Influence of the Entomopathogenic Fungus, *Verticillium lecanii* on the Whitefly Predator, *Axinoscymnus cardilobus* (Coleoptera: Coccinellidae) Under Laboratory Conditions

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**Abstract.**—The effects of entomopathogenic fungus *Verticillium lecanii* on the biological characteristics and life table of the whitefly predator, *Axinoscymnus cardilobus* Pang and Ren (Coleoptera: Coccinellidae) were studied by using five different conidial concentrations under laboratory conditions. The total developmental period (from egg to adult) among the treatments did not differ between fungus treatments and control. The longest total development period for *A. cardilobus* was observed when treated with $1 \times 10^7$ spore/ml. No significant difference was found for *V*. *lecanii* on the percent survival of all immature stages of *A. cardilobus*. The treatment with *V. lecanii* did not elicit any significant effect on mean generation time, intrinsic rate, the finite rate of increase and longevity of *A. cardilobus* when compared with control treatment. It can be concluded that control strategies tested are compatible to a greater extent and incorporation of these have promising prospect for control of whitefly.

**Key words:** Entomopathogenic Fungus, whitefly predator, *Axinoscymnus cardilobus*, *Verticillium lecanii*

**INTRODUCTION**

*Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) is a very severe pest in all tropical and subtropical regions of the world (Brown, 1994; Oliveira *et al*. 2001). Isolates of *Zoophthora radicans* (Brefeld) Batko (*Pell et al*., 1993), *Paecilomyces fumosoroseus* (Wize) Brown & Smith (*Altre et al*., 1999), and *Beauveria bassiana* (Balsamo) Vuillemin can infect whitefly under greenhouse or field conditions.

Of these fungal species, *Verticillium lecanii* (Zimm) Viegas is one of the most promising fungal species for control of whiteflies, and other insect pests. Wang *et al.* (2005) studied virulence of six strains of *V. lecanii* against *Bemisia tabaci*. Their results indicated that strains V16063, V3450 and Vp28 were the most virulent with LC$_{50}$ values of 2.57×$10^{5}$, 6.03×$10^{5}$ and 6.05×$10^{5}$ conidia/mL, respectively.

Whiteflies are also attacked by a variety of predatory coccinellids (Nordlund and Legaspi, 1996; Gerling *et al*., 2001; Ren *et al*., 2004). Earlier studies have indicated that the coccinellid predators belonging to the genus *Axinoscymnus* (Coleoptera: Coccinellidae) are consistently considered the most suitable whitefly predators under field as well as laboratory conditions (Ren and Pang, 1992; Huang *et al*., 2003, 2006a, b, 2008). *A. cardilobus* feeds upon all stages of whitefly and had shown a great potential as an effective biocontrol agent against *B. tabaci* (Huang *et al*., 2003, 2006a, b). Mycoses in nature have been observed in a number of predatory insects (Goettel *et al*., 2000); however little is known about their epizootology and resultant effects on predators particularly the non target species. Many species of predatory insects seems refractory to fungal infection when challenged under laboratory conditions (Porawaski *et al*., 1998).

The infectivity and pathogenicity of entomopathogenic fungi have been screened on coccinellids under laboratory conditions besides developing a standard bioassay protocol (James and Lighthart, 1992; Todorova *et al*., 1994). However, effects of insect control agents (entomopathogens
and chemical pesticides) other than direct kill may also inhibit the beneficial capacity of non-target natural enemies of pests. Although, many reports are available on short term detrimental effects of entomopathogenic fungi on non target organisms (Goettel et al., 1990), little is known about their indirect effects. This study therefore, aimed at evaluating the direct impacts of \textit{V. lecanii} on the survival and reproductive biology of \textit{A. cardilobus} by using life table analysis for quantitative evaluations of natural enemies in terms of detailed description of age-specific mortality of individuals in the whitefly population.

**MATERIALS AND METHODS**

**Insects**

\textit{Bemisia tabaci} and \textit{Axinoscymnus cardilobus} were collected from the stock colony kept in greenhouse of the Engineering Research Center of Biological Control, South China Agricultural University (SCAU) on poinsettia. Plants were grown in plastic pots having a diameter of 15-cm. Slow release fertilizer (N: P: K=13:7:15) was added as required to maintain normal plant growth. Intact plants were maintained in another greenhouse. \textit{A. cardilobus} used in the experiments were moved into an air-conditioned room at 26 ± 2 \(^\circ\)C, RH 80~90\%, L : D = 14 : 10, after maintaining on host plants for several generations. A large number of \textit{B. tabaci} adults were put into the plastic bags with small holes for ventilation. The leaves of poinsettia were fixed in plastic bags. Six hours later, \textit{B. tabaci} adults were removed. Nymphs of \textit{B. tabaci} were kept on host plants in an air-conditioned room, when nymphs of whiteflies entered into second instar, 100 second instar nymphs per leaf were marked in a circle with colour pen for treatment.

