# Effect of *Verticillium lecanii* on biological characteristics and life table of *Serangium japonicum* (Coleoptera: Coccinellidae), a predator of whiteflies under laboratory conditions

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**Abstract** Effects of entomopathogenic fungus *Verticillium lecanii* on biological characteristics and life table of *Serangium japonicum*, a predator of whiteflies against five different conidial concentrations  $(1 \times 10^4, 1 \times 10^5, 1 \times 10^6, 1 \times 10^7, \text{ and } 1 \times 10^8 \text{ conidia/mL})$  were studied under laboratory conditions. The developmental periods for all immature stages (from eggs, 1st, 2nd, 3rd, 4th instar nymph and pupae up to emergence) among the treatments were significantly different when compared to that of control, and the longest development period was observed as treated with  $1 \times 10^8$  spore/mL. However, no significant difference on the percent survival of all immature stages was observed among the treatments and control. Also, there were no significantly different effects of *V. lecanii* on mean generation time, intrinsic rate, the finite rate of increase and longevity of *S. japonicum* among the treatments and control.

Key words biological characteristics, life table, Serangium japonicum, Verticillium lecanii

## Introduction

Studies regarding biological control of whitefly, a serious pest in all tropical and subtropical regions of the world (Brown, 1994) 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 (Goolsby *et al.*, 1996; Legaspi *et al.*, 1996; Ren *et al.*, 2004; Yao, 2003). *Serangium parcestosum* larvae can consume 25 to 50 whitefly eggs or nymphs in 24 h depending on the larval stage (Legaspi *et al.*, 1996).

Verticillium lecanii (Zimm.) Viegas is a major microbial biocontrol agent of whiteflies and aphids. Wang et al.

Correspondece: Shun-Xiang Ren, Engineering Research Center of Biological Control, Ministry of Education, College of Natural Resource and Environment, South China Agricultural University, Guangzhou 510642, China. Tel: +86 20 85283508; fax: +86 20 85280293; email: rensxcn@yahoo.com.cn, hzscau@scau.edu.cn, alientomologist@gmail.com (2004) studied virulence of six strains of *V. lecanii* against sweet potato whitefly *Bemisia tabaci*. Their results indicated that strains V16063, V3450 and Vp28 were virulent against *B. tabaci* having LC<sub>50</sub> values of  $2.57 \times 10^5$ ,  $6.03 \times 10^5$  and  $6.05 \times 10^5$  conidial/mL, respectively.

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 (Fazal, 2004). 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 (Roy & Pell, 2000). 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 (Brooks, 1993). 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.

Life table studies provide a powerful technique for

quantitative evaluations of natural enemies in terms of detailed description of age-specific mortality of individuals in the population (Luck *et al.*, 1988). When information on the insect's fecundity and age-specific mortality is available, the effect of the natural enemy can be easily expressed in terms of its effects on the pest population growth rate (van Driesche & Bellows, 1996). Nevertheless, there is very little information on the compatible utilization of *V. lecanii* and *S. japonicum* for integrated management of *B. tabaci*, by using the life table method. The global objectives of this work were to evaluate the impact of *V. lecanii* on the survival and reproduction of *S. japonicum* in relation to determining the role of natural mortality factors, including natural enemies and their efficient ratio on whitefly population dynamics.

## Material and methods

# Host plant

Eggplants, *Solanum melongena* L. var *dafeng* were grown individually in plastic pots (16 cm diameter). The leaves with *B. tabaci* were used as substrate nutrition for the beetles.

#### Serangium japonicum

Serangium japonicum adults were obtained from greenhouses of the South China Agricultural University, Guangzhou China. The adults were cultured on eggplants under laboratory conditions at  $24 \pm 2$  °C and  $75\% \pm 5\%$  RH. The insects were paired ( $9 \pm 3$ ) and transferred in plastic Petri dishes ( $9 \text{ cm} \times 9 \text{ cm}$ ). Eggplant leaves bearing different immature stages of *B. tabaci* were used as substrate nutrition and a moist filter paper was placed to maintain humidity inside the Petri dishes. The culture was placed in a chamber at a temperature of  $26 \pm 1$  °C and humidity of  $80\% \pm 5\%$  RH. Females were allowed to lay eggs. Hatched larvae were transferred into plastic Petri dishes having substrate food (an eggplant leaf with *B. tabaci*).

