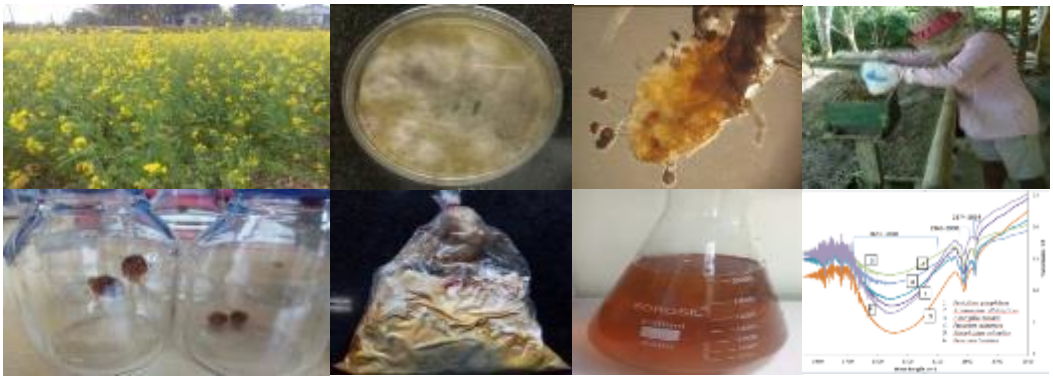
Final Completion Report

Project Report No. 2013NE01

DST REF NO: SR/SO/PS/0039/2012 Dated 24.09.2012

Bioformulation of indigenous entomopathogenic fungi of Assam for control of mustard aphid (*Lipaphis erysinbi* Holt)

Supported by
Department of Science and Technology,
Government of India, New Delhi sponsor



Suggested format for citation

T E R I.2016
Bioformulation of indigenous entomopathogenic fungi of Assam for control of mustard aphid (*Lipaphis erysinbi* Holt)
New Delhi: The Energy and Resources Institute.57pp.
[Project Report No.2013NE01]

For more information

Project Monitoring Cell
T E R I
Darbari Seth Block
IHC Complex, Lodhi Road
New Delhi – 110 003
India

Tel. 2468 2100 or 2468 2111
E-mail pmc@teri.res.in
Fax 2468 2144 or 2468 2145
Web www.teriin.org
India +91 • Delhi (0)11

Table of contents

12. Detailed analysis of results indicating contributions made towards increasing the state of knowledge in the subject:
    12.1 Assessment of selected fungal strain for virulence against the pest *Lipaphis erysinbi* Holt.
        12.1.1 Isolation and purification of of fungal strains
            12.1.1.1Identification of isolates (Morphological)
            12.1.1.2Identification of isolates (Molecular tools)
        12.2.1 Pathogenicity study of fungal strains against mustard aphids
            12.2.1.1 Pathogenicity of *Acremonium cellulolyticus*
            12.2.1.2 Pathogenicity of *Aspergillus niger*
            12.2.1.3 Pathogenicity of *Aspergillus tamarii*
            12.2.1.4 Pathogenicity of *Fusarium culmorum*
            12.2.1.5 Pathogenicity of *Penicillium pinophilum*
            12.2.1.6 Pathogenicity of *Metarhizium anisopliae*
            12.2.1.7 Pathogenicity of *Verticillium lecanii*
            12.2.1.8 Pathogenicity of *Beauveria bassiana*
            12.2.1.9 Pathogenicity of *Acremonium breve*
            12.2.1.10 Pathogenicity of *Nomuraea releyi*
            12.2.1.11 Pathogenicity of *Penicillium chrysogenum*
            12.2.1.12 Pathogenicity of *Aspergillus fumigates*
        12.2.2 FTIR spectrum of selected fungal isolate extract
        12.2.3 LC50 & LT50 study of entomopathogenic fungi
    12.3 Pathogenicity study of fungal strains against Lady Bird beetle (predator) and Honey bee (pollinator).
    12.4 Bioformulation of the virulent fungal isolates
        12.4.1 Granule formulation
        12.4.2 Liquid formulation
        12.4.3 Viable population of fungal isolates
    12.5 Field evaluation of Bioformulation on mustard aphid.
13. Conclusions summarizing the achievements and indication of scope for future work:
14. New Observation
15. Innovation
16. Application Potential

1. S&T benefits accrued
    i. List of Research publications
    ii. Manpower trained on the project
        a) Research Scientists or Research Associates
        b) No. of Ph.D. produced
        c) Other Technical Personnel trained
    iii. Patents taken, if any
2. Financial Position
3. Procurement/ Usage of Equipment

b) Plans for utilising the equipment facilities in future

List of Tables

List of Figures

List of Plates

PROJECT COMPLETION REPORT

1. Title of the project: Bioformulation of indigenous entomopathogenic fungi of Assam for control of mustard aphid (*Lipaphis erysinbi* Holt)

2. File number: DST No: SR/SO/PS/0039/2012 of Dated 24.09.2012

3. Names and addresses of the investigation:

   3.1 PI (Name & Address):
   Dr Alak Chandra Deka,
   Fellow, Environmental and Industrial Biotechnology Division,
   TERI-North Eastern Regional Centre, Hengrabari,
   Express Highway, Guwahati-781036,
   Phone: 0361-2334790, Fax: 0361-2334869,
   E-mail: alak.deka@teri.res.in; alakdeka@gmail.com

   3.2 Co-PI (Name & Address)
   Dr Swapan Kumar Sinha,
   Senior Fellow, Environmental and Industrial Biotechnology Division,
   TERI-North Eastern Regional Centre, Hengrabari,
   Express Highway, Guwahati-781036,
   Phone: 0361-2334790, Fax: 0361-2334869,
   E-mail: sksinha@teri.res.in

4. Implementing Institution(s) and other collaborating Institution(s): The Energy and Resources Institute (TERI)-North Eastern Regional Centre, Guwahati-
5. Date of commencement: 21.05.2013
6. Planned date of completion : 20.05.2016
7. Actual date of completion: 20.05.2016
8. Objectives as stated in the project proposal:

   8.1 Assessment of selected fungal strain for virulence against the pest *Lipaphis erysinbi* Holt.
   8.2 Study the impact of virulent fungal isolates on predators(Lady bird beetles *Coccinella septempunctata*) and pollinator(Honey bees)
   8.3 Bioformulation of the virulent fungal isolates
   8.4 Field evaluation of Bioformulation on mustard aphid.

9. Deviation made from original objectives if any, while implementing the project and reasons thereof: Nil

10. Experimental work giving full details of experimental set up, methods adopted, data collected supported by necessary table, charts, diagrams & photographs:

11. Methodology:

11.1 Isolation and pure culture establishment of fungal strains

Fungal strains were isolated following three different methods i) direct isolation from mycosed cadavers of mustard aphids, ii) soil samples on agar plates and iii) soil samples using insect baiting methods.

11.1.1 Isolation methods

11.1.1.1Direct isolation from mycosed cadavers of mustard aphids

The collected cadavers of the mustard aphid were surface sterilized and then transferred to PDA (potato dextrose agar) media containing different concentrations of streptomycin. The mycosed aphids collected from the fields were surface sterilized with 1 % sodium hypochlorite for 2 mins. followed by rinsing with sterilized double distilled water for 5 times.

After 1 week of incubation in BOD incubator at 28^0C temperature and 70% humidity external sporulation of the fungal hyphae were observed on the surface of the cadavers of both larvae and adult mustard aphids. In the subsequent week primary cultures of these sporulating fungi were established by scraping the surface of cadavers using sterilized needle and inoculated into PDA medium for further growth. Wherever sporulation was not noticed, the cadavers were homogenized using micro-pestle and small portions of the homogenate were inoculated into PDA medium. After 10 days of incubation, the fungal isolates were sub-cultured several times till purified colonies are established. Thereafter PDA slants of each culture were prepared from purified culture and microscopic observations were carried out for morphological characters of mycelium and conidia.

11.1.1.2 Soil samples on agar plates

Fungal strains were also isolated from soil sample by following serial dilution methods. Two to three drops of diluted soil suspension sample were spread over the sterilized antibiotics fortified PDA plates and incubated in the BOD incubator at 28°C for 5 days. Pure cultures were established by repeatedly sub-culturing these fungal isolates.

11.1.1.3 Soil samples using insect baiting methods

Fifteen numbers of adult mustard aphids were placed on moistened soil containing petriplates that were kept in ambient temperature for 3 days. Thereafter these cadavers were surface sterilized and placed on PDA medium and incubated as above. In the subsequent week fungal mycilial growth were noticed on cadaver surface as well as on the PDA medium. Subsequent purification of fungal strains was followed as mentioned above.

11.1.1.4Identification of isolates (Morphological)

Morphological characteristics of entomopathogenic fungal isolates were reviewed from literature for microscopic identification. Fungal isolates were identified according to Samson *et al.* (1988) and Humber (1997) based on morphological characteristics considering both vegetative and reproductive characters.

Dead pests were examined under a microscope for external symptoms and fungal reproductive structures. Insect cadavers or portion of mycelium were also mounted on slides, stained with lacto phenol and cotton blue.
The virulent isolates were also sending to validated at NCFT (National Centre for Fungal Taxonomy), New Delhi.

11.1.1.5Identification of isolates (Molecular tools)

Additionally, along with the morphological characteristics, molecular techniques were also used for accurate identification of the entomopathogenic isolates. DNA isolation of the Entomopathogenic fungal isolates was carried out by growing the isolates in liquid PD medium for 96-120 hours at 26°C-28°C, after which mycelia were collected by filtration, washed three times with sterile distilled water, freeze-dried and grounded in liquid nitrogen. Five volumes of extraction buffer (50 mM Tris-HCl pH 7.2; 50 mM EDTA pH 8; 3% SDS; 1% beta-mercaptoetanol) were added to 0.5 g of powdered fungal mycelia (Lee & Taylor, 1990), and kept at 65°C for 20 min. An equal volume of phenol : chloroform : isoamyl alcohol (25:24:1) were added to the mixture, stirred gently and then centrifuged at room temperature for 20 min at 5,000 rpm. The aqueous phase were re-extracted with an equal volume of chloroform: Isoamyl alcohol (24:1), and then DNA was precipitated by adding 0.6 volume of isopropanol and 100 mM NaCl. DNA was spooled out with a glass hook, washed twice with 70% (v/v) ethanol and eluted in distilled water.
rDNA analysis of the fungal isolates: Recognition of distinct clades or lineage of organisms from isolates was based on internal transcribed spacer (ITS) regions, 5.8S rDNA. ITS region amplification carried out using universal primers are given below
Primers: ITS1 (5′ - TCC GTA GGT GAA CCT GCG G - 3′) and ITS4 (5′ - TCC TCC GCT TAT TGA TAT GC - 3′)
PCR Conditions : Amplification reactions was achieved in a total volume of 50 µL containing 0.2 µM of each primer; 20 mM Tris-HCl; 50 mM KCl; 2.5 mM MgCl2; 0.1 mM each deoxinucleotide (dATP, dCTP , dGTP and dTTP); 1 U Taq DNA polymerase; and 50 ng genomic DNA. Samples were amplified using an initial step of 15 s at 94°C, followed by 40 cycles (94°C at 15 s, 50°C at 30 s, 72°C at 30 s), and one final extension of 7 min at 72°C.

