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# COLLECTION, ISOLATION, MORPHOLOGICAL CHARACTERIZATION AND PATHOGENICITY OF *LECANICILLIUM LECANII*

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**ABSTRACT**

Entomopathogenic fungi play a significant role in sustainable insect pest management due to their natural pathogenicity against insect hosts. The present study was conducted to collect, isolate, identify and morphologically characterize the entomopathogenic fungus *Lecanicillium lecanii* from soil samples and insect cadavers collected from different agro-climatic parts of Chhattisgarh. Isolation was carried out using serial dilution and insect cadaver techniques followed by cultural and microscopic identification on PDA medium. Three isolates of *L. lecanii* ($Li_1$, $Li_2$ and $Li_3$) were morphologically characterized based on colony appearance and conidial features. Further, the pathogenic potential of these isolates was evaluated against third instar larvae of rice moth (*Corcyra cephalonica*) under laboratory conditions at different conidial concentrations. Significant variation among isolates and concentrations, with isolate $Li_3$ showing the highest larval mortality. Mortality increased with increasing spore concentration, indicating a dose-dependent effect. The study highlights the potential of *L. lecanii*, particularly isolate $Li_3$, as an effective bio-control agent against *C. cephalonica* and supports its possible use in eco-friendly pest management strategies.

***Keywords*:** Entomopathogenic fungi, *Lecanicillium lecanii,* Pathogenicity

## Introduction

Entomopathogenic fungi (EPF) are pathogenic to insects and play a vital role in insect population dynamics making it the earliest insect pests control agents. Soil is a natural habitat for several important insect pathogenic fungi such as *Beauveria spp*., *Metarhizium spp*., *Paecilomyces spp*. and *Lecanicillium spp*. and is acting as a buffered medium against extreme biotic and abiotic influences (Keller and Zimmerman,1989); (Porte *et al.*, 2025). Entomopathogenic fungi occur naturally in orchard soil, vegetable field and infected insects. However, several entomopathogenic fungi only occur as infection in living hosts for a relatively short period of time during their life cycle. The dead host cadavers will mostly fall to the ground and thus a reservoir of fungal material is present in the soil environment. Further, dispersal from cadavers as focal points presumably occur due to weather (rain and wind), soil manipulation and also insect activity (Meyling *et al*., 2006). The entomopathogenic fungi were also isolated from the forest soils samples using the *Galleria mellonella* insect bait method (Zimmermann, 1986 and Nirmalkar *et al*., 2020[c]).

*Corcyra cephalonica* (Stainton) is a serious pest of some important stored food commodities such as rice, wheat, maize, sorghum, groundnut, cotton seeds, coffee, spices and cocoa beans. *Corcyra cephalonica* is a notorious pest of stored cereals and cereal commodities in India as well as in other tropical and subtropical region of the world. (Meena and Bhargava, 2003).

## Materials and Methods

### Collection, isolation and morphological characterization of entomopathogenic fungi *i.e., Lecanicillium lecanii*.

### Field survey

The field survey was conducted from different district of Chhattisgarh region to collect the soil samples and fungus infected larvae.

### Collection of the soil samples

For the isolation of entomopathogenic fungi from the soil, the soil samples were collected from the various location of Chhattisgarh region viz., Agricultural College and KVK Farm Bhatapara, Balod, Mungeli, Bilaspur, KVK Kawardha, Agriculture college farm and KVK Bemetara, etc. Soil samples were collected using a screw auger from the rhizosphere zone at a depth of 5 to 15 cm below the soil surface. Each sample, weighing approximately 200 grams, was placed in an individual polythene bag and appropriately labelled. Samples with excess moisture was air-dried in the shade. Once dried, the soil was finely ground using a mortar and pestle, and then passed through a 100-mesh sieve to ensure uniformity. The processed samples were then ready for microbial isolation procedures.

### Collection of infected larvae

During the month of September, insect cadavers of different insects was collected, exhibiting characteristic whitish fungal growth were collected from various crop fields, including soybean, groundnut, maize, rice, moong bean. For the isolation of entomopathogenic fungi, infected insects or cadavers were collected directly from the field. Each sample was stored in a 6 × 4 cm plastic container and taken to the laboratory for isolation.

