	48.88 ±2.93b	90	63	68.88 ± 4.8a
1×10 <sup>6</sup>	90	41	45.55± 4.00 b	90	61	67.77 ±6.75a
1×10 <sup>7</sup>	90	26	33.33± 4.84c	90	56	67.77 ±2.22a
1×10 <sup>8</sup>	90	30	28.88 ± 3.8c	90	61	62.22 ±2.93a

Means compared by one-way ANOVA, number within same column followed by different letters are significantly different (LSD Test,  $P < 0.05$ ). Modified from Fatiha et al[30]

#### 4.2 Effect of *L. muscarium* on survival of adult *Eretmocerus* sp. nr. *fulvipes*

Significant differences in adult parasitoid survivorship were observed among conidial concentrations of  $1 \times 10^6$ ,  $1 \times 10^7$ ,  $1 \times 10^8$  conidia/ml and the control after 3 (df=5,  $F=2.4$ ,  $P=0.057$ ) and 5 days (df=5,  $F=4.36$ ,  $P=0.0058$ ) of confinement in test tubes (Table-2). After 3 days, highest average parasitoid survivorship (96.67%) was observed in the control, and it was lowest (76.66%) at  $1 \times 10^8$  conidial/ml. Similarly maximum survival after 5 days (90%) was observed in the control and it was lowest (66.66%) at  $1 \times 10^7$  and  $1 \times 10^8$  conidia/ml. There were no significant differences in adult parasitoid survivorship after 7 days of confinement into test tubes treated with different conidial concentrations and control (df=5,  $F= 1.06$ ,  $P=0.1026$ ). Maximum survivorship (73.33%) was observed in the control and it was minimum (60%) at  $1 \times 10^8$  conidia/ml.

**Table 7** Survivorship of *Eretmocerus* sp. nr. *fulvipes* adults after 24 h exposure to increasing dry conidial concentrations of *L. muscarium*

Treatments (conidia/ml)	Percent survival at indicated days		
	3 day	5 day	7 day
0.03%Tween-80	96.67± 3.33 a	90.00± 3.97 a	73.331 ± 4.02 a
1×10 <sup>4</sup>	93.32 ± 4.08 a	86.66 ± 2.72 ab	73.331 ± 3.64 a
1×10 <sup>5</sup>	89.99 ± 3.97 ab	73.33 ± 1.26 bc	69.99 ± 2.78 a
1×10 <sup>6</sup>	83.331± 2.23 b	66.66 ± 3.33 c	63.33 ± 3.23 a
1×10 <sup>7</sup>	79.99 ± 1.08 b	66.66 ± 2.79 c	63.33 ± 3.33 a
1×10 <sup>8</sup>	76.66 ± 2.79 b	66.66 ± 3.26 c	60.00 ± 4.26 a

Means compared by one-way ANOVA, number within same column followed by different letters are significantly different (LSD Test,  $P < 0.05$ ). Modified from Fatiha et al[30]

#### 4.3 Effect of *L. muscarium* on next offsprings and longevity of adult *Eretmocerus* sp. nr. *Fulvipes*

*L. muscarium* showed a non significant effect on longevity and next offsprings of female parasitoids (Table.8, 9). *E.sp.nr. fulvipes* females emerging from the treatment of *L. muscarium* 12 days after oviposition, when tested for their reproductive capacity during 48 h, showed no significant differences (df=5,  $F=1.45$ ,  $P=0.3527$ ). The percentage emergence of parasitoids from the whitefly nymphs produced by the females emerged from treated pupae was almost similar. Maximum emergence (69.77%) was observed at  $1 \times 10^6$  conidia/ml and it was lowest (61.02%) at  $1 \times 10^8$  conidia/ml.

There were no significant differences in the longevity of adult *Eretmocerus* emerging from whitefly nymphs treated after 12 days of postoviposition among the treatments and control (df=5,  $F=0.12$ ,  $P=0.9863$ ). Maximum longevity (5 days) was observed at  $1 \times 10^5$  conidia/ml whereas the lowest longevity was 4.9 days observed at  $1 \times 10^8$  conidia/ml.

**Table 8** Total number of *Bemisia tabaci* parasitized by *Eretmocerus* sp. nr. *furuhashii* emerged from *L. muscarium* treatment after 12 days of parasitoid oviposition.

