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 biopesticides 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 esterase's 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-decenoic 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]

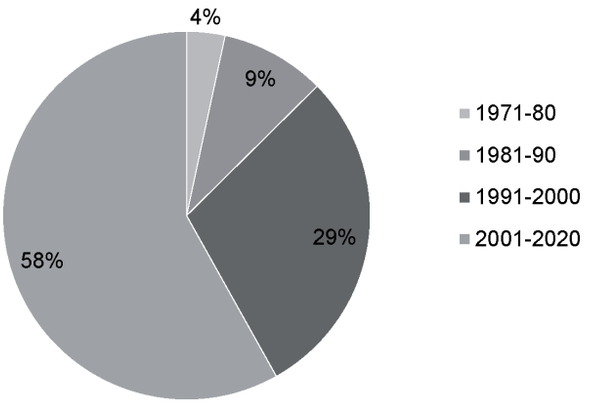

Figure 1.
Per cent R & D publications related to Lecanicillium *spp.*

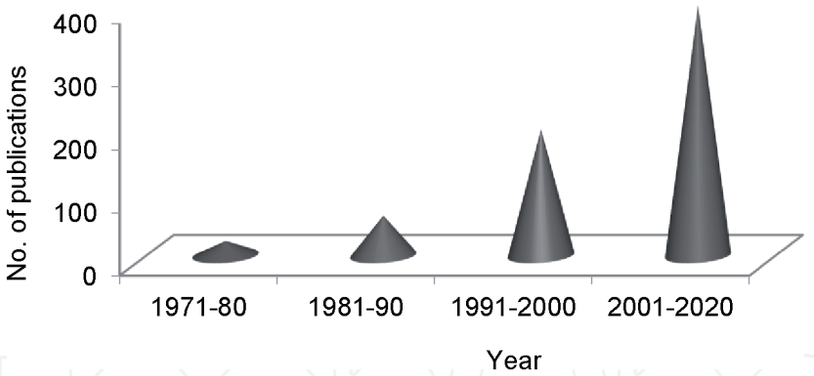

Figure 2.
R & D publications on Lecanicillium *spp.*

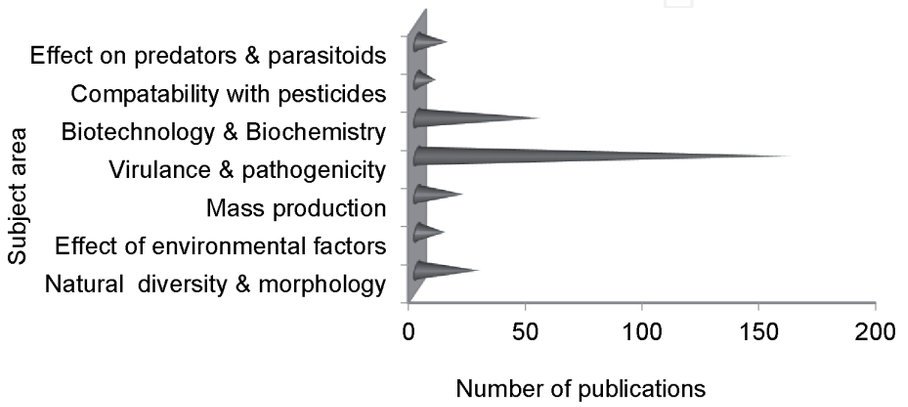

Figure 3.
R & D publications on various aspects of Lecanicillium *spp.*

the publications were completely nil, but during 1981–91^{s}, 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]

Strain/isolate	Host	Location	Reference
L. lecanii (Vl6063)	*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 ribisnigri* in lettuce	Madrid	[16]
L. lecanii (ICAL6)	*M. persicae* 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 (HRI 1.72)	*Macrosiphoniella sanbornii*	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. lecani (ICAL3)	*Macrosiphum euphorbiae* in tomato	Madrid, Spain	[19]
L. lecanii (ITEM 3757)	*Brevicorne brassicae* in Cabbage	Bari, Italy	[20]
L. lecanii	*S. bispinosus* on tea	Tamil Nadu, India	[21]
L. sabanense sp. nov	*Pulvinaria caballeroramose*	Bogota (Columbia)	[22]
L. attenuatum ZJLSP07 and *L. psalliotae* ZJLA08	*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.

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**.

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	—

Temperature	**5 °C**	**10°C**	**15°C**	**20°C**	**25°C**	**30°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	[25]
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	[26]
Vertalec	5.0	18.6	34.1	50.2	52.1	5.0	
Mycotal	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.

