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Chapter

Lecanicillium spp. for the Management of Aphids, Whiteflies, Thrips, Scales and Mealy Bugs: Review

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Abstract

*Lecanicillium* spp. are potential microbial bio-control agent mainly used for the management of sucking insect pests such as aphids, whiteflies, scales, mealy bugs etc. and gaining much importance at present for management of pests. Due to indiscriminate use of chemical pesticides which results in development of resistance, resurgence, outbreak of pests and residue problem, the farmers/growers are forced to use bio-pesticides for sustainable agriculture. *Lecanicillium* spp. is promising biocontrol agent against sucking insect pests and can be used as one of the components in integrated pest management (IPM). However, optimum temperature and relative humidity are the major environmental factors, for the performance of *Lecanicillium* spp. under protected/field conditions. The present review is mainly focused on nomenclature of *Lecanicillium* spp., mode of infection, natural occurrence, influence of temperature and humidity on the growth, factors influencing the efficacy, virulence/pathogenicity to target pests, substrates used for mass production, safety to non-target organisms, compatibility with agrochemicals and commercially available products. This review is mainly useful for the researchers/students to plan their future work on *Lecanicillium* spp.

Keywords: entomopathogenic fungus, aphid, whitefly, virulence, mass production, safety

1. Introduction

The increased use of conventional chemical pesticides over the years has not only contributed to an increase in food production, but also has resulted in adverse effects on the environment and non-target organisms. In view of these side effects, the necessity for sustainable crop production through ecofriendly pest management technique is being largely felt in the recent times. Few biopesticides are available in the market, among them *Lecanicillium* spp. based microbial bio-pesticide gaining much importance for sucking pests for organic and sustainable agriculture [1–4]. Myco pesticides are potential microbial alternative to chemical pesticides and offer a number of benefits such as facility of growth on a variety of substrates, high virulence, trans cuticular penetration, broad host range, less expensive, safe to humans, animals and the environment. Therefore, this review is prepared by compiling the research work done on *Lecanicillium* spp. by various research groups on various
aspects viz., nomenclature, mode of infection, natural occurrence, effect of temperature and humidity for the growth, factors influencing its efficacy, virulence and pathogenicity against target pests under laboratory/greenhouse/field, substrates used for mass production, safety to non-target organisms, compatibility with agrochemicals and commercially available products were discussed and presented.

2. Nomenclature of *Lecanicillium* spp.

The genus *Verticillium* contains diverse host ranges including arthropods, nematodes, plants and fungi [5]. The genus has been redefined using rDNA sequencing, grouping insect pathogens into the new genus *Lecanicillium* which includes *L. attenuatum*, *L. lecanii*, *L. longisporum*, *L. muscarium* and *L. nodulosum*, which were all formerly classified as *V. lecanii* [5–7].

3. Mode of infection

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 or grow over the surface of the epicuticle. The germ tube penetrates by lysing both the epicuticle and the procuticle [8, 9]. This is accomplished by the mechanical pressure exerted by appresorium (penetration peg) and secretion of enzymes viz., proteases, chitinases and esterases which plays an important role during cuticle penetration of insect host and also serve as cuticle degrading enzymes. The fungus proliferates throughout the insect’s body, draining the insect of nutrients, and eventually killing it in around 48–72 hours. The mycotoxins produced by *L. lecanii* are bassianolide [10, 11], vertilecanin-A1, decenedioic acid and 10-hydroxy-8-decenolic acid) [12–14]. As the host nutrients are depleted, the blastopores 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. Under favourable environmental condition, conidiophores mature giving rise to conidia which continues the disease cycle further.

4. R & D publications on different aspects of *Lecanicillium* spp.

The number of publications related to *Lecanicillium* spp. from 1971 to 2020 was presented in the Figures 1 and 2. The data clearly indicated that, during 1971–80’s...
the publications were completely nil, but during 1981–91, the R&D work has been initiated in the entire world and the publications were increased gradually reaching 58% during 2001–2020 (Figure 2). While, considering the number publications on various aspects of Lecanicillium spp., more research work has been done on virulence and pathogenicity (Figure 3) followed by biotechnology and biochemistry as compared to morphology, diversity, ecology, mass production. The number of publications was meagre on effect of environmental factors (temperature and humidity), safety to natural enemies and compatibility with pesticides [15].

5. Natural occurrence of Lecanicillium spp.

Lecanicillium spp. is the most widely distributed and generally found on infected insects both in temperate and tropical areas throughout the world. There are number of reports on natural infection of Lecanicillium spp. on different insect pests but out of the reported insects and pests, maximum are sucking pests belonging to Hemiptera, Thysanoptera and Acarina which indicates its possible spectrum for use as a biocontrol agent for pest management. Reports of natural occurrence of Lecanicillium spp. on sucking insects presented in the Table 1.

| Strain/isolate | Host          | Location | Reference |
|----------------|---------------|----------|-----------|
| L. lecanii (Is-2, Is-5) | M. persicae | Israel   | [14]      |
| L. lecanii (Is-6)    | Acrithosiphon pisum | Israel   | [14]      |
| L. lecanii (R-1)     | T. vaporariorum | Russia   | [14]      |
6. Effect of temperature and humidity on the growth of Lecanicillium spp.