### Isolation of entomopathogenic fungi from soil

Isolation of *L. lecanii* fungi from soil samples was performed according to Soil sample was air dried and coarse particles were removed by passing through sieve. One gram of sieved soil sample was dissolved in 9 ml of sterilized distilled water in a tubes and shaken for five minutes and serially diluted. 0.1 ml of diluted sample was pipetted out from $10^{-2}$ and $10^{-3}$ dilution and spread over the selective agar medium (SDA) supplemented with chloramphenicol. Plates were incubated at 25±2$^0$C for 3-5 days. The white and sporulating colonies were selected and further cultured on Potato Dextrose medium (PDA) supplemented with chloramphenicol.

### Isolation of entomopathogenic fungi from insect cadavers

Collected the infected cadavers will be surface sterilized with sodium hypochlorite solution of 1% and then rinsing with sterile distilled water and drying on filter paper. The cadavers were then aseptically cut into small pieces and infected tissue fragments were transferred onto selective media plates Sabouraud Dextrose Agar (SDA). It was incubated at 25±2$^0$C for growth and inspected daily to observe the fungal growth that was purified and to confirm the pathogens, then stored on slant of PDA artificial media at 4$^o$C until used in subsequent experiments (Nirmalkar *et al*., 2020[a]).

### Identification of fungal isolate

Petri dishes containing PDA media were inoculated with the isolated fungi. The plates were maintained at room temperature and morphological identification was done with the help of microscopy.

### Morphological characterization of entomopathogenic fungi *i.e., L. lecanii*.

Insect-harming fungus viz., *L. lecanii* was culturally examined based on its colony characters, i.e., colony texture, colony colour, spore shape, colony pigmentation, spore colour, spore size and growth pattern on PDA. The fungus was grown on PDA and allowed to evaluate colony characters at the 7$^{th}$ DAI. A tiny thread from a pure culture of *L. lecanii* of 15$^{th}$ days old was taken on glass slides with a sterile needle for morphological studies. Colony texture and colony colour of EPF were recorded on PDA at 8$^{th}$ DAI. While, for spore, a small amount of 15$^{th}$ day old pure culture of EPF (*L. lecanii*) was taken on glass slide with a sterile needle and observed under microscope. The mycelium was then examined under a microscope. Spores were measured under compound microscope at 40x magnification after the ocular micrometre was calibrated with a stage micrometre. The presence of conidiophores and phialides, two similar exterior morphological features, was recorded. Employed by followed formula: -

$$\text{One division of ocular micrometer(g)} = \frac{\text{No.of division of stage mirometer}}{\text{No.of division of ocular micrometer}} \times 10$$

Size= Calibration factor × No. of division of ocular micrometre

$$\text{Mycelial growth} = \frac{\text{Length} + \text{width}}{2}$$

No. of conidia (cm$^2$) = Average no. of spore × dilution factor × 4000 ×1000

(Nirmalkar *et al*.,2020[c])

**Study the pathogenicity of isolated entomopathogenic fungi *i.e., L. lecanii.***

**Rearing of rice moth (*Corcyra cephalonica*)**

The moth *Corcyra cephalonica* was reared on a medium comprising bold, clean grains of rice, wheat, sorghum and other oil seeds were grinded in a domestic grinder by making 2 to 3 pieces of each grain. (Nirmalkar *et.al*. 2020a) Each culture contained 600 g of the particular food commodity, infested initially with randomly selected insects obtained from the laboratory cultures. All equipment used in handling the insects was dry heat sterilised at 100˚C for at least 3 hrs. while, the food media were sterilised in autoclave at 121˚C for 2 h before experimentation. All cultures and experimental setups were maintained at ambient laboratory conditions (temperature range 28˚C±2) and relative humidity range 73% with a photoperiod of 12:12 h, L:D). All the cultures in jars were held in trays with supports immersed in engine oil to prevent insects from crawling into them.(Nirmalkar *et al*.,2020[a])

**Preparation of PDA broth media**

Composition for 1 Liter of broth media

Peeled Potato : 200g

Dextrose : 20g

Distilled water : 1 lit.

**Procedure of broth media preparation**

Take 200g of peeled potatoes and cut into small slices, after heating, 1 litre distilled water on high flame half cooked potatoes that starch present in it comes out then separate the extracted starch and add 20g dextrose to it.

