

## “*LECANICILLIUM LECANII* (ZIMM.) ZARE AND GAMES” AN IMPORTANT BIOCONTROL AGENT FOR THE MANAGEMENT OF INSECT PESTS – A REVIEW

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### ABSTRACT

The Entomopathogenic fungus, *Lecanicillium lecanii* (Zimm.) Zare and Games is one of the potential microbial biocontrol agents which have wide host range. The present review article contains the information related to its taxonomic position, mode of action, toxins and extracellular enzymes produced by the fungus. The various approaches for mass multiplication, carriers for development of commercial formulations and shelf life of formulated products of the fungus are reviewed and discussed. The factors like genetic variability, tritropic interaction, temperature, humidity, formulation base, inoculum level, isolation host, stage of insect host, compatibility with chemical pesticides which affect the performance of the fungus are presented and discussed. Response of *L. lecanii* to different insect pests, under protected conditions and field conditions, and its effect on non targeted organisms are reviewed and discussed.

**Key words** : Entomopathogenic fungus, *Lecanicillium lecanii*, *Verticillium lecanii*, Mass multiplication, Virulence, Performance.

Microbial bio-agents are the important components in IPM system. Entomopathogenic fungi are emerging as potential bio-agents. Pathogens of insects and other invertebrates have been identified in more than 100 fungal genera with >700 species recognized that attack insect hosts (Roberts and Humber, 1981; Hajek and St. Leger, 1994). The fungi like *Metarhizium anisopliae* (Metch.) Sorokin., *Beuveria bassiana* (Bals.) Vuillemin, and *Lecanicillium lecanii* (Zimm.) Zare and Games have gained the great scope as a biological control agents for the insect pest management. *L. lecanii* is one of the important entomopathogenic fungi, which was formerly known as *Verticillium lecanii* Zimm . It is

known by name “White-halo fungus” because of white mycelial growth on the edges of infected scale insects. It was first reported in 1939 by Viegas, who referred to the characteristic white halo formed by the fungus on the scale insect *Coccus viridis* (Green) as ‘the farmers friend’. The effectiveness of *L. lecanii* was studied and demonstrated first in India by Easwaramoorthi and Jayaraj (1978). It is effective for the control of sap feeding pest like aphids, whiteflies, scale insects, thrips, mealy bugs (Hom, 1915; Ekbohm, 1979; Kanagaratnam *et al.*, 1982). The available articles on entomopathogenic fungi mostly devoted to fungi *Metarhizium anisopliae* and *Beuveria bassiana*, so the present article solely

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focused on review of research work done on *Lecanicillium lecanii* by various researchers which will be helpful for planning and strengthening the future research pertaining to entomopathogenic fungus *L. lecanii*.

#### Taxonomic position:

According to Zare and Gams (2001), the new classification of *Lecanicillium lecanii* is Super kingdom: Eukaryota, Kingdom: Fungi, Phylum: Ascomycota, Subphylum: Pezizomycotina, Class: Sordariomycetes, Order: Hypocreales, Family: Hypocreaceae, Genus: *Lecanicillium*, Species: *lecanii*

#### Synonyms of *L. lecanii*:

*L. lecanii* was reported earlier by different names viz., *Lecanicillium muscarium* (Petch) Zare and Gams, *L. attenuatum* Zare & Gams, *L. longisporum* (Petch) Zare & Gams, *Verticillium lecanii* Zimm., *V. coccorum*, (Petch), Westerd, *Verticillium aphidum* (Oeder) Ellis, *V. hemileiae* (Bouriquet), *V. minutissimum* (Corda), *Cephalosporium lecanii* Zimm., *C. aphidicola* (Petch), *C. muscarium* (Petch), *C. thripidium* (Petch), *C. coccorum* (Petch), *C. dipterigenum* (Petch), *C. subclavetum* (Petch), *C. nodulosum* (Petch), *C. lefroyi* (Horne), *C. lanoso-niveum* (Beyma), *C. thripsidium* (Petch), *C. tumefaciens* (Winter), *C. verticicola* (Petch), *Sporotrichum lichnicola* (Berk and Broom), *S. kirchneri* (Rostr.), *Acrostalagmus aphidum* (Oudem), *A. coccidicola* (Gueg.), *Hirsutella kirchneri* (Rostr.) Minter, Brady and Hall, *Botrytis eriophytis* (Messe) Leath, *Acremonium kirchneri* (Rostr.) Muller, *Paecilomyces eriophytis* (Messe) Leath, *Oospora necans* (Sacc. and Trotter), *Spicaria mucoricola* (Speg.), and *Torula epistromata* (Cif.)

#### Important identification characters of *L. lecanii*:

1. Fungus colony on potato dextrose agar (Fig. 1a) were white, spreading slightly floccose and developed light yellow

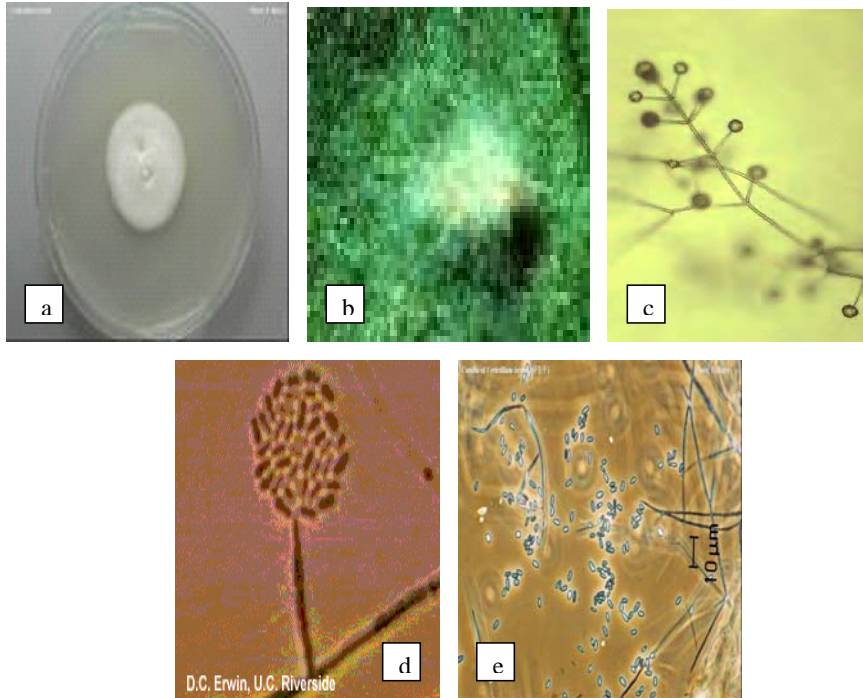
colour in the medium. (3.3 x 2.8 cm growth at 23°C) (Nagaich, 1973)

2. Spores and hyphal structures visible on exterior or host, few or no spores form inside host cadaver. (Fig. 1b), hyphae hyaline, 2.8  $\mu\text{m}$ , in diameter (Nirwan and Upadhyaya, 1976)
3. Phialides typically awl shaped and forming many conidia in heads, phialides in whorls, born on verticillately branched conidiophores (Fig. 1c)
4. Conidia (Fig. 1d) were hyaline, elliptical or cylindrical with slight depression in the centre, sometimes with slightly pointed ends [3-7 x 1-2  $\mu\text{m}$  in size (Nagaich, 1973)] or oval to elongated, 1.8-5 x 1.4-2.8  $\mu\text{m}$  in size (Nirwan and Upadhyaya, 1976) which were held together in a drop of easily soluble mucilage or slimy head (Fig. 1e) on the tip of phialides.