Temperature and humidity are the main factors influencing the growth of the fungus. Effect of different temperature on conidial germination, growth rate, colony size and mycelial growth was discussed and presented in Table 2.

| Strain/isolate         | Host                  | Location                 | Reference |
|------------------------|-----------------------|--------------------------|-----------|
| L. lecanii (V6063)     | T. vaporariorum       | Halifax, Canada           | [2]       |
| L. lecanii (V0175)     | B. tabaci             | Guangdong, China         | [2]       |
| L. lecanii (Vp28)      | Pseudococcus sp.      | Guangdong, China         | [2]       |
| L. lecanii (ICAL4)     | Nasonovia ribisnigeri in lettuce | Madrid | [16] |
| L. lecanii (ICAL6)     | M. persica in pepper  | Madrid                   | [16]      |
| L. lecanii (41185)     | M. persicae, T. vaporariorum | Korea       | [17]      |
| L. longisporum (6541)  | Aphis gossypii   | UK                       | [17]      |
| L. longisporum (6543)  | M. persicae       | UK                       | [17]      |
| L. longisporum (4078)  | M. persicae       | Denmark                  | [17]      |
| L. longisporum (HR1.72) | Macrospodiella tanbournii | UK            | [18]      |
| L. lecanii (ARSEF 7207) | T. vaporariorum     | Argentina                | [16]      |
| L. longisporum (ARSEF 7461) | T. vaporariorum     | Argentina                | [16]      |
| L. muscarium (ARSEF 7460) | T. vaporariorum     | Argentina                | [16]      |
| L. lecanii (ICAL3)     | Macrospodiella tanbournii in tomato | Madrid, Spain | [19] |
| L. lecanii (ITEM 3757) | Brevicornes brassicae in Cabbage | Bari, Italy | [20] |
| L. lecanii              | S. bispinosus on tea | Tamil Nadu, India        | [21]      |
| L. sabaterus sp. nov    | Pulvinaria caballeronoe | Bogota (Columbia) | [22]      |
| L. attenuatum ZJLSP07 and L. psalliotae ZJLLA08 | Diaphorina citri | Taizhou (Zhejiang Province, China | [23] |
| L. lecanii (FI 2482) and L. muscarium (FI 2481) | Thaumastocoris peregrinus | South-East Uruguay | [24] |

Table 1. Natural occurrence of Lecanicillium spp. on different sucking insect pests.

| Temperature | 5°C | 10°C | 15°C | 20°C | 25°C | 30°C |
|-------------|-----|------|------|------|------|------|
| Water activity (aw) | —   | 0.985 | 0.99 | 0.98 | 0.975 | [25] |
| Strain/isolate | % Conidial germination |
| L. longisporum (Vertalec) | —   | —    | 98   | 98   | 28.7 | —    |
| L. muscarium (Mycotal) | —   | 20.6 | 98   | 98   | 98   | —    |
| PFC 1        | —   | —    | —    | 50.6 | 47   | —    |
| PFC 3        | —   | —    | —    | 49.7 | 86.6 | —    |
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6.1 Temperature

Temperature affects the Lecanicillium spp. in different ways by influencing the germination, growth and viability of the fungus in the host insect and environment. High temperature inactivates the fungus before contact with the pest insect or may reduce or accelerate the growth within an insect depending on the temperature requirements of the fungus and the host insect. In contrast, low temperatures reduce or stop the germination and growth. Optimal germination and growth rates of Lecanicillium spp. range between 23°C and 28°C, growth rapidly slows >30°C and ceases at 34 to 37°C. Similarly, conidial germination is adversely affected by temperatures above 30°C. Temperature below 16°C increasingly slows germination and growth and thus affects efficacy in terms of a longer survival of the target population.

Lecanicillium strains showed optimum growth at 25°C; the aerial conidia of Lecanicillium strains germinate in a broad temperature range (15–30°C) and L. lecanii 41,185 was the only strain with conidial germination at 35°C [27].