**Procedure of preparation of different spore loads of entomopathogenic fungi (*L. lecanii.*)**

Firstly, the isolate culture was grown fully in a 90 mm petri plates after that, the fully growth culture was moved to the laminar air flow along with broth and equipment's (needle, cork borer and Streep lamp) are sterilized under UV light for 15 to 20 minutes after that, different isolates of fully grown EPF were cut into 5mm diameter discs and transferred into the broth with the help of a needle after that the culture was kept in BOD for growing after 15-20 DAI culture fresh mycelial was mixed than treatment prepared. Third instar larvae of *Corcyra cephalonica* was used to treat the virulence study.

The different treatment of *Lecanicillium lecanii*, products kept in room temperature for virulence study. For the virulence study five larvae were used for each replication and replicated thrice. Moisture filter paper was used to provide favourable condition for conidial growth and germination. Artificial food material was also put in each petri plates and different concentration of *L. lecanii* were sprayed once over the insect then incubated at 25±2˚C and observed 96 hours after inoculation until insect was dead and calculate mortality percentage by given formula

$$\text{Mortality \%} = \frac{\text{Total dead larvae}}{\text{Total no. of larvae inoculated}} \times 100$$

Different isolates used for virulence test that is $Li_1$- *L. lecanii*- isolate 1, $Li_2$- *L. lecanii*-isolate 2 and $Li_3$- *L. lecanii*- isolate 3.

## Results and Discussion

**Collection, isolation and morphological characterization of entomopathogenic fungi *i.e., Lecanicillium lecanii*.**

**Isolation and identification of entomopathogen**

Twelve Insect cadavers were collected from various parts of Chhattisgarh from different crops i.e. Moong (*Spodoptera litura*), Soybean (*Spodoptera litura*) and Moong (*Helicoverpa armigera*). Amongst all cadavers one species of *L. lecanii* were isolated during the process and different others pathogen i.e. *Aspergillus* and *Fusarium* were isolated which was lesser pathogenic or non-pathogenic to insect and cannot consider for further study (Table -2, Plate -1). Various thirty-five soil samples were collected from different parts of Chhattisgarh for isolation of EPF using serial dilution method. Among all collected soil samples two isolates of *L. lecanii* was identified. Different workers also reported entomopathogenic fungi by larva of *Spodoptera litura* and others(Table -1, Plate -1).

The isolated entomopathogenic fungi were identified based on their cultural, morphological and key characters (Humber, 1997) and reconfirmed from ITCC, ICAR New Delhi.

Many reserchers have also isolated *L. lecanii* from different agricultural pest cadavers i.e., thrips, mealy bug, white fly and other insects (Banu (2013a) isolated *L. lecanii* from cotton mealy bug insect cadavers and Gokak *et al*., (2017) collected 30 soil sample and isolated seven *L. lecanii* isolate, Nirmalkar *et al.,* (2020[b]); Verma *et al.,* (2022); Patel *et al*, (2025) and Rajwade *et al.,* (2022), also isolated different entomopathogen from field and horticultural insect pests.

**Morphological characterization of entomopathogenic fungi *i.e., Lecanicillium lecanii*.**

Three isolates of the entomopathogenic fungus *L. lecanii* ($Li_1$, $Li_2$ and $Li_3$) were morphologically characterized based on colony appearance and conidial features (Table -3). Isolate $Li_1$ produced a regularly circular colony, white in colour with a smooth texture. It's conidia were large and lanceolate, measuring 9 µm in length and 1.9 µm in width. Isolate $Li_2$ formed a raised cottony colony, white and smooth, with small cylindrical conidia having half constrictions and rounded ends, and measured 8 µm × 2.4 µm and Isolate $Li_3$ showed a slightly raised cottony colony, white and smooth and produced short ellipsoidal conidia measuring 8.4 µm in length and 2.3 µm in width (Plate -2).

All isolates exhibited smooth-walled, hyaline conidia typical of *L. lecanii*, with shapes ranging from lanceolate to cylindrical and short-ellipsoidal. These morphological characteristics are consistent with descriptions of the species, where conidia are unicellular, hyaline and produced on conidiophores that may occur singly or in small whorls (Plate -3).

**Study the pathogenicity of isolated entomopathogenic fungi *i.e., L. lecanii.***

**Mortality Percent of Rice Moth (*Corcyra cephalonica*) at 96 HAT against Isolates of *L. lecanii***

The data presented in table - 4 show that different isolates of *L. lecanii* exhibited variable mortality against third instar larvae of rice moth (*Corcyra cephalonica*) at 96 hours after treatment (HAT) under laboratory conditions. Among the three isolates, $Li_3$ recorded the highest mean mortality (37.69%) followed by $Li_1$ (35.10%), whereas the lowest mortality (32.26%) was observed in $Li_2$. The variation in mortality among isolates was statistically significant, indicating distinct pathogenic potential of each isolate against the rice moth larvae.