**Fungi preparing**

\textit{V. lecanii} used for bioassays was isolated from \textit{Trauleurodes} sp, maintained in tubes containing Sabouraud Dextrose Agar (SDA) and kept in the Engineering Research Center of Biological Control, SCAU, was cultured on potato dextrose agar (PDA) and incubated at 26 ± 2\(^\circ\)C for 10 days. Conidia were harvested with deionized water containing 0.02% Tween 80 and sieved through filter paper into sterile vials. Conidia were counted in a compound microscope using a hemocytometer (0.0625mm\(^2\); Fuchs-Rosenthal Merch Eurolab) to calibrate a suspension of \(1 \times 10^7\) conidia/ml of \textit{V. lecanii}. Lower concentrations of \(1 \times 10^6\) to \(1 \times 10^3\) conidia/ml were prepared by serial dilutions.

Spore viability was determined before preparation of suspension by spreading 0.2 ml of \(1 \times 10^4\) conidia/ml suspension on PDA and estimating the number of germinated propagules after 24 hrs of incubation at room temperature. Propagules were considered viable when the germ tube lengths correspond to the width. The viability of conidia was assessed immediately just prior to the start of experiment and percentage germination was estimated to > 95% for all experiments.

**Influence of \textit{V. lecanii} on immature stages of \textit{A. cardilobus}**

The different life stages of \textit{A. cardilobus} (eggs, 1st instars, 2nd instars, 3rd instars, 4th instars and pupae) on poinsettia leaves having eggs and immatures of \textit{B. tabaci} as food were directly dipped into the prepared fungal suspensions (\(1 \times 10^3\), \(1 \times 10^4\), \(1 \times 10^5\), \(1 \times 10^6\), \(1 \times 10^7\) conidia/ml) for 15 seconds and then dried up on filter paper. Each filter paper with treated stages was placed into a Petri dish. To maintain nearly saturated humidity, the lids of the Petri dishes were closed with strip of parafilm and incubated for 24 hours at 26 ± 2\(^\circ\)C and 14:10 (L:D). Twenty four hours later, treated stages of \textit{A. cardilobus} were removed to new Petri dish with poinsettia leaves having mixed population of \textit{B. tabaci} eggs and nymphs as food and kept at 60 – 70% RH under similar conditions of temperature and light. Petri dishes were covered with plastic sheet having small holes for aeration.

The mortality of beetles was recorded at 24 h intervals until adult emergence. The dead larvae were sterilised 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. Beetles showing mycelia and conidia of \textit{V. lecanii} on the cadavers were considered dead from infection of the fungus. The
Petri dishes were incubated at 26±2°C and 80%±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, 40 individuals of every life stage of A. cardilobus were used for each fungal concentration and the entire experiment was repeated 5 times.

**Influence of V. lecanii on A. cardilobus females**

Pairs of sexually mature beetles (4 days old) collected from the stock culture were dipped into five different conidial concentrations (1×10³, 1×10⁴, 1×10⁵, 1×10⁶, 1×10⁷ conidia/ml) of V. lecanii for 15 seconds while adults of A. cardilobus were treated with water mixed with 0.1% Tween 80 served as a control. The beetles were then transferred to plastic Petri dishes with poinsettia plant leaves. The Petri dishes were incubated at 26 ± 2°C, 80% ± 5% for 24 h. The leaves were changed every day and the numbers of eggs laid by each pair were recorded until adult died. For each conidial concentration six pairs of the beetles were used against each conidial concentration and the entire experiment was repeated 5 times.

**Life table analysis**

Life and fertility tables were calculated from the cohort of eggs according to the method of Fatiha et al. (2008). 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 (lx) was also calculated. The intrinsic rate of population increase (rm) was calculated using the Fatiha et al. (2008):

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

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 developmental period, percentage of survival, duration of oviposition, longevity and fecundity of the beetles treated with fungal suspension of different concentrations were compared using Analysis of Variance (ANOVA). The difference between the means among the different concentrations were compared using Duncan’s Multiple Range Test (DMRT \( P = 0.05 \)). All the analyses were done using SAS program (SAS, 2000).