Effect of different temperature on conidial germination, growth rate, colony size and mycelial growth of L lecanii was discussed and presented in Table 2. At 25°C and 0.975 a_w (water activity) conidial germination occurred in all the isolates ranging from 28.7 to 98% whereas isolate PFC 10 no conidial germination had. Percent germination decreased from highest values at 25°C to the lowest trend at 10°C in Mycotol (20.6°C). Maximum germination of conidia was observed between 15 and 25°C [25]. Most of the isolates showed growth at 5 and 30°C and mean growth rate increased as temperature increased. Optimum growth rate occurred at 25°C (1.64 to 2.07 mm) for all isolates) [25]. Colony size of the fungus was influenced by temperature and strain/isolate.

| Temperature | 5°C | 10°C | 15°C | 20°C | 25°C | 30°C | 35°C |
|-------------|-----|------|------|------|------|------|------|
| PFC 10      | —   | 64.7 | 47.7 | 14.7 | —    | —    | —    |
| PFC 11      | —   | —    | 98   | 98   | 49.3 | —    | —    |
| PFC 13      | —   | 88   | 98   | 98   | 98   | —    | —    |

Mean radial growth rates (mm/day)

| Strain/isolate | 5°C | 10°C | 15°C | 20°C | 25°C | 30°C | 35°C |
|----------------|-----|------|------|------|------|------|------|
| L. longisporum (Vertalec) | 0.21 | 0.66 | 1.10 | 1.31 | 1.86 | 0.55 | —    |
| L. muscarium (Mycotal) | 0.22 | 0.59 | 1.03 | 1.59 | 2.03 | 0.59 | —    |
| PFC 1 | 0.16 | 0.43 | 0.90 | 1.13 | 1.64 | 0.69 | —    |
| PFC 3 | 0.15 | 0.54 | 1.03 | 1.35 | 1.86 | 0.83 | —    |
| PFC 10 | 0.18 | 0.63 | 1.02 | 1.40 | 2.07 | 0.05 | —    |
| PFC 11 | 0.17 | 0.58 | 1.03 | 1.25 | 2.05 | 0.05 | —    |

Mean colony size (diameter: mm)

| Strain/isolate | 5°C | 10°C | 15°C | 20°C | 25°C | 30°C | 35°C |
|----------------|-----|------|------|------|------|------|------|
| Vertalec       | 5.0 | 18.6 | 34.1 | 50.2 | 52.1 | 5.0  | —    |
| Mycotol       | 12.1 | 20.5 | 31.5 | 42.1 | 47.3 | 8.3  | —    |
| B-2           | 11.6 | 21.3 | 25.4 | 46.2 | 53.6 | 26.9 | —    |

Table 2. Effect of temperature on growth of Lecanicillium spp.
by the temperature, the colony growth is maximum at 25°C (47.3 to 53.6 mm) as compared to the temperature between 5 to 20°C [26]. The optimum temperature for the mycelial growth of L. lacanii CA-1-G was 23°C (37.57 mg/cm²) and 26°C (39.43 mg/cm²) as compared to 20°C (29.43 mg/cm²), 29°C (20.7 mg/cm²) and 32°C (20.63 mg/cm²). Similarly, L. lacanii grew and sporulated over a wide range of temperatures (20–32°C). The optimum temperature for growth was 23°C (46.45 x10⁵ conidia cm⁻²) or 26°C (33.76 x10⁵ conidia cm⁻²) for L. lacanii CA-1-G [28]. Virulence of Lecanicillium spp. isolates was evaluated against third instar T. vaporariorum on tomato plants at 23°C. Colony radial growth, conidial production and germination decreased with the reduction in water activity, while 32°C was extremely detrimental for all fungal isolates. However, some isolates were able to grow and produce conidia at low water activity and high temperature [29]. L. muscarium can multiplied in temperature range of 15–30°C but optimum temperature against M. persicae between 20 to 30°C [30].

6.2 Humidity

Humidity is another important environmental factor affecting the efficacy and survival of Lecanicillium. Spore germination on the insect cuticle and sporulation after outgrowth of the dead host insect require high moisture. Generally high humidity is required for germination of spores under in vitro, insects can become infected at much lower humidity. Under fluctuating humidity, daily saturated humidity requirement of at least 16 h for causing death in Trialeurodes vaporariorum (Westwood) infected with L. lecanii [31]. Several previous studies provided evidence that a threshold time period at high humidity was required for infection. Conidia of L. lecanii required at least 72 h at 100% RH and 20°C before removal to 70% RH to reach >90% infectivity of Myzus persicae (Sulzer) [32]. Similarly, at 25°C temperature and 75% relative humidity (RH), L. lecanii 41,185 showed highly virulent pathogenicity (100%) against M. persicae and Aphis gossypii Glover [27]. Application of L. longisporum against A. gossypii on cucumber in controlled environment (Temperature; 19–26°C and humidity; 80–98%) resulted in 100% mortality [32, 33]. L. muscarium grow at optimum temperature but higher mortality observed against M. persicae between 55 and 90% humidity [30].

7. Factors influencing the efficacy of Lecanicillium spp. against sucking insect pests

The virulence and pathogenicity of Lecanicillium spp. vary with strain, stage of the insect and dose of the fungus.