(For detailed identification keys of *L. lecanii*, see paper by Viegas, 1939; Zare and Gams, 2001)

#### Mode of action :

When *L. lecanii* conidia comes in contact with the host integument, it gets adhere to the epicuticle and germinate. Germinated conidia form germ tubes that penetrate cuticle directly (Hughes and Gillespie 1985) or grow over the surface of the epicuticle (Fig.2). The germ tube penetrates by lysing both the epicuticle and the procuticle. The fungus produces number of extra cellular enzymes which plays an important role during cuticle penetration of insect host. It produces different extracellular enzymes viz., Proteases, Chitinases (St. leger *et al.*, 1986; St. leger *et al.*, 1987) Esterase, N-Acetylglucosamine, Endoprotease, Chitinase, Aminopeptidase, Carboxypeptidase A, Lipase and Pr1 – Chymoelastase serine protease (Goettel *et al.*, 1989) reported. All these enzymes serve as



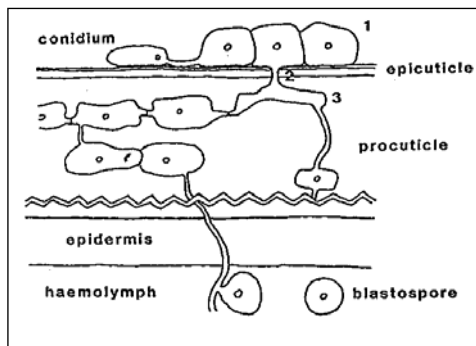
**Fig.1:** Colony characters of *L. lecanii*: a- growth on PDA, b- fungal growth over infected whitefly nymph, c- Verticillately branched conidiophore, d- slime conidial head and e- conidia

cuticle degrading enzymes. Among these enzymes, Pr-1 serves as major cuticle degrading enzyme as its concentration increased at the site of penetration peg in comparison to other enzymes. An active digestion and absorption of cuticular components occurs, colonizing hosts tissues and producing elongated hyphal bodies (blastospores). The pathogen causes infection symptom of colour change of aphid from original yellow to red which turns to dark brown before the visible fungal growth over the body surface of *Aphis gossypii* Glover (Shinde *et al.*, 2010). An insect dies due to mechanical pressure exerted by excessive fungal growth and action of mycotoxins (Ferron, 1981). The mycotoxins produced by *L. lecanii* are *viz.*, beauvericin, bassianolide (Kanaoka *et al.*, 1978; Suzuki *et al.*, 1977), dipicolinic acid

(Claydon and Grove, 1982) vertilecanin-A1, decenedioic acid and 10-hydroxy-8-decenoic acid (Soman *et al.*, 2001). As the host nutrients are depleted, the blastospores differentiate into elongated hyphae which extend outward from the body forming a mycelial mat of conidiophores over the surface of the integument resulting in mummification (Yeo, 2000). Under proper environmental condition, conidiophores mature giving rise to conidia which continues the disease cycle further (Roberts, 1989).

#### Natural infection of *L. lecanii* on different insects and pests:

There are number of reports of natural *L. lecanii* infection to different insects and pests (Table. 1), but out of the reported insects and pests, maximum are sucking insect and pests (belonging to Homoptera, Hemiptera, Thysanoptera and



**Fig.2** : General mode of infection of entomopathogenic fungi (1=Deuteromycetes entomopathogens appressorial complex, 2 = penetration peg, and 3 = penetration plate)

Acari), which indicates its possible spectrum for use as a biocontrol agent for sucking insects and pests management, which was also reported by various researchers *Viz.*, Horn, 1915; Ekbom, 1979 and Kanagaratnam *et al.*, 1982.

#### Mass Production of *V. lecanii*:

The entomopathogenic fungi can be mass multiplied by solid state and liquid state fermentation using different growth media. For solid state fermentation mostly sorghum and rice have been used as a growth media for obtaining conidial mass of the fungus. While for liquid state fermentation, molasses is the chipset and best medium for getting blastospores and mycelial biomass of the fungus. Kybal and Vleek (1976) used polyethylene cushion made of large thin walled polyethylene tubing sealed into sections which were partially filled (1 cm high layer absolutely horizontal) with submerged culture of *V. lecanii* after 2+1 days cultivation and inflated with sterile air. The harvested product after 14-16 days by discarding the medium and retaining the mad, yielded in to  $1 \times 10^{12-13}$  conidia/  $1m^2$  from medium composed of 0.8% peptone and 1% sorbitol. The use of sorghum broken grain as a solid grain media, produced  $15 \times 10^8$  conidia /g (Lakshmi *et al.*, 2001) while cooked rice and bran gave higher spore production of  $1.5$  and  $1.4 \times 10^9$  spores/g substrate (Feng *et al.*, 2000). Malt extract peptone medium used for mass

production, yielded as  $5.23 \times 10^7$  conidia/cm<sup>2</sup>, while Dextrose peptone yeast extract agar medium, produced  $4.58 \times 10^7$  conidia /cm<sup>2</sup> (Kamp and Bidochka, 2002 ). In liquid fermentation, Molasses yeast broth (MYB) supported maximum sporulation ( $8.33 \times 10^8$  spores/ml) and biomass production (746 mg /100ml), which can be maximized by addition of molasses at 4% concentration to MYB, to spore count of  $8.56 \times 10^8$  spores/ml and biomass 776 mg /100ml, while rice grains as a solid substrates gave spore production of 1.14 g /100g, so the biphasic state production by using MYB and rice grains produced the spore count of 1.70g /100g (Derakhshan *et al.*, 2008a). In another research spore production of four isolates of *V. lecanii* in biphasic system (PDB and rice) after 10 days, ranges from 0.23 to  $1.75 \times 10^9$  spores /ml (Nirmala *et al.*, 2006a). Combination of different adjuvants with the fungus, 1) *V. lecanii* + glycerol 2% + Tween-80 1% + arachid oil 0.5% and 2) *V. lecanii* + glycerol 5% + Tween-80 1% + arachid oil 2% covers the surface area of PDB medium more than 91%, with biomass production more than 31gm /40ml of medium after 10 days of incubation and found superior to *V. lecanii* incubation on PDB medium alone in conical flasks (Chavan and Kadam, 2009a). Shi *et al.* (2009) developed a solid-state fermentation with sugarcane bagasse as carrier absorbing liquid medium to propagate *V. lecanii* spores. They first used one factor at a time design to identify corn flour and yeast extract as the best carbon and nitrogen sources for the spore production of *V. lecanii*. Then, they used two-level fractional factorial design to confirm corn flour, yeast extract, and  $KH_2PO_4$  as important factors significantly affecting *V. lecanii* spore production. Finally, they optimized these selected variables using a central composite design and response surface method. The optimal medium composition was (grams per liter): corn flour 35.79, yeast 8.69,  $KH_2PO_4$  1.63,  $KH_2PO_4$  0.325 and  $MgSO_4$  0.325. They reported that under optimal conditions, spore production reached  $1.1 \times 10^{10}$  spores/g dried carrier, much higher than that on