Treatments (conidia/ml)	Total Hosts	Emerged Adults	Emergence (%)
0.03%Tween-80	167	109	65.540 ± 3.72 a
1×10 <sup>4</sup>	172	120	69.509 ± 4.88 a
1×10 <sup>5</sup>	169	103	61.291 ± 2.29 a
1×10 <sup>6</sup>	165	115	69.774 ± 2.57 a
1×10 <sup>7</sup>	157	104	65.674 ± 4.23 a
1×10 <sup>8</sup>	168	103	61.021 ± 2.51 a

Means compared by one-way ANOVA, number within same column followed by different letters are significantly different (LSD Test,  $P < 0.05$ ). Modified from Fatiha et al [30]

**Table 9** Longevity of emerged *Eretmocerus* sp. nr. *furuhashii* adults from parasitized pupae treated with *L. muscarium* after 12 days of parasitoid oviposition

Treatments (conidia/ml)	Individuals	Longevity± SEM (d)
0.03%Tween-80	30	4.90 ± 0.27 a
1×10 <sup>4</sup>	30	4.80 ± 0.57a
1×10 <sup>5</sup>	30	5.00 ± 0.47a
1×10 <sup>6</sup>	30	4.60 ± 0.26 a
1×10 <sup>7</sup>	30	4.70 ± 0.42 a
1×10 <sup>8</sup>	30	4.90 ± 0.40a

Means compared by one-way ANOVA, number within same column followed by different letters are significantly different (LSD Test,  $P < 0.05$ ). Modified from Fatiha et al[30]

## 5. Interaction effect of *Lecanicillium muscarium* and *Serangium japonicum* (Coleoptera;Coccinellidae) controlling *Bemisia tabaci* (Homoptera;Aleyrodidae)