7.1 Strains

Virulence of the Lecanicillium spp. varies with strain to strain or isolate to isolate. The isolate ICA L6 was more virulent (LC₅₀ = 1.05 x 10⁷ conidia mL⁻¹) to nymphs of M. persicae than Macrosiphum euphorbiae (Thomas) (LC₅₀ = 1.26 x 10⁷ conidia mL⁻¹) and Nasonovia ribisnigrri (Mosley) (LC₅₀ = 2.78 x 10⁷ conidia mL⁻¹) [19]. The strain VI 6063 imported from Canada was more virulent to Bemisia tabaci (Gennadius) (2.57 x 10⁵ conidia mL⁻¹) than the domestic strains V3450 and Vp28 (LC₅₀ = 6.03 x 10⁵ conidia mL⁻¹) [2]. L. lecanii @ 1x10⁷ conidia mL⁻¹ is more effective against nymphs of Planococcus citri (84% mortality) after six days
of treatment as compared to \textit{L. longisporum} (59\% mortality) [34]. \textit{L. muscarium} isolate FI 2481 \textsuperscript{1} \texttimes 10\textsuperscript{7} conidia mL\textsuperscript{−1} was more effective against \textit{Thaumastocoris peregrinus} (72\% mortality) as compared to \textit{L. lecanii} isolate FI 2482 which reported 50\% mortality [24]. Similarly, \textit{L. lecanii} hybrid strain 2aF4 was more promising (\textit{LC}_{50} = 5.3 \times 10\textsuperscript{4} conidia mL\textsuperscript{−1}) for the management of \textit{Trialeurodes vaporariorum} than \textit{L. lecanii} 2aF4 (\textit{LC}_{50} = 7.8 \times 10\textsuperscript{4} conidia mL\textsuperscript{−1}) [35].

7.2 Stage of the insect

Stage of host plays important role in the success of \textit{Lecanicillium} spp. and not all stages of 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 stages of the particular pest become dominant among population.

First and third instar nymphs of \textit{B. tabaci} (38 and 65\% mortality) were significantly more susceptible to \textit{L. muscarium} than the fourth instar (15\%) in verbena plants. Similarly, first and second instars \textit{B. tabaci} was more susceptible (50 and 55\% mortality) than the third and fourth instar (25 and 20\% mortality) on tomato foliage [36]. \textit{L. lecanii} (ARSEF 7460) showed higher mortality against nymphs of \textit{T. vaporariorum} followed by \textit{L. longisporum} (ARSEF 7207) and \textit{L. muscarium} (ARSEF 74601) \textsuperscript{1} \times 10\textsuperscript{7} conidia mL\textsuperscript{−1}) [16]. The pathogenicity of \textit{L. lecanii} strains was more in pupae (59–72.5\%) than adults (34–52.6\%) after 6 days of inoculation [14]. \textit{L. lecanii} (2.8 \times 10\textsuperscript{7} conidia/ml) isolated from \textit{Scirtothrips bispinosus} (Bagnall) in tea showed higher mortality against larvae (60\%) than adults (30\%) of \textit{S. bispinosus} under laboratory at same dose [21]. Mortality of nymphs of \textit{Plannococcus citri} were more susceptible (84\% mortality) after six days of treatment to \textit{L. lecanii} \textsuperscript{1} \times 10\textsuperscript{7} conidia mL\textsuperscript{−1} as compared to adults which showed 40\% mortality [34]. \textit{L. lecanii} hybrid strain 2aF43 \textsuperscript{1} \times 10\textsuperscript{7} conidia mL\textsuperscript{−1} showed more efficacy against first instar nymphs of \textit{T. vaporariorum} (68\% mortality) as compared to 4th instar nymphs (30\% mortality) and adults (60\% mortality). Similarly, \textit{L. lecanii} hybrid strain 2aF4 is more effective against first instar nymphs (46\% mortality) as compared to 4th instar nymphs (30\% mortality) [35].

7.3 Dose/inoculums 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. Higher dose of \textit{L. lecanii} (1.2 \times 10\textsuperscript{9} conidia ha\textsuperscript{−1}) was caused 92.30 and 80.93\% mortality of \textit{Brevicoryne brassicae} Linnaeus and \textit{Aleurodicus disperses} (Russell) respectively at 10 days after treatment in the laboratory, whereas in field conditions \textit{L. lecanii} (V13) at 2 \times 10\textsuperscript{13} conidia ha\textsuperscript{−1}) causing 61.16\% and 66.50\% mortality of \textit{B. brassicae} and \textit{A. craccivora} respectively [2].