**Table 1** : Reports of natural infection of *L. lecanii* on different pests.

Sr.No.	Name of the pest	Researcher
1	<i>Myzus persicae</i> Suzl. (Homoptera : Aphididae) <i>Aphis craccivora</i> Koch (Homoptera : Aphididae) <i>Aphis rumicis</i> Linn. (Homoptera : Aphididae) <i>Brevicoryne brassicae</i> Linn. (Homoptera : Aphididae) <i>Macrosiphoniella sanborni</i> Gill (Homoptera : Aphididae)	Nagaich, 1973
2	<i>Lecanium hesperidum</i> L. (Homoptera: Coccidae)	Kobiashvili, 1972
3	<i>Coccus viridis</i> Green (Homoptera: Coccidae)	Gopalakrishnan, 1973;Easwaramoorthy and Jayaraj, 1976; Kolher, 1980
4	<i>Coccus</i> species (Homoptera: Coccidae)	Oncuer, 1974
5	<i>Lecanium tiliae</i> L. (Homoptera: Coccidae)	Rubin and Beirme, 1975
6	<i>Saissetia oleae</i> Olivier (Homoptera: Coccidae)	Ahmad, 1975;Mendal <i>et al.</i> , 1984
7	<i>Malacosoma neustria</i> L. (Lepidoptera:Lasiocampidae)	Machowicz, <i>et al.</i> , 1976
8	<i>Scolytus scolytus</i> Fabricius (Coleoptera: Curculionidae)	Barson, 1976
9	<i>Idiocerus(Amritodus) atkinsoni</i> Lethierry (Hemiptera: Cicadellidae)	Nirwan and Upadhaya, 1976;Shashidhar <i>et al.</i> , 1994
10	<i>Cecidophyopsis ribis</i> Westw. (Acari: Eriophyidae)	Kanagaratnam, 1981
11	<i>Synanthedon salmachus</i> L. (Lepidoptera: Sesiidae)	Baker, 1981
12	<i>Hetreodera schachtii</i> Schmidt (Tylenchida: Heteroderidae)	Hanssler and Hermanns, 1981
13	<i>Cydia pomonella</i> L. (Lepidoptera: Tortricidae)	Glen, 1982
14	<i>Idioscopus clypealis</i> Lethierry (Hemiptera: Cicadellidae)	Kumar, 1983
15	<i>Aedes triseriatus</i> Say (Diptera: Culicidae)	Ballard and Knapp, 1984
16	<i>Phlephherda tuberculosa</i> (Nakahara & Gill)(Homoptera: Coccidae)	Pena and McMillan, 1986
17	<i>Bombyx mori</i> L. (Lepidoptera: Bombycidae)	Zhu <i>et al.</i> , 1988
18	<i>Holotrichia consanguinea</i> Blanch (Coleoptera: Scarabaeidae)	Gour and Dabi, 1988
19	<i>Thaumetopoea pityocampa</i> Den. Et Schif (Lepidoptera: Thaumetopoeidae)	Paparatti and Fabozzi, 1988
20	<i>Archips pomivora</i> Meyrick (Lepidoptera: Tortricidae)	Gopalakrishnan, 1989
21	<i>Taeniothrips inconsequense</i> Uzel (Thysanoptera: Thripidae)	Skinner <i>et al.</i> , 1991; Brownbridge <i>et al.</i> , 1999
22	<i>Rhopalosiphum rufiabdominalis</i> Sasaki.(Hemiptera: Aphididae)	Etzel and Pettitt, 1992
23	<i>Aphis gossypii</i> Glover (Homoptera : Aphididae)	Sancher-Pena, 1993
24	<i>Myzus persicae</i> Sulzer (Homoptera : Aphididae)	Kish <i>et al.</i> , 1994
25	<i>Ixodes ricinus</i> L. (Acari: Ixodidae)	Kalsbeek <i>et al.</i> , 1995
26	<i>Phytoptus avellanae</i> Nal. (Acari: Eriophyidae) <i>Cecidophyopsis vermiformi</i> Nal. (Acari: Eriophyidae)	Oezman, 1998
27	<i>Lymantria dispar</i> L. (Lepidoptera: Lymantriidae)	Hajek <i>et al.</i> , 1997
28	<i>Adelges tsugae</i> Annand (Homoptera: Adelgidae)	Gouli <i>et al.</i> , 1997
29	<i>Hypothenemus hempei</i> Ferrari (Coleoptera: Curculionidae)	Balakrishnan <i>et al.</i> , 1995
30	<i>Eriosoma lanigerum</i> Hausmann (Homoptera : Aphididae)	Asante, 1997
31	<i>Ixodes scapularis</i> Say (Acari: Ixodidae)	Zhioua <i>et al.</i> , 1999
32	<i>Toxoptera citricidae</i> Kirbaldy (Hemiptera : Aphididae)	Michaud, 1999
33	<i>Diuraphis noxia</i> Mord. (Homoptera: Aphididae)	Hatting <i>et al.</i> , 1999
34	<i>Pentalonia nigronervosa</i> f. caladii van der Goot(Hemiptera : Aphididae)	Mathew, 1999
35	<i>Bemisia tabaci</i> Gennadius (Homoptera : Aleyrodidae)	Lourencao <i>et al.</i> , 2001, Ben-Ze'ev <i>et al.</i> , 1994
36	Whiteflies (Homoptera: Aleyrodidae)	Lopez <i>et al.</i> , 2001
37	<i>Musca domestica</i> L. (Diptera: Muscidae) <i>Stomoxys calcitrans</i> L. (Diptera: Muscidae)	Skovgard and Steenberg,2002
38	<i>Trialeurodes vaporariorum</i> Westw. (Homoptera: Aleyrodidae) <i>Bemisia tabaci</i> Gennadius (Homoptera : Aleyrodidae)	Nier <i>et al.</i> , 1991; Drummond <i>et al.</i> , 1987 Lacey <i>et al.</i> , 1996; Ana Clara Scorsetti <i>et al.</i> , 2008
39	<i>Lepidosaphes beckii</i> Newman (Homoptera: Diaspididae)	Nirmala, <i>et al.</i> , 2006a
40	<i>Aleurolobus barodensis</i> Maskell (Homoptera: Aleyrodidae)	Shinde, <i>et al.</i> , 2010

wheat bran ( $1.7 \times 10^9$  spores/g initial dry matter). In another research sorghum was found to be ideal for the mass production ( $11.31 \times 10^{10}$  spores/100 g) while, pearl millet also found suitable media for the spore production ( $10.17 \times 10^{10}$  spores/100g). Among the liquid media, coconut water produced significantly higher spore production ( $5.27 \times 10^8$  spores/100 ml) and biomass production (0.51 g/100g). Among the non synthetic solid media, jack seeds produced significantly higher spores ( $4.11 \times 10^8$ ) and biomass (0.48 g/100g) followed by the ladies finger which produced  $3.12 \times 10^8$ /100g spores and 0.46g/100g biomass (Sahayaraj *et al.*, 2008)