Similarly, the results at different spore concentrations ($1\times10^5$, $1\times10^6$, $1\times10^7$ and $1\times10^8$ CFU $ml^{-1}$) revealed a clear dose-dependent relationship between conidial concentration and larval mortality. The maximum mean mortality (45.09%) was recorded at $1\times10^8$ CFU $ml^{-1}$ followed by $1\times10^7$ CFU $ml^{-1}$ (40.27%) and $1\times10^6$ CFU $ml^{-1}$ (29.26%), while the minimum mean mortality (25.44%) was observed at the lowest concentration ($1\times10^5$ CFU $ml^{-1}$). Overall, the results demonstrated that increasing spore load significantly enhanced mortality of *C. cephalonica* larvae. Among the isolates tested, $Li_3$ proved to be the most virulent strain of *L. lecanii* at 96 HAT.

**Mortality at ($10^5$ conidia $ml^{-1}$)**

The results presented in table - 4 revealed that at the conidial concentration of $1\times10^5$ conidia $ml^{-1}$, the mortality of rice moth (*Corcyra cephalonica*) larvae varied slightly among the tested isolates of *L. lecanii*. The highest larval mortality (28.07%) was recorded in isolate $Li_3$ followed by $Li_1$ (25.28%), while the lowest mortality (22.98%) was observed in $Li_2$. Statistical analysis indicated that the differences in mortality among isolates were non-significant, suggesting that at lower spore concentration, all isolates exhibited comparable levels of virulence.

**Mortality at $1x10^6$ conidia $ml^{-1}$**

Among the evaluated isolates of *L. lecanii*, differences in larval mortality of *Corcyra cephalonica* were observed at the concentration of $1x10^6$ conidia $ml^{-1}$. The isolate $Li_3$ exhibited the highest mortality rate (31.47%) followed by $Li_1$ (28.92%), whereas $Li_2$ (27.38%) recorded the lowest mortality percentage. Although variation was apparent among the isolates, the statistical analysis indicated that these differences were not significant.

**Mortality at $1x10^7$conidia $ml^{-1}$**

At the conidial concentration of $1x10^7$ conidia $ml^{-1}$, a noticeable increase in larval mortality of *Corcyra cephalonica* was observed across all *L. lecanii* isolates. The isolate $Li_3$ exhibited the highest mortality rate (42.68%) followed closely by $Li_1$ (40.49%), while $Li_2$ (37.63%) recorded the lowest mortality. Although slight numerical variations were observed among the isolates, these differences were statistically significant.

**Mortality at $1x10^8$ conidia $ml^{-1}$**

At the highest conidial concentration of $1x10^8$ conidia $ml^{-1}$, all isolates of *L. lecanii* showed a marked increase in pathogenicity against *Corcyra cephalonica* larvae. The isolate $Li_3$ recorded the maximum mortality (48.53%) followed by $Li_1$ (45.71%), while $Li_2$ (41.04%) exhibited the lowest mortality percentage. Although the mortality values differed numerically among isolates, statistical analysis revealed that the differences were significant. Similar result was also found by Ibrahim *et al.,* (2016) they isolate and identify the entomopathogenic fungi as biocontrol agents and to evaluate their pathogenicity against the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) and concluded that *Beauveria bassiana* and *Paecilomyces lilacinus* were the most virulent isolates causing 98.0 and 87.5% larval mortality.

Similar trends of results was also found by many researcher i.e. Urkude *et al.,* (2023); Verma *et al*., (2022); Midori *et al*., (2002) and Verma *et al*., (2022) recorded 75.6% mortality and Urkude *et al*., (2023) recorded 79.97% mortality of 3rd instar larvae of rice moth (*Corcyra chepholanica*), Midori *et al*. (2002) reported over 90% mortality of green peach aphids by *L. lecanii*, reported against mortality of *M. persicae* by the entomopathogenic fungi (95%). Kumar *et al*., (2023) found 88.89,84 and 82.22% mortality at $10^7$,$10^8$ and $10^9$ concentrations of spore load respectively Rajwade *et al*., (2022) reported 85% mortality by *P. lilacinus*.