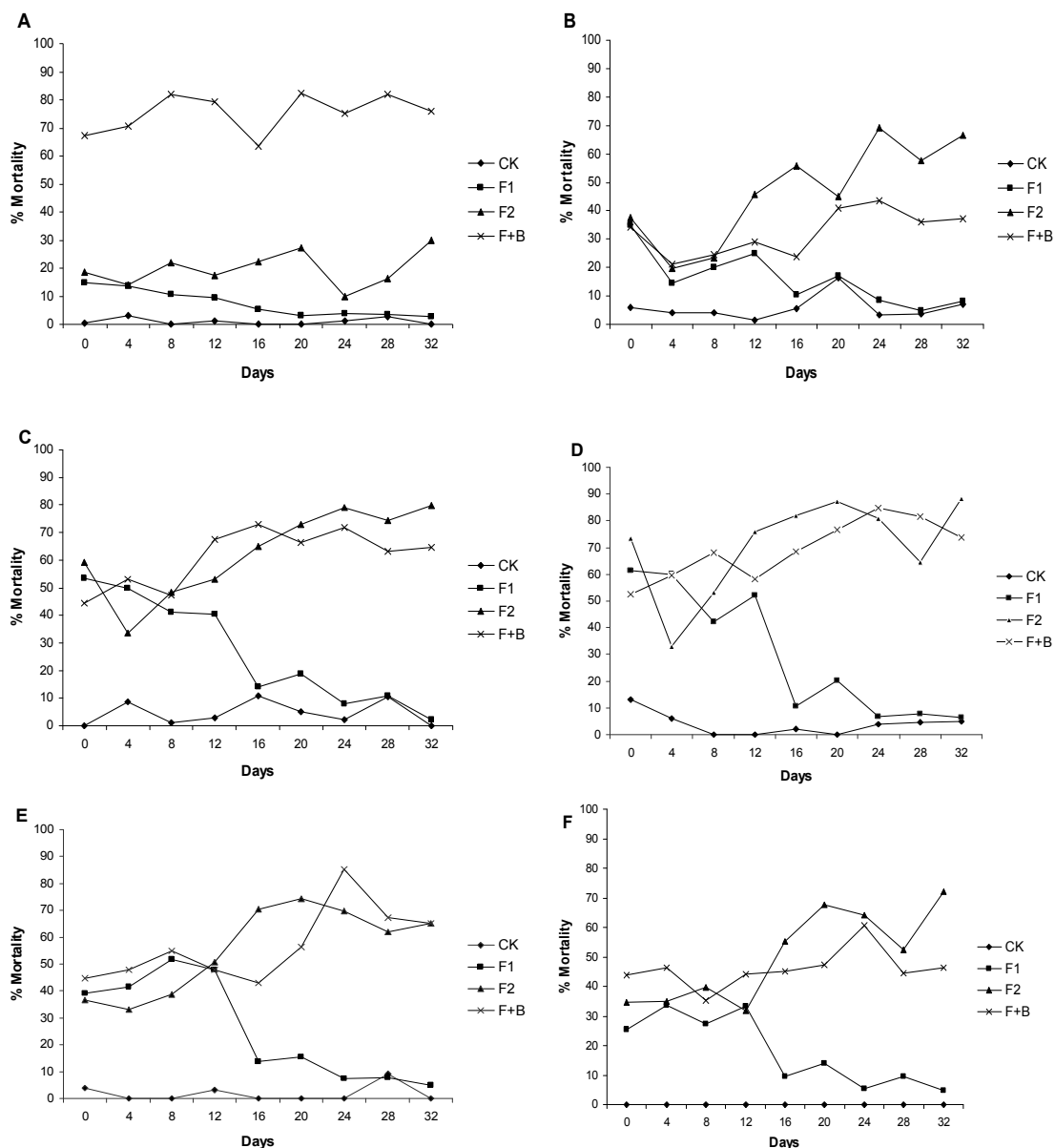
Biological control of *B. tabaci* in greenhouses, as compared to that of *Traileurodes vaporariorum*, remains problematic and may demand more supervision, increased releases of parasitoids and the use of additional natural enemies [31,32]. Increasing experimental evidences have suggested that biological control can be disrupted by direct and indirect interactions such as competition, interguild predation and behavioral interference between natural enemies [33, 34, 35]. Understanding and exploiting interactions among natural enemies are therefore helpful for implementing effective pest control strategies. The compatibility between *L. muscarium* (strain V<sub>20</sub>) and *S.japonicum* observed to assess the mortality of different instars of whitefly when both biocontrol agents were applied in different combinations. This study was also designed to study the interaction of both species at large scale green house level. The effects of different treatments were compared for their efficiency to control different stages of *B. tabaci*.

### 5.1 Mortality of *B.tabaci* eggs

The percentage mortality of *B.tabaci* eggs against different treatments at different sampling dates has been shown in figure-1A. All the treatments showed a significant effect on the mortality when compared to the control (df = 3, F=786.98, p<0.001). At the end of trial period T4 (fungi + beetles) proved to be the most effective giving an overall mortality of 75.92%. Similarly a significant effect was also observed for the interaction between different treatments and sampling dates (df=24, F=2.46, p=0.0018). Maximum mortality of whitefly eggs (82%) was observed after 28 days for T4. Incase of T1(control) there was a gradual decrease in % age mortality of eggs with the passage of time and there was almost 7 folds decrease in the mortality of eggs at the ed of experimental period (Figure-1A).

### 5.2 Mortality of *B.tabaci* nymphal instars

Data regarding the percentage mortality of different *B.tabaci* nymphal instars is shown if figure 2-5. All the treatments showed a significant effect on the mortality of different nymphal instars. ( First instar df= 3, F=80.53; P<0.0001; Second instar df= 3, F=216.98, P<0.0001; Third instar df= 3, F=356.48, P<0.0001; Fourth instar df= 3, F= 171.86, P<0.0001). First three nymphal instars were most susceptible to T3 (having 3 funagal applications, each application at



**Fig 1** Whitefly mortality (%) on eggplants over time in response to *L. muscardium* and *S. japonicum* (A), the mortality of eggs (%) (B) First instar mortality (%), (D) Second instar mortality (%), (C) third instar mortality (%), (E) Fourth instar mortality (%) and (F) pupae mortality (%).

\*CK = control, F1= single application of fungi, F2 = three fungal applications and F+B= single application of fungi + beetles (4 adults/plant)

15 days interval) showing an overall 46.07%, 62.81% and 71% mortality at the end of experimental period (Figure 1B,1C &1D) whereas T4 (fungi + beetles) caused maximum mortality of 56.96% against 4<sup>th</sup> instar at the end of trail(Figure-1E).