#### **Factors affecting the performance of *L. lecanii*:**

##### **Genetic variability:**

The fungus from different geographical areas of different environmental conditions (abiotic conditions), insect-pest hosts (biotic conditions) and inherent virulence carries great morphological and genetic diversity. Sugimoto and Koike (2002) studied 46 isolates of *L. lecanii* from different geographical locations at molecular level using molecular markers RFLP (Restricted Fragment Length Polymorphism), SSCP (Single Stranded Conformational Polymorphism) and found great genetic variation among them. Internal transcribed spacer (ITS) & intergenic spacer (IGS) region of ribosomal DNA (rDNA) and mitochondrial small subunit rDNA (mt-SrDNA) of *V. lecanii* isolates, analyzed by PCR-RFLP & results shows no relationships among the RFLP, SSCP, isolation source and location. However amplified product size of IGS shows relationship with conidia size and sporulation of *V. lecanii* i.e., six isolates (out of 46) produces conidia of large size and number of conidia with lower quantity as compared to conidia of other isolates. Three isolates out of six were highly virulent (over 90%) against green peach aphids (*Myzus persicae*, Sulzer). Double stranded RNA (dsRNA) was detected in 22 out of 35 *V. lecanii* isolates which related with the amplicon size of IGS, though not with virulence or isolation location. Isolates

containing dsRNA were divided into six distinct types based on banding pattern.

##### **Tritropic interaction:**

The resultant impact of fungus against particular pest on particular host plant is the output of inter relation of pathogen, its targeted insect host and the host plant from which the pest get the nourishment. Meeke (2001) studied the tritropic interaction of *L. lecanii*, greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) and glasshouse crops and found that the *L. lecanii* is more effective on cucumber, gerbera and tomato against the whitefly (50 % mortality) as compared to poinsettia (20 % mortality) which indicates the crop vegetation and microclimate of the crop affects the performance of fungus.

##### **Temperature and relative humidity:**

Among the abiotic factors temperature and relative humidity are the important limiting factors which have great impact on survival as well as success or failure of the entomopathogenic fungus against the insect pest. Temperature affects the longevity of *V. lecanii* conidia with half-life in distilled water varies, both at 2°C (110-160 days) and 17°C (60-120 days), blastospores on the whole are even shorter-lived and more variable (100-150 days at 2°C). When conidia were kept at a range of humidities at 20°C, only high humidity permitted good survival. In contrast, dried conidia (separated from slime heads from their parent mycelium or washed) at 58 % R.H. died in less than 24 hours. However, conidia in slime heads still attached to the parent mycelium on aphids or on culture mycelium (without agar) survived for up to 13 days at 58 % R.H. (Hall, 1976; Hall, 1979 and Hall, 1981). Kim *et al.* (2001) reported that *L. lecanii* strain CS-626 causes the 98% mortality of greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) at the temperature rang of 25°C with least  $LT_{50}$  of 3.7 days, while it causes 80% mortality at 15 and 35 °C on the 7<sup>th</sup> day.

Virtually all fungi require humidity for spore germination, growth and sporulation. To ensure

maximum germination of spores and thereby highest possible levels of infection to insects, spore sprays should be synchronized with optimal humidity, which occur in the evening as ambient temperature falls in most crops. A favorable microclimate humidity is probably responsible for good survival of spores on aphid bodies killed by *V. lecanii* in glasshouse, 80 to 90 % of conidia survived for at least 30 days after death of aphids despite daytime air temperature of well over the upper temperature limit for growth (Khalil *et al.*, 1983). In many high humid areas of Vermont sugar maple forest, the fungal infection of *V. lecanii* occurred sometimes at levels exceeding 20% in thrips (Skinner *et al.*, 1991). *V. lecanii* causes more than 90% mortality of Coffee green scale (*Coccus viridis* Green) during monsoon and the colder months of the year when relative humidity was above 76% in Karnataka, India (Reddy *et al.*, 1997). *V. lecanii* shown considerable potential for managing whiteflies and thrips in greenhouse crops, where infection and penetration of aphids by *V. lecanii* occur under conditions of reduced humidity (Hsiao *et al.*, 1992), but sporulation and transmission requires high (~100%) humidity (Milner and Luton, 1986). Helyer *et al.* (1992) observed that four consecutive nights of high humidity per week or a cycle of two nights of high humidity and two nights of ambient humidity resulted in excellent control of aphids, thrips and whiteflies by the *V. lecanii* with no adverse impact on the crop.

#### Formulation base and surface active agents:

The base used for preparation of the fungal formulation at manufacturing stage and the base used during application of entomopathogenic fungi have a great effect on viability of the fungal propagule under storage condition and after spray application in field conditions. Naik and Shekharappa (2009) conducted a field experiment to study the bioefficacy of entomopathogenic fungal formulations *viz.*, crude, wettable powder and oil based formulation of *V. lecanii* against sucking pests of okra during kharif 2007-08. In leafhopper

management, *V. lecanii* oil based formulation recorded 7.75 leafhopper /3 leaves after second spray. In aphid management, oil based formulation of *V. lecanii* was best and recorded 7.75 aphids/3 leaves, after second spray. Oil based formulation of *V. lecanii* recorded the mean number of 2.70 whiteflies/3 leaves, after second spray. Similarly oil based formulations of *V. lecanii* recorded 3.00 thrips/3 leaves. However, all these formulations were next best to standard check. The yield of okra was significantly higher in oil based formulation of *V. lecanii* (38.50 q/ha) with monetary returns of Rs. 14480/ha with the highest benefit cost ratio of 18.40:1 were recorded in *V. lecanii* wettable powder formulation. Derakhshan *et al.* (2008b) studied the effect of cultural media, storage temperature and moisture content on viability. Among the media evaluated, Molasses yeast broth (MYB) plus 2% Polyethylene glycol (PEG) followed by MYB plus 1% PEG and rice powder maintained the fungal viability higher than other media. Viability in refrigerator temperature was significantly higher than in room temperature. Among three moisture levels tested, viability at 5 and 10% were on par and were significantly higher than at 15%. Viability over time decreased and the differences in viability among the three storage times were significant. Lee *et al.* (2006) studied the effect of UV protective agents on fungus performance. They found that fungal spores ( $10^8$  spores ml<sup>-1</sup>) suspended in 1% (w/v) montmorillonite SCPX-1374 and 1% (w/v) of the wetting agent, EM-APW#2, which is a polyoxyethylene, had approx. 80% survival after exposure to UV-C for 30 min and about 93% after exposure to UV-B for 6 h. In greenhouse testing, cotton aphid densities increased 14-fold over their initial density in 15 d without spore application. However, initial cotton aphid densities were decreased by 60% of the initial level when plants were treated with the spore formulation. Erkilic (1992) reported that Cod acid as formulation base gives 88.9% viability of fungal conidia for 72 hr., where as addition of Triton 0.05% along with  $16 \times 10^6$  conidia /ml dose of fungus gave 79.9%

mortality of Coffee green bug (*Coccus viridis* Green). Easwaramoorthi and Jayaraj (1977) noted increased mortality of bugs from 47.5 to 62.6% due to *V. lecanii* with glycerol 0.1%. Easwaramoorthi *et al.* (1978) also found that Tween-20 at 0.05% in spray suspension of *V. lecanii* increased the mortality (92.6%) of *C. viridis*. Verhaar *et al.* (1999) reported that arachnid oil (0.5%) gave the best development of fungus on mildewed cucumber leaves. They also reported that arachid oil or maize oil with Tween-80 stimulated the germination of fungus and considerably improved the biocontrol potential of it at reduced humidity's.

