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# Impact of entomopathogenic fungus, *Verticillium lecanii* (Zimmerman) Viegas on natural enemies of cabbage aphid, *Brevicoryne brassicae* (Linnaeus) and other beneficial insects

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**ABSTRACT:** The entomopathogenic fungus, *Verticillium lecanii* (Zimm.) Viegas was evaluated in the laboratory for its safety to natural enemies of cabbage aphid, *Trichogramma chilonis* Ishii and two species of silkworms using different formulations and inoculation methods. The fungus was not pathogenic to *Chrysoperla carnea* (Stephens), *Coccinella septempunctata* (Linnaeus), *Episyrphus balteatus* (De Geer) and *Samia cynthia ricini* (Boisduval), but was found to be pathogenic to *Bombyx mori* (Linnaeus). Aphid mummification and *Diacretiella rapae* adult emergence were significantly affected by the fungus. Parasitism, adult emergence and adult longevity of *Trichogramma chilonis* were affected by fungal treatments. The adverse effects of the fungus on all insects tested were less than 25 percent. Our results suggest that *V. lecanii* is compatible with natural enemies of cabbage aphid and *T. chilonis* and is harmless to silk worm, therefore can be used for integrated management of cabbage aphid.

**KEY WORDS:** Beneficial insects, cabbage aphid, natural enemies, safety, *Verticillium lecanii*

## INTRODUCTION

Biopesticides are inherently different from conventional pesticides with fundamentally different modes of action and lower risk of adverse effects from their use (Geetha and Rabindra, 2001). Once a commercial mycoinsecticide formulation has been developed, it must meet specific safety criteria before it can be registered, marketed and distributed (Lockwood, 1993). Fungi within the class Hyphomycetes are generally considered to have much wider host ranges than Entomophthorales fungi (Goettel and Inglis, 1997) and may infect both target pest species and their insect natural enemies (Roy and Pell, 2000). However, although many species of these fungi have been recorded from numerous different insect orders, isolates or strains within a species are frequently only virulent to a few arthropod species (Feng *et al.*, 1994). The impact of entomopathogens upon arthropod natural enemies, specially the predaceous coccinellids, is poorly understood and scarcely has been studied (Cottrell and Shapiro, 2003). In order to protect invertebrate biocontrol agents of insect pests, the possible adverse effects of insect fungal pathogens on these organisms must be considered (Laird *et al.*, 1990). Roy and Pell (2000) demonstrated that although *Verticillium lecanii* has been reported from a number of insects and mite hosts, in glasshouse trials, target-derived isolates against aphids and whitefly did not infect *Tetranychus urticae* (red spider mite), *Phytoseiulus persimilis* (spider mite predator) or *Encarcia formosa* (whitefly parasitoid). Askary and Brodeur (1999) indicated that *V. lecanii* is pathogenic to the aphid parasitoid, *Aphidius nigripes*, but only when aphid populations are heavily infected by the fungus. Similarly, *Paecilomyces fumosoroseus* infects the aphid parasitoid, *Aphelinus asychis*, but only at high pathogen doses and at high humidity (Lacey *et al.*, 1997). To ensure maximum safety to invertebrate non-target organisms, fungal

control agents must not become just a replacement for chemical insecticides, but should be integrated with other control strategies (Fuxa, 1987).

In a previous study (Derakhshan *et al.*, 2006), V.I-7 isolate of *V. lecanii* was found to be the most virulent isolate to *B. brassicae*. In this study we have evaluated the safety of the fungus to natural enemies of cabbage aphid and *Trichogramma chilonis* which are used as parasitoids in cabbage fields to control other pests of cabbage and two species of silkworms.

## MATERIALS AND METHODS

Laboratory bioassay studies with V. I-7 isolate of *V. lecanii* were conducted to study its infectivity to four natural enemies of cabbage aphid, *viz.*, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae), *Episyrphus balteatus* (De Geer), (Diptera: Syrphidae) and *Diaeretiella rapae* (M. Intosh) (Hymenoptera: Braconidae), an egg parasite *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) and two species of silkworms namely, mulberry silkworm (*Bombyx mori* (Linnaeus) (Lepidoptera: Bombycidae)) and Eri silkworm (*Samia cynthia ricini* (Boisduval) (Lepidoptera: Saturniidae). All tests were conducted by using a spore concentration of $1 \times 10^8$ spores/ml ($1 \times 10^8$ CFU/g in case of talc powder).