8. Efficacy of \textit{Lecanicillium} spp. against sucking pests under laboratory/ greenhouse/field

Efficacy of \textit{Lecanicillium} spp., against aphids, whiteflies, thrips, scales and mealy bugs in the laboratory/greenhouse/field conditions w.r.to its mortality, \textit{LC}_{50} and \textit{LT}_{50} values were presented in the Table 3.
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| Strain/isolate               | Conditions (Lab, GH, F) | Pest                        | Mortality/LC50/LT50 | Temperature (°C) | Humidity (%) | References |
|------------------------------|-------------------------|----------------------------|---------------------|------------------|--------------|------------|
| Lecanicillium lecanii        | Lab                     | *Bemisia argentifolii*     | 95-98%              | 20–25            | 100          | [14]       |
| *L. lecanii* (HRI 1.72)      | Lab                     | *A. fabae*                 | LT50 (2.79 d)       | 10–23            |              | [23]       |
| *L. lecanii* (HRI 1.72)      | Lab                     | *M. persice*               | LT50 (3.39 d)       |                |              | [37]       |
| *L. lecanii* (V16063)        | Lab                     | *B. tabaci*                | (94.9%) LC50 = 2.57 x 10^5 Conidia mL^-1 | 25               | 95           | [2]        |
| *L. lecanii* (V3450)         | Lab                     | *B. tabaci*                | 86.9 (LC50 = 6.03 x 10^5 conidia mL^-1) | 25               | 95           | [2]        |
| *L. longisporum*             | Lab                     | *M. persice, Macrosiphum euphorbiae, Aulacorthum solani* (LT50 = 2.4;1.8; 2.0 d) | 100% mortality    | 25               | 95           | [38]       |
| *L. longisporum* (HRI 1.72)  | Lab                     | *M. persice, A. fabae, Acrithosiphon pisum, Stinktoniaveneae* | LT50 = 74–78 h   |                |              | [18]       |
| *L. longisporum*             | Cucumber                | *A. gossypii*              | 100% (LT50 = 6.9 d) | 25.8             | 80.6         | [33]       |
| *L. longisporum or L. muscarium* | Lab                  | *Frankliniella occidentalis* | 95%                | 20               | 70%          | [39]       |
| *L. lecanii*                 | Lab                     | *A. cracciva*              | (LC50 = 2.5 x 10^4 spores mL^-1) (LT50 = 3.9 x 10^4 spores mL^-1) |                |              | [40]       |
| *L. muscarium* (1x10^7 spores/ml) | Verbana, tomato (GH)   | *B. tabaci*                | 65 and 55% mortality | 20               | 95           | [41]       |
| *L. muscarium* (1x10^7 conidia/ml) | Verbana, tomato (GH)   | *B. tabaci*                | 85 and 80%         | 20               | 85           | [36]       |
| *L. longisporum*             | Cucumber (GH)           | *A. gossypii*              | 100%                | 19.0             | 80.2         | [42]       |
| *L. lecanii*                 | Tea (F)                 | *S. bispinosus*            | 30–60%              |                  |              | [21]       |
| *L. attenuatum* Z1SLSP07 and L. pullistate ZJLA08 | Lab                     | *Diaphorina citri*         | 100% (1x10^7 conidia/ml) | 25               | 90           | [23]       |
| *L. attenuatum* (SD-16, SDMP1 and 2) | Lab                     | *M. persice*               | 100%                | 25               | >90          | [43]       |
| Strain/isolate | Conditions (Lab, GH, F) | Pest                  | Mortality/LC/LT<sub>50</sub> | Temperature (°C) | Humidity (%) | References |
|---------------|-------------------------|-----------------------|-----------------------------|------------------|--------------|------------|
| L. lecanii    | Lab                     | M. persicae, A. gossypii | 100% (1x10<sup>8</sup> conidia/ml) | 20               | >90          | [44]       |
| L. lecanii (JMC-01) | Lab                     | B. tabaci             | 82.2% (1x10<sup>8</sup> conidia/ml) | 25               | 70           | [45]       |
| L. lecanii (FI 2482) and L. muscarium (FI 2481) | Lab                     | Thaumastocoris peregrinus | 50 and 72%  | 25               | 65           | [24]       |
| L. lecanii 2aF4 3 and 2aF4 | Lab                     | T. vaporariorum       | 83% (LC<sub>50</sub> = 5.3x10<sup>4</sup> conidia/ml) and 84% (LC<sub>50</sub> = 7.8 x10<sup>4</sup> conidia/ml) | 23               | 99.6         | [35]       |

GH; Green house, F; Field, LC<sub>50</sub>; Lethal concentration to kill 50% insects, LT<sub>50</sub>; Lethal time to kill 50% insects.

Table 3.
Efficacy of Lecanicillium spp. against sucking pests under laboratory/greenhouse/field.
9. Substrates used for mass production of *Lecanillium* spp.

*Lecanillium* spp. can be mass multiplied by solid state fermentation (SSF) and liquid state fermentation (LSF) using different growth media. In SSF, different grains, agars and non-synthetic solid media were used for mass production of *Lecanillium* spp. (Table 4).