**Suggestions**

- Molecular characterization of *Lecanicillium lecanii* isolates using DNA-based techniques (e.g., ITS sequencing) should be carried out to confirm species identity and assess genetic diversity among isolates.
- Further bioassays on different life stages and other economically important insect pests should be conducted to explore the broader biocontrol potential of *L. lecanii*.
- Investigation into environmental factors such as temperature, humidity, and UV radiation on fungal virulence and persistence would help optimize field application strategies.

**Table 1 :** Details of soil samples collected for isolation of entomopathogenic fungi *L. lecanii*.

| S.N. | Month | Locations | Lattitude and longitude | Standing Crop | Soil type | Sample code |
|---|---|---|---|---|---|---|
| 1. | August | Belgahana Bilaspur | 22.437900°N 82.037800°E | Green gram | Sandy loam | $S_1$-$S_{10}$ |
| 2. | August | Karwa Raipur | 22.494577°N 82.000794°E | Soyabean | Sandy loam | $S_{11}$-$S_{14}$ |
| 3. | August | Alesur Bhatapara | 21.729558°N 81.987413°E | Green gram | Loamy | $S_{15}$-$S_{18}$ |
| 4. | August | Khunta Mungeli | 22.115616°N 82.518716°E | Sugarcane | Loamy | $S_{19}$-$S_{20}$ |
| 5. | August | Pendri Kalan Kawardha | 22.120551°N 81.443864°E | Sugarcane | Loamy | $S_{21}$-$S_{25}$ |
| 6. | August | Jeodan Khurd Kawardha | 22.059028°N 81.296237°E | Redgram | Sandy loam | $S_{26}$ |
| 7. | August | Dholiya Bemetara | 21.773089°N 81.545342°E | Green gram | Loamy | $S_{27}$ |
| 8. | August | Jhal Bemetara | 21.807380°N 81.558912°E | Soyabean | Sandy loam | $S_{28}$ |
| 9. | August | BTC CARS Farm Bilaspur | 22.105576°N 82.138553°E | Green gram | Sandy loam | $S_{29}$ |
| 10. | August | Umarkhohi Bilaspur | 22.601389°N 82.062455°E | Groundnut | Sandy | $S_{30}$ |
| 11. | August | Gaurela-Pendra-Marwahi | 22.718084°N 81.897714°E | Black gram, Green gram | Loamy | $S_{31}$-$S_{35}$ |

**Table 2 :** Details of insects' cadavers collected for isolation of *Lecanicillium lecanii*.

| S.N. | Month | Locations | Lattitude and longitude | Standing Crop | Insect | No. of cadavers collected | Sample code |
|---|---|---|---|---|---|---|---|
| 1 | October | BTC CARS Bilaspur | 21.105576°N 82.138553°E | Moong | *Helicoverpa armigera* | 3 | Li1-Li3 |
| 2 | October | IGKV Field | 21.241389°N 81.719444°E | Soyabean | *Spodoptera litura* | 3 | Li4-Li6 |
| 3 | October | IGKV Field | 21.238611°N 81.716389°E | Moong | *Spodoptera litura* | 6 | Li7-Li12 |

**Table 3 :** Morphological characterizations of entomopathogenic fungi *i.e., L. lecanii*

| S.N. | Isolates | Colony observation | | | Conidial shape | Size of conidia | |
|---|---|---|---|---|---|---|---|
| | | Growth pattern | Colour | Texture | | Length (µm) | Width (µm) |
| 1 | **Li1** | Regularly circular | White | Smooth | Large conidia, lanceolate shape | 9 | 1.9 |
| 2 | **Li2** | Raised cottony | White | Smooth | Small conidia, cylindrical with half constrictions and rounded ends | 8 | 2.4 |
| 3 | **Li3** | Slightly raised cottony | White | Smooth | Short-ellipsoidal shape | 8.4 | 2.3 |