A significant interaction effect between the treatments and sampling dates was also observed on the mortality of all the nymphal instars (First instar df = 24, F=3.11; P<0.0001; Second instar df= 24, F=7.37, P<0.0001; Third instar df = 24, F=12.39, P<0.0001; Fourth instar df= 24, F= 7.11, P<0.0001).The maximum mortality of 1<sup>st</sup> instar nymphs (6.66%) was observed for T3 (having 3 funagal applications, each application at 15 days interval) and lowest mortality of (3.4%) was observed after 24 days in case of control. Similarly 2<sup>nd</sup> nymphal instar also showed 79.66% mortality for T3 (having 3 funagal applications, each application at 15 days interval) after 32 days whereas for T4 (fungi + beetles) it was 64.66% on the same sampling date (Figure-1C).3<sup>rd</sup> nymphal instar was also most effected by T3 and maximum mortality(87.33%) was observed after 20 days whereas T4 caused a maximum mortality of 84.66% after 24 days (Figure-1D).T4 (fungi + beetles) proved most effective against 4<sup>th</sup> nymphal instar showing 85.33% mortality on 22<sup>nd</sup>



December whereas for T3 maximum mortality of 74% was observed after 20 days and after that there was a gradual decrease in mortality for this treatment.

### 5.3 Mortality of *B. tabaci* pupae

All the treatments had a significant effect on the mortality of *B. tabaci* pupae (df=3, F=172.77 p<0.0001). At the end of trial period 18.09, 50.25 and 45.88% mortality was observed for T1, T2 and T3 respectively. The interaction effect between different treatments and sampling dates showed significant differences. (df=24, F=4.4 p<0.0001). For T2 a constant mortality was observed up to 10<sup>th</sup> December and then a gradual decrease was observed till the end of experimental period. T3 (having 3 fungal applications, each application at 15 days interval caused a maximum mortality of 72% after 32 days. In case of T4 60.66% mortality was observed after 24 days and after this mortality started to decrease till the end of these trials. (Figure 1F).

## 6. Conclusion and future prospects

The results suggest that *L. muscarium* could be considered for the microbial control of *B. tabaci*. Further studies are now required to determine pathogenicity and virulence of the isolates are independently from the original host and geographical location. The susceptibility of *S. japonicum* to infection by *L. muscarium* was not significant, but infection may have been an important factor in predator mortality. It is apparent from our research results that no influence of *L. muscarium* combined application on predator. Our result suggested that combined use of both *V. lecanii* and *S. japonicum* for integrated pest management of the whitefly *B. tabaci*. The use of *L. muscarium* and *S. japonicum* for integrated pest management of the whitefly *B. tabaci*, was successful to control this pest, but fungal spores should as much as possible be timed to coincide with later developmental stages of the predators to conserve the predator within system.

**Acknowledgements:** This research was funded by grants from the Chinese National Basic Research Program (called 973 Program) (No. 2006CB102005), Public sector specific research projects (No. 200803005) and the eleventh five year forest support program of China (No. 2006BAD08A1903).