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. Per cent 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 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/cm^2) and 26°C (39.43 mg/cm^2) as compared to 20°C (29.43 mg/cm^2), 29°C (20.7 mg/cm^2) and 32°C (20.63 mg/cm^2). Similarly, *L. lecanii* grew and sporulated over a wide range of temperatures (20–32°C). The optimum temperature for growth was 23°C (46.45 x10^5 conidia cm^{-2}) or 26°C (33.76 x10^5 conidia cm^{-2}) for *L. lecanii* 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 ICAL6 was more virulent (LC_{50} = 1.05 x 10^7 conidia mL^{-1}) to nymphs of *M. persicae* than *Macrosiphum euphorbiae* (Thomas) (LC_{50} = 1.26 x 10^7 conidia mL^{-1})) and *Nasonovia ribisingri* (Mosley) (LC_{50} = 2.78 x 10^7 conidia mL^{-1}) [19]. The strain Vl 6063 imported from Canada was more virulent to *Bemisia tabaci* (Gennadius) (2.57 x 10^5 conidia mL^{-1})) than the domestic strains V3450 and Vp28 (LC_{50} = 6.03 x 10^5 conidia mL^{-1}) [2]. *L. lecanii* @ 1x10^7 conidia mL^{-1} is more effective against nymphs of *Plannococcus citri* (84% mortality) after six days

of treatment as compared to *L. longisporum* (59% mortality) [34]. *L. muscarium* isolate FI 2481 @ $1x10^7$ conidia mL^{-1} was more effective against *Thaumastocoris peregrinus* (72% mortality) as compared to *L. lecanii* isolate FI 2482 which reported 50% mortality [24]. Similarly, *L. lecanii* hybrid strain 2aF4 was more promising (LC_{50} = $5.3x10^4$ conidia mL^{-1}) for the management of *Trialeurodes vaporariorum* than *L. lecanii* 2aF4 (LC_{50} = $7.8x10^4$ conidia mL^{-1}) [35].

7.2 Stage of the insect

Stage of host plays important role in the success of *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 *B. tabaci* (38 and 65% mortality) were significantly more susceptible to *L. muscarium* than the fourth instar (15%) in verbena plants. Similarly, first and second instars *B. tabaci* was more susceptible (50 and 55% mortality) than the third and fourth instar (25 and 20% mortality) on tomato foliage [36]. *L. lecanii* (ARSEF 7460) showed higher mortality against nymphs of *T. vaporariorum* followed by *L. longisporum* (ARSEF 7207) and *L. muscarium* (ARSEF 74601) @ 1 x 10^7 conidia mL^{-1}) [16]. The pathogenicity of *L. lecanii* strains was more in pupae (59–72.5%) than adults (34–52.6%) after 6 days of inoculation [14]. *L. lecanii* (2.8 x 10^7 conidia/ml) isolated from *Scirtothrips bispinosus* (Bagnall) in tea showed higher mortality against larvae (60%) than adults (30%) of *S. bispinosus* under laboratory at same dose [21]. Mortality of nymphs of *Plannococcus citri* were more susceptible (84% mortality) after six days of treatment to *L. lecanii* @ $1x10^7$ conidia mL^{-1} as compared to adults which showed 40% mortality [34]. *L. lecanii* hybrid strain 2aF43 @ $1x10^7$ conidia mL^{-1} showed more efficacy against first instar nymphs of *T. vaporariorum* (68% mortality) as compared to 4th instar nymphs (30% mortality) and adults (60% mortality). Similarly, *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 *L. lecanii* (1.2 x 10^9 conidia ha^{-1}) was caused 92.30 and 80.93% mortality of *Brevicoryne brassicae* Linnaeus and *Aleurodicus disperses* (Russell) respectively at 10 days after treatment in the laboratory, whereas in field conditions *L. lecanii* (Vl3) at 2 x 10^{12} conidia ha $^{-1}$) causing 61.16% and 66.50% mortality of *B. brassicae* and *A. craccivora* respectively [2].

8. Efficacy of *Lecanicillium* spp. against sucking pests under laboratory/greenhouse/field

Efficacy of *Lecanicillium* spp., against aphids, whiteflies, thrips, scales and mealy bugs in the laboratory/greenhouse/field conditions w.r.to its mortality, LC_{50} and LT_{50} values were presented in the **Table 3**.