RFLP analysis and sequencing of the ITS fragment: Restriction Fragment Length Polymorphism (RFLP) analysis of the ITS fragment was carried out using various 4 base pair cutter Restriction enzymes such as TaqI, Sau3A, HaeIII, AluI etc. to find the rapid genetic diversity among genus and species. PCR-amplified ITS fragments of the isolates were purified using the gel extraction kit, and each ITS fragment was sequenced in both directions with the primers ITS1 and ITS4. Amplified ITS sequences were compared with the GenBank Nucleotide Database (http://www.ncbi.nlm.nih.gov) using the algorithm Blast N. The entire sequence was aligned using the Clustal W 1.8 software, and grouped with other sequences deposited in the GenBank. Molecular identification of the 5 fungal strains was carried out using ITS1-ITS4 region. The sequences were blasted in NCBI database for identification.

11.1.1.6Mass Culture of fungi

Potato Dextrose Broth (PDB) was used for large scale production of fungal spores. Conidial suspension were harvested by washing off the Petri plates of pure culture with sterilized distilled water for inoculation into the PDB substrate and incubated at 28° C in BOD incubator for 7-10 days.
All cultures were grown as 100 ml cultures volume in 250 ml Erlenmeyer flasks at 28° C and placed in the rotary shaker incubator at 300 rpm.

11.1.2 Maintenance of culture

A loopful of inoculum from subcultured plate of isolates were transferred to Potato dextrose agar (PDA) and Sabouraud dextrose agar with yeast extract (SDAY) slants and maintained as pure culture.

11.2 Laboratory rearing of pest and predator

11.2.1 Rearing of aphids

Mustard aphids (wingless female, nymph) collected from various mustard crop fields were placed in ventilated plastic jars (10 × 6 cm) and fed the tender Brassica leaves. Simultaneously samples were also transferred to experimental plot of mustard crop at TERI NE campus.

11.2.2 Rearing of lady bird beetle (*Coccinella septempunctata*Linnaeus)

Lady bird beetle (*Coccinella septempunctata*) (20 pairs) were collected from the farmers fields, Boko, Kamrup, Assam and reared in the specimen jars (15 cm Length × 15 cm Width × 25 cm Height) in laboratory condition at 25 ± 1°C with relative humidity 65 ± 5%. The mustard twigs infested with mustard aphid were provided as food for the lab reared lady bird beetles. The experiment was replicated 5 times for each set during the rabi crop season. The eggs were collected gently from the specimen jars and reared in other jars till they become adults by providing mustard twigs infested with mustard aphid. Adult male and female beetle were taken for the bioassay treatment to evaluate the pathogenicity against the selected potent entomopathogenic fungal strains.

11.2.3 Rearing of honey bees

Experimental bee colonies were Indian hive bee type collected in typical movable hive from the referred farmers of Khadi Board, Hengrabari, Guwahati and maintained at experimental plot- Khetri of TERI, Guwahati. It comprises of wooden boxes with two holes one is for entrance and other for exit of the bees. The hives containing 10 frames (44.4 x 23 cm) are used for the bee rearing. These frame containing boxes were placed nearby tree in open area on a wooden stand where base are with water to prevent insect.Trees serve as wind belts for protection of hives. The selected site is near the crop field like vegetable crops, oilseed crops, fruits and nuts that yield pollen and nectar to the bees. Clean water source also provided nearby the hive.

11.3 Pathogenicity study of fungal strains against mustard aphids

11.3.1 Screening of pathogenicity

Aphid's population were raised and treated with isolated fungal strains following bioassay method. The fungal strains showing >70% aphid mortality within 3 days of treatments were chosen to understand their virulence on friendly insects lady bird beetle and honey bees.

11.3.2 Screening of virulence against Mustard aphids

Healthy aphids were used in pathogenicity assays.

The established pure culture of the fungal isolates from the primary isolation plates were applied to the laboratory reared mustard aphid by leaf bioassay method and observed their efficacy. Six different conidial suspensions i.e.,1×10^4, 1×10^5, 1×10^6, 1×10^7 1×10^8 and 1×10^9 were prepared utilizing serial dilution method (Waksman and Fred, 1922) from 14 days old fungus culture mixed with 0.02% Tween 80 solution. For each treatment the whole leaf was separated from a healthy mustard plant and dipped into 5ml of conidial suspension for 10 s while excess suspension was removed by placing the leaf on sterilized filter paper for 5 minutes, and the control leaf were treated with 0.05% Tween-80 only. These leaves were then placed on moist filter paper in petri plates. Twenty-five healthy aphids per replication were placed on treated and untreated (control) leaf and incubated at room temperature.

The numbers of dead aphids were recorded from the day of inoculation.

11.4 Pathogenicity study of fungal strains against Lady Bird beetle (predator) and Honey bee (pollinator).

11.4.1 Selected Fungal strains virulence bioassay against friendly insect

11.4.1.1**Bioassay of Lady bird beetle**:

The beetle were collected from mustard field and maintained in laboratory condition. Bettles were fed with aphid larvae before the day of experiment. Lady bird beetles were placed into glass bottle singly and these bottles were capped with parafilm or plug to prevent escape and placed into refrigerator at 6±2°C for approximately 20 minutes to minimize the beetle activity for application of fungal treatments. Bioassay of predators was carried out with adult beetles by using the dip method. For the treatment, the beetles were taken out from the refrigerator and 1 ml of conidial suspension was transferred to the glass bottle containing the pre-treated beetle for five second with slow agitation of these conidial suspensions for ensuring proper adhesion of the conidial suspension to the lady bird beetle. After that the conidial suspension was drain out from the glass bottle and the treated beetle were gently tapped out of the glass bottle into sterile filter paper to remove any extra droplets on the beetle. After removal of extra droplets the treated beetles were gently transferred to sterile petri dishes (3 beetles/petridishe). The beetles with similar treatments were grouped on trays and maintained at 25°C and 14:10 light and dark ratio with 88% relative humidity. Mortality of these treated beetles was checked daily for the next 7 days. The dead beetles were maintained on the same petri-dishes and examined daily till the visual sign of mycosis observed.

11.4.1.2Bioassay of Honey bees:

Feeding method:

Fifty young bees collected from the colony maintained at experimental plot-Khetri of TERI, Guwahatiwere kept in the hoarding case at room temperature varying between 25° to 30°C. The bio-agents (10^8 cfu/ml of each) mixed in 20% sucrose solution were fed to bees by keeping the bio-agent containing sucrose absorbed pre-sterilized cotton on petri-dishes in a corner of the box as described by Soni and Thakur (2011). Mortality of bees in the hoarding cages was recorded daily till seven days. In control bees were fed with 20% sucrose solution only.

11.5 **Standardization of substrate for Bioformulation**.

11.5.1 Bioformulation of fungal bioagents:

11.5.1.1**Bioagent preparation:**

The seven days old fungal cultures was washed with sterile distilled water to obtain the conidial suspension and diluted to obtain 10^8 spores/ml. Carboxymethyl cellulose (0.5% prepared in distilled water), and white flour gum(2%) were used as adhesives for the formulation. Carrier organic substrate Rice bran (75micron size) was mixed with the adhesive(CMC and white flour gum) and 3% mannitol. The moisture content adjusted to 25 per cent by adding distilled water. The pH of the mixture was adjusted to 7 by adding required amount of CaCO3.

100 g of the formulated mixture were weighed in autoclavable polybags (8 cm x 6.5 cm), adding cotton plug tied with rubber band /sealed and autoclaved at 121°C for 30 minutes. After proper cooling the sterilized polybags were placed under laminar air flow hood. Subsequently, the *Penicillium pinophyllium* and *Acremonium cellulolyticus* fungal conidial suspension of 10^8 cfu/ml concentration was transferred separately to the 100 g sterilized mixture at 1:10 (v/w ratio) and thoroughly mixed with the substrate. The punctured holes were sealed with adhesive tape to avoid contamination. The produced bioformulations were stored at normal room temperature. Viable population of fungal isolates in the powder formulation was determined at 7, 15, 30, 60, 90, 120, 150 days at room temperature.

11.5.1.1.1 Procedure-1

Rice bran, rice straw, wheat bran, decomposed mustard oil cake, vermincompost were screened as carrier substrate for bioformulation. The air dried organic substrates were grinded and sieved with 200 mesh (ASTM) that allows 75 micron sized particles and filled 100 g mixture in autoclavable polythene (6x8.5 cm).

11.5.1.1.2 Procedure-2

Secondly, the bioagents (*Penicillium pinophyllium* and *Acremonium cellulolyticus*) were cultured in a liquid growth medium and incubated (at 28°C, 180 rpm) for seven days followed by allowing static sporulation at 28°C without shaking for another 7 days. After that fungal conidia were harvested from the highly sporulated mycelial mate and suspended in pre-sterilized 10% sucrose water and

finally adjusting at 10^{12}conidia/ml. The resulting spore suspensions were then mixed with 2% humic acid and 0.1% Glycerol for formulation.

11.5.1.2Viability assessment of Fungal bioagents in Bioformulation:

The viability of formulated bioagents were assessed and compared to the viability of non-formulated spore suspension (control) to determine the effects of formulated materials on viability of fungal spores. Viability assessment was conducted at every monthly interval for 3 months storage period. For each assessment 1.0 g of the bioformulation was sampled and suspended in 15 ml of sterile distilled water. After that a serial dilution was undertaken until 10^4 dilution times. An aliquot of 0.02 ml of each diluted sample was spread on the Chloramphenicol (25 mg/l) fortified PDA plates under sterile condition and then incubated at 28°C for 2 days. The fungal colonies formed on the PDA plates were then enumerated. For control (spore suspension alone) the same procedure was repeated by substituting the bioformulation with 0.5 ml of fungal spore suspension. Similar procedure was also followed for the bioformulations exposed to 6h sunlight (UV rays) for viable fungal spore enumeration.

11.6 Field evaluation of the Bioformulation.

Field experiment: The field experiment was laid out in Khetri, Kamrup district, Assam. The randomized block design (RBD) with 4 replications in plots measuring 2.5 x 2.5 m with a spacing of 60 cm between rows and 20 cm between plants was maintained. Mustard crop was raised during rabi season by following all the recommended package and practices except the plant protection measures. Two entomopathogenic fungal wettable organic formulations of the conidial strength of 1 x 10^8 were prepared from the laboratory produced bioformulations. One untreated control was kept as water spray. The treatment dose @ 2.5 g/l for the bioformulation was used for spraying. High volume knapsack sprayer was used for spraying different formulations with a spray volume of 500 Litre / hectare at 30 and 50 days after sowing for the first and second spray. A day before spraying the pre count of the mustard aphids per plant was made on 10 tagged plants from each replication of treatment forming a quadrate design. The subsequent observations were made at 3, 7 and 10 days after each spray. The observations of the numbers of aphids of both pre and post count on three top, middle and bottom leaves of ten randomly selected plants from each replication for the sucking pest mustard aphid were recorded and analyzed.

11.7 FTIR Analysis of the potent entomopathogenic fungal isolates

FT-IR spectrum also studied to investigate the functional group of the screened potent entomopathogenic fungal isolates against mustard aphids. FTIR (Fourier transform infrared) spectra was analyzed by KBr pellets methods using FTIR spectrophotometer [Perkin Elmer, Spectrum two FTIR, Standard DTC (Dithiocarbamates) KBr (Potassium bromide)] to investigate the functional groups present in the MIR (Mid Infrared) region of 400 – 4000 cm^{-1} in the fungal secrete. The functional groups were assigned referring FTIR data available in published literature. The samples were prepared by harvesting fungal biomass after incubation at Potato Dextrose (PD) broth by filtering through filter paper

followed by repeated washing with de-ionized water to remove any medium component from the biomass. For the FTIR spectra study the vacuum dried samples (2.0mg) were mechanically mixed with 20 mg KBr in reduced pressure and performs analysis.