#### Inoculum level:

Fungal inoculum level is the important factor which affects the performance. It is general trend that the higher fungal inoculum level gives higher insect mortality. However, sufficient inoculum level should be worked out for the particular pest to prevent the over inoculum wastage and to achieve higher mortality. Joshi *et al.* (2003) found that higher inoculum level ( $10^8$  conidia /ml) of fungus, resulted in to highest mortality (40%) of mustard aphid, *Lipaphis erysimi* (Kalt.), while at lower inoculum level of  $10^7$  conidia /ml and  $10^6$  conidia /ml, it caused 33.3 and 23.3 % mortality, respectively. Kim *et al.* (2001) reported that lower concentration ( $1 \times 10^3$ - $10^7$  conidia /ml) of fungus exhibited relatively low mortality in adult whitefly, *Bemisia tabaci* (Gennadius); while higher concentrations ( $10^9$  and  $10^8$  conidia /ml) exhibited almost 100% mortality of *B. tabaci*, after 7 and 9 days of treatments under greenhouse conditions. Chavan and Kadam (2009b) reported effects of two liquid formulations (A & B- both having  $8 \times 10^8$  CFU /ml) and one WP formulation ( $2 \times 10^8$  CFU /gm) of *V. lecanii* at different concentrations *viz.*, 0.15, 0.30, 0.45, 0.60, 0.75, 1% (for formulation A & B) and 0.2% (for WP formulation) of spray solution on nymphs of spiraling whitefly (*Aleurodicus disperses* Russell). They found that all the concentrations of three formulations gave more than 50 % nymphal mortality after 7 days of treatments. They further reported that 0.60, 0.75 and

1% concentrations of liquid formulations A & B and 0.2% concentration of WP formulation of *V. lecanii* gave more than 90% mortality of nymphs after 14 days of treatments. In another research, Vertalec (*V. lecanii*) significantly increased aphid mortality (mean mortality because of mycosis increased from  $45.55 \pm 6.93$  % at  $10^4$  conidia/ml to  $95.55 \pm 4.45$  % at  $10^8$  conidia/ml). The  $LC_{50}$  value for pathogen was  $5.14 \times 10^4$  conidia/ml.  $LT_{50}$  values for  $10^5$ ,  $10^6$ ,  $10^7$  and  $10^8$  conidia/ml were 10, 8, 6.5 and 5 days, respectively (Safavi *et al.*, 2002).

#### Isolation host:

Findings of different workers showed that the fungal strain isolated from particular pest performs best against the same pest as it may possible that the genetic information related to virulence against the same host transferred in fungal propagule multiplied on the same insect host. Hall (1982) studied the effectiveness of 2 isolates of the fungus (one from whiteflies and the another from aphids) for the control of *Trialeurodes vaporariorum* (Westw.) and *Aphis gossypii* Glov., on cucumbers in the glasshouse was evaluated in studies in the UK in 1977-1980 and found that control of homologous hosts was better than that of heterologous hosts. A single spray of a formulation containing a commercial substrate of the 'whitefly' isolate controlled established whitefly populations satisfactorily on sprayed foliage and also when the whitefly moved up to younger untreated foliage. In addition, because the substrate permitted growth and sporulation of the fungus on leaf surfaces, whitefly populations introduced after a fungal application were also controlled. Control of whitefly by the 'aphid' isolate alone was unsatisfactory although control could be obtained in conjunction with the parasite *Encarsia formosa* Gah. control of *A. gossypii* by the 'aphid' isolate was obtained by spray application of a commercial preparation containing a substrate but not by that of pure spore suspensions. The 'whitefly' isolate did not control aphids satisfactorily. Hincapie *et al.* (1990) reported that *V. lecanii* isolate obtained from their natural



host (*Myzus persicae*, Sulzer) gives 100% mortality of *M. persicae* (Sulzer) under laboratory trial and 76.25 % mortality under Greenhouse trail against the same host *M. persicae* as compared to mortality obtained by *V. lecanii* isolated from other insect pest, *Erinnyis ello* L. and *Trialeurodes vaporariorum* (Westwood). Nirmala *et al.* (2006b) tested the cultures isolated from different host insects on different growth media and found that isolate from *Meconellicoccus hirsutus* (Green) collected from Pune, Maharashtra gave maximum colony diameter (5.7 cm) after 15 days of incubation on PDA medium plates which was significantly higher than the colony diameter of isolates VI 3a isolated from *Coccus viridis* (Green), VI 2a isolated from *Lepidosaphes beckii* (Newman), collected from Madikeri and VI 1 isolated from *Spodoptera litura* (Fabricius) collected from Bangalore. They further reported that isolate VI 2a gave highest biomass production (0.77g /100 ml) on Potato Dextrose Broth medium, compared to biomass produced by other isolates *i.e.*, 0.73, 0.74 and 0.47g /100ml respectively in case of isolate, VI 1, VI 3a and VI 5 after 10 days of incubation in stationary culture at 25°C. They further reported that in shake culture of PDB medium, the isolate VI 2a gave highest biomass production (1.03 g/100ml) as compared to biomass produced by other isolates VI 1, VI 3a and VI 5 (0.90, 0.60 and 0.86 g/100ml, respectively) after 10 days incubation at 25 °C. VI 2a gave highest spore production ( $7.28 \times 10^8$  spores/ml) in stationary culture and in shake culture. It also produces highest blastospores ( $17 \times 10^6$  /ml) as compared to other isolates after 10 days of incubation at 25°C. Hirte *et al.* (1989) also reported that fungus originated from whiteflies, aphids or thrips were most virulent to the host from which the isolate was collected.