# Fungal material

*Verticillium lecanii* used for bioassays was isolated from *Traileurodes* sp, kept by the Engineering Research Center of Biological Control, Ministry of Education, South China Agricultural University, Guangzhou, China. After the passage of the fungus through *B. tabaci*, the *V. lecanii* V<sub>20</sub> strain was cultured on Sabouraud Dextrose Agar media (SDAY). Application of *V. lecanii* was made with aque-

ous suspension of 15-day-old conidia. The series of suspension tested was  $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$ ,  $1 \times 10^7$  and  $1 \times 10^8$  conidia/mL. The blastospores were quantified using a standard hemocytometer at 40 × magnification. Spore viability was determined by culturing 0.1 mL aliquot of the stock suspension onto three potato dextrose agar (PDA) plates. The plates were incubated at 25 °C for 24 h. Three hundred spores were examined and scored for viability (Goettel & Inglis, 2000).

#### Influence of V. lecanii on egg and larvae of S. japonicum

The different life stages of *S. japonicum* (eggs, 1st instars, 2nd instars, 3rd instars and 4th instars) were dipped into 20 mL of the prepared suspensions  $(1 \times 10^4, 1 \times 10^5, 1 \times 10^6, 1 \times 10^7, 1 \times 10^8$  conidia/mL) for 10 s and were dried by placing on filter paper. The insects were transferred to plastic Petri dishes containing eggplant leaves having substrate nutrition. To maintain the humidity and aeration, the eggplant leaves were placed on moistened filter paper and dishes were covered with plastic with small holes.

The Petri dishes were incubated for 24 h at  $25 \pm 2$  °C,  $80\% \pm 5\%$  RH and 16:8 (L:D). After 24 h of incubation S. japonicum individuals were transferred into test tubes (10 cm long) with discs of eggplant kept in a culture chamber maintained at  $25 \pm 2$  °C and  $75\% \pm 5\%$  RH. The mortality of beetles was recorded at 24-h intervals until adult emergence. The dead larvae were sterilized with 2% sodium hypochlorite for 1 min and were dried by using filter paper (Fazal, 2004). After drying by aeration, dead insects were cultured on PDA media. It was decided the beetles died from infection of the fungus if the mycelia and conidia of V. lecanii were observed on the cadavers. The Petri dishes were incubated at 25  $\pm$  2 °C and 80%  $\pm$  5% RH. The egg hatchability and developmental time of each stage until the next molt was also recorded. As a control, 0.03% Tween 80 (Whiga Chemicals, Guangzhou, China) was used (Fazal, 2004). For each conidial concentration 20 individuals of every life stage of the beetles were used against each conidial concentration and the entire experiment was repeated 10 times.

# Influence of V. lecanii on S. japonicum females

Pairs of sexually mature beetles (4 days old) collected from the stock culture were dipped into five different conidial concentrations ( $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$ ,  $1 \times$  $10^7$ ,  $1 \times 10^8$  conidia/mL) of *V. lecanii* strain (V20) for 10 second. The beetles were then transferred to plastic Petri dishes with eggplant leaves. The Perti dishes were incubated at  $25 \pm 2 \,^{\circ}$ C,  $70\% \pm 5\%$  for 24 h. The leaves were changed every day and the numbers of eggs laid by each pair were recorded. For each conidial concentration six pairs of the beetles were used against each conidial concentration and the entire experiment was repeated 10 times.

# Life table analysis

Life and fertility tables were calculated from the cohort of eggs according to the method of Andrewartha and Birch (1954). The death and survival rates were recorded daily for all the immature stages. The probability of surviving from birth (cohort eggs) to age x for every immature stage  $(l_x)$  was also calculated. The intrinsic rate of population increase  $(r_m)$  was calculated using the Birch (1948):

 $R_0 = \sum l_x m_x,$   $T = 1/R_0 \sum l_x m_x,$   $r_m = \ln R_0/T,$  $\lambda = \exp(r_m),$ 

where  $l_x$  is the survivorship at the corresponding time,  $m_x$  is the number of female eggs laid according to sex ratio laid per female per day. The net productive rate  $R_0$  is the mean number of female progeny produced by a single female during its mean life span.

This parameter expresses the generation growth rate of the population and is related to discrete daily growth rate and the finite rate of increase ( $\lambda$ ).

#### Data analysis

The duration of developmental period, the percentage of survival, the duration of oviposition, longevity and fecundity of the beetles treated with fungal conidial suspension of different concentrations were compared using analysis of variance (ANOVA). The difference between the means among the different concentrations were compared by using LSD test (P = 0.05). All the analyses were done using SAS program (SAS, 2000).