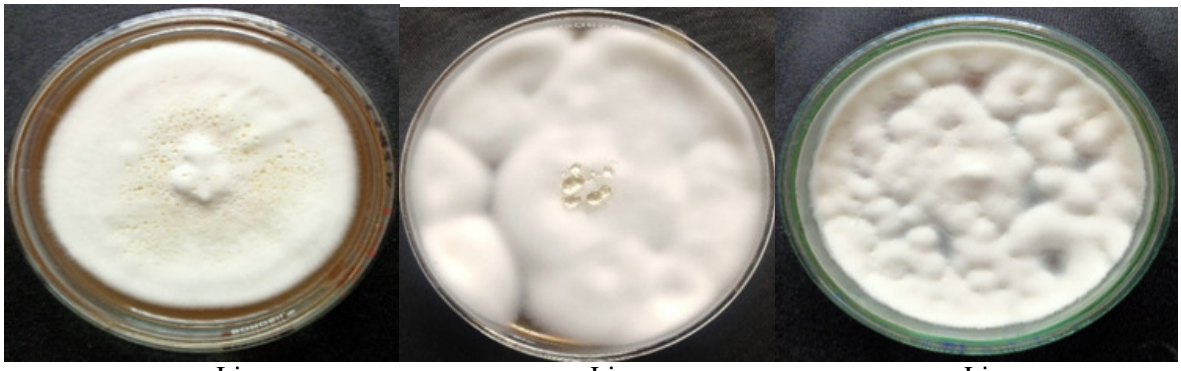
**Table 4 :** Pathogenicity of isolated entomopathogenic fungi *i.e., L. lecanii* against rice moth *(Corcyra cephalonica)* at 96 HAI.

| Isolates | Mortality % | | | | |
|---|---|---|---|---|---|
| | Conidial Concentration ($CFU^{-ml}$) | | | | |
| | $1 \times 10^5$ | $1 \times 10^6$ | $1 \times 10^7$ | $1 \times 10^8$ | Mean |
| **Li1** | 25.28 | 28.92 | 40.49 | 45.71 | **35.10** |
| **Li2** | 22.98 | 27.38 | 37.63 | 41.04 | **32.26** |
| **Li3** | 28.07 | 31.47 | 42.68 | 48.53 | **37.69** |
| **Mean** | **25.44** | **29.26** | **40.27** | **45.09** | |
| | CD | SE(m) | CV | | |
| Factor (A) (Isolates) | 0.73 | 0.25 | 2.45 | | |
| Factor (B) (Concentration) | 0.84 | 0.29 | | | |
| Factor (A X B) | 1.46 | 0.50 | | | |