Strain/isolate	Conditions (Lab, GH, F)	Pest	Mortality/LC_{50} /LT_{50}	Temperature (°C)	Humidity (%)	References
Lecanicillium lecanii	Lab	*Bemisia argentifolii*	95–98%	20–25	100	[14]
L. lecanii (HRI 1.72)	Lab	*A. fabae*	LT_{50} (2.79 d)	10–23		[23]
L. lecanii (HRI 1.72)	Lab	*M. persicae*	LT_{50} (3.39 d)	23		[37]
L. lecanii (Vl6063)	Lab	*B. tabaci*	(94.9%) LC_{50} = 2.57 x 10^5 Conidia mL^{-1}	25	95	[2]
L. lecanii (V3450)	Lab	*B. tabaci*	86.9 (LC_{50} = 6.03 x 10^5 conidia mL^{-1})	25	95	[2]
L. longisporum		*M. persice, Macrosiphum euphorbiae, Aulacorthum solani*	(LT_{50} = 2.4;1.8; 2.0 d) 100% mortality	25	95	[38]
L. longisporum (HRI 1.72)	Lab	*M. persicae, A. fabae, Acrithosiphon pisum, Sitobion avenae*	LT_{50} = 74–78 h			[18]
L. longisporum	Cucumber	*A. gossypii*	100% (LT_{50} = 6.9 d)	25.8	80.6	[33]
L. longisporum or L. muscarium	Lab	*Frankliniella occidentalis*	95%	20	70%	[39]
L. lecanii	Lab	*A. craccivora*	(LC_{50} = 2.5 x 10^4 spores mL^{-1}) (LT_{50} = 3.9 x 10^8 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 ZJLSP07 and *L. psalliotae* ZJLA08	Lab	*Diaphorina citri*	100% (1x10^8 conidia/ml)	25	90	[23]
L. attenuatum (SD-16, SDMP1 and 2)	Lab	*M. persicae*	100%	25	>90	[43]

Strain/isolate	Conditions (Lab, GH, F)	Pest	Mortality/LC_{50} /LT_{50}	Temperature (°C)	Humidity (%)	References
L. lecanii	Lab	*M. persicae, A. gossypii*	100% ($1x10^8$ conidia/ml)	20	>90	[44]
L. lecanii (JMC-01)	Lab	*B. tabaci*	82.2% ($1x10^8$ 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_{50} = $5.3x10^4$ conidia/ml) and 84% (LC_{50} = $7.8 x10^4$ conidia/ml)	23	99.6	[35]

GH; Green house, F; Field, LC_{50}; Lethal concentration to kill 50% insects, LT_{50}; 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 *Lecanicillium* spp.

Lecanicillium 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 *Lecanicillium* spp. (**Table 4**).

Substrates	Conidia/Spores	References
Media		
Sabaroud dextrose agar	2.87×10^7 conidia cm^{-2}	[46]
Malt extract agar	5.23×10^7 conidia cm^{-2}	
Nutrient agar	1.07×10^7 conidia cm^{-2}	
Corn meal agar	0.09×10^7 conidia cm^{-2}	
Yeast peptone dextrose agar	4.58×10^7 conidia cm^{-2}	
Potato dextrose agar	2.91×10^7 conidia cm^{-2}	
Grains		
Rice	8.43×10^8 spores g^{-1}	[47]
Wheat	9.13×10^8 spores g^{-1}	
Sorghum	11.31×10^8 spores g^{-1}	
Pearl millet	10.17×10^8 spores g^{-1}	
Finger millet	9.76×10^8 spores g^{-1}	
Maize	7.54×10^8 spores g^{-1}	
Rice	1.97×10^9 spores g^{-1}	[48]
Sorghum	1.90×10^9 spores g^{-1}	
Finger millet	1.66×10^9 spores g^{-1}	
Wheat	1.65×10^9 spores g^{-1}	
Corn	1.84×10^9 spores g^{-1}	
Polished rice	5.7×10^8 conidia g^{-1}	[49]
Cooked rice	1.5×10^9 conidia g^{-1}	
Rice bran	1.4×10^9 conidia g^{-1}	
Crushed bajra +1% yeast extract (YE)	17.49×10^8 conidia g^{-1}	[4]
Crushed sorghum +1% YE	10.34×10^8 conidia g^{-1}	
Crushed navane +1% YE	3.52×10^8 conidia g^{-1}	
Crushed maize +1% YE	4.80×10^8 conidia g^{-1}	
Crushed rice +1% YE	24.59×10^8 conidia g^{-1}	
Crushed wheat +1% YE	3.54×10^8 conidia g^{-1}	
Rice bran	24×10^7 conidia g^{-1}	[34]
Agro wastes		
Crushed maize cobs +10% molasses	10.07×10^4 conidia/g^{-1}	[4]
Wheat bran +10% molasses	18.76×10^4 conidia g^{-1}	
Rice bran +10% molasses	30.86×10^4 conidia g^{-1}	
Baggase +10% molasses	10.88×10^4 conidia g^{-1}	

