The Pharma Innovation Journal 2022; 11(9): 500-503

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(9): 500-503

www.thepharmajournal.com
Received: 13-06-2022
Accepted: 18-07-2022

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Isolation of *Lecanicillium* spp., its compatibility with different insecticides and bioassay against cotton aphids

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Abstract
Lecanicillium species are important entomopathogenic fungi ubiquitously distributed in soils, although these fungi are mainly isolated from insects. *Lecanicillium* species have a wide host range including insects, phytopathogenic fungi and plant-parasitic nematodes. The test fungus was isolated from soil by serial dilution method and from infected sucking pest. Detailed morphological studies of effective isolate (Vl2) show that the fungus grew well in SDAY medium. To test the pathogenicity for isolated *Lecanicillium* spp., a bioassay test was conducted against cotton aphids. The efficacy of *Lecanicillium spp*. against *A. gossypii* was determined by using the detached leaf method. Highest insect mortality was recorded at 0.25×10^7 conidia/ml, least mortality recorded at 0.100×10^7 conidia/ml after 2, 4, 6 and 8 days after treatment. Four different semi synthetic media namely PDA, SDAY, CMA and MEA were evaluated for better growth of *Lecanicillium* spp. and it was observed that SDAY medium showed highest growth (53.00 mm) of isolates. Compatibility of *Lecanicillium* spp. with commonly used insecticides was tested. Acetamiprid was seen non-toxic to *Lecanicillium* spp. as no significant reduction in radial growth was noticed.

Keywords: *Lecanicillium* spp, insecticides, bioassay against

1. Introduction
The fungus *Lecanicillium* appears to have been first observed in Ceylon (Sri Lanka) about 1861, on diseased *Lecanium coffeae*. It was subsequently found by Zimmermann on *Lecanium viride* on coffee, in Java (Indonesia) and was briefly described by him under the name *Cephalosporium lecanii*, in 1898. He stated that each dead scale was surrounded by a white fungus. He cultivated the fungus on nutrient agar and directed attention to the possibility that this fungus can be utilized for controlling the scale-insect (Petch, 1925) [6]. *Verticillium* morphology includes a characteristic verticillate arrangement of the 3 to 5 asexual spore producing cells (phialides) forming branches at each node of the conidiophores. *V. lecanii* conidia require high humidity to germinate and possibly do so in water film only (Hall, 1981) [4]. At 5 °C, spores of *V. lecanii* can germinate and grow slowly and above 30 °C its germination and growth may cease. The modern cotton production technology relies predominantly on use of chemicals to control insect pests. Keeping in view the negative aspects of chemical insecticidal usage, biological control of insect pests has gained greater momentum. Under biological control, use of entomopathogenic microorganisms especially entomopathogenic fungi occupies a predominant position. The entomopathogenic fungus, *Lecanicillium lecanii* naturally infects a wide range of sucking pests such as thrips, whiteflies, aphids, etc. The effect of entomopathogenic fungi depends not only on the strain and favorable environmental conditions, but also on their interaction with other factors such as sprays of pesticides, micronutrients, hormones, etc. used by man in his attempt to increase productivity.
The establishment of *L. lecanii* as an endophyte or as the casual agent of an aphid epizootic in the field might be affected by a wide range of insecticides directed against aphids. Compatibility between entomopathogens and insecticides used against aphids in cotton would enable their complementary use to be considered as part of an integrated control strategy (Gurulingappa, 2011) [3].

2. Materials and methods

2.1 Source of culture

The pure culture of fungus *Lecanicillium* spp. was isolated from naturally infected sucking pests on different field conditions from different places, soil by serial dilution and from *Galleria* bait method.

2.2 Test insect for bioassay

Aphis gossypii (Cotton Aphids) were used as test insects. They were collected from cotton crops from agronomy and entomology field of Agriculture College, Nagpur and from CICR-Nagpur. The culture of test insects were maintained on cotton plants planted in pot under net house conditions. The adults were taken from the culture, as and when required. All the experiments were conducted in biocontrol laboratory, Department of Plant Pathology, Dr. PDKV College of Agriculture, Nagpur.