**Effect of the fungus on *C. carnea***

The test was carried out with six different methods of inoculations:

1- Dipping *C. carnea* larvae in talc formulation (dip larva method),

2- Dipping *C. carnea* larvae in oil in water emulsion formulation (1% kerosene,0.05% Tween 80),

3- Dipping *C. carnea* larvae in spore suspension only,

4- Allowing *C. carnea* larvae to crawl on dried spore suspension (Dry film method),

5- Spraying spore suspension on host aphid (*B. brassicae*),

6- Dipping *C. carnea* larvae in 0.05% Tween-80 solution only as control.

The eggs of *C. carnea* were taken from mass production unit of PDBC. Each egg was transferred to individual house of plastic louvers. The bottom and the top surface of the louvers were closed tightly using transparent acrylic sheet. The sides were locked with the use of metal clips to avoid the escape of the predator. The eggs were allowed to hatch and third instar larvae were used for pathogenicity tests. The larvae were fed with fresh mixture of cabbage aphid nymphs till pupation. Each treatment was replicated three times, and each replication consisted of 10 larvae. Observations on larval mortality, pupation, adult emergence and mycosis were recorded.

**Effect of the fungus on *C. septempuntata* and *E. balteatus***

The treatments and bioassay were conducted as described above except that instead of plastic louvers, plastic Petri-plates were used. Coccinellid and syrphid eggs were taken from mass production unit of PDBC. Each treatment was replicated three times and five larvae per treatment were used. The larvae in each treatment were provided daily with fresh cabbage aphid nymphs till pupation. Observations on larval mortality, pupation, adult emergence and mycosis were recorded.

**Effect of the fungus on parasitoid, *D. rapae***

Effect of the fungus on parasitism and adult emergence of *D. rapae* were evaluated when either aphids were first exposed to the parasitoid then treated with the fungus or aphids first treated with fungus and then exposed to the parasitoid. This experiment was conducted with 4 replications and for each replication two freshly mated female parasitoids were released for 24 hours. Observations on mortality, mycosis, parasitism and adult emergence of aphid parasitoid were recorded. Per cent parasitism was calculated based on mummification of aphids.

**Effect of the fungus on *T. chilonis***

Tricho cards (*T. chilonis*) and *Corcyra* eggs (*Corcyra cephalonica*) were obtained from mass production unit of PDBC. Fresh eggs of *C. cephalonica* were exposed to UV light for 20 minutes to kill the developing embryo and then glued over strips of cards (2.5 x 10 cm) @ 200/card strips. Each card strip was put in a test tube. The tubes were then plugged with cotton wads. A streak of honey (50%) provided on the walls of tubes served as food for parasitoids after emergence. Treatments had five such cards as five replicates. Two experiments were conducted, where in the first set of experiments, the *Corcyra* eggs were treated with the fungus before releasing the wasps and in another set of

experiments, the *Corcyra* eggs were treated with the fungus after one day of releasing the wasps. Newly emerged parasitoids of *T. chilonis* were released at the rate of 50 parasitoids per treatment for 24 hours. In case of second set of experiments, the tricho cards exposed to parasitoids for 24 hours were treated with fungal formulations. Treatments were given as follows:

i) Dipping of tricho cards in talc powder suspension for 5 seconds and then the cards allowed to shade-dry and kept in test tubes.
ii) Dipping the tricho cards in oil in water emulsion formulation (1% kerosene, 0.05% Tween 80) as mentioned above.
iii) Dipping the tricho cards in spore suspension
iv) Dry film method, spraying the spore suspension into test tubes and allowed them to shade-dry and releasing wasps in the test tubes for two hours and then transferring them to new tubes provided with *Corcyra* egg cards.
v) Dipping the tricho cards in Tween-80 (0.05%) solution as control.