12. Detailed analysis of results indicating contributions made towards increasing the state of knowledge in the subject:

12.1 Assessment of selected fungal strain for virulence against the pest *Lipaphis erysinbi* Holt.

12.1.1 Isolation and purification of of fungal strains

Twelve fungal isolates of 8 genera such as *Acremonium breve*, *Acremonium cellulolyticus*, *Aspergillus fumigates*, *Aspergillus niger*, *Aspergillus tamari*, *Beauveria bassiana*, *Fusarium culmorum*, *Metarhizium anisopliae*, *Nomuraea releyi*, *Penicillium chrysogenum*, *Penicillium pinophilum* and *Verticillium lecanii* were isolated from mycosed cadavers of mustard aphids, soil sample on agar plate and insect baiting methods (Table 1). Out of these 12 isolates only 3 isolates such as *Acremonium breve, Fusarium culmorum* and *Penicillum pinophilum* were isolated from mycosed cadavers of mustard aphids. Whereas 7 isolates such as *Acremonium cellulolyticus*, *Aspergillus fumigates*, *Aspergillus niger*, *Aspergillus tamarii*, *Nomuraea releyi*, *Penicillium chrysogenum* and *Verticillium lecanii* were isolated from soil sample on agar plate and 3 isolates such as *Aspergillus fumigates*, *Beauveria bassiana* and *Metarhizium anisopliae* by insect baiting methods. Further, out of these 12 isolates the only common isolates found was *Aspergillus fumigates* between soil sample on agar plate and insect baiting methods.

Table 1: Isolation and pure culture establishment of fungal bio-agents

Sl no	Strains	Method of isolation		
		Mycosed cadavers of mustard aphids	Soil samples on agar plates	Soil samples using insect baiting methods
1.	*Acremonium breve*	√	X	X
2.	*Acremonium cellulolyticus*	X	√	X
3.	*Aspergillus fumigates*	X	√	√
4.	*Aspergillus niger*	X	√	X
5.	*Aspergillus tamarii*	X	√	X
6.	*Beauveria bassiana*	X	X	√
7.	*Fusarium culmorum*	√	X	X
8.	*Metarhizium anisopliae*	X	X	√
9.	*Nomuraea releyi*	X	√	X
10.	*Penicillium chrysogenum*	X	√	X
11.	*Penicillium pinophilum*	√	X	X
12.	*Verticillium lecanii*	X	√	X

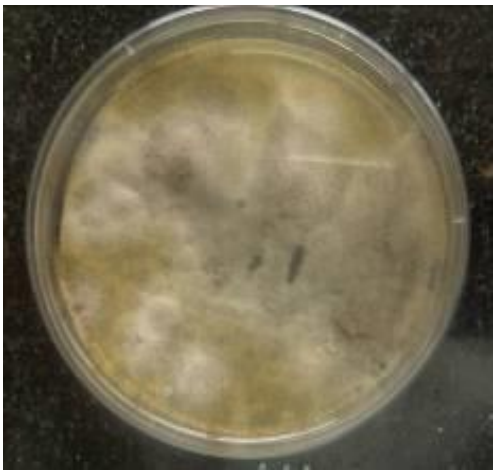
12.1.1.1Identification of isolates (Morphological)

The morphological characters of 12 fungal isolates are described in the table 2. The colony features and microscopic observation are illustrated in Plate 1and 2 respectively.

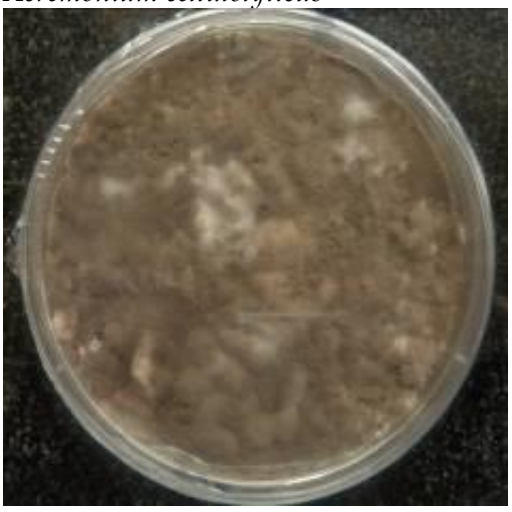
Table 2: Morphological characteristics of fungal bio-agents used for bioassay treatment

Sl No	Fungal Isolate	Morphological Characteristics
1	*Acremonium cellulolyticus*	Initially compact and moist. Hyphae fine. Conidia one-celled, pigmented, globose to cylindrical and arranged in slimy heads.
2	*Penicillium pinophilum*	Macroscopic feature of P. pinophilum on petridish containing PDA media showed rapid growth, dark green colour, granular powdery colony.
3	*Fusarium culmorum*	Colony colour- Pale to brown-greenish to white-greenish, Conidiogenous cell- long and branched monophides
4	*Aspergillus tamarii*	Rapid growth, surface rough walled, conidial shape-spherical, conidia surface - smooth
5	*Aspergillus niger*	Rapid growth, Stripes colour- slightly brown with smooth walled surface, conidia shape-glubose, conidia surface – rough and irregular.
6	*Metarhizium anisopliae*	Rapid growth in PDA media, Colony colour- pale to dark green, conidia spherical
7	*Verticillium lecanii*	Initially witish to creamy colony, compact, less dense hyphae, rapid growth in PDA, conidia colour-green.
8	*Beauveria bassiana*	Rapid growth, crèmes white colony, globose to flask-shaped conidiogenous cells , one-celled terminal holoblastic conidia, conidia-smooth walled, hyaline, globose to cylindrical in nature.
9	*Acremonium breve*	Moderate growth in PDA, colony margin relatively flat, fasciculate, conidia-dark.
10	*Nomuraea releyi*	White mycelia growth, Spore-green, globose, surface-smooth walled.
11	*Penicillium chrysogenum*	Penicillium chrysogenum showed moderate growth, green colour center of colony found to be white and back side of colony was yellow in colour in PDA media.
12	*Aspergillus fumigates*	Moderate growth, stipes colour- grayish near apex, surface-smooth walled, shape- glubose small in colums, conidia-smooth

Acremonium cellulolyticus

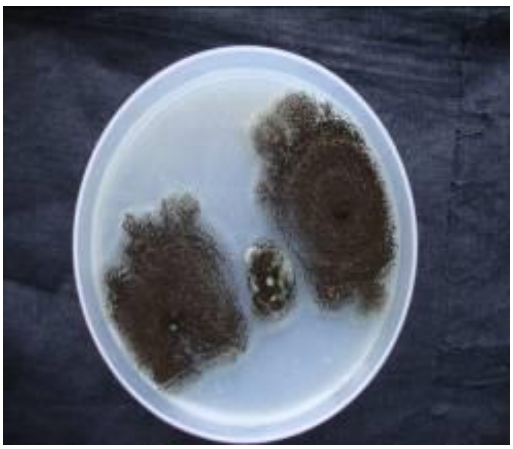


Penicillum pinophilum

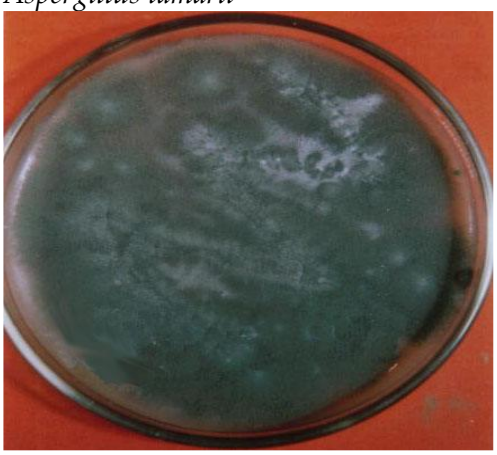


Fusarium culmorum

Aspergillus tamarii

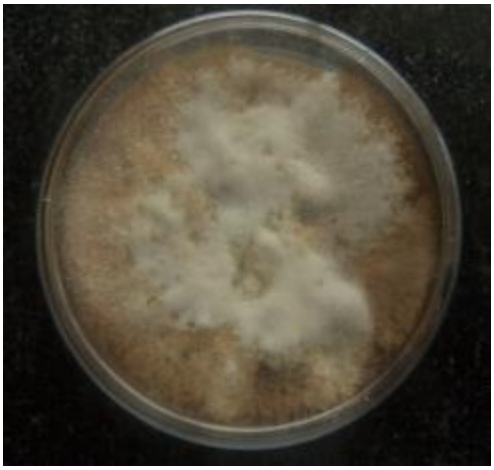


Aspergillus niger



Metarhizium anisopliae

Verticillium lecanii

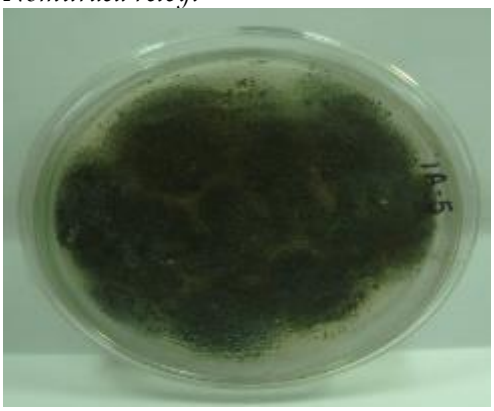


Beauveria bassiana

Acremonium breve

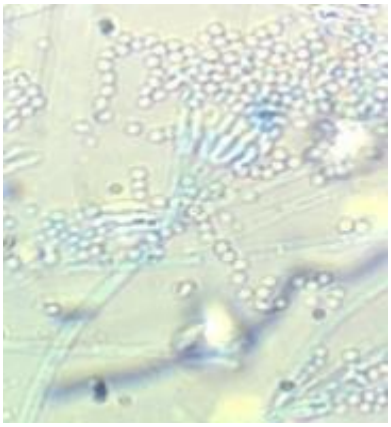
Nomuraea releyi

Penicillium chrysogenum

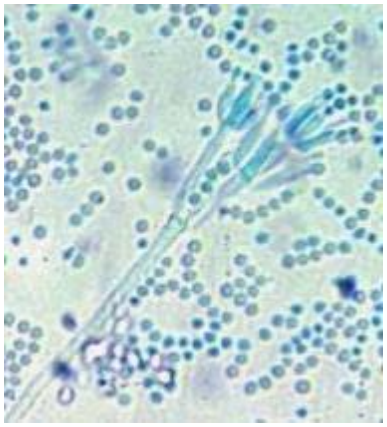


Aspergillus fumigates

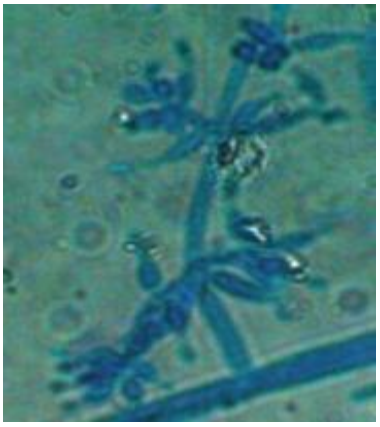
Plate 1: The colony features of 12 fungal isolates.