| Substrates | Conidia/Spores | References |
|------------|----------------|------------|
| **Media**  |                |            |
| Sabaroud dextrose agar | $2.87 \times 10^7$ conidia cm$^{-2}$ | [46] |
| Malt extract agar      | $5.23 \times 10^7$ conidia cm$^{-2}$ |            |
| Nutrient agar          | $1.07 \times 10^7$ conidia cm$^{-2}$ |            |
| Corn meal agar         | $0.09 \times 10^7$ conidia cm$^{-1}$ |            |
| Yeast peptone dextrose agar | $4.58 \times 10^7$ conidia cm$^{-2}$ |            |
| Potato dextrose agar   | $2.91 \times 10^7$ conidia cm$^{-2}$ |            |
| **Grains**            |                |            |
| Rice                  | $8.43 \times 10^7$ spores g$^{-1}$ | [47] |
| Wheat                 | $9.13 \times 10^7$ spores g$^{-1}$ |            |
| Sorghum               | $11.31 \times 10^7$ spores g$^{-1}$ |            |
| Pearl millet          | $10.17 \times 10^7$ spores g$^{-1}$ |            |
| Finger millet         | $9.76 \times 10^7$ spores g$^{-1}$ |            |
| Maize                 | $7.54 \times 10^7$ spores g$^{-1}$ |            |
| Rice                  | $1.97 \times 10^7$ spores g$^{-1}$ | [48] |
| Sorghum               | $1.90 \times 10^7$ spores g$^{-1}$ |            |
| Finger millet         | $1.66 \times 10^7$ spores g$^{-1}$ |            |
| Wheat                 | $1.65 \times 10^7$ spores g$^{-1}$ |            |
| Corn                  | $1.84 \times 10^7$ spores g$^{-1}$ |            |
| Polished rice         | $5.7 \times 10^7$ conidia g$^{-1}$ | [49] |
| Cooked rice           | $1.5 \times 10^7$ conidia g$^{-1}$ |            |
| Rice bran             | $1.4 \times 10^7$ conidia g$^{-1}$ |            |
| Crushed bajra +1% yeast extract (YE) | $17.49 \times 10^7$ conidia g$^{-1}$ | [4] |
| Crushed sorghum +1% YE | $10.34 \times 10^7$ conidia g$^{-1}$ |            |
| Crushed navane +1% YE | $3.52 \times 10^7$ conidia g$^{-1}$ |            |
| Crushed maize +1% YE | $4.80 \times 10^7$ conidia g$^{-1}$ |            |
| Crushed rice +1% YE  | $24.59 \times 10^7$ conidia g$^{-2}$ |            |
| Crushed wheat +1% YE | $3.54 \times 10^7$ conidia g$^{-1}$ |            |
| Rice bran             | $24 \times 10^7$ conidia g$^{-1}$ | [34] |
| **Agro wastes**       |                |            |
| Crushed maize cobs +10% molasses | $10.07 \times 10^8$ conidia/g$^{-1}$ | [4] |
| Wheat bran +10% molasses | $18.76 \times 10^7$ conidia g$^{-1}$ |            |
| Rice bran +10% molasses | $30.86 \times 10^8$ conidia g$^{-1}$ |            |
| Bagasse +10% molasses  | $10.88 \times 10^7$ conidia g$^{-1}$ |            |
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Among grains, rice is most suitable for mass production (1.97 x 10^9 spores g^-1) followed by sorghum (1.90 x 10^9 spores g^-1) as compared to finger millet, wheat and corn (1.6–1.80 x 10^9 spores g^-1) [48]. Similarly, crushed rice +1% yeast extract recorded higher spore yield (24.59 x 10^8 conidia g^-1) followed by crushed bajra +1% yeast extract (17.49 x 10^8 spores g^-1) as compared to crushed sorghum, maize and wheat [4].

Among different agro wastes used for multiplication of *L. lecanii*, the growth and sporulation were found to be better on rice bran +10% molasses (30.86 x 10^6 conidia g^-1) followed by wheat bran +10% molasses (18.76 x 10^6 conidia g^-1) and rice bran (15.98 x 10^6 conidia g^-1). Complete inhibition of growth and reproduction of the fungus was noticed on bagasse and pressmud with 1 per cent yeast extract alone. However, growth was recorded when bagasse and press mud was supplemented with 10% molasses (10.88 and 7.90 conidia g^-1) respectively [4]. Among synthetic media, jack seeds produced high spore yield (4.11 x 10^8 spores g^-1) followed by ladies’ finger (3.12 x 10^8 spores g^-1), carrot (2.17 x 10^8 spores g^-1) and rice husk (1.27 x 10^8 spores g^-1) [47].

| Substrates                        | Conidia/Spores          | References |
|-----------------------------------|-------------------------|------------|
| Press mud +10% molasses           | 7.90 x 10^9 conidia g^-1|            |
| Sugarcane molasses 3%             | 8.35 x 10^7 spores ml^-1| [48]       |
| Sugarcane molasses 4%             | 8.56 x 10^7 spores ml^-1|            |
| Sugarcane molasses 5%             | 8.42 x 10^7 spores ml^-1|            |

### Non synthetic solid media

| Substrates | Conidia/Spores          | References |
|------------|-------------------------|------------|
| Carrot     | 2.17 x 10^9 spores g^-1 | [47]       |
| Jack seeds | 4.11 x 10^9 spores g^-1 |            |
| Ladies finger | 3.12 x 10^9 spores g^-1|            |
| Rice husk  | 1.27 x 10^9 spores g^-1 |            |
| Saw dust   | 0.69 x 10^9 spores g^-1 |            |
| Beet pulp  | 23 x 10^7 conidia g^-1  | [34]       |