Observations for first set of experiments were made on parasitism, parasitoid emergence and adult longevity, and for second set of experiments observations were recorded on parasitoid emergence and adult longevity.

**Effect of the fungus on silkworms**

To test the pathogenicity of this fungus on silkworms, two species of silkworms *viz.*, Mulberry silkworm (*B. mori*) and Eri silkworm (*S. cynthia ricini*) were used. Silkworm larvae (third instar) used in the experiment were obtained from the Sericulture Department,UAS, Bangalore. The bioassay had 6 treatments. For each treatment 3 replications and for each replication 10 larvae were used. The larvae were maintained in individual Petri-plates and enough mulberry and castor leaves as food were given twice a day. The treatments were as follows:

i) Dipping larvae in suspension of talc powder formulation
ii) Dipping larvae in suspension of oil in water emulsion formulation (1% kerosene + 0.05 % Tween-80 solution)
iii) Dipping larvae in spore suspension only
iv) Allowing silkworm larvae to crawl on dried spore suspension
v) Dipping leaves in spore suspension
vi) Dipping larvae in 0.05% Tween-80 solution as control.

Observations were made on larval mortality, pupation, weight of pupa and adult emergence.

## RESULTS AND DISCUSSION

**Effect on *C. carnea***

Larval mortality (10 to 16.67 %), pupal period (9.38 to 10.4 day) and adult emergence (97.5 to 100.0 %) were not significantly different among the treatments (Table 1). Larval period was significantly affected by treatments ($P = 0.05$). It was lowest in control treatment (9.65 days) and highest in oil in water formulation treatment (10.6 days). In none of the cases of larval mortality, mycosis occurred. Thus, *V. lecanii* either as formulations or as unformulated spore suspension did not have any harmful effect on *C. carnea*. Pavlyushin (1996) reported that *V. lecanii*, *Beauveria bassiana* and *P. fumosoroseus* had an entomocidal effect on larvae of *C. carnea* and *C. nipponensis*. He concluded that larval mortality depended on the infection dosage. At a spore concentration of $5 \times 10^6$ and $2.5 \times 10^7$ spores/ml, mortality of *Chrysoperla* spp. was 4 percent and at $1 \times 10^8$ spores/ml mortality reached 28 percent. In our study, highest larval mortality (16.67%) was observed in oil in water emulsion treatment (Table 1).

**Effect on *C. septempunctata***

There was no significant difference in larval mortality (11.11 to 15.53%), larval period (8.50 to 9.26 days), pupal period (5.66 to 6.33 day), pupal weight (18.00 to 18.66 mg), and adult emergence (96.66 to 98.33) among the fungal treatments and control (Table 2). Although larval mortality was observed in all treatments, none of them was due to mycosis. This indicates that the mortality is not due to direct infection of the fungus. Cottrell and Shapiro (2003) reported that *B. bassiana* is pathogenic to two coccinellid species. They concluded that native species of lady beetle was more susceptible than exotic one to the fungus. Although coccinellids are susceptible to various entomopathogenic fungi (Cottrell and Shapiro, 2003; Ceryngier and Hodek, 1996), in our study it was found that not only *V. lecanii* (isolate V.I-7) is not pathogenic to this natural enemy but also has no significant adverse effect on its biological parameters (Table 2).

**Effect on *E. balteatus***

Larval mortality (6.67 to 13.33%), larval period

**Table 1. Effect of *V. lecanii* on some biological parameters of *C. carnea* (mean± SE)**

| Treatment | Larval mortality (%) | Larval period (days) | Pupal period (days) | Adult emergence (%) |
|---|---|---|---|---|
| Larval dip Talc powder | 13.33±3.33[a] | 9.77±0.29[ab] | 9.21±0.29[a] | 100.00±0.00[a] |
| Larval dip in Oil in water emulsion | 16.67±4.81[a] | 10.60±0.23[a] | 9.04±0.21[a] | 100.00±0.00[a] |
| Larval dip in Spore suspension | 13.33±3.33[a] | 10.50±0.40[ab] | 9.16±0.39[a] | 97.50±1.67[a] |
| Dry film (Spore suspension) | 13.33±3.33[a] | 9.70±0.18[b] | 8.86±0.30[a] | 98.75±1.67[a] |
| Treated aphid (Spore suspension) | 13.33±3.65[a] | 9.90±0.21[ab] | 9.24±0.28[a] | 97.50±3.33[a] |
| Control ( 0.05%Tween-80 solution) | 10.00±3.33[a] | 9.65±0.15[b] | 8.88±0.14[a] | 98.75±1.67[a] |

Means followed by similar letters in a column are not significantly different at 5%.