#### Stage of insect host:

Stage of host is an important factor playing role in the success of entomopathogens. Not all stages in an insect life cycle are equally susceptible to fungal infection. So the fungal application can be successful against the particular pest when it can

be done at the condition where the susceptible stage or weaker stage of the particular pest become dominant among population. Kim (2007) investigated the influence of fungus on development and reproduction of cotton aphid (*Aphis gossypii*) and found that increased spore concentration did not significantly affect each nymphal stage, total nymphal period, pre-reproductive period and the age of first larviposition while the significant dose effect on reduction of life span, reproductive period and fecundity was observed in 1st and 3rd instars after spore application. When conidia were applied to 1st instars, life span was significantly reduced to 10.8 and 8.4 days at  $1 \times 10^4$  and  $1 \times 10^8$  conidia/ml, respectively from 12.2 days in the control. During the life span, total fecundity was  $41 \pm 7.3$ ,  $26 \pm 0.8$  and  $22 \pm 5.7$  nymphs per female at  $1 \times 10^4$ ,  $1 \times 10^6$  and  $1 \times 10^8$  conidia/ml, respectively compared with  $51 \pm 2.0$  nymphs per untreated female. Reproduction period was also significantly shortened with increasing spore concentration. Application of spores to 3<sup>rd</sup> instars showed a similar trend. However, daily fecundity of individual aphids was not affected by spore dose. He concluded that the isolate of *L. attenuatum* is able to affect populations of cotton aphid by reducing life span and total fecundity as well as by killing the aphids directly. Vestergaard *et al.* (1995) also reported that adult western flower thrips (*Frankliniella occidentalis* Pergande) were more susceptible to *V. lecanii* than larvae. Gopalkrishnan (1989) found that early instars of *Plutella xylostella* L. were more susceptible to the fungus as compared to late instars at  $2.8 \times 10^9$  conidial /ml dose at 25 °C temperature. In another research, the nymphal stages of *B. tabaci* found highly susceptible to infection by *V. lecanii* while there was no differential susceptibility among 1st, 2nd, and 3rd instar *B. tabaci* treated with fungus. (Meade and Byrne, 1991).

#### Compatibility of *L. lecanii* with chemical pesticides:

Chemical pesticides may have antagonistic or synergistic effect on the potentiality of *V. lecanii*

and may have disrupt natural epizootic. Under such epizootic condition, it is expected to enhance effectiveness through joint action of pathogen and compatible insecticides, which would reduce not only the cost of protection but also reduce the contamination of the environment. Growth inhibition of pathogenic fungi is useful criterion for initial testing of its compatibility.

Wilding (1972) reported that the fungicide dimethirimol as harmless to *V. lecanii* while benomyle and triarimol inhibited the mycelial growth of fungus on agar plates under *in vitro* condition. He further reported that neither triarimol nor dimethirimol inhibited the fungus in aphid fed on plants treated with the fungicides and suggested that it could be used to control aphids on plants when plant pathogenic fungi are being treated with triarimol or dimethirimol. Eswaramoorthi *et al.* (1978) showed that *V. lecanii* alone caused the mortality of 28.4% in *Coccus viridis* and with fenthion (0.1%) and phosphomidan caused antagonistic effect, reduced radial growth, mycelial dry weight and germination of spores, due to high concentration of chemical insecticides. Hall (1981) studied the effect of fungicides (benomyle, oxycarboxin, dinocap and ipropodine), insecticides (imidacloprid, carbaryl, tetradifonand permethrin) and acaricides (dicofol and fenarimol) on *V. lecanii* which were found relatively harmless to fungus. Gardner *et al.* (1984) reported that the foliar sprays of benomyl inhibited Vertalec (Commercial formulation of *V. lecanii*) activity when the fungicide was applied at 3 days and immediately before Vertalec applications. Spray intervals of 7 days between a Vertalec application and a previous benomyl application appeared to have no deleterious effects on Vertalec. Khalil *et al.* (1985) revealed that spore germination and mycelial growth of the entomopathogenic fungus were little affected by benomyl, cypermethrin, fenbutatin oxide, formothion, mevinphos, copper oxychloride, oxamyl, permethrin, pirimicarb, thiophanate-methyl or triadimefon at the recommended concentration.

Fenitrothion, mancozeb and methomyl were partially incompatible, while metiram, bitertanol and dichlofluanid were completely incompatible.

Alves *et al.* (1993) studied the effect of some pesticides on *V. lecanii* and found that fenbutatin oxide, fenpropathrin and chinomethionat were most selective insecticides while propargite and dicofol were most toxic pesticides to *V. lecanii*. Fiume (1993) tested *V. lecanii* against *M. persicae* on *Capsicum annum* in greenhouses, where *V. lecanii* and methomyl were applied separately and in combination and found that almost all treatments gave good control of aphids except pirimicarb. The best result was achieved with application of methomyl, followed by inoculation with *V. lecanii*. Ravensberg *et al.* (1994) noted that the chitin bio synthesis inhibitor, teflubenzuron inhibited only the chitin of insect but not fungi and consistent synergism was established. Rebollar *et al.* (1994) reported that mancozeb, benomyl, captan, copper oxychloride + mancozeb and oxadixyl + mancozeb inhibited mycelial growth by cent percent at the recommended dose, while chlorothalonil, anilazine, iprodione and zineb had less harmful. Rebollar *et al.* (1996) found that manzate 200, benlate PH, captan 50 PH, cupravit Mix PH and recoil PH inhibited cent percent growth of the fungus at recommended dosage while treatment with daconil 2787 PH, dyrene 50 PH, rovril 50 PH and zineb 80 PH resulted in lower inhibition percentage. Saito and Yabuta (1996) found that fungicides like polyoxin, sulfur, mepronil, procymidone, copper hydroxide, dithianon and zineb were harmless to mycelial growth at the recommended dose, while triflumizole, chinomethionat, anilazine and benomyle were found toxic to mycelial growth. Quintala and Mycoy (1997) reported that the confidential formulation of imidacloprid spreaded and increased the conidial germination on both agar and insect cuticle. Debnath (1997) studied the effect of different concentrations of endosulfan 35 EC at 0.0001, 0.001, 0.01 0.1% against *V. lecanii* and found that endosulfan has pronounced inhibitory effect on *V.*

*lecanii* which increased with increased concentration. Kim *et al.* (2001) reported that Fenbuconazole + thira and propineb strongly inhibited both spore germination and mycelial growth while azoxystrobin and chlorothalonil strongly inhibited spore germination but did not seriously impair mycelial growth. They further reported that fungicides dimethomorph and procymidone did not affect either spore germination or mycelial growth. Batista *et al.* (2001) reported that thiamethoxam as a compatible insecticide with *V. lecanii* at *in vitro* as well as field conditions. Shelke (2001) reported that dichlorvos 0.035%, imidacloprid 0.002% and chloropyrifos 0.003% combined with *V. lecanii* and *C. montrouzieri* could be the most promising treatments against grape vine mealy bug, *Macronellicoccus hirsutus* (Green).