### Non synthetic liquid media

| Substrates | Conidia/Spores          | References |
|------------|-------------------------|------------|
| Coconut water | 5.27 x 10^8 spores g^-1 | [47]       |
| Rice cooked water | 2.11 x 10^8 spores g^-1 |            |
| Rice wash water   | 3.12 x 10^8 spores g^-1 |            |
| Wheat wash water  | 1.21 x 10^8 spores g^-1 |            |

### Liquid media

| Substrates          | Conidia/Spores          | References |
|---------------------|-------------------------|------------|
| Potato carrot broth | 6.50 x 10^7 spores mL^-1| [48]       |
| Potato dextrose broth | 3.95 x 10^7 spores mL^-1|            |
| Potato sucrose broth | 6.30 x 10^7 spores mL^-1|            |
| Jaggery yeast broth | 2.45 x 10^7 spores mL^-1|            |
| Sucrose yeast broth | 2.50 x 10^7 spores mL^-1|            |
| Molasses yeast broth | 8.33 x 10^7 spores mL^-1|            |

Table 4. Substrates (media, grains, agro wastes) used for mass production of *Lecanicillium* spp.
In LSF, molasses yeast broth (MYB) supported maximum spore production of *L. lecanii* (8.33 x 10⁷ spores ml⁻¹) followed by potato carrot broth (6.5 x 10⁷ spores/ml) and potato sucrose broth (6.3 x 10⁷ spores ml⁻¹) as compared to Sucrose yeast broth, Jaggery yeast broth and Potato dextrose broth (2.45–3.95 x 10⁷ spores mL⁻¹). Among non-synthetic liquid media, coconut water produced higher spores (5.27 x 10⁷ spores g⁻¹) and biomass production than rice wash water (3.12 x 10⁷ spores g⁻¹) as compared to rice cooked water and wheat wash water (1.21–2.11 x 10⁷ spores g⁻¹) [24]. The growth of *L. longisporum* conidial spores are higher in rice bran [24 x 10⁴ conidia g⁻¹] as compared to beet pulp [23 x 10⁷ spore g⁻¹] [34].

10. Safety of *Lecanicillium* spp. to parasitoids/predators/pollinators

The safety of any bio control agent to parasitoids/predators/pollinators is the important aspect which should be studied thoroughly before its commercialization to avoid the hazards and disturbance of ecological balance. Effect of *L. lecanii* on aphid parasitoid *Aphidius colemani* (Viereck) which showed the normal development (approximately 90% adult emergence) when its cotton aphid, *A. gossypii* host was treated with *L. lecanii* conidia 5 or 7 days after parasitization. Fungus exposure 1 day before or up to 3 days after parasitization, however, reduced *Aphidius colemani* (Viereck) emergence from 0 to 10%. They suggested that the parasitoid and fungus may be used together for aphid bio control [50]. *L. lecanii* showed pathogenicity against predatory mite, *Phytoseiulus persimilis* Athias-Henriot but its effect was lower than that of spider mite, *Tetranychus urticae* (Koch) [51]. *L. lecanii* is safer to predatory coccinellid, *Coccinella septempunctata* Linnaeus and predatory mites, *Amblyseius ovalis* (Evans) and *Amblyseius longispinosus* (Evans) under field conditions [52]. The fungus *L. lecanii* was not pathogenic to *Crypsoperla carnea* (Stephens), *Coccinella septempunctata* (Linnaeus), *Epipyrhus baliatus* (De Geer) and *Samia cynthia ricini* (Boisdouval), but was found to be pathogenic to *Bombyx mori* (Linnaeus). Parasitism, adult emergence and adult longevity of *Trichogramma chilonis* (Ishii) were affected by fungal treatments. Aphid mummification and *Diaeretiella rapae* adult emergence were affected by the fungus. Results suggest that *L. lecanii* is compatible with natural enemies of cabbage aphid, *T. chilonis* and is harmless to silk worm [53]. *L. muscarium* at 10⁶ and 10⁷ spores mL⁻¹ was safer to predatory mite *P. persimilis* [54]. Number of parasitized larvae of *Eretmocerus sp. nr. furushashii* survival decreased with increasing concentrations of *L. muscarium* and only 29% emergence of pupae was observed at a conidial concentration of 1 x 10⁸ conidia mL⁻¹. Similarly, 67% emergence of adult *E. sp. nr. Furushashii* was observed [55]. Parasitoid (*Diaeretiella rapae*) emergence was affected by application of *L. longisporum* before or after parasitism and longevity decreased in female F1 populations [56]. In the laboratory conditions, application of *L. muscarium* (1 x 10⁸ conidia/ml) against *A. colemani* had not affected longevity and fertility of the female *A. colemani*. The combination of *Aphidius colemani* with *L. muscarium* reduced the aphid infestation in the semi field conditions as compared to *A. colemani* alone [30].