2.3 Isolation of *Lecanicillium* spp. from infected pests

Isolation from infected pest was done simultaneously by two methods. In plating of surface sterilized insect, the symptomatic insect specimens for mycoses were brought to the laboratory from various fields. Upon death or mycosis, the specimens were surface sterilized using 1% sodium hypochlorite, followed by 70% alcohol and 3 repeated changes of sterile distilled water. They were then inoculated on selective media and kept under incubation for one week under room temperature. On development of fungal mycelia, they were transferred to fresh media for further growth. The pure cultures thus developed were stored under refrigeration for identification (Reji *et al*., 2015) [7]. In Direct insect plating the samples were directly plated on the media and allowed for solidification. After solidification the plates were wrapped with para film wax paper and kept for incubation under room temperature.

2.4 Isolation of *Lecanicillium* spp. from soil

In serial dilution method 0.2 g soil sample was placed in a 1.5 ml m Fshinicro centrifuge tube with 1.3 ml of 0.02% Tween-80 solution and vortexed for 15 min. The resulting suspension was serially diluted (10^{-9}) and plated on selective medium After incubation for six days at 25 ºC, the putative entomopathogenic fungi were selected by morphological characteristics (aspects of the colonies, such as color, diameter and mycelia texture) (Shin *et al*., 2010) [8].

2.5 Isolation of *Lecanicillium* spp. by *Galleria* bait method

Collected soil samples were sieved and filled in plastic boxes. Larvae of greater wax moth were successfully used for the detection of fungal pathogens. Depending on the size of the larvae and the amount of soil, 5, 10 or 15 larvae of the bait insects were added to each of the moistened soil samples The boxes were stored at room temperature and inspections were made after 1 week and again after about 2 weeks. The larvae were surface sterilized with 1 per cent mercuric chloride for 3 min and rinsed with sterile water for few minutes. Finally all specimens with signs and symptom of disease were placed in a moist chamber for outgrowth of fungi then mycelia growth transferred to petridish containing PDA and kept at 25 ºC temperature and 90±5 per cent relative humidity. (Zimmermann, 1986) [9].

2.6 Pathogenicity studies

To test the pathogenicity and determine their median lethal concentration value for isolated *Lecanicillium* spp., a bioassay test was conducted by modifying previously reported bioassay procedures (Asi *et al*., 2009; Diaz *et al*., 2009) [1, 2]. A preliminary experiment was run in order to decide the final concentrations for the bioassay. Serial dilutions were prepared in 1000 ml distilled water for each formulation ($0.15X10^7$, $0.20X10^7$ and $0.25x10^7$ spores/1000 ml D.W). The efficacy of *Lecanicillium* spp. against *A. gossypii* was determined by using the detached leaf method. A 2.0 ml from each concentration were smeared on the detached fresh cotton leaf with cotton wrapped to its petiole and later it was shade dried and placed on Petridish containing a thin layer of water agar which is a non-nutritive just to maintain moisture. A batch of 30 laboratory reared *A. gossypii* nymphs were released to each Petri dish and were covered with the double layered muslin cloth. The petri dishes were maintained at room temperature 27±1 ºC and the relative humidity of 70.0 ± 5.0%. Another group of 30 *A. gossypii* was released on the leaf smeared with double distilled water and maintained under the above-mentioned conditions, served as control. Each treatment was replicated thrice with a 30 aphids/replication. Mortality was counted after 2, 4, 6 and 8 days of treatment (DAT). Moribund aphids were counted as dead.

2.7 Compatibility studies of *Lecanicillium* spp. with different insecticides

The study was taken up to assess the impact of different insecticides on radial growth, conidial concentration and conidial viability of *Lecanicillium* spp. Following insecticides were taken as treatments to ascertain the compatibility of *Lecanicillium* spp. with commonly used insecticides.

The required components of SDAY medium were weighed and dissolved in 100 ml of distilled water. Four flasks of with 100 ml media respectively were prepared for four different test insecticides. After cotton plugging, wrapped with the paper media were kept for autoclaving at 121 degrees with 15 lbs pressure for 15 to 20 minutes. After sterilization, the media was allowed to cool to tolerable temperature for handling. After cooling of medium the insecticides were added in given concentrations. The media then was poured into Petri plates were allowed for solidification. After solidification the plates were inoculated with the pure culture of *Lecanicillium* spp. Control plate (without insecticide) was also maintained for comparison purposes. The plates were kept for incubation at 25±5 ºC temperature for 3 to 5 days.