Olan and Cortez (2003) studied the effect of copper oxychloride, copper sulphate and chlorothalonil on the development of seven strains of the fungus *V. lecanii* *in vitro*. The inhibition percentages were 79.24, 68.53, and 28.39 per cent, for copper oxychloride, copper sulphate and chlorothalonil, respectively. All fungicides had an adverse effect on the development of the fungus, which varied depending upon the strain and the product used. Small conidia strains appeared to be more susceptible to the fungicides. Li *et al.* (2003) investigated the effect of six insecticides and six fungicides at recommended rates on mycelial growth of *V. lecanii* for field spraying. They found that Imidacloprid 10 per cent, methomyl 90 per cent, beta-cyfluthrin 2.5 per cent and fenpropathrin 20 per cent showed more than 10 per cent growth inhibition, while fenpropathrin 20 per cent had an inhibition rate of 17.67 per cent. It was also suggested that for the control of insect pests with *V. lecanii*, it is necessary to have an interval period after the use of fungicides. Sterk (2003) found that the treatments of captan, azoxystrobin, kresoxim-methyl, trifloxystrobin, mepanipirim, procymidone, sulphur, tolylfuanid, imazalil, pyrimethanil, thirum and bitertanol were less toxic for mycelium of the

fungus. Whereas, imazalil was found very toxic to this fungi in all cases. Germinating spores were more sensitive to pesticides than mycelium. Cuthbertson *et al.* (2003) reported that abamectin, nicotine, deltamethrin and imidacloprid were compatible pesticides with *L. lecanii*. Derakhshan (2006) reported that the insecticides, endosulfan caused maximum inhibition of germination and growth of *V. lecanii*, while spinosad and imidacloprid were less toxic. Among the three fungicides tested, mancozeb inhibited cent per cent spore germination at field recommended dose with *in vitro* condition. Manjunatha *et al.* (2006) tested the compatibility of *V. lecanii* with five insecticides at field recommended dose by food poison technique on SMAY agar media. Among the five insecticides, NSKE was the safest followed by cypermethrin and carbaryl, whereas chlorpyrifos inhibited the fungal growth greatly, followed by endosulfan. Xu *et al.* (2006) tested the effects of 10 fungicides, including thiophanate-methyl, mancozeb, iprodione, carbendazim, Sporgon [prochloraz], triadimefon, chlorothalonil, feniminosul and oligosaccharins; and 6 insecticides, including dichlorvos, deltamethrin, dimethoate, omethoate, methamidophos and fenvalerate, on the growth of *V. lecanii* strain KM9803. They found that the all pesticides controlled fungal growth except oligosaccharins and methamidophos. Where as the fungal growth inhibition was strongest for Sporgon, triadimefon and DDVP [dichlorvos].

#### **Response of *L. lecanii* to different insect hosts under protected conditions / field conditions:**

The mortality response to *L. lecanii* in insect pests varies with their defense mechanisms and habitats. Samsinakova and Kalalova (1976) recommended the use of preparation of conidia ( $41 \times 10^7/l$ ) of *V. lecanii* for the control of scale insects (*Coccus hesperidum*) which gave 80-100 % mortality of *C. hesperidum* in 3 weeks. Easwaramoorthy and Jayaraj (1979) found that Guava scale, *Palvinaria psidii* Mask (69.0%

mortality) and coffee aphid, *Toxoptera aurantii* Boy. Def (48.4% mortality) were more susceptible to *V. lecanii* as compared to banana aphid, *Pentalonia nigronervosa* Coq. (34.1% mortality) and coffee scale, *Saissetia coffee* Walker (19.2% mortality) at the dose  $10^6$  conidia/ml after 7 days of treatment. Gour and Dabi (1988) reported that soil inoculation with fungal suspension of *V. lecanii* gave maximum control of white grub (*Holotrichia consanguinea*, Blanch) under pot culture experiment. (Van der Scaaf *et al.*, 1990) used *V. lecanii* as a wettable powder (Mycotal, Koppert Pvt. Ltd. Netherlands) for the control of *Trialeurodes vaporariorum* (Westwood) on cucumber and tomato and *Frankliniella occidentalis* Pergande on cucumber in greenhouse and found that the weekly spray with Mycotal at  $10^3$  spores/ml, resulted in 90% reduction of population of *T. vaporariorum*, while the infection rates of up to 60% of *F. occidentalis* were also recorded. Ghelani *et al.* (2006) tested *V. lecanii* (Vertisoft with  $2 \times 10^8$  cfu/g) at 5.0 g/litre) under field conditions against *Aphis gossypii* on Bt cotton (cv. MECH 162) and recorded percent pest reduction between 50 and 70 percent, as an effective treatment for *A. gossypii* management. Phadke and Phadke (2000) conducted trails with commercial formulation of *V. lecanii* (Verti-Guard, Ajay Farm Chem Pvt. Ltd. Pune, India) against brinjal whitefly (*Bemisia tabaci*) under greenhouse conditions during year 1998 and 1999. They reported the promising results of 93% nymphal mortality of *B. tabaci* during year 1998 and 67% nymphal mortality during year 1999 after five days of fungal spray of Verti-Guard at the rate of 1 g/litre. Kim *et al.* (2001) reported that the conidial formulation of *V. lecanii* strain CS-625 gives 100% mortality of cotton aphids (*Aphis gossypii* Glover) while blastospores formulation gave 97% mortality with  $LT_{50}$  of 2.74 and 3.31 days, respectively. Kadam *et al.* (2008) used the application of *V. lecanii* (Phule Bugicide Liquid)  $6 \times 10^5$  cfu/ml-1 0.3% for the management of sucking pests of gerbera and after 14 days of treatment, recorded appreciable mortality (95.45, 93.44, 91.67

and 82.40%) of white fly (*Trialeurodes vaporariorum*), aphid (*Myzus persicae*), thrips (*Thrips tabaci*) and red spider mite (*Tetranychus urticae*) under polyhouse condition. Kim *et al.* (2008) tested commercial preparation of *Lecanicillium longisporum* (= *Lecanicillium lecanii*), Vertalec Reg. for simultaneous suppression of cotton aphid *Aphis gossypii* and cucumber powdery mildew (*Sphaerotheca fuliginea*) on potted cucumber plants. They applied Vertalec onto cucumber plants that had been infested with either cotton aphid, spores of *Sphaerotheca fuliginea* or both. They also applied Irradiation-inactivated Vertalec (II Vertalec) was also to an identical series of cucumber plants as a control. They found that Vertalec was highly pathogenic against adult aphids with an  $LT_{50}$  of 6.9 days. II Vertalec did not affect aphid survival. Application of either active or II Vertalec significantly suppressed spore production of *S. fuliginea* compared to the water control. For dual control assays, Vertalec applications were made one day after infestation of both aphid and *S. fuliginea* onto potted cucumbers. Fifteen days after the Vertalec treatments, the numbers of surviving aphids and the production of powdery mildew spores were significantly reduced compared with the water control. The presence of aphids also suppressed *S. fuliginea* spore production. They suggested the potential of a dual role for Vertalec (*L. lecanii*) as a microbial control agent of aphids and powdery mildew in cucumber. Saito (1993) reported that the fungus was as efficient as buprofezin in controlling nymphs of *B. tabaci* infesting tomatoes.

#### **Effect on non-targeted organisms :**

The safety of any biocontrol agent to non targeted organisms is the important aspect which should be studied thoroughly before its commercialization to avoid the hazards and disturbance of ecological balance of agricultural ecosystem. Kim *et al.* (2005) studied the effect of *V. lecanii* on aphid parasitoid *Aphidius colemani* Viereck which showed the normal development (approximately 90% adult emergence) when its

**Table 2** : List of commercial products of *L. lecanii*, available in India and some other countries along with their producer companies/ supplier companies.