# **Results**

Influence of Verticillium lecanii 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  $1 \times 10^6$ ,  $1 \times 10^7$  and  $1 \times 10^8$  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  $1 \times 10^8$  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  $(1 \times 10^4, 1 \times 10^5, 1 \times 10^6, 1 \times 10^7, 1 \times 10^8 \text{ conidia/mL})$  as shown in Table 1.

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 2).

**Table 1** Developmental period (Mean  $\pm$  SEM) for different life stages of Serangium japonicum treated with different concentrationsof Verticillium lecanii (days).

Treatments (conidia/mL)	0.03% Tween-80	$1 \times 10^4$	$1 \times 10^{5}$	$1 \times 10^{6}$	$1 \times 10^7$	$1 \times 10^{8}$	<i>F</i> , df, <i>P</i>
Egg	17.93 c	18.59 b	18.71 b	18.87ab	19.09 a	19.15 a	0.11, 5,
	$(\pm 0.08)$	(± 0.10)	(± 0.11)	(± 0.10)	(± 0.12)	$(\pm 0.11)$	0.0473
1st instar	13.83 c	13.93 c	14.01 c	14.28 b	14.28 b	14.62 a	0.10, 5,
i st mstar	$(\pm 0.07)$	(± 0.03)	$(\pm 0.07)$	(± 0.12)	(± 0.06)	$(\pm 0.06)$	0.0195
2nd instar	11.94 d	12.14 cd	12.33 bc	12.43 b	12.48 b	12.71 a	0.06, 5,
	$(\pm 0.08)$	$(\pm 0.05)$	(± 0.04)	$(\pm 0.09)$	$(\pm 0.07)$	$(\pm 0.08)$	0.001
3rd instar	10.19 b	10.30 b	10.35 b	10.86 a	10.96 a	10.96 a	0.06, 5,
	(± 0.03)	(± 0.03)	(± 0.04)	$(\pm 0.07)$	(± 0.03)	(± 0.21)	0.0376
4th instar	8.70 c	8.82 bc	8.88 b	9.17 a	9.21 a	9.24 a	0.07, 5,
	$(\pm 0.05)$	$(\pm 0.04)$	$(\pm 0.04)$	$(\pm 0.05)$	$(\pm 0.05)$	$(\pm 0.04)$	0.0284
Pupa	4.53 c	4.58 c	4.72b	4.91 a	4.94 a	4.99 a	0.09, 5,
	$(\pm 0.04)$	(± 0.03)	$(\pm 0.04)$	$(\pm 0.70)$	$(\pm 0.03)$	$(\pm 0.03)$	0.0275

Means compared by one-way ANOVA, number within same row followed by different letters are significantly different (LSD Test, P < 0.05).

# Fecundity

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) were 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 3).

## Pre-ovipoition period

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.

# Adult longevity

Longevity of adult females treated with different concentrations  $(1 \times 10^4, 1 \times 10^5, 1 \times 10^6, 1 \times 10^7, 1 \times 10^8 \text{ conidia/} \text{mL})$  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 (Table 3). *V. lecanii* showed no effect on the longevity of *S. japonicum* females.

# Life table characteristics

The values of  $l_x$  (survivorship at corresponding time) and  $m_x$  (number of female eggs laid according to the sex ration per female per day) were almost similar among all the treatments as well as control (Fig. 1).

The value of the net reproduction rate observed for the control was significantly higher when compared to differ-

**Table 2** Survival ratio (Mean  $\pm$  SEM) of different stages of Serangium japonicum treated with different concentrations of Verticilliumlecanii.