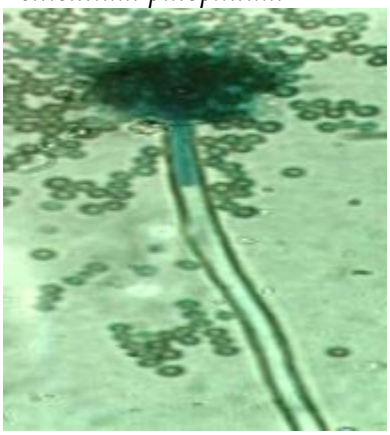
Penicillium pinophilum



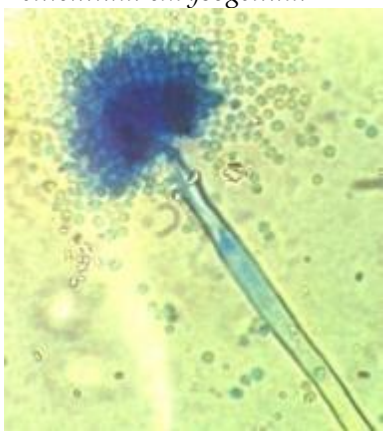
Penicillium chrysogenum



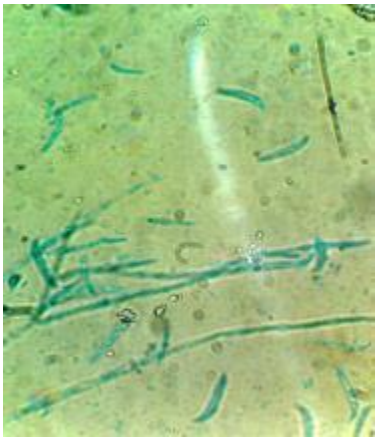
Trichoderma viridae



Aspergillus niger



Aspergillus fumigatus



Fusarium culmorum

Plate 2: Microscopic image of hyphae and spores of 6 fungal isolates

12.1.1.2Identification of isolates (Molecular tools)

Out of 12 molecular fungal isolates 5 strains were amplified and sequenced at ITS1-4 region and BLAST were carried out for alignment with the global database in NCBI and the similarity index are presented in table 3 and 4.

Table 3: DNA sequence(>ITS-1-4) of different entomomathogenic fungal strains

Sl no	Strains	ITS1-4 sequence
1.	*Acremonium cellulolyticus*	GGGGTTTTCCGGTTAATGCTATGCGGGGCTCGCGGCCAACCTCCCACCCTTGTCTCTATAC ACCTGTTGCTTTGGCGGGCCCACCGGGGCCACCTGGTCGCCGGGGGACGCACGTCTCCGG GCCCGCGCCCGCCGAAGCGCTCTGTGAACCCTGATGAAGATGGGCTGTCTGAGTACTGTG AAAATTGTCAAAACTTTCAACAGATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAG CGAAATGCGATAAGTAATGTGAATTGCAGCAATTCACGTGAATCATCGAATTGCGCTGCG TTCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGGGGCATGCCTGTCCGAGCGTCATT TCTGCCAGTAACTCAAGCAGCCCCGGCTTGTGTCAGTTGGGTGTGGTCCCCCCGGGGACC TGCCCGAAAGGCAGCGGCGACGTCCGTCTGGTCCTCGAGCGTATGGGGCTCTGTCACTCG CTCGGGAAGGACCTGCGGGGGTTGGTCACCACCATGGGTGAGGGTTTTACCACGGTTGAC CTCGGACTCAGGTAGGAGTTACCCGCTGAACTTAAGCATATCAATAACCCCGGAAAAGA TCATTTACCGAGTGCGGGCCCTCGCGGCCCCAACTCCCCCCCCCTTGTCTCTATACACCTGT TGCTTTGGCGGGCCCACCGGGGCCACCTGGTCGCCGGGGGACGCACGTCTCCGGGCCCGC GCCCGCCGAAGCGCTCTGTGAACCCTGATGAAGATGGGCTGTCTGAGTACTGTGAAAAAT TGTCAAAACTTTCAACAATGGGATCTCTTGGGTTCGGCATCGATGAAGAACGCAGCGAAA TGCGATAAGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTG CGCCCCCTGGCATTCCGGGGGGCATGCCTGTCCGAGCGTCATTTCTGCCCTCAAGCACGG CTTGTGTGTTGGGTGTGGTCCCCCCGGGGACCTGCCCGAAAGGCAGCGGCGACGTCCGTC TGGTCCTCGAGCGTATGGGGCTCTGTCACTCGCTCGGGAAGGACCTGCGGGGGTTGGTCA

		CCACCATGTTTTACCACGGTGACCTCGGATCAGGTAGGAATATCAAAATT
2.	*Aspergillus niger*	CGGCCAATCCTACAGAGCATGTGACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGC CGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGAGGGGGACGGCGACCCAACACACAAGC CGGGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCCGGAATACCAGGGGGCG CAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCAT TTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGAT TGCATTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTGGGGTCTCCGGCGG GCACGGGCCCGGGGGGCAGAGGCGCCCCCCCGGCGGCCGACAAGCGGCGGGCCCGCCG AAGCAACAGGGTACAATAGACACGGATGGGAGGTTGGG
3.	*Aspergillus tamarii*	TACGGAGGGTGGACTTTCCCCGGGGGTTCCTAGGTACGGAGCCCAACCTCCCACCCGTGT TTACTGTAACCTTAGTTGCTTCGGCGGGCCCGCACTTTAAGGCCGCCGGGGGGCATCAGC CCCCGGGCCCTTGGCGCCCGCCGGAGACACCACTAACTCTGTCTGATCTAGTGAAGTCTG AGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTACCCGCGCAT GCATCGATGACCATGACGCAGCGAAATGCGATACACACTAGTGTGAATTGCAGAATTCC GTGAATCATCGAGTCTTTGAACGCATATTGAGCCCCCTGGTATTCCGGGGGCCTTGCCTGT CCAAGCGTCATTGCTGCCCATCAAGCTCTCCCCTGTGTGTTGGGTCGTCTTCCCCTCTTTGG GGAGATCCGACCCAATTGGTGGTGGCCGCACATATTCCTACCCCCGCGGCTAGAGGGCTC TGTTCCCCCCTTCTGTGGGCACGGCCCGCCGGAGCTAGCTAAGGTAAAACTAACATTTCA TTTCCAGGTTGGGCTTCGGATCGGGACCCCGACTACCCGTTGATGATTCTTCAGATGATAA TGTGGACACAAAGAAGGATCATTACCGAGTGTAGGGTTCCTAGCGAGCCCAACCTCCAC ACCCGTGTTTACTGTAACCTTAGTTGCTTCGGCGGGCCGGCCTTTAAGGACGCCGGGGGG GCATCAGCCCCGGGGGCCCGCGCCGCGCCGGAGACACCACAAACTCTGTCTGATCTAGT GAAGTCTGAGTTGATTGTATCGCCATCAGTTTAAAACTTTCAACAATGGGATCTCTTGGGT TCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGT GAATCATCGAGTCTTTGAACGCACAATTGCGCCCCCTGGTATTCCGGGGGGCATGGCTGG TCGAGAGCGCTATATTGCGTCTCCTCACACGCGGCCTTGTGGTGGTGTGGGGCCCCCGTCC TCTCTCTTGGGGGGGAGGAGAGGCACAAAAGAGAGCGGGACGCACGGCATTCATATCAT CTGAAGTGGTATAGGGGTGTTGTGTTTACGCTCTGTTATAGGCCGCGCCGGCGCTGTGGCC TAACGCAAAACAACCATTCTTTCCAGGTGGACTCGATAGGAGAGATTACCGCTGCATCTA CGTTTCGAGGAGAGAGAAATT
4.	*Fusarium culmorum*	CCGGATATCGAGTTTCACTCCCAACCCCTGTGAACATACCTATACGTTGCCTCGGCGGATC AGCCCGCGCCCCGTAAATGTGACGGGACGGCCCGCCCGAGGCACCCCTAAACTCTGTTTT TAGTGGAACTTCTGAGTAAAACAAACAAATAAATCAAAACTTTCAACAACGGATCTCTTG GTTCCCCACACACAATGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAA TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGTCACATTGCGCCCGCCAGTCATTCTG GCGGGCATGCCTGCTTCGAGCGTCATTTCAGCGACCCTCAAGCTCAGAACCTTGGTGTTG GGACTCAAAAAAAAAAGGGGGCGGTAACCCGCAGGAGGTTCCCACAAATCGATTGGCG GTCACGTCGAGCTTCCATAGCGTAGTAATCATACACCTCGTTACTGGTAATCGTCGCGGC CACGCCGTAGGTAAACCCCAACTTCTGAATGTTGACCTCGGATCTGGAGGTAGGAATACC CGCTGACTTTAGCCATATCATTTGGCCCCGGAGGCGGAGGGTTTTTTTACGGAATTTCCCA CCCCCCAACCCCTGGTGAACATACCTATACGTTGGCTCGGGGGATTAGCGGGCGTACCGT AAAAGGGGACGGGCCCCCCGAGGACCCCTAAATTTTGTTTTTTTAGTGGGGACTTCCGAA TAAAACAAAAAAAATAATTTAAAATTTTTTACTACGGGGCTCTTGTGTTCTGGGCATCAAT GAAAAAAACAACCAAAAGCGGTAAGTTATGTGGAGTTGTGGAAATTATTGAAATATTGA ATCTTTTTTTTCATATTGGGCCCGCCACTATTTTGGGGGGGGAGCCTGTTTAGGCATTATAT TAACCCCCACACCCGGCTTGGTGTTGTGGTACGAGGGAACCCGCGCTCCTCAAATCGATT GGCGGTCACGTCGAGCCTACATAGCGTAGTAATCATACACCTCGTTACTGGTAATCGTCG CGGCCACGCCGTTAAACCCCAACTTCTGAATGTGACCTCGGATCAGGTAGAATGCCCCGA GG
5.	*Penicillium pinophilum*	CTGGACCGCTGCCCCGCCGACTGGCCGGGGCACAGTGCGTAAGAGTTGGTGATACAGAT TTCCACAATCCACATTACTTATCGCATTGCGCTGCTTCTTAATGGACGCCGAACACAAGA GATCCCATGAGAGAGTTTGACAATTTTTCACAGTACTCAGACAGCCCATCTTTCATCAGTT TCACAGAGGGTTCGGCGGGCCCCCGGAGAGTGGCGTCCCCCCCGCGACCCGTGCCCCCG TGGCCCCGCCCAAAGCAACAAGTTTAAAAAGAGAACAAGGTGGAGGTTGGCCGCGAGG GCCCCCCACTCGGTAATGATTTCCTCGTTTAGGGTGTACCTGGGGAAGGATCATTACGGA GTGCGGGGGCCTCGCGGGCCCAACCTCCCCACCCTTGTCTCTATACACCTGTTGCTTTGGC GGGCCCACCGGGGCCACCTGGTCGCCGGGGGACGCACGTCTCCGGGCCCGCGCCCGCCG AAGCGCTCTGTGAACCCTGATGAAGATGGGCTGTCTGAGTACTGTGAAAATTGTCAAAAC TTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT AATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGG CATTCCGGGGGGCATGCCTGTCCGAGCGTCATTTCTGCCCTCAAGCACGGCTTGTGTGTTG GGTGTGGTCCCCCCGGGGACCTGCCCGAAAGGCAGCGGCGACGTCCGTCTGGTCCTCGA GCGTATGGGGCTCTGTCACTCGCTCGGGAAGGACCTGCGGGGGTTGGTCACCACCATGTT TTACCACGGTTGACCTCGGATCAAGGTAGGAGTTACCCGCTGAACTTAAGCATATCAATC