The compatibility was calculated by using following formula.

$$\% \text{ of inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

3. Results and Discussion

3.1 Collection and Isolation of *Lecanicillium* spp.

A roving survey was conducted in Nagpur district of Maharashtra state, a total of 10 places were covered for collection of soil samples and different suspected sucking insect samples infected with EPF. Ten soil samples and three insect samples were collected from ten villages. The soil samples were serially diluted in sterile water and also used in *Galleria* bait method for isolation of EPF, whereas insects were surface sterilized and plated on agar media for growth of fungi. Three isolates of the ten soil samples and two isolates of the three insect samples were identified as *Lecanicillium* spp. based on their morphological and colony characteristics

viz., white or creamy color with thin cottony outgrowth on upper surface, ventrally pale/dark yellow with diameter of 2.4 cm at room temperature and later were confirmed by microscopic examination, which has the hyaline, elliptical or cylindrical conidia with slight depression in the centre, sometimes with slightly pointed ends which were held together on the tip of phialides.

3.2 Compatibility of *Lecanicillium* spp. with different insecticides

The effect of four Insecticides *viz.*, Imidacloprid, Acetamiprid, Thiodicarb and Thiamethoxam was tested on the radial growth of *Lecanicillium* spp. The overall radial growth in different treatments revealed that the Acetamiprid, treated SDAY media recorded highest radial growth of *Lecanicillium* spp. with 35.66 mm followed by Imidacloprid (32.33 mm), Thiamethoxam (29.66 mm) and Thiodicarb (24.00 mm) respectively, as against 40 mm in control. All the treatments including control were significantly different from each other, which indicated that all the treatments were not safe to *Lecanicillium spp.*, but among them the insecticides, acetamiprid was safer when compared to other insecticides followed by Imidacloprid (Table 1). The per cent inhibition of radial growth over control was the lowest in Acetamiprid (10.85%), Imidacloprid (19.17%), Thiamethoxam (25.85%) and Thiodicarb (40%) respectively and all the above insecticides were significantly different from each other. The results are in agreement with Gurulingappa and Sword (2011) [3] who studied the compatibility of *Lecanicillium lecanii* with nine different insecticides used against *Aphis gossypii*.

Table 1: Compatibility of *Lecanicillium* spp. with different insecticides

Sr. No.	Treatment	Conc. (µl or µg)	Radial mycelia growth after 14 days in (mm)*	Per cent inhibition of radial growth over control
1.	Imidacloprid	112.5	32.33	19.17
2.	Acetamiprid	225	35.66	10.85
3.	Thiodicarb	36	24.00	40
4.	Thiamethoxam	157	29.66	25.85
5.	Control	-	40	-
SE(m)±			0.516	
CD (P=0.01)			1.150	

Bioassay against *Aphis gossypii*

It was observed that with the increase in spore intensities, the larval mortality also increased. The data indicates that highest mortality was noticed in spore concentration 0.25 x 10^7 conidia per ml, recording 8.66% mortality in second day and it rapidly increased to 96.66 per cent on eighth day. The mortality increased as incubation period increased and decreased as dilution decreased. The lowest mortality 44.33 per cent at 0.15 x 10^7 spores per ml after eight days of treatment (Table no. 2). This was in accordance with Karthikeyan and Selvanarayanan (2011) [5].

4. Conclusion

Compatibility of commonly used insecticides namely imidacloprid, acetamiprid thiodicarb and thiamethoxam with *Lecanicillium* spp. was tested by poison food technique *in vitro*. The result of the present study marked that acetamiprid can be used with *Lecanicillium* spp. in management of sucking pests. Acetamiprid were non-toxic to *Lecanicillium* spp., as no significant reduction in radial growth noticed. The insecticide thiodicarb recorded 40 per cent inhibition of growth and was toxic to *Lecanicillium* spp. Among isolate studied, isolate Vl2 proved to be a most effective isolate as compared to other isolates hence promising for mycopesticides preparation for control of crop pest. Integration of selected strain of entomopathogenic fungi with selective insecticides can improve the control efficiency. Nowadays the resistance of insect against insecticides increase in that case entomopathogenic fungi gives us selective insect control. The application of entomopathogenic fungi for insect control is increasing largely because of greater environmental awareness, food security concern, availability in cheaper rate, so save the money as compare to chemicals and not hazardous to human lives.