## References

- [1] Gerling D, Motro U, Horowitz R. Dynamics of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) attacking cotton in the coastal plain of Israel. *Bulletin of Entomological Research*. 1980; 70: 213–219.
- [2] Nomikou M, Janssen A, Schraag R, Sabelis MW. Phytoseiid predators as potential biological control agents for *Bemisia tabaci*. *Experimental and Applied Acarology*. 2001; 25: 271–291.
- [3] Xu RM. The occurrence and distribution of *Bemisia* in China. In: Gerling D, Mayer RT, eds. *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*. Andover, UK: Intercept; 1996: 125–131.
- [4] Ren SX, Wang ZZ, Qiu BL, Xiao Y. The pest status of *Bemisia tabaci* in China and non-chemical control strategies. *Entomologia Sinica*. 2001; 8: 279–288.
- [5] Qui BL, Ren SX, Mandour NS, Lin L. Effect of temperature on the development and reproduction of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Entomologica Sinica*. 2003; 10: 43–49.
- [6] Gorman K, Hewitt F, Denholm I, Devine GJ. New developments in insecticide resistance in the glasshouse whitefly (*Trialetrodes vaporariorum* Westw.) and the two-spotted spider mite (*Tetranychus urticae*) in the UK. *Pest Management Science*. 2002; 58: 123–130.
- [7] Lacey LA, Fransen JJ, Carruthers R. Global distribution of naturally occurring fungi of *Bemisia*, their Biologies and use as Biological control agents. In: Gerling D, Mayer RT, eds. *Bemisia 1995: Taxonomy, Biology, Damage Control and Management*. Andover, Intercept Ltd. 1996; 401–433.
- [8] Osborne, LS, Landa, Z. Biological control of whiteflies with entomopathogenic fungi. *Florida Entomologist*. 1992; 75: 149–471.
- [9] Clarkson JM, Charnley AK. New Insights into Mechanisms of Fungal Pathogenesis in Insects. *Trends in Microbiology*. 1996; 4: 197–204.
- [10] Meekers ETM, Fransen JJ and van Lenteren JC. Pathogenicity of *Aschersonia* spp. against whiteflies *Bemisia argentifolii* and *Trialetrodes vaporariorum*. *Journal of Invertebrate Pathology*. 2002; 81: 1–11.
- [11] Altre JA, Vandenberg JD, Cantone FA. Pathogenicity of *Paecilomyces fumosoroseus* Isolates to Diamondback Moth, *Plutella xylostella*: Correlation with Spore Size, Germination Speed, and Attachment to Cuticle. *Journal of Invertebrate Pathology*. (1999); 73: 332–338.
- [12] James RR, Buckner JS, Freeman TP. Cuticular Lipids and Silverleaf Whitefly Stage Affect Conidial Germination of *Beauveria bassiana* and *Paecilomyces fumosoroseus*. *Journal of Invertebrate Pathology*. 2003; 84: 67–74.
- [13] Ali S, Huang Z, Ren SX. Media composition influences on growth, enzyme activity and virulence of the entomopathogen hyphomycetes *Isaria fumosoroseus*. *Entomologia Experimentalis et Applicata*. 2009; 131: 30–38.
- [14] Wang L, Huang J, You M, Liu B. Effects of toxins from two strains of *Verticillium lecanii* (Fungi: Hyphomycetes) on bioattributes of a predatory ladybeetle, *Delphastus catalinae* (Coleoptera: Coccinellidae). *Journal of Applied Entomology*. 2005; 129: 32–38.