	ATCCCGGAAGGAGATACATTACCGAGTGACGGGCCTCCGGGTCAACCTCCACCTTGTCTC TATTACACTGTTTGCTTTTGGGCGGGCCACGGGGCACTGGTCGCGGGGGACGCCCGTCTC GGGGCGCCGCGAAACGCTCTGTGAAACTGATGAAAGATGGGCTGTCTGAGTACTGTGAA AATTGTCAAACTTTCATCATGGGATCTCTTGGGTTCGGCATCGATGAAGAACCAGCGAAA TGGATAGTTAATGTGTAATTGTGAAATCTGTATCATCTAATCTTTGAAAGCCATTGGGCCC GCGCATTCGGGGGGGCAGCCGTTGAGATCATTTTTTCTTCAACACGCATGTGTGTGTTGCG TT

Table 4: BLAST results of different fungal isolates

Description	Max score	Total score	Query cover	E value	Identity	Accession
Acremonium cellulolyticus strain Cs/9/1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1160	2352	96%	0.0	91%	JN624892.1
Aspergillus niger 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	843	843	100%	0	100%	KM979493.1
Aspergillus tamarii isolate UPM A16 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	588	1123	80%	5e-164	86%	HM116372.1
Fusarium culmorum isolate HN15 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	664	1051	98%	0.0	89%	KJ572170.1
Penicillium pinophilum isolate H4284 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1005	1517	93%	0.0	99%	GU595046.1

12.5.1 Pathogenicity study of fungal strains against mustard aphids

12.5.1.1 Pathogenicity of *Acremonium cellulolyticus*

The effect of entomopathogenic fungus *Acremonium cellulolyticus* against Mustard aphid showed significant results from the 3rd day and 100% mortality was obtained at 3rd day in the spore load 1 x 10^9 while in case of spore load 1 x 10^8 the 100% mortality was recorded on 4th day after treatment. The results showed that mortality was dose dependent which increased with enhancing the concentration while no mortality was recorded in control (Fig. 1, Plate-3).

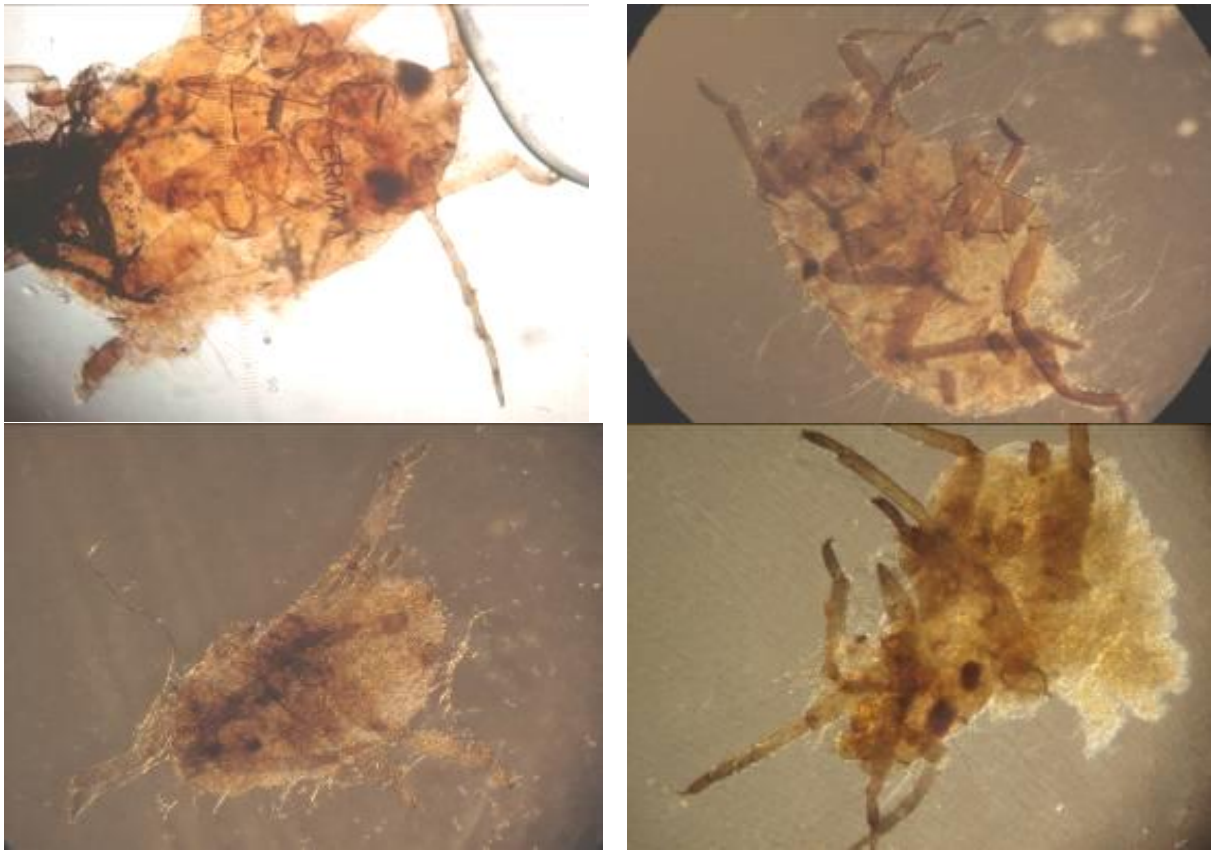
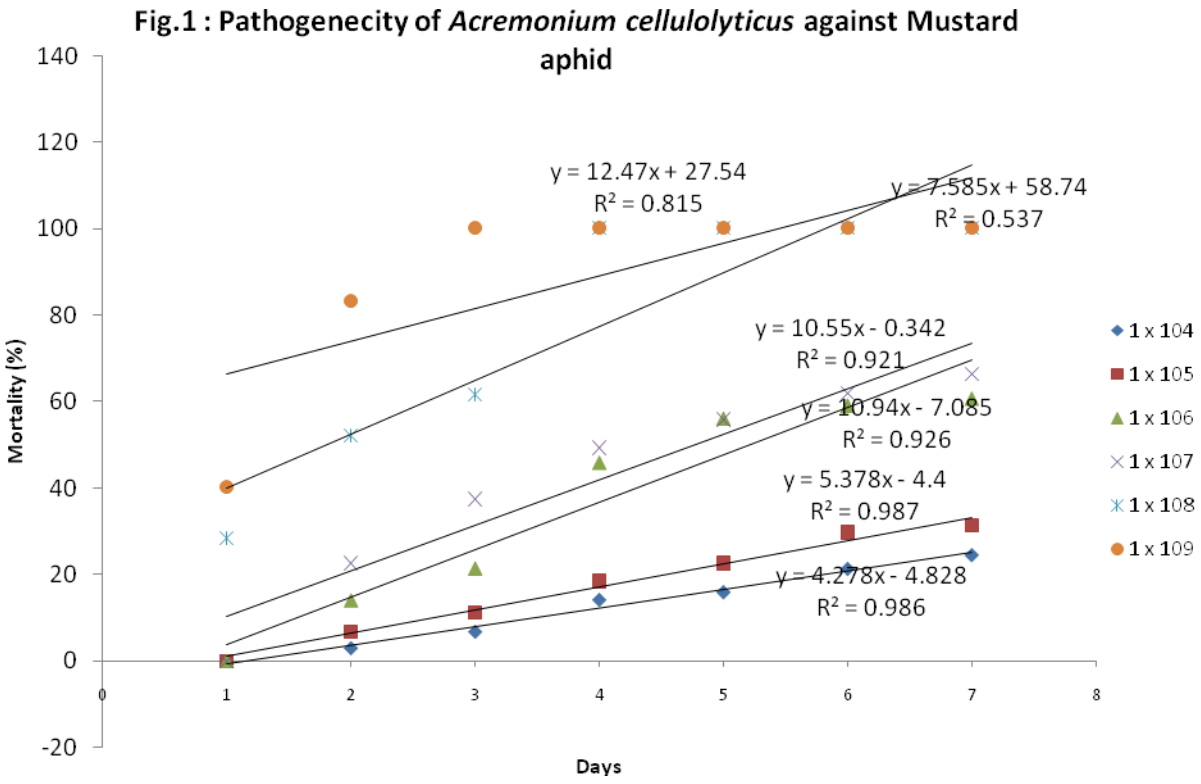


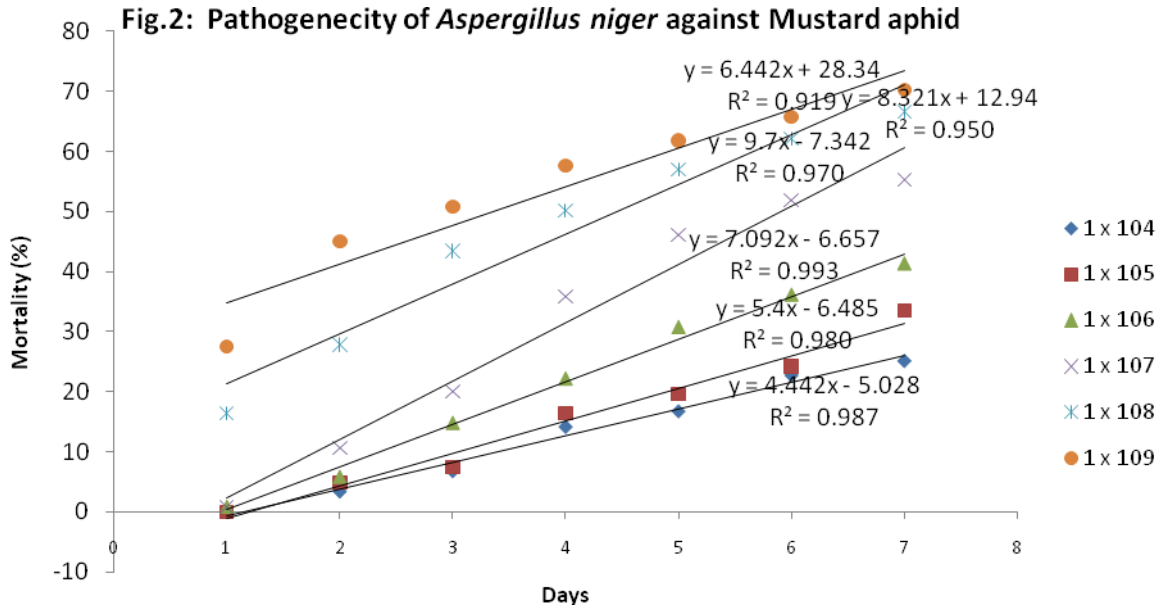
Plate 3: Mycelial outgrowth and degradation of body parts of Mustard aphid after treatment with *Acremonium cellulolyticus* (after 2, 4, 5 and 7 days of post treatment).