Table 2: Mean cumulative per cent mortality of adult of *Aphis gossypii* with *Lecanicillium* spp. at different concentration

Conidial concentration (Conidia ml^{-1})	Percent cumulative mortality of *Aphis gossypii* after treatment (DAT)*			
	2 Days**	4 Days***	6 Days***	8 Days***
$0.15x10^7$	0.00 (00)	12.2 (20.44)	23.33 (28.88)	44.33 (41.74)
$0.20x10^7$	3.33 (10.51)	17.66 (24.84)	58.66 (49.98)	75.33 (60.21)
$0.25x10^7$	8.66 (17.11)	43.33 (41.16)	77.76 (61.86)	96.66 (79.46)
Control	0.00	0.00	0.00	0.00
SE(m) ±	0.192	0.430	0.981	0.861
C D (P=0.01)	0.679	1.518	3.462	3.036

*Average of three replication.
**Figures in parenthesis are square root mean transformed values.
*** Figures in parenthesis are arcsine transformed value.

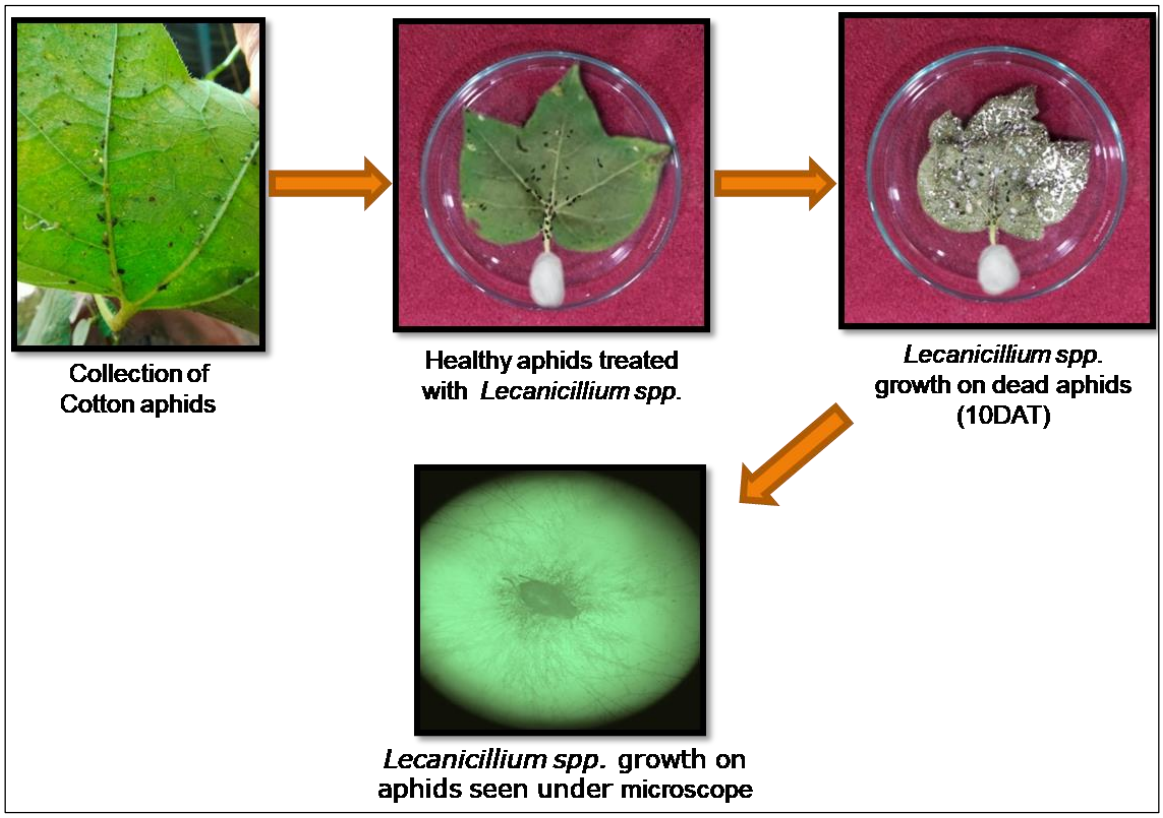

Fig 1: Bioassay by Detached Leaf method against *A. gossypii*

5. Acknowledgement
The authors are grateful to the Director, Central Institute of Cotton Research, Nagpur and Dr. PDKV College of Agriculture, Nagpur for the facilities and encouragement provided during the course of the study.

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