Product Name	Content	Recommendations of respective company for Targeted Pests	Producer Company/ Supplier
Mycotal	<i>L. lecanii</i>	whitefly, thrips	Koppert, The Netherlands
Vartalac	<i>L. lecanii</i>	aphids	Koppert, The Netherlands
Biocatch	<i>L. lecanii</i>	whitefly	T- Stanes & Company Ltd. India
Nutri-Life Myco-Force	<i>L. lecanii</i> + <i>B. bassiana</i>	Whitefly, thrips, aphids, scale insects, mealy bugs	Nutri Tech Solutions, Australia
Ago biocontrol	<i>L. lecanii</i>	Homoptera & Diptera	AgoBiocontrol, Colombia
Verticillium 50			
Probiovert	<i>L. lecanii</i>	coccids, whitefly	Probioma, Santacruse
Verticel	<i>L. lecanii</i>	aphids, whitefly, thrips, mealy bugs, scale insects	Excel Industries Ltd., India
Vertisol	<i>L. lecanii</i>	Hemiptera, Homoptera & Thysonoptera	Biotropic, S.A., Jalisco, Mexico
Microgermin	<i>L. lecanii</i>	Thrips	Chr. Hansen, Denmark
Vertilec	<i>L. lecanii</i>	All soft bodied insects	Funducion, Agrotecnologica, Colombia
Spider	<i>L. lecanii</i>	Sucking pests	Smruti Biotech, Mumbai, India
Vertisoft	<i>L. lecanii</i>	Whitefly, thrips, aphids, scale insects, mealy bugs	Agriland Biotech, Pvt. Ltd. Vadodara, Gujarat, India
BioPlus-Verticillium	<i>L. lecanii</i>	Scales, aphids, thrips, bugs, mites, jassids, hoppers	Deepa Farm Inputs (P) Ltd. Trivendrum, Kerala, India
Bioline	<i>L. lecanii</i>	thrips, bugs, mites, jassids, hoppers	Biotech International, New Delhi, India
Verti-Guard	<i>L. lecanii</i>	Sucking pests	Ajay Farm Chem Pvt. Ltd. Pune, Maharashtra, India
Varcitile	<i>L. lecanii</i>	Scales, aphids, thrips, jassids and hoppers	Indore Biotech Inputs and Research, Pvt. Ltd. Indore, M.P., India
Vertimust	<i>L. lecanii</i>	Whitefly, thrips, pyrilla, aphids, scale insects, mealy bugs	Jai Biotech Industries, Nashik, Maharashtra, India
EUVERT	<i>L. lecanii</i>	Aphids, whitefly, thrips, red spider mites, Powder mildew, rust and nematodes	Akshaya Biosciences, United States
Abtec Verticillium	<i>L. lecanii</i>	Whitefly, thrips, aphids, scales, mealy bugs, red spider mites and nematodes	Jai Biotech Industries, Nashik, Maharashtra, India
Sun Agro Verti	<i>L. lecanii</i>	Thrips, pyrilla, mealy bugs, aphids, scales,	Sun Agro Biosystem Pvt. Ltd., Chennai, Karnataka, India
Brigade VMB	<i>L. lecanii</i> + <i>M. anisopliae</i> + <i>B. bassiana</i>	Homoptera, Lepidoptera, Coleoptera and mites	Krishi Mitra, India
Phule Bugicide	<i>L. lecanii</i>	Whitefly, thrips, aphids, scales, mealy bugs and mites	MPKV, Rahuri, Ahmadnagar Maharashtra, India

cotton aphid (*Aphis gossypii* Glover) host was treated with *V. lecanii* conidia 5 or 7 days after parasitization. Fungus exposure 1 day before or up to 3 days after parasitization, however, reduced *A. colemani* emergence from 0 to 10%. Also, numbers of spores and mycelial fragments in aphid homogenates were much higher in aphids exposed to the fungus up to 3 days after parasitization than in aphids treated after 5 or 7 days. They suggested that the parasitoid and fungus may be used together for aphid biocontrol as long as fungus applications are timed to allow late-instar development of the parasitoid. Shaw *et al.* (2002) reported that the conidial dose of  $1 \times 10^8$ /ml, gave cent percent control of mite, *Varroa destructor* (Anderson and Trueman) an ectoparasite of honey bee, *Apis mellifera* (L.) after 7 days; while the mortality of honey bees due to *V. lecanii* was less than 20% after 14 days of treatment. Koike *et al.* (2005) studied the effects of *V. lecanii* on two-spotted spider mite (*Tetranychus urticae*, Koch), and its predatory mite (*Phytoseiulus persimilis*, Athias-Henriot) and found that, all isolates of *V. lecanii* (Vertalec, Mycotal, A-2, B-2) produced pathogenicity in both the mites, but the effect was less in predatory mite than spider mite at low relative humidity (66%), which resulted into synergistic effect against the field colony of *T. urticae*, when *V. lecanii* used with predatory mite *P. persimilis*. Cuthbertson *et al.* (2005) reported that,  $1 \times 10^7$  conidia/ml dose can be applied in combination with entomopathogenic nematode, *Steinernema feltiae* (Filipjev) at 5000 IJs/ml and neonectonoids, imidacloprid at 0.2 g/l water, as there was no adverse effect on EPN and also resulted into 83% mortality of *Thrips palmi* (Karny) under laboratory conditions. Wang *et al.* (2005) tested crude insecticidal toxins extracted from two strains of *V. lecanii*, V3450 and Vp28 against the grub of ladybird beetle, *Delphastus catalinae* (Horn), showed low toxicity to with  $LC_{50}$  values of 1942 (133-2710) and 2471 (1291-4731) ppm; which is approximately 10 and 12 fold higher than field application rate of 200 ppm. Further more they found

that the adult beetles has less sensitivity to crude toxin with  $LC_{50}$  values of 4260 (3376-5375) and 4426 (1734-11298) ppm, which is approximately 20 and 22 fold higher than field rate of 200 ppm. They further reported that the consumption and foraging capacity were significantly impaired especially in the second instar larva beetles which took longer time (more than twice of the control beetles) to consume whitefly eggs after exposure to toxins. However, there was no significant effect on fecundity and longevity. Broza *et al.* (2001) reported that collombolan, *Folsomia candida* (Willem) is not affected by *V. lecanii* even after consumption of fungus and act as scavengers of biological insecticides when artificially introduced into the environment. Thus, findings of different researchers showed that *L. lecanii* have no adverse effect on non targeted organisms which makes *L. lecanii* as an important biocontrol agent for pest management strategy.

#### Commercial Formulations:

The commercial formulations/products of *Lecaniillum lecanii*, available in India and some other countries are presented with their producer companies/ supplier companies in Table 2.

#### CONCLUSION

*L. lecanii* is a promising biocontrol agent and can be used as an important component of integrated pest management under field and green house conditions. It is most effective for the control of sucking insect pests. However, temperature and relative humidity are the major environmental factors, which affect the performance of *L. lecanii* under field conditions. It can be mass multiplied on most of the conventional pathological media, but it can be mass multiplied at cheaper rate on solid grain media of sorghum and rice and liquid media of molasses. It can be used effectively in conjunction with other natural enemies and compatible insecticides. If it is applied at appropriate dose and time using virulent strain and suitable adjuvant under sufficient pest population condition, it may prove as an important biocontrol tool in IPM strategy.

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