12.5.1.2 **Pathogenicity of Aspergillus *niger***

The effect of *Aspergillus niger* against Mustard aphid showed significant results from the 3rd day onward and more than 50% mortality was obtained at 3rd day but with the passage of time at 7th day 66.6 and 70.2% mortality was observed at $1x10^8$ to $1x\ 10^9$ concentrations respectively. The results showed that mortality was dose dependent and increased with higher spore concentration of *Aspergillus niger*. No mortality was recorded in the control (Fig.2, Plate-4).

Plate 4: Mustard aphid with mycelial outgrowth after treatment with *Aspergillus niger* (after 4, 6, 8 and 9 days of post treatment)



12.5.1.3 Pathogenicity of *Aspergillus tamarii*

The efficacy of *Aspergillus tamarii* against Mustard aphid showed significant results from the 3rd day onwards. It was observed that more than 50% mortality was obtained at 4th day and recorded highest mortality of 74.6% at 7^{th} day which was followed by 73.2% at $1x10^8$ and $1x\ 10^9$ concentrations respectively. The results showed that mortality was dose dependent and increased with higher fungal spore concentration. No mortality was recorded in the control (Fig.3, Plate -5).

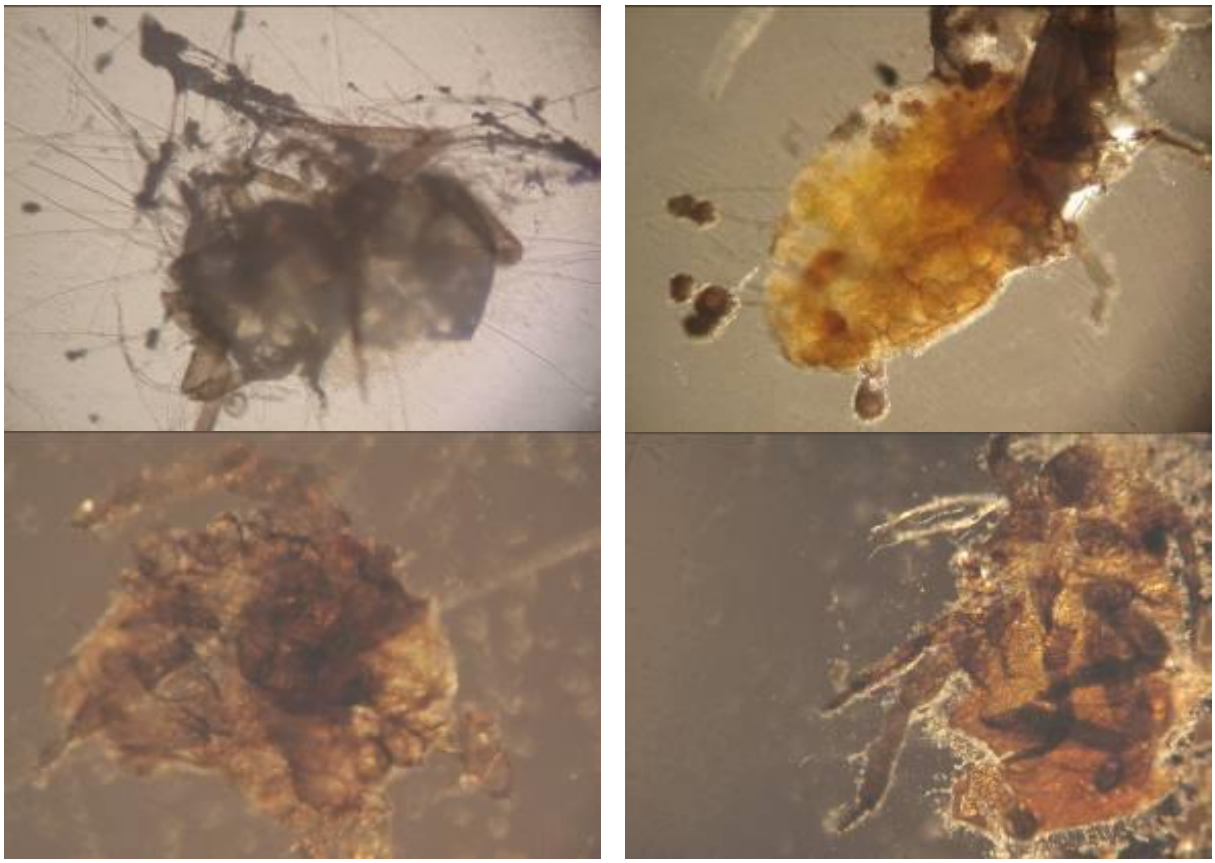
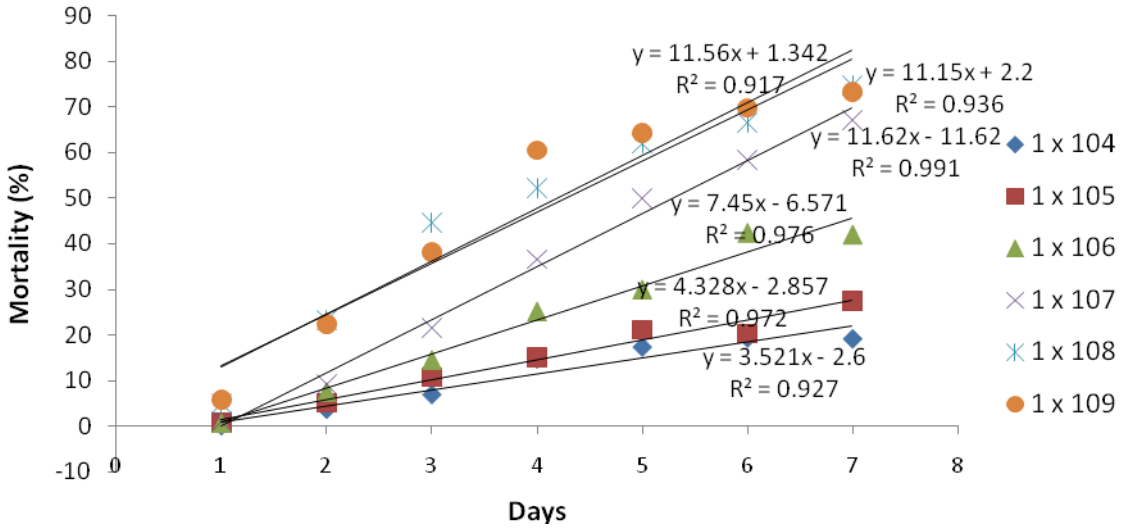


Plate 5: Fungal sporulation and degradation of Mustard aphid after treatment with *Aspergillus tamarii* (after 4, 6, 8 and 9 days of post treatment)

Fig. 3: Pathogenecity of *Aspergillus tamarii* against Mustard aphid



12.5.1.4 **Pathogenicity of *Fusarium culmorum***

The pathogenicity of *Fusarium culmorum* was evaluated against *Lipaphis erysinbi* which showed significant results. The maximum mortality per cent of 77.4 was recorded after 7th day of treatment with a concentration of 1×10^{9}, which was followed by 73.2% in the spore load 1×10^{8}. In contrast to this minimum mortality per cent of 44.4% was obtained by the application of lowest concentration i.e., 1×10^{4} while no mortality was recorded in the control (Fig. 4, Plate-6).

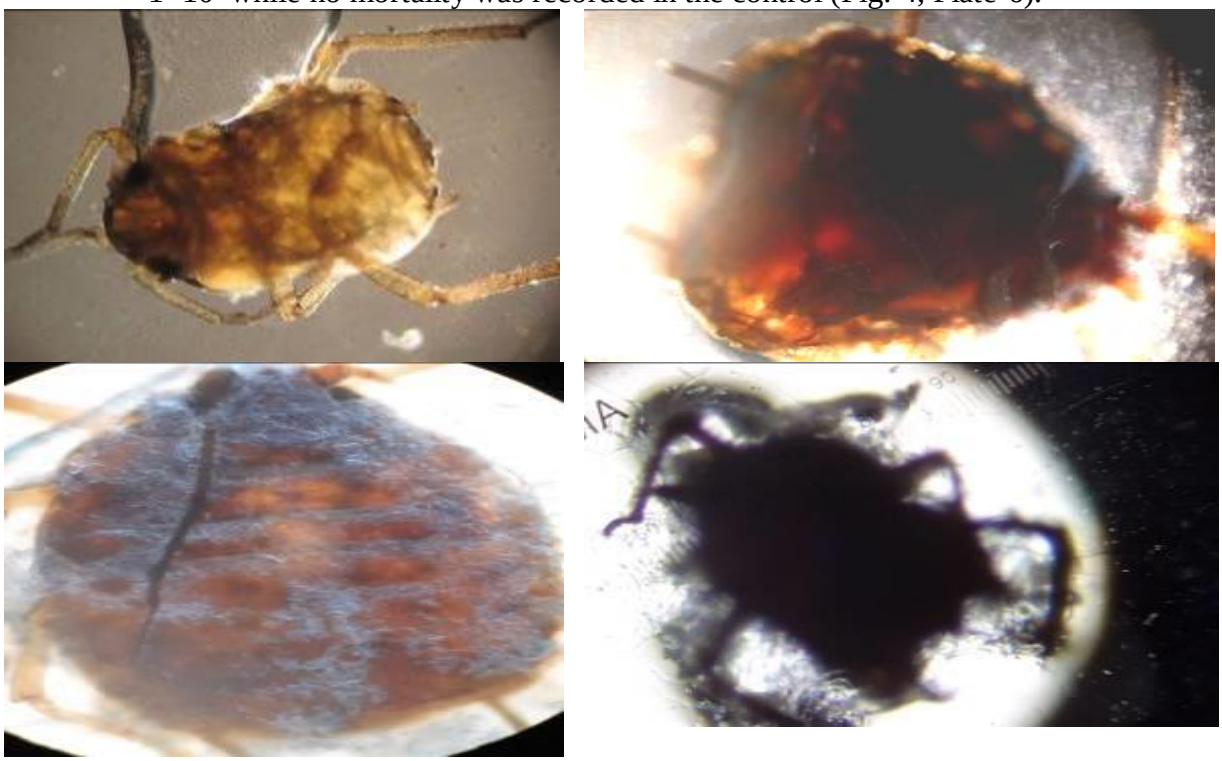
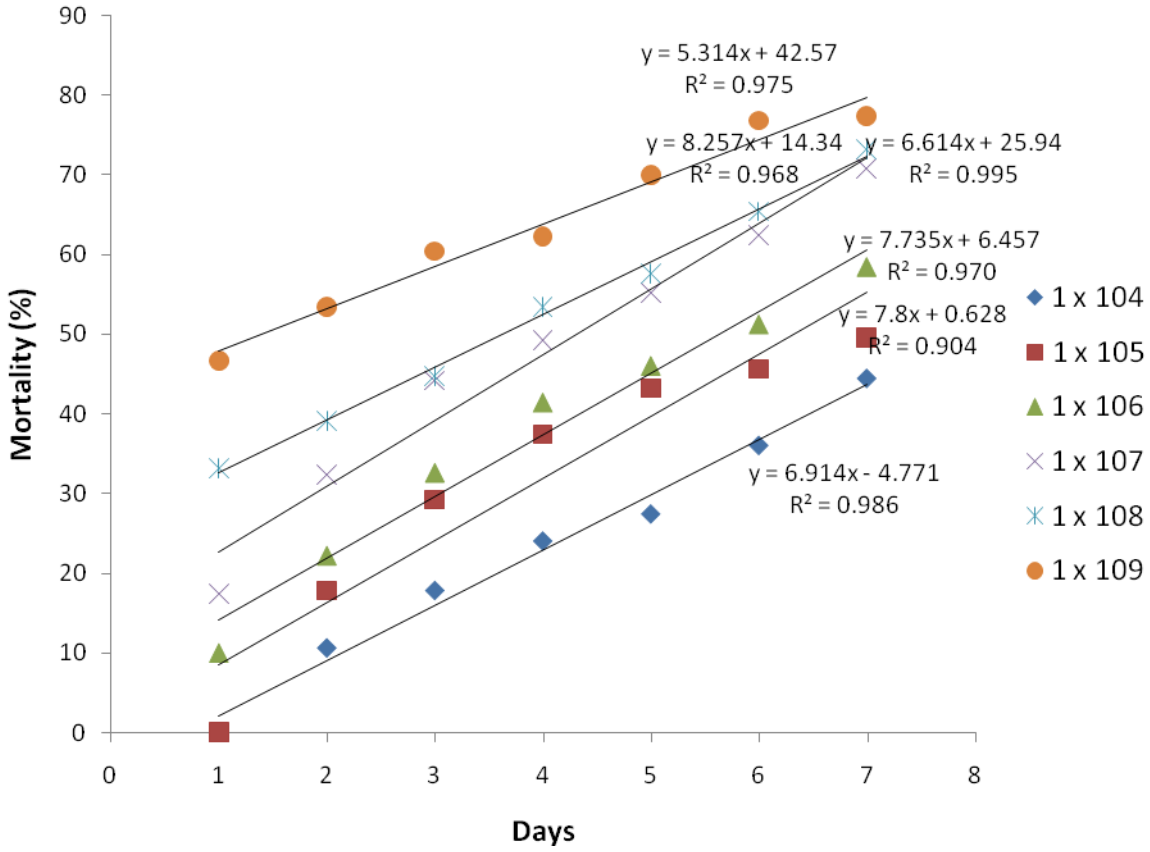