- [15]. Fazal S. Biological control of *Bemisia tabaci*(Homoptera: Aleyrodidae) on poinsettia with *Eretmocerus* sp. (Hymenoptera: Aphelinidae), *Serangium japonicum*(Coleoptera: Coccinellidae) and *Paecilomyces fumosoroseus*(Deuteromycotina: Hyphomycetes). PhD Thesis, South China Agricultural University, Guangzhou, China, 2004.
- [16]. Parker BL, Skinner M, Costa SD, Gouli S, Ried W, El-Bouhsini M. Entomopathogenic fungi *Eurygaster integriceps* Puton (Hemiptera: Scautelleridae): collection and characterization for development. *Biological Control*. 2003; 27: 260-272.
- [17]. Mor H, Gindin G, Ben-Zeev IS, Geschtovt NU, Arrkhozina N, Barash I. Diversity among isolates of *Verticillium lecanii* as expressed by DNA polymorphism and virulence towards *Bemisia tabaci*. *Phytoparasitica*. 1996; 24: 111-118.
- [18]. Gindin G, Barash NI, Raccach B. Effect of endotoxic compounds isolates from *Verticillium lecanii* on the sweet potato whitefly, *Bemisia tabaci*. *Phytoparasitica*. 1994; 22: 189-196.
- [19]. Fatiha L, Ali S, Ren SX, Afzal M. Biological characteristics and pathogenicity of *Verticillium lecanii* against *Bemisia tabaci* (Homoptera: Aleyrodidae) on egg plant. *Pakistan Entomologist*. 2007; 29: 63-71.
- [20]. Brown JK. Current status of *Bemisia tabaci* as plant pest and virus vector in agroecosystem worldwide FAO. *Plant Protection Bulletin*. 1994; 42: 3-32.
- [21]. Goolsby JA, Legaspi JC, Legaspi BC. Quarantine evaluation of exotic parasitoids of the sweetpotato whitefly *Bemisia tabaci* (Gennadius). *Southwestern Entomologist*. 1996; 21: 13-21.
- [22]. Ren SX, Yao SL, Huang ZH. The morphological characters and biology of *Serangium japonicum*. *Natural Enemies of Insects*. 2004; 26: 22-27. (in Chinese)
- [23]. Legaspi JC, Legaspi BCRL, Meagher JR, Ciomperlik MA. Evaluation of *Serangium parcestosum* (Coleoptera: Coccinellidae) as a biological control agent of the silverleaf whitefly (Homoptera: Aleyrodidae). *Environmental Entomology*. 1996; 25: 1421-1427.
- [24]. Fatiha L, Zhen H, Ren SX, Ali S. Effect of *Verticillium lecanii* on biological characteristics and life table of *Serangium japonicum* (Coleoptera: Coccinellidae), a predator of whiteflies under laboratory conditions. *Insect Science*. 2008; 15: 327-333.
- [25]. Qui BL, Ren SX, Xiao Y, Mandour NS. Effectiveness of *Eretmocerus* sp and *Aschersonia aleyrodis* in controlling *Bemisia tabaci* population. *Bulletin of Entomological Research*. 2004; 14:2251-2254.
- [26]. Gerling D, Tremblay E, Orion T. Initial stages of vital capsule formation in the *Eretmocerus*—*Bemisia tabaci* association. *Redia*. 1991; 74:411-415.
- [27]. Roudrigues MD, Moreno R, Tellez MM. *Eretmocerus mundus* (Mercet), *Encarsia lutea* (Masi) *Encarsia transcera* (Timberlake) (Hymenoptera: Aphelinidae) parasitoides de *Bemisia tabaci* (Homoptera: Aleyrodidae) en los cultivos horticolas protegidos almerienses. *Bol Sanid Veg, Plagas*. 1994; 20:695-702.
- [28]. Gerling D, Fried R. Biological studies with *Eretmocerus mundus* (Hymenoptera: Aphelinidae). *Journal of Economic Entomology*. 2000; 87:117-123.
- [29]. Kirk AA, Lacey LA, Brown JK, Ciomperlik MA, Goolsby JA, Vacek DC. Variation in the *Bemisia tabaci*, 1. Species complex (Hemiptera: Aleyrodidae) and its natural enemies leading to successful biological control of *B. tabaci* biotype B in the USA. *Bulletin of Entomological Research*. 2000; 90:317-327.
- [30]. Fatiha L, Zhen H, Ali S, Ren SX. Effect of *Lecanicillium muscarium* on *Eretmocerus* sp.nr.furuhashii (Hymenoptera: Aphelinidae), a parasitoid of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Journal of Pest Science*. 2009;
- [231]. Goettel MS, Poprawski TJ, Vanderberg JD, Li Z, Roberts DW. Safety to non-target invertebrates of fungal biocontrol agents. In: Laird M, Lacey LA, Davidson EW. Eds. *Safety of Microbial Insecticides*. CRC Press, Boca Raton, 1990; pp 209-232.
- [32]. Gabarra R, Bersi M. Tomatoes. In: Albajes R, Gullino ML, Van Lenteren JC, Elad Y. eds. *Integrated Pest and Disease Management in greenhouse Crops*. Kluwer, Dordrecht, Netherlands, 1999; 420-434.
- [33]. van Lenteren JC, Martin NA. Biological control of whiteflies In: Albajes R, Gullino M L, Van Lenteren, JC, Elad Y. eds. *Integrated Pest Management in Greenhouse Crops*. Kluwer, Dordrecht, Netherlands, 1999; 202-216.
- [34]. Rosenheim JA, Kaya HK, Ehler LE. Intraguild predation among biological-control agents: theory and evidence. *Biological Control*. 1995; 5: 303-335.
- [35]. Symondson WOC, Sunderland KD, Greenstone MH. Can generalist predators be effective biocontrol agents?. *Annual Review of Entomology*. 2002; 47: 561-594.