**Table 2. Effect of *V. lecanii* on some biological parameters of *C. septempunctata* (mean±SE)**

| Treatments | Larval mortality (%) | Larval period (day) | Pupal period (day) | Pupal weight (mg) | Adult emergence (%) |
|---|---|---|---|---|---|
| Larval dip in Talc powder formulation | 15.55±2.22a | 9.10±0.61a | 5.67±0.33a | 18.67±0.33a | 98.33±1.67a |
| Larval dip in Oil in water emulsion | 13.33±0.00a | 8.50±0.63a | 5.83±0.16a | 18.33±0.33a | 98.33±1.67a |
| Larval dip in Spore suspension | 13.33±0.00a | 9.26±0.54a | 5.67±0.16a | 18.00±0.57a | 98.33±1.67a |
| Dry film (Spore suspension) | 15.55±2.22 | 9.05±0.50a | 5.83±0.16a | 18.67±0.89a | 98.33±1.67a |
| Treated aphid (Spore suspension) | 11.11±2.22 | 9.00±0.53a | 6.33±0.44a | 18.33±0.33a | 96.67±3.33a |
| Control ( 0.05%Tween-80 solution) | 11.11±2.22 | 9.16±0.00a | 5.67±0.16a | 18.33±0.89a | 98.33±1.67a |

Means followed by similar letters in a column are not significantly different at 5%.

**Table 3. Effect of *V. lecanii* on some biological parameters of *Episyrphus balteatus* (mean±SE)**

| Treatments | Larval mortality (%) | Larval period (day) | Pupal period (day) | Pupal weight (mg) | Adult emergence (%) |
|---|---|---|---|---|---|
| Larval dip in Talc powder formulation | 13.33±6.67a | 9.43±0.35a | 9.28±0.22a | 9.50±0.28a | 100.00±0.00a |
| Larval dip in Oil in water emulsion | 6.67±6.67a | 9.57±0.35a | 9.30±0.20a | 9.67±1.17a | 100.00±0.00a |
| Larval dip in Spore suspension | 6.67±6.67a | 9.77±0.17a | 9.22±0.14a | 10.00±0.44a | 98.33±1.67a |
| Dry film | 6.67±6.67a | 9.65±0.15a | 9.00±0.29a | 9.50±0.29a | 98.33±1.67a |
| Treated aphid | 6.67±6.67a | 9.60±0.20a | 9.33±0.44a | 9.83±0.17a | 96.67±3.33a |
| Control ( 0.05%Tween-80 solution) | 6.67±6.67a | 9.67±0.17a | 8.77±0.14a | 10.17±0.44a | 98.33±1.67a |

Means followed by similar letters in a column are not significantly different at 5%.

**Table 4. Effect of *V. lecanii* on some biological parameters of *D. rapae* (mean± SE)**

| Treatment | Mummification (%) | Parasitoid emergence(%) | Aphid mortality (%) | Corrected aphid (%) mortality | Mycosis (%) |
|---|---|---|---|---|---|
| *V. lecanii* | 0.00 | 0.00 | 87.50±1.44b | 85.51b | 77.21±2.08a |
| *D. rapae* | 65.00±2.04a | 78.94±1.51a | 72.50±2.50c | 68.12c | 0.00 |
| *D. rapae* + *V. lecanii* | 68.75±2.39a | 56.21±3.47b | 98.75±1.25a | 98.55a | 36.71±1.17c |
| *V. lecanii* +*D. rapae* | 45.00±2.04b | 61.81±5.59c | 97.50±1.44a | 97.10a | 51.38±2.68b |
| Control ( 0.05%Tween-80 solution) | 0.00 | 0.00 | 13.75±1.25d | 0.00 | 0.00 |

Means followed by similar letters in a column are not significantly different at 5%.