**RESULTS**

**Influence of V. lecanii on the survival and development of A. cardilobus**

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 when compared with that of the control. The increasing concentrations of conidia also did not have any significant effect on survival (Table I).

The total developmental periods for all immature stages (from eggs to adult emergence) at different concentrations were not significantly different from the control (Table II). The total development period was longest for the colony treated with conidial concentrations of 1×10⁷ spore/ml, and the shortest development period for different life stages was observed in the control colony. The pre-imaginal developmental time was longest for eggs and 1st instar larvae and pupae at different concentrations (1×10³, 1×10⁴, 1×10⁵, 1×10⁶, 1×10⁷ conidia/ml), while it was shortest for fourth instar larvae and pupae (Table II).

**Fecundity**

Data indicated that the application of the pathogenic fungus V. lecanii had no significant effect on the fecundity of A. cardilobus. The lowest number of eggs was observed when the predator was treated with 1×10⁶ conidia/ml with an average
Table I.- Percentage survival (Mean ± SE) of *A. cardilobus* immature stages treated with different concentrations of *V. lecanii*.

<table>
<thead>
<tr>
<th>Treatments (conidia/ml)</th>
<th>Egg</th>
<th>1st instar</th>
<th>2nd instar</th>
<th>3rd instar</th>
<th>4th instar</th>
<th>Pupa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.91±0.011 a</td>
<td>0.92 ±0.025a</td>
<td>1.00 ±0.000 a</td>
<td>1.00 ±0.000 a</td>
<td>1.00 ±0.000 a</td>
<td>0.89 ±0.023 a</td>
</tr>
<tr>
<td>1×10³</td>
<td>0.90±0.014 a</td>
<td>0.91 ±0.074 a</td>
<td>1.00 ±0.000 a</td>
<td>1.00 ±0.000 a</td>
<td>1.00 ±0.000 a</td>
<td>0.89±0.028 a</td>
</tr>
<tr>
<td>1×10⁴</td>
<td>0.88±0.074a</td>
<td>0.90 ±0.012 a</td>
<td>0.98 ±0.011 a</td>
<td>1.00 ±0.000 a</td>
<td>1.00 ±0.000 a</td>
<td>0.88±0.020 a</td>
</tr>
<tr>
<td>1×10⁵</td>
<td>0.88±0.074a</td>
<td>0.90 ±0.013 a</td>
<td>0.97 ±0.022 a</td>
<td>1.00 ±0.000 a</td>
<td>1.00 ±0.000 a</td>
<td>0.86±0.025 a</td>
</tr>
<tr>
<td>1×10⁶</td>
<td>0.87±0.030a</td>
<td>0.89 ±0.022 a</td>
<td>0.96 ±0.034 a</td>
<td>0.98 ±0.000 a</td>
<td>0.98±0.021 a</td>
<td>0.86±0.031 a</td>
</tr>
<tr>
<td>1×10⁷</td>
<td>0.86 ±0.031a</td>
<td>0.88 ±0.041 a</td>
<td>0.96 ±0.023 a</td>
<td>0.98±0.000 a</td>
<td>0.98±0.017 a</td>
<td>0.86±0.042 a</td>
</tr>
</tbody>
</table>

Means compared by one way ANOVA, number within the same column followed by the same letters are not significantly different (DMRT, \( P > 0.05 \)).

Table II.- Developmental periods (Mean±SEM days) of immature stages of *A. cardilobus* treated with different concentrations of *V. lecanii*.

<table>
<thead>
<tr>
<th>Treatments (conidia/ml)</th>
<th>Egg-Adult</th>
<th>1st instar-Adult</th>
<th>2nd instar-Adult</th>
<th>3rd instar-Adult</th>
<th>4th instar-Adult</th>
<th>Pupa-Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.5±1.69a</td>
<td>14.6 ±1.25a</td>
<td>13.1±1.14 a</td>
<td>11.3±1.26 a</td>
<td>10.1±1.61 a</td>
<td>7.5 ±1.05a</td>
</tr>
<tr>
<td>1×10³</td>
<td>18.5 ±1.21a</td>
<td>14.6 ±1.02a</td>
<td>13.2±1.37 a</td>
<td>11.4±1.19 a</td>
<td>10.1±1.48 a</td>
<td>7.5 ±0.88 a</td>
</tr>
<tr>
<td>1×10⁴</td>
<td>18.5 ±1.55a</td>
<td>14.7±1.36a</td>
<td>13.2±1.05a</td>
<td>11.4±1.05a</td>
<td>10.1±1.02 a</td>
<td>7.5 ±1.00 a</td>
</tr>
<tr>
<td>1×10⁵</td>
<td>18.6 ±1.29a</td>
<td>14.7±1.11 a</td>
<td>13.4±1.77a</td>
<td>11.4±1.04a</td>
<td>10.1±0.95 a</td>
<td>7.5 ±0.95 a</td>
</tr>
<tr>
<td>1×10⁶</td>
<td>18.8 ±1.42a</td>
<td>14.8±1.41a</td>
<td>13.6±1.01a</td>
<td>11.5±1.41a</td>
<td>10.3±1.06 a</td>
<td>7.6 ±1.00 a</td>
</tr>
<tr>
<td>1×10⁷</td>
<td>18.8 ±1.16a</td>
<td>15.0±1.06a</td>
<td>13.7±1.23a</td>
<td>11.5±1.29a</td>
<td>10.3±1.25 a</td>
<td>7.6 ±1.02 a</td>
</tr>
</tbody>
</table>