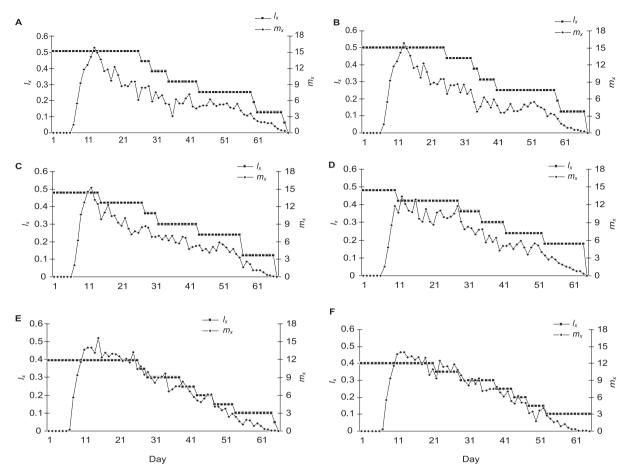
Treatments (conidia/mL)	0.03% Tween-80	$1 \times 10^4$	$1 \times 10^{5}$	$1 \times 10^{6}$	1 × 10 <sup>7</sup>	$1 \times 10^{8}$	<i>F</i> , df, <i>P</i>
Egg	0.75 a	0.70 a	0.71 a	0.71 a	0.71 a	0.65 a	0.87, 5,
	(± 0.028)	(± 0.050)	(± 0.016)	(± 0.016)	(± 0.033)	(± 0.050)	0.5308
1st instar	0.80a	0.81 a	0.80 a	0.75a	0.76 a	0.73a	1.55, 5,
	(± 0.028)	(± 0.016)	(± 0.028)	(± 0.028)	(± 0.016)	(± 0.016)	0.2478
2nd instar	0.81 a	0.86 a	0.83 a	0.80 a	0.83 a	0.78 a	0.80, 5,
	(± 0.033)	(± 0.044)	(± 0.016)	(± 0.013)	(± 0.044)	(± 0.033)	0.5705
3rd instar	0.86 a	0.90 a	0.86 a	0.86 a	0.83 a	0.88 a	0.62, 5,
	(± 0.016)	(± 0.028)	(± 0.016)	(± 0.044)	(± 0.016)	(± 0.033)	0.6852
4th instar	0.95 a	0.98 a	0.93 a	0.93 a	0.88 a	0.90 a	0.53, 5,
	(± 0.050)	(± 0.016)	(± 0.044)	(± 0.044)	(± 0.072)	(± 0.050)	0.7520
Pupa	1.00 a	0.95 a	0.96 a	0.95 a	0.93 a	0.95 a	0.57, 5,
	(± 0.000)	(± 0.028)	(± 0.033)	(± 0.028)	(± 0.044)	(± 0.028)	0.7220

Means compared by one-way ANOVA, number within same row followed by different letters are significantly different (LSD Test, P < 0.05).

**Table 3** The fecundity, preoviposition and longevity (Mean  $\pm$  SEM) of *Serangium japonicum* treated with different concentrations of *Verticillium lecanii*.

Treatments (conidia/mL)	0.03% Tween-80	$1 \times 10^4$	$1 \times 10^{5}$	$1 \times 10^{6}$	$1 \times 10^7$	$1 \times 10^{8}$	<i>F</i> , df, <i>P</i>
Fecundity	569.70	556.20	552.20	538.60	526.33	510.18	1.66, 5,
	(± 8.01 a)	(± 2.14 a)	(± 9.07 a)	(± 7.35 ab)	(± 2.60 b)	(± 4.62 b)	0.0490
Preoviposition	6.58	6.54	6.62	6.50	6.87	6.75	0.82, 5,
(days)	$(\pm 0.19 \text{ a})$	$(\pm 0.18  ext{ a})$	$(\pm 0.18  ext{ a})$	(± 0.18 a)	$(\pm 0.12 \text{ a})$	$(\pm 0.16 \text{ a})$	0.5427
Longevity	66.62	67.74	63.87	63.87	65.50	64.62	0.05, 5,
(days)	$(\pm 6.28 \text{ a})$	$(\pm 5.87  ext{ a})$	$(\pm 6.82  ext{ a})$	(± 7.14 a)	$(\pm 5.30 \text{ a})$	$(\pm 5.49 \text{ a})$	0.9985

Means compared by one-way ANOVA, number within same row followed by different letters are significantly different (LSD Test, P < 0.05).



**Fig. 1** Trends of *Serangium japonicum* treated with different concentrations of *Verticillium lecanii*,  $(l_x)$ : age-specific survival rates and  $(m_x)$ : mortality. (A) 0.03% Tween 80; (B)10<sup>4</sup> conidia/mL; (C) 10<sup>5</sup> conidia/mL; (D) 10<sup>6</sup> conidia/mL; (E) 10<sup>7</sup> conidia/mL; (F) 10<sup>8</sup> conidia/mL.

ent treatments (F = 12.02, df =5, P = 0.004 4). 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  $(1 \times 10^4, 1 \times 10^5, 1 \times 10^6, 1 \times 10^7, 1 \times 10^8 \text{ conidia/mL})$  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).