(9.43 to 9.77 days), pupal period (8.77 to 9.33 days), pupal weight (9.50 to 10.17 mg) and adult emergence (96.67 to 100 %) were not affected by treatments (Table 3). Among larval cadavers, none showed mycosis.

**Effect on *D. rapae***

The results of this experiment revealed that aphid mortality caused by combination of the fungus and parasitoid either first aphid treated by the fungus and then exposed to the parasitoid or vice versa were significantly higher than when these two biocontrol agents were used alone. Parasitoid emergence were significantly affected by the fungus. Emergence of the parasitoid was highest in parasitoid alone treatment and it was lowest in fungus + parasitoid treatment, 78.94 and 61.81% respectively. Per cent mycosis was maximum in fungus alone treatment and minimum in parasitoid + fungus treatment (Table 4). Most interactions between the parasitoid and entomopathogenic fungi have been recorded as being asymmetrical, favoring the pathogens (Brooks, 1993). Kim *et al.* (2005) indicated that combined use of both aphid parasitoid, *Aphidius colemani*, and *V. lecanii* for integrated pest management of aphids is a viable control option, but fungal spore applications should as much as possible be timed to coincide with the later developmental stages of the parasitoid to conserve the parasitoids within the system.

**Effect on *T. chilonis***

Two sets of experiments were conducted to test the impact of the fungus on *T. chilonis*. In the first experiment in which *Corcyra* eggs treated with the fungus were exposed to the parasitoid, per cent parasitism was affected by fungal treatments (P = 0.08). There was no significant difference among spore suspension and dry-film treatments with control but other treatments decreased parasitism (Table 5).

The parasitoid emergence was not affected by dry-film treatment while in other treatments significant differences were seen. Similar trend was observed in adult period. In second experiment, parasitoid emergence slightly decreased in fungal treatments compared to control (P = 0.018). In this experiment there was no significant difference among treatments in adult longevity (P = 0.61).

**Effect on *B. mori***

Larval mortality was not significantly affected by treatments (P = 0.51). Maximum and minimum larval mortality of 13.99 and 5.99 percent were observed in oil in water emulsion and dip leaf treatments, respectively. Highest and lowest reductions in weight gained were seen in oil in water and dip-leaf treatments, 6.85 and 1.07 percent respectively (Table 6). Because maximum larval mortality (13.99%) and reduction in weight gained (6.85%) are less than 25 percent, the fungus can be considered as a harmless biopesticide to mulberry silk worm. Mukunthan (2004) reported that two isolates of *Fusarium* (GM14 and GM15) were not pathogenic to silkworm larvae. Indications are that, through careful selection of both pathogen and silkworm strains, it may be possible to use fungal control agents in the immediate vicinity of sericulture farms without endangering the silkworms.

**Effect on *S. cynthia ricini***

Weight gained (779 to 805.62 mg) and larval mortality (16.66 to 26.67 %) were not significantly affected by treatments (P = 0.92 and 0.60, respectively) (Table 7).

**Table 5. Effect of *V. lecanii* on some biological parameters of *T. chilonis* (mean±SE)**

| Treatment | *Corcyra* eggs treated with fungus exposed to parasitoid | | | Parasitized eggs treated with fungus | |
|---|---|---|---|---|---|
| | Parasitism(%) | Parasitoid emergence (%) | Adult duration (day) | Emergence (%) | Adult duration (day) |
| Talc formulation | 74.75±0.77ab | 93.13±0.73b | 2.65±0.02ab | 91.53±0.70ab | 2.64±0.04a |
| Oil in water emulsion | 73.75±0.46b | 89.67±0.39c | 2.60±0.04ab | 89.85±0.38c | 2.48±0.20a |
| Dry film (Spore suspension) | 74.88±0.43ab | 94.83±0.41a | 2.71±0.04a | 92.73±0.40a | 2.59±0.03a |
| Spore suspension | 75.75±0.25a | 92.90±0.39b | 2.47±0.14b | 90.56±0.54bc | 2.55±0.05a |
| Control ( 0.05%Tween-80 solution) | 75.88±0.77a | 95.07±0.73a | 2.80±0.02a | 93.13±0.48a | 2.69±0.02a |

Means followed by similar letters in a column are not significantly different at 5%.