Substrates	Conidia/Spores	References
Press mud +10% molasses	7.90 x 10^4 conidia g^{-1}	
Sugarcane molasses 3%	8.35 x 10^8 spores ml^{-1}	[48]
Sugarcane molasses 4%	8.56 x 10^8 spores ml^{-1}	
Sugarcane molasses 5%	8.42 x 10^8 spores ml^{-1}	
Non synthetic solid media		
Carrot	2.17 x 10^8 spores g^{-1}	[47]
Jack seeds	4.11 x 10^8 spores g^{-1}	
Ladies finger	3.12 x 10^8 spores g^{-1}	
Rice husk	1.27 x 10^8 spores g^{-1}	
Saw dust	0.69 x 10^8 spores g^{-1}	
Beet pulp	23 x 10^7 conidia g^{-1}	[34]
Non synthetic liquid media		
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		
Potato carrot broth	6.50 x 10^7spores mL^{-1}	[48]
Potato dextrose broth	3.95 x 10^7spores 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.

Among grains, rice is most suitable substrates 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 10^4 conidia g^{-1}) followed by wheat bran +10% molasses (18.76 x 10^4 conidia g^{-1}) and rice bran (15.98 x 10^4 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 agars, malt extract agar (MEA) yields high conidia production (5.23 x 10^7 conidia cm^{-2}) followed yeast peptone dextrose agar (4.58 x10^7 conidia cm^{-2}) as compared to potato dextrose agar and sabaroud dextrose agar (2.91 and 2.87 x 10^7 conidia cm^{-2} respectively) [46]. In non-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].

In LSF, molasses yeast broth (MYB) supported maximum spore production of *L. lecanii* (8.33 x 10^7 spores ml^{-1}) followed by potato carrot broth (6.5 x 10^7 spores/ml) and potato sucrose broth (6.3 x 10^7 spores ml^{-1}) as compared to Sucrose yeast broth, Jaggery yeast broth and Potato dextrose broth (2.45–3.95 x 10^7 spores mL^{-1}). Among non-synthetic liquid media, coconut water produced higher spores (5.27 x 10^7 spore's g^{-1}) and biomass production than rice wash water (3.12 x 10^7 spore's g^{-1}) as compared to rice cooked water and wheat wash water (1.21–2.11 x 10^7 spores g^{-1}) [24]. The growth of *L. longisporum* conidial spores are higher in rice bran [24 x 10^7 conidia g^{-1}] as compared to beet pulp [23 x 10^7 conidia 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 *Chrysoperla carnea* (Stephens), *Coccinella septempunctata* (Linnaeus), *Episyrphus balteatus* (De Geer) and *Samia cynthia ricini* (Boisduval), but was found to be pathogenic to *Bombyx mori* (Linnaeus). 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 10^6 and 10^7 spores mL^{-1} was safer to predatory mite *P. persimilis* [54]. Number of parasitized larvae of *Eretmocerus sp. nr. furuhashii* survival decreased with increasing concentrations of *L. muscarium* and only 29% emergence of pupae was observed at a conidial concentration of 1 × 10^8 conidia mL^{-1}. Similarly, 67% emergence of adult *E. sp. nr. Furuhashii* 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 10^8 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 triadimefom 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, Nicaragua, Jamaica	Verzam	Whiteflies, aphids, thrips, mites	Escuela Agrícola Panamericana Honduras	—
Colombia	Vercani WP	Whiteflies	Colombia	www.ica.gov.co
Uruguay	Lecafol	Whiteflies	Lage y Cía. S.A., Uruguay	www.lageycia.com
L. muscarium				
Denmark, Finland, Italy, UK, Netherlands, Italy, Turkey, Switzerland, Japan, France, India	Mycotal	Whiteflies, thrips	Koppert Biological Systems, Netherlands	www.koppert.com

Country	Trade Name	Target pest	Country	Source
V. lecanii				
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

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

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.

Acknowledgements

Author is grateful to Director, Institute of Himalayan Bioresource Technology (Council of Scientific and Industrial Research, New Delhi), Ministry of Science and Technology, Government of India, Palampur, Himachal Pradesh, India for encouragement and support.

Conflict of interest

Author declares that no conflict of interest is reported.

DOI: http://dx.doi.org/10.5772/intechopen.94020

IntechOpen

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