# Discussion

Little information is available on sub-lethal and chronic effects of entomopathogenic fungi on the developmental

time of beetles when a fungus is directly applied. The total developmental period from egg to adult of *S. japonicum* feeding on nymphs of *B. tabaci* on eggplant (17.42 days) recorded by Yao (2003) is almost same as that recorded in this study in the control. The developmental period of each immature stage of *S. japonicum* was within 15–16 days and remained unaffected by the fungi (Fazal, 2004). Similar to Poprawaski *et al.* (1998), eggs, larval and pupal developmental times were not significantly different for all application dosages with respect to controls. Thus, it can be concluded that *V. lecanii* had no sub-lethal effects on developmental biology of *S. japonicum* surviving direct contamination by the entomopathogenic fungi.

Fertility of females varied substantially over the different concentrations (Table 3). This decrease in the rate of fertilization might be derived from a decline in the female physiological state related to: (i) fungal colonization of tissues, such as fat body (source of vitellogeins) and ovaries (Blay & Yuval, 1999); (ii) fungal toxin production

Treatments (conidia/mL)	0.03% Tween-80	$1 \times 10^4$	$1 \times 10^5$	$1 \times 10^{6}$	$1 \times 10^7$	$1 \times 10^{8}$	<i>F</i> , df, <i>P</i>
$R_0$	102.10	96.40	93.53	92.48	84.37	84.07	12.02, 5,
(Progeny/female)	(± 2.73 a)	(± 1.96 ab)	$(\pm 0.74 \text{ b})$	$(\pm 2.43 \text{ b})$	$(\pm 1.11 \text{ c})$	$(\pm 2.32 c)$	0.0044
r	0.0998	0.0996	0.0986	0.0983	0.0965	0.0969	1.61, 5,
(Progeny/female)	$(\pm 0.0013 \text{ a})$	(± 0.1241 a)	$(\pm 0.0005 \text{ a})$	$(\pm 0.0008 \text{ a})$	$(\pm 0.0018 \text{ a})$	(± 0.0015 a)	0.2873
Т	46.32	45.84	45.99	46.00	45.92	45.74	0.17, 5,
(days)	$(\pm 0.32 \text{ a})$	(± 0.16 a)	(± 0.34 a)	(± 1.22 a)	$(\pm 0.85 a)$	(± 0.60 a)	0.9636
λ	1.105	1.104	1.103	1.103	1.101	1.101	1.62, 5,
(days)	$(\pm 0.001 \text{ a})$	$(\pm 0.000 a)$	$(\pm 0.006 a)$	$(\pm 0.009 a)$	(± 0.001 a)	$(\pm 0.002 a)$	0.2868

**Table 4** Life table parameters (Mean  $\pm$  SEM) of Serangium japonicum treated with different concentrations of Verticillium lecanii.

Means compared by one-way ANOVA, number within same row followed by different letters are significantly different (LSD Test, P < 0.05).

to overcome insect cellular and humoral immune reactions (Inglis et al., 2001; Quesada-Moraga & Vey, 2004); and (iii) depletion of resources needed for vital egg production such as proteins (Carey et al., 1998). Sewify and El Arnaouty (1998) studied the effect of infection of Chrysoperla carnea larvae with the fungus Verticillium lecanii (Zimm.) Viegas in the laboratory with two fungal isolates under relative humidities of 65% and 95%. One isolate was highly pathogenic to third instar larvae, impaired their feeding and searching capacity, and decreased emergence of adults. Feeding of the larvae with infected aphids had similar effects, and also decreased fecundity. Wang et al. (2005) studied the effect of two strains on Delphastus catalinae, a predator of whitefly. They observed that D. catalinae suffered no significant effects on fecundity and longevity, when exposed to V. lecanii. The net reproduction rate in the control was more than that observed for different concentrations, the mean generation time (T) was not significant, and the  $r_m$  values were similar for different concentrations. These results are also in-line with the findings of Neilson et al. (2005), who studied the effect of M. anisopilae on survival and reproduction of Spalangia cameroni. They showed that by testing the sensitivity of  $r_m$ , where the infection rate of fungal treated females  $(1 \times 10^8 \text{ conidia/mL})$  was increased from 50% to 100%. A pronounced decline in survival and reproduction from day 7 onwards had no impact on the value of  $r_{\rm m}$ , which was changed from 0.1350 to 0.1321 days<sup>-1</sup>. However, if the mortality of 100% infected females had occurred 3 days earlier, the value of  $r_m$  would have been reduced to 0.1137 days-1.

It is apparent from our research results that there are no effects of *V. lecanii* application on predators. It can be concluded that *V. lecanii* control strategies tested are compatible with natural predators and their incorporation has promising prospects for the control of whitefly. The

results mentioned above indicate that the interaction among biocontrol agents is positive to a greater extent with minimum risk hazards.

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