Means compared by one way ANOVA, number within the same column followed by the same letters are not significantly different (DMRT, \( P > 0.05 \)).

of 126.2±34.85 eggs/female, whereas maximum number of eggs (133.2±32.18 eggs) was recorded in control beetles (Table III).

Table III.- Fecundity (Mean±SE) preoviposition and longevity of *A. cardilobus* female treated with different concentrations of *V. lecanii*.

<table>
<thead>
<tr>
<th>Treatments (Conidia/ml)</th>
<th>Fecundity</th>
<th>Preoviposition (days)</th>
<th>Longevity (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>133.2±29.9 a</td>
<td>7.8±1.17 a</td>
<td>71.7±8.5 a</td>
</tr>
<tr>
<td>1×10³</td>
<td>130.2±39.5 a</td>
<td>7.9±1.36 a</td>
<td>70.2±7.5 a</td>
</tr>
<tr>
<td>1×10⁴</td>
<td>128.5±36.8 a</td>
<td>7.9±1.41 a</td>
<td>69.8±6.9 a</td>
</tr>
<tr>
<td>1×10⁵</td>
<td>127.8±32.3 a</td>
<td>8.0±1.01 a</td>
<td>68.2±7.2 a</td>
</tr>
<tr>
<td>1×10⁶</td>
<td>126.2±34.9 a</td>
<td>8.1±1.53 a</td>
<td>66.8±5.5 a</td>
</tr>
<tr>
<td>1×10⁷</td>
<td>126.7±30.9 a</td>
<td>8.3±1.61 a</td>
<td>64.7±6.8 a</td>
</tr>
</tbody>
</table>

Means compared by one way ANOVA, number within the same column followed by the same letter are not significantly different (DMRT, \( P > 0.05 \)).

**Pre-oviposition period**

The duration of the pre-oviposition period of *A. cardilobus* showed no significant difference among the treatments compared to the control (Table III). The longest lowest pre-oviposition period (8.3±1.61 days) was recorded in 1×10⁷ conidia/ml while the shortest one was observed in the control with a mean value of 7.8±1.97 days.

**Adult longevity**

Longevity of adult females treated with different concentrations (1×10³, 1×10⁴, 1×10⁵, 1×10⁶, 1×10⁷ conidia/ml) did not differ significantly as compared to the control, while, the longest longevity of 71.7±8.45 days was observed in control, the shortest longevity of 64.7±6.81 days was recorded in 1×10⁷ conidia/ml (Table III).

**Life table parameters**

The observed values of the net reproduction rate were not significantly different from among
Table IV.- Life table parameters (Mean ± SE) of A. cardilobus treated with different concentrations of V. lecanii

<table>
<thead>
<tr>
<th>Treatments (Conidia/ml)</th>
<th>$R_o$ (Progeny/female)</th>
<th>$r_m$ (Progeny/female)</th>
<th>$T$ (days)</th>
<th>$\lambda$ (Finite rate of increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>49.8 ± 9.01 a</td>
<td>0.0571 ± 0.06 a</td>
<td>67.9 ± 12.47 a</td>
<td>1.061 ± 0.43 a</td>
</tr>
<tr>
<td>1×10^3</td>
<td>48.0 ± 8.24 a</td>
<td>0.0576 ± 0.10 a</td>
<td>67.4 ± 20.26 a</td>
<td>1.059 ± 0.32 a</td>
</tr>
<tr>
<td>1×10^4</td>
<td>47.8 ± 7.73 a</td>
<td>0.0574 ± 0.10 a</td>
<td>66.7 ± 13.89 a</td>
<td>1.059 ± 0.51 a</td>
</tr>
<tr>
<td>1×10^5</td>
<td>46.1 ± 8.13 a</td>
<td>0.0580 ± 0.23 a</td>
<td>65.6 ± 13.31 a</td>
<td>1.060 ± 0.38 a</td>
</tr>
<tr>
<td>1×10^6</td>
<td>45.2 ± 8.56 a</td>
<td>0.0584 ± 0.21 a</td>
<td>65.1 ± 11.45 a</td>
<td>1.060 ± 0.29 a</td>
</tr>
<tr>
<td>1×10^7</td>
<td>44.8 ± 6.21 a</td>
<td>0.0587 ± 0.09 a</td>
<td>64.8 ± 10.09 a</td>
<td>1.060 ± 0.48 a</td>
</tr>
<tr>
<td>df, F, P</td>
<td>5, 0.75, 0.5816</td>
<td>5, 0.52, 0.5821</td>
<td>5, 1.06, 0.5083</td>
<td>5, 0.64, 0.6002</td>
</tr>
</tbody>
</table>