**CFUs**- Colony forming units, **HAT**- Hours after treatment.

a b c d e

**Plate 1:** Soil sample and insect cadavers collected from Blackgram, Greengram and Soyabean field (A-E)



$Li_1$ $Li_2$ $Li_3$

**Plate 2 :** Colony characterization of different isolates of *Lecanicillium lecanii* ($Li_1$-$Li_3$)

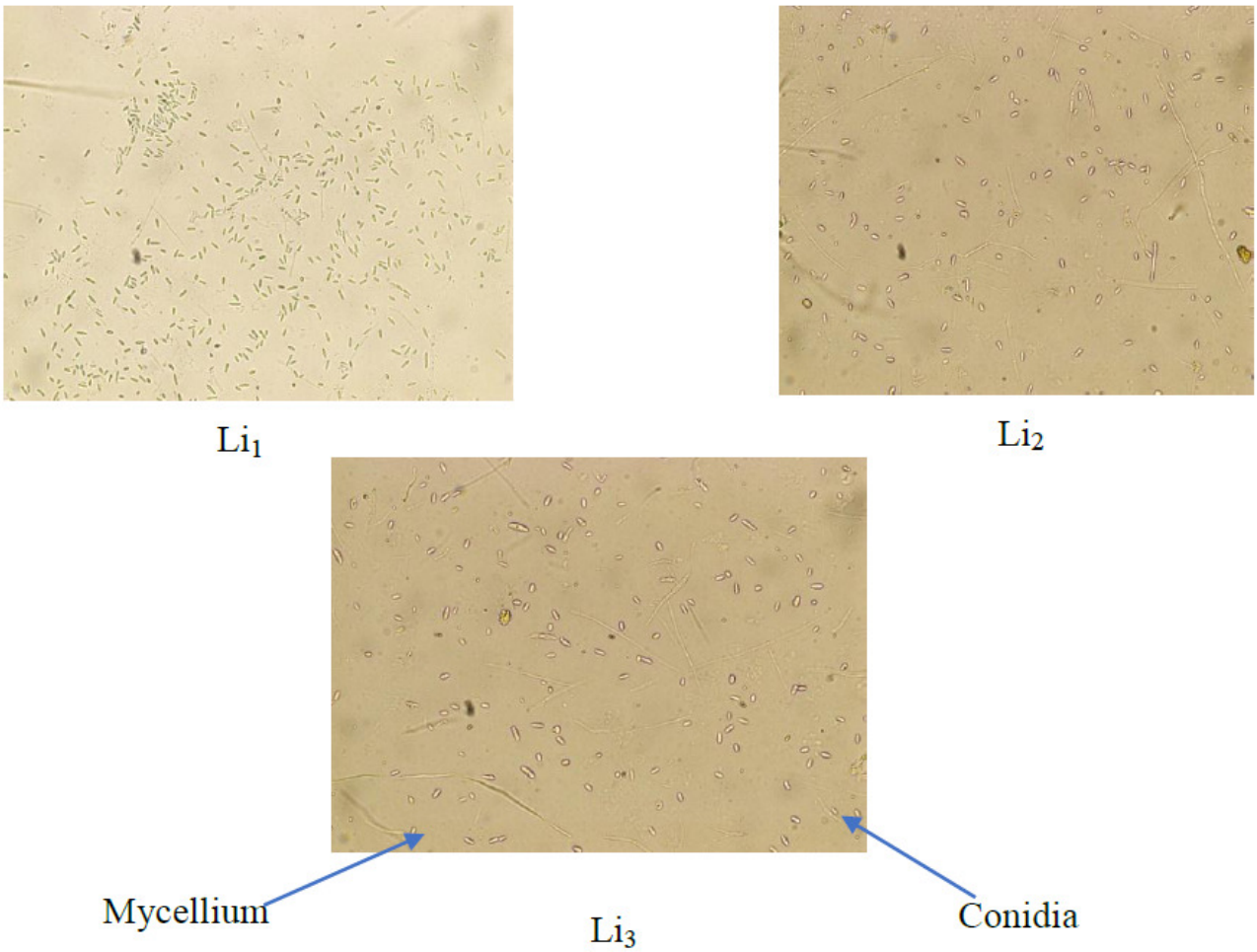
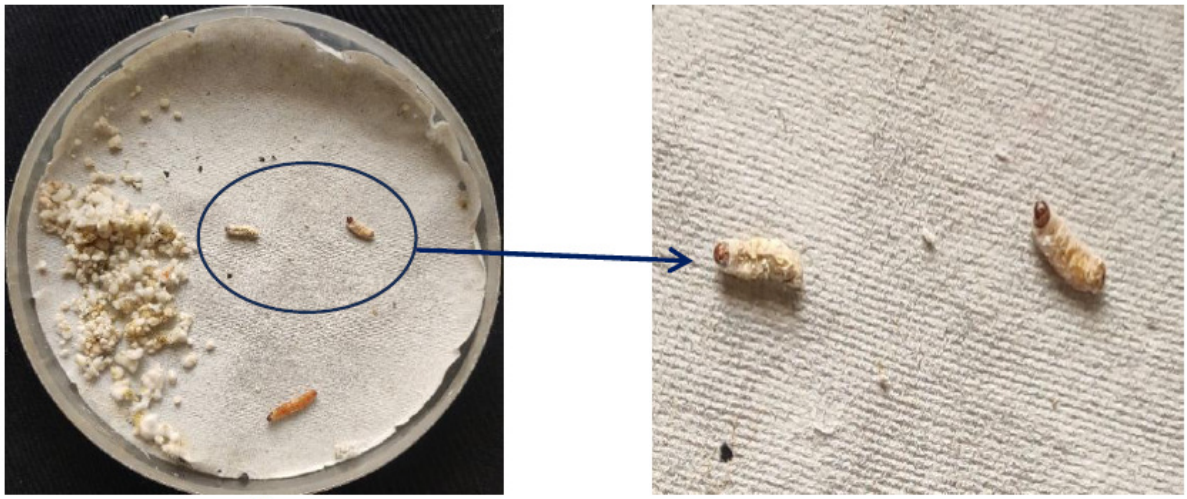



**Plate 3 :** Mycelium and conidia of different isolates of *L. lecanii* at 40x ($Li_1$-$Li_3$)

$Li_1$



$Li_2$

$Li_3$

**Plate 4 :** Pathogenicity of *L. lecanii* against rice moth *(Corcyra cephalonica)* at 96 HAI.

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