**Table 6. Effect of different formulations of *V. lecanii* on some biological parameters of *B. mori* (mean± SE)**

| Treatment | Initial weight | Cocoon weight(mg) | Weight gained(mg) | Reduction in weight gained over control (%) | Larval mortality (%) | Corrected mortality (%) | Mycosis (%) |
|---|---|---|---|---|---|---|---|
| 1. Larval dip in Talc powder formulation | 186.63±1.04a | 665.59±4.53b | 478.87±4.89b | 3.11 | 25.00±5.69a | 9.99 | 68.75±11.97a |
| 2. Larval dip in Oil in water emulsion | 187.54±0.76a | 655.02±4.22c | 460.37±4.09c | 6.85 | 28.33±3.19a | 13.99 | 54.17±85.83a |
| 3. Larval dip in Spore suspension | 188.58±0.94a | 666.27±1.05b | 477.50±1.17b | 3.38 | 23.33±4.30a | 7.99 | 54.58±8.75a |
| 4. Dry film (Spore suspension) | 187.03±0.89a | 667.31±4.37b | 480.02±3.81b | 2.87 | 23.33±4.30a | 7.99 | 69.17±10.83a |
| 5. Leaf dip (Spore suspension) | 188.72±0.88a | 674.51±1.82ab | 485.87±2.71ab | 1.70 | 21.67±3.19a | 5.99 | 7.69±6.25b |
| 6. Control ( 0.05%Tween-80 solution) | 187.22±1.30a | 682.10±3.61a | 494.24±3.40a | 0.00 | 16.67±3.34a | - | 0±0.00b |

Means followed by similar letters in a column are not significantly different at 5%.

**Table 7. Effect of different formulations of *V. lecanii* on some biological parameters of *S. cynthia ricini* (mean± SE)**

| Treatment | Initial weight (mg) | Cocoon weight (mg) | Weight gained (mg) over control (%) | Reduction in weight gained (%) | Larval mortality (%) | Corrected mortality |
|---|---|---|---|---|---|---|
| 1. Larval dip in Talc powder formulation | 84.40±1.34 | 885.23±26.35 | 800.83±27.66a | 0.98 | 21.67±3.19a | 4 |
| 2. Larval dip in Oil in water emulsion | 86.05±1.43 | 880.69±15.31 | 794.64±15.57a | 1.66 | 26.67±1.92a | 10 |
| 3. Larval dip in Spore suspension | 90.69±0.54 | 896.32±10.78 | 805.62±10.48a | 0.30 | 18.33±1.67a | 0 |
| 4. Dry film (Spore suspension) | 86.98±0.95 | 895.05±12.32 | 800.78±20.56a | 0.90 | 18.33±1.67a | 0 |
| 5. Leaf dip (Spore suspension) | 88.55±0.44 | 868.62±15.32 | 779.87±15.58a | 3.49 | 16.66±1.93a | 0 |
| 6. Control ( 0.05% Tween-80 solution) | 88.56±1.67 | 889.44±19.84 | 808.06±11.59a | 0.00 | 18.33±3.19a | 0 |

Means followed by similar letters in a column are not significantly different at 5%.

No mycosis among dead larvae was seen. Results of this experiment indicate that the fungus is not pathogenic to eri silkworm. Sitch and Jackson (1997) evaluated the pathogenicity of two species of *V. lecanii* to non-target invertebrates and found that there was no evidence of infection in any of the 20 non-target invertebrates tested. Our results suggest that *V. lecanii* is compatible with the natural enemies of cabbage aphid and *T. chilonis* and is harmless to silkworm, therefore can be used for integrated management of cabbage aphid.

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