Means compared by one way ANOVA, number within the same column followed by the same letter are not significantly different (DMRT, $P>0.05$)

fungal treatments as compared to the control. The net reproductive rate was lowest in $1 \times 10^7$ conidia/ml with a mean value of 44.8 ± 6.21 progeny/female while the highest net reproductive (49.8 ± 9.01 progeny/female) was observed for the control (Table IV).

The values of $r_m$ were significantly similar among different treatments ($1 \times 10^3$, $1 \times 10^4$, $1 \times 10^5$, $1 \times 10^6$, $1 \times 10^7$ conidia/ml) and the control. The mean generation time ($T$) was also significantly similar among the treatments when compared with the control.

**DISCUSSION**

To evaluate the effect of V. lecanii used to control whiteflies, we looked how a direct application of conidia to a predatory coccinellid, A. cardilobus affects the survival. The findings of current research work clearly suggest that different conidial concentrations of V. lecanii had a very low pathogenic effect against immatures of A. cardilobus when compared with control. Our present results are in accordance with Poprawaski et al. (1998), who reported only 2.2% corrected mortality of 2nd instar of another coccinellid predator Serangium parcesetosum (Coleoptera: Coccinellidae) up to adult emergence when sprayed with low, medium and high dosages of P. fumosoroseus. They further reported that neither Beauveria bassiana nor P. fumosoroseus had sublethal effects on biology of S. parcesetosum. Also, James and Lighthart (1992) treated the 1st instar of Hippodemia convergens (Coleoptera: Coccinellidae) for 10 seconds in five concentrations of four entomopathogenic fungi. Two B. bassiana strains caused 75% to 95% mortality (Quesada-Moraga and Vey, 2004), Metarhizium anisopliae caused up to 56% mortality (Nielsen et al., 2005). They emphasize that further research is needed to determine how direct effects observed in laboratory play out in field environment.

Little information is available on sublethal and chronic effects of entomopathogenic fungi (when applied directly to the insects) on developmental time of A. cardilobus. Development time of Serangium japonicum (Coleoptera: Coccinellidae) reported by Yao (2003) for larvae feeding on eggs and 1st instar of B. tabaci was 14-15 days at 26°C. In the present work, the developmental time of each immature stage was within 7-19 days and was remained unaffected by the fungi. Similar to Poprawaski et al. (1998), eggs, larval and pupal developmental times were not significantly different for all application dosages with respect to control. Thus, it can be concluded that P. fumosoroseus had no sublethal effects on developmental biology of S. japonicum surviving the direct contamination by the entomopathogenic fungi.

In the present work, fertility, longevity and life table parameters of females were almost similar over the different concentrations (Tables III, IV). Our data are in agreement with those reported by Wang et al. (2005) who found that Delphastus catalinae suffered no significant effect on fecundity and longevity when exposed to V. lecanii, the net reproduction rate in the control was more than that observed for different concentrations. Also, the mean generation time ($T$) and the $r_m$ values were
similar in the different concentrations. These results are also in-line with the findings of Fatiha et al. (2008), who studied the effect of *V. lecanii* biological characteristics and life table of *S. japonicum*. They showed non significant 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. Sewify and El Arnaouty (1998) studied the effect of 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.

It can be concluded that control strategies tested are compatible to a greater extent and incorporation of these have promising prospect for control of whitefly. The predatory species (*A. cardilobus*) was not highly susceptible to *V. lecanii* when spores were applied directly to the predator and thus beneficial capacity of predator was not affected dramatically. Further knowledge is needed about the timing adjustments for various releases of both biological control agents to obtain maximum additive effectiveness.

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