Plate 6: Dead Mustard aphids at different stages of infection after treatment with *Fusarium culmorum* (after 2, 4, 6 and 9 days of post treatment)

Fig.4: Pathogenecity of *Fusarium culmorum* against Mustard aphid



12.5.1.5 **Pathogenicity of *Penicillium pinophilum***

The efficacy of *Penicillium pinophilum* was evaluated against *Lipaphis erysinbi* which showed significant results at all concentrations. Among all the treatments the application of *Penicillium pinophilum* was found to be the best with higher efficacy for the control of mustard aphid.

The fungal application of *Penicillium pinophilum* on *Lipaphis erysinbi* showed significant results at all concentrations. Effectiveness after 2nd day when above 90% of tested aphid population was killed but with the passage of time at 2nd day onwards 100% mortality was observed at 1x10^{8}.Similar results were also observed at the highest spore concentration at 1 x 10^{9} while no mortality was recorded in the control. The present findings also establish that mortality of mustard aphid was dose dependent which increased with increasing the spore concentration. There was no mortality recorded in the control (Fig.5, Plate-7).

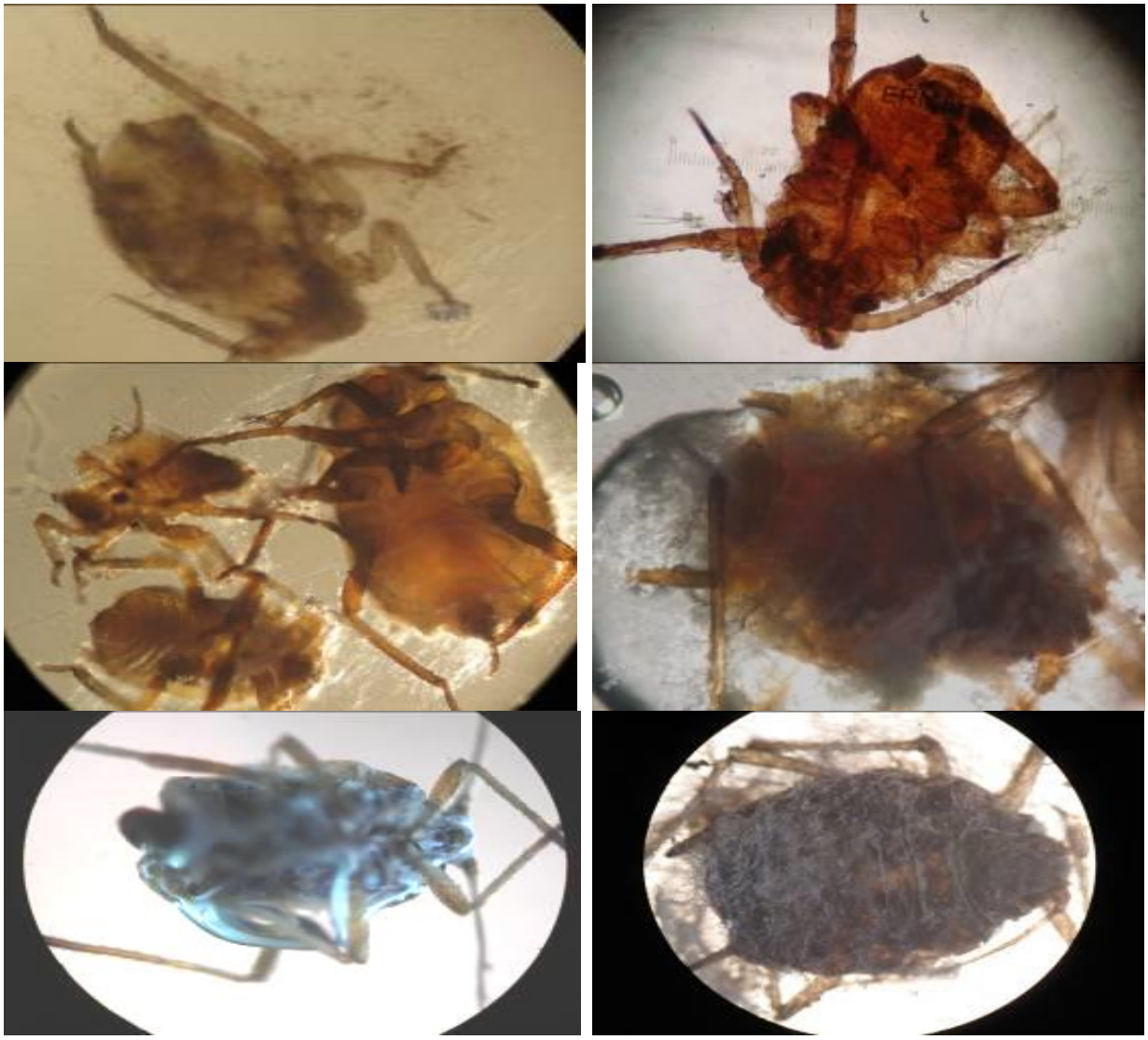
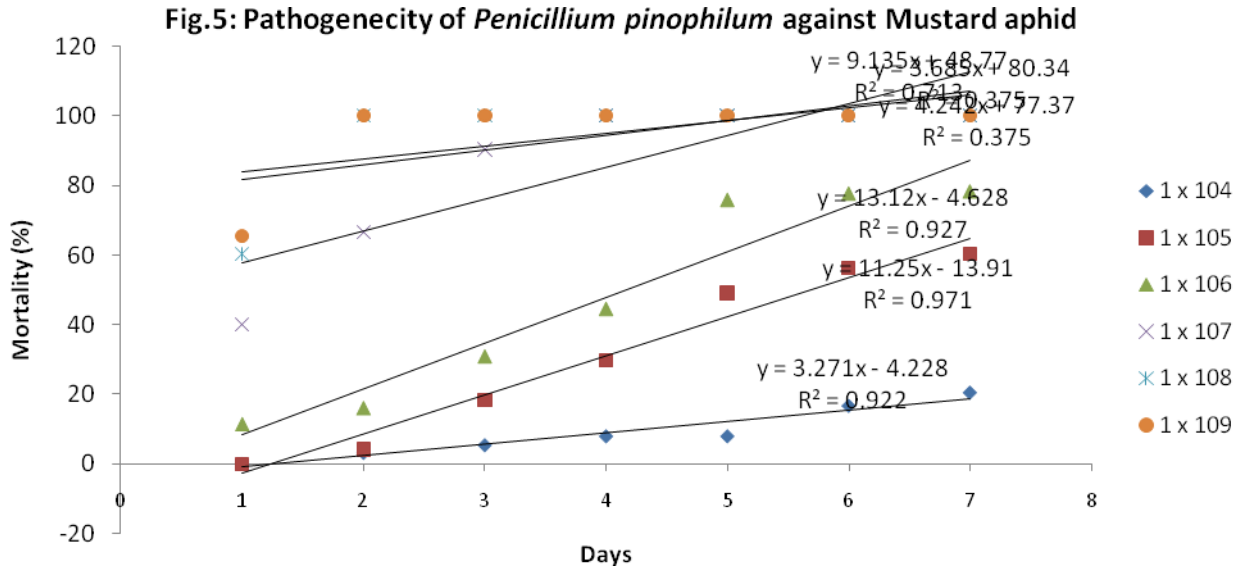


Plate 7: Different stages of infection after treatment with *Penicillium pinophilum* (after 2, 4, 5, 7, 9 and 11 days of post treatment)



12.5.1.6 **Pathogenicity of *Metarhizium anisopliae***

The application of *Metarhizium anisopliae* on *Lipaphis erysinbi* showed significant result. Higher mortality was observed at higher concentration of spores and recorded ≥50% mortality from the 5th day onwards. Highest mortality per cent was recorded in the treatment 1x10^{8} and 1x 10^{9} and recorded 69.2% mortality. In contrast no mortality was observed in the control (Fig.6, Plate-8).