The *Lecanicillium* spp. is not harmful to humans during handling in the laboratory and field for the control of pests.

11. Compatibility of *Lecanicillium* spp. with agro chemicals

Chemical pesticides may have antagonistic or synergistic effect on the potentiality of *Lecanicillium* spp. and may 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. The literature on compatibility of *Lecanicillium* spp. with agrochemicals is lacking.

Among different insecticides studied for their effect on *L. lecanii* under in-vitro, malathion was significantly detrimental (69.18% inhibition) than all other insecticides except quinalphos (66.76%). Conversely, endosulfon and chlorpyriphos were significantly safer (37.31 to 44.37%), followed by oxydemeton methyl and dimethoate (45.33 to 48.27% inhibition) [4]. Similarly, endosulfan completely inhibited the germination of conidia and hyphal growth. In contrast, diafenthiuron, thiamethoxam, imidacloprid, thiodicarb, primicarb, omethoate, acetamiprid, and pymetrozine were compatible with *L. lecanii* in planta [57]. Imidacloprid and cyromazine were compatible with *L. lecanii* in terms of vegetative growth, sporulation, conidial viability and pathogenicity against *T. urticae*. At the recommended concentration, the fungicides carbendazim, chlorothalonil, propiconazole, mancozeb and wettable sulphur completely inhibited the germination of candida (100%) except iprodione and triadimefon allowed 37.38 and 41.62% conidia to germinate respectively [4].

### 12. Commercial formulations

The commercial formulations based on *Lecaniillium* spp. are available in India and other countries are presented in Table 5. Number of manufacturers based on *Lecanicillium* spp. products is more in India however; the production is very low and not available to the farmers/stakeholders/growers on time as compared to synthetics due to dominant in pesticides market and lack of awareness to farmers/growers about biopesticides. In India, the efficacy of *Lecanicillium* spp., based products was less due to high temperature and low humidity as compared to temperate countries, even though in India, these products were used as one of the components in IPM and also used for the management of sucking pests of flowers and vegetables in greenhouse.

| Country                   | Trade Name | Target pest                      | Country            | Source                        |
|---------------------------|------------|----------------------------------|--------------------|-------------------------------|
| *Lecanicillium* spp.      |            |                                  |                    |                               |
| Honduras, El Salvador,    | Verzam     | Whiteflies, aphids, thrips, mites | Escuela Agrícola   |                                |
| Nicaragua, Jamaica        |            |                                  | Panamericana       |                                |
| Colombia                  | Vercani WP | Whiteflies                        | Colombia           | www.ica.gov.co                |
| Uruguay                   | Lecafol    | Whiteflies                        | Lage y Cia. S.A.,  | www.lageycia.com              |
|                           |            |                                  | Uruguay            |                               |
| *L. muscarium*            |            |                                  |                    |                               |
| Denmark, Finland, Italy,  | Mycotal    | Whiteflies, thrips                | Koppert Biological | www.koppert.com               |
| UK, Netherlands, Italy,   |            |                                  | Systems, Netherlands|                               |
| Turkey, Switzerland,      |            |                                  |                    |                               |
| Japan, France, India      |            |                                  |                    |                               |
13. Conclusions

*Lecanicillium* spp. is promising biocontrol agent and can be used as one of the components of integrated pest management under green house and field conditions against sucking insect pests. *Lecanicillium* is multiplying on commercially available media (potato dextrose agar and broth etc.) till date but it can be mass multiplied at cheaper rate on solid grain media of sorghum and rice; liquid media of sugar cane molasses. It can be used effectively in conjunction with other natural enemies and compatible pesticides.

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Conflict of interest

Author declares that no conflict of interest is reported.

### Table 5.
Commercially available products based on *Lecanicillium* spp. [58, 59].

| Country | Trade Name | Target pest | Country | Source |
|---------|------------|-------------|---------|--------|
| India   | Bio-Catch  | Whitefly, Aphids, Thrips, Mealy bugs | M/s T. Stanes & Company, India | www.tstanes.com |
| Multiplex Varsha | aphids, thrips, mealy bug, whitefly, scales mites | Multiplex Biotech Pvt. Ltd., Bengaluru, Karnataka, India | www.multiplexgroup.com |
| Verti Guard | ---Do--- | Lokmangal Bio Tech Maharashtra, India | www.lokmangalbiotech.com |
| Sun Bio Verti | ---Do--- | Sonkul Agro Industries Pvt. Ltd. Maharashtra, India | www.bioorganic.co.in |
| Vertisterk | Scales, mealy bugs | Vijaya Agro Industries, Maharashtra, India | www.vijayaagro.com |
| Green Basivert | Aphids, thrips, whitefly, mealy bug, scales | Greentech Biotech Laboratory, Tamil Nadu, India | www.agrizone.in |
| Vertocoz-P | Whitefly, mealy bug | Utkarsh Agrochem Pvt. Ltd., Surat, India | www.utkarshagrochem.com |
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