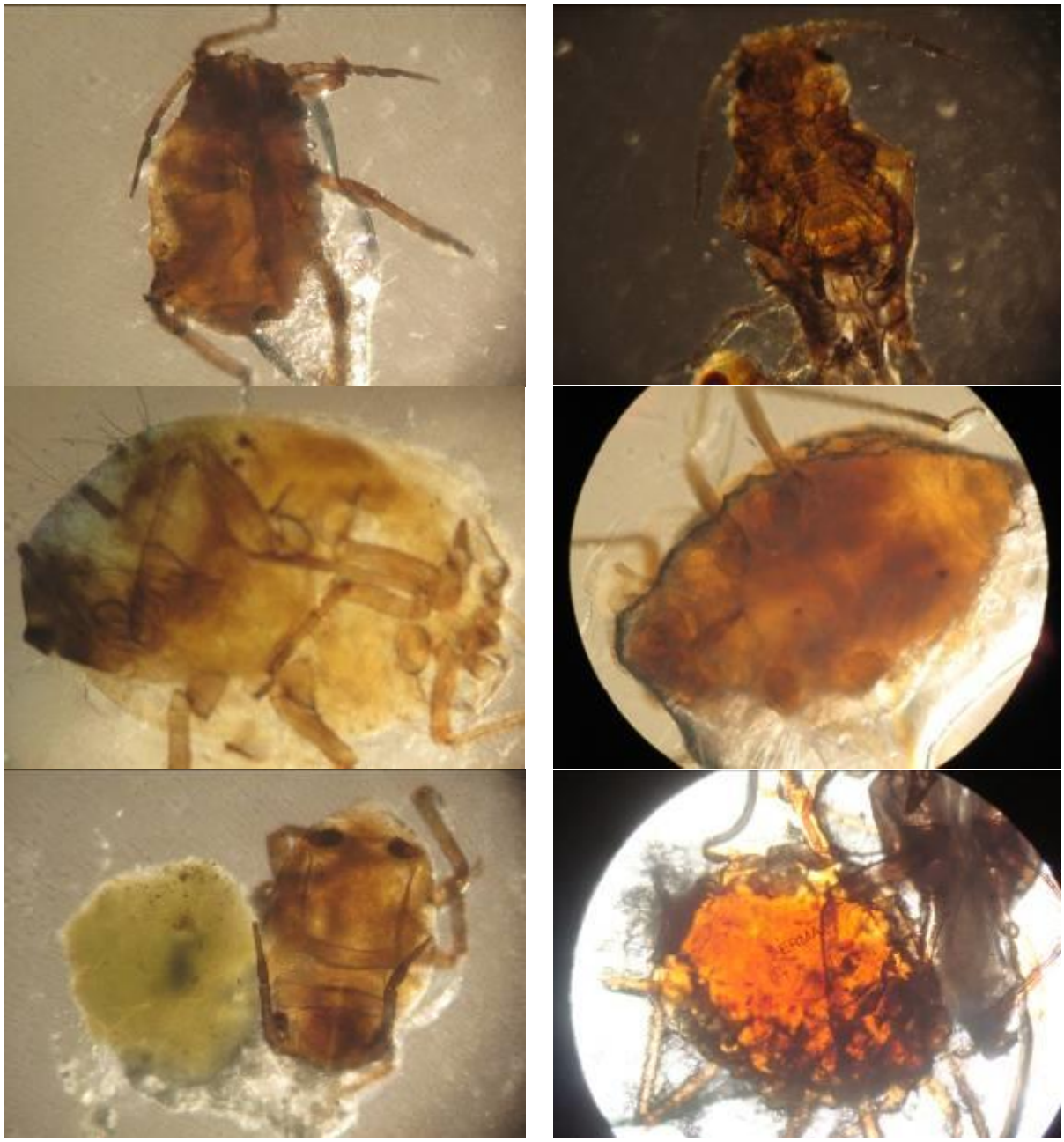
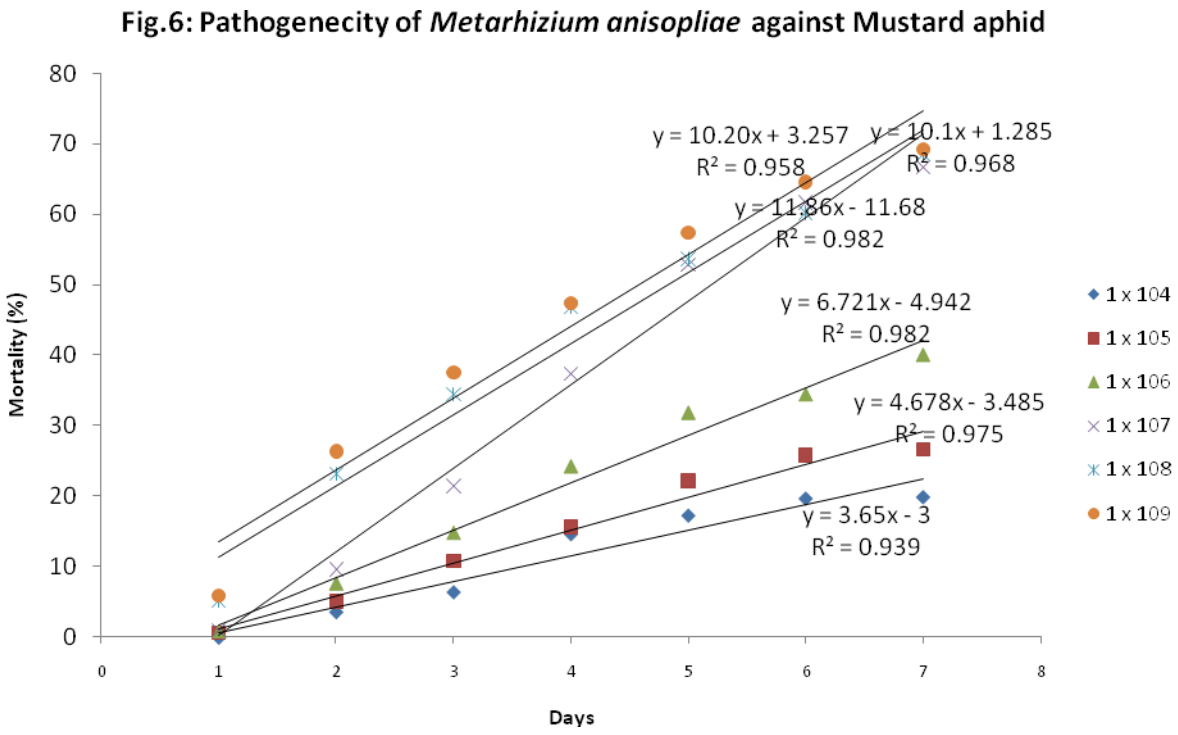


Plate 8 : Different stages of infection after treatment with *Metarhizium anisopliae.* (after 3, 4, 5, 6 8 and 10 days of post treatment)



Pathogenicity of *Verticillium lecanii*

The effect of entomopathogenic fungus *Verticillium lecanii* against Mustard aphid showed significant results from the 4th day and more than 60% mortality was obtained at 6th day at the spore load 1x10^{8} and above. The results showed that mortality was dose dependent which increased with enhancing the concentration while no mortality was recorded in control (Fig.7, Plate-9).

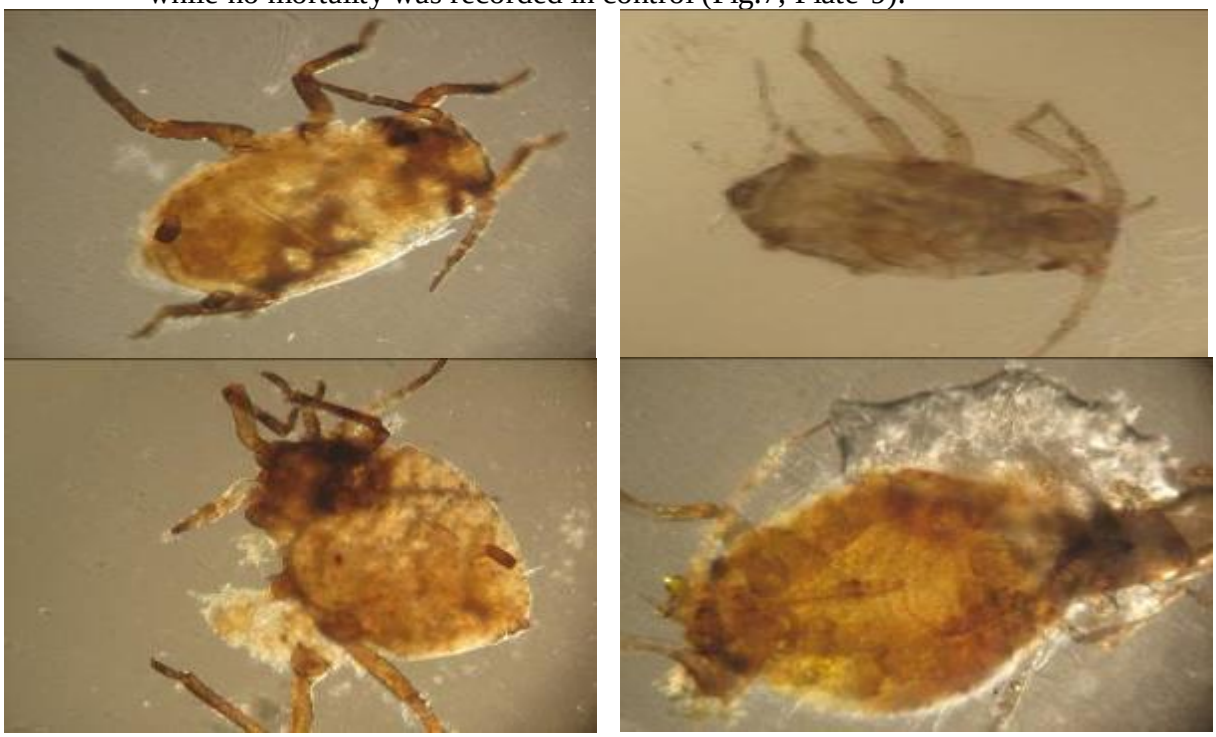
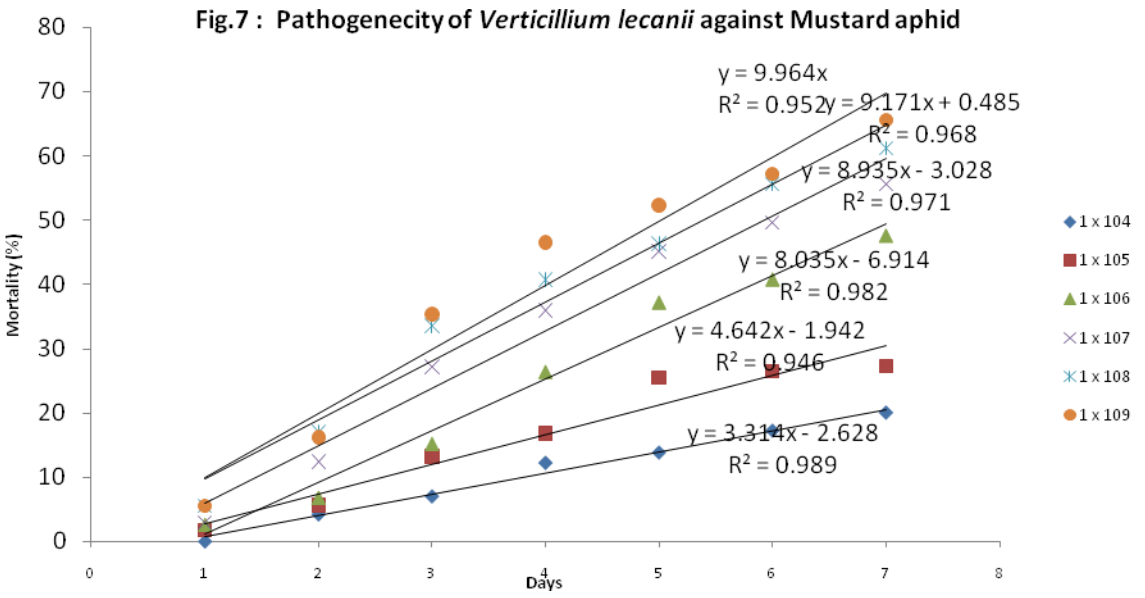


Plate 9 : Infected and degraded Mustard aphid after treatment with *Verticillium lecanii*. (after 2, 3, 5 and 8 days of post treatment)



12.5.1.7 **Pathogenicity of *Beauveria bassiana***

The fungal spore application of *B. bassiana* on *L. erysimi* showed effectiveness after 2nd day onwards. Higher mortality was observed at higher concentration of spores and recorded ≥50% mortality from the 5th day onwards. Highest mortality per cent of 69.6% was recorded in the treatment $1x10^9$ which was followed by 65% in the treatment of $1x\ 10^9$ spore load after 7days of post treatment. Lowest pest mortality (21.6%) was recorded in the lower concentration ($1\ x\ 10^4$) of fungal spores. There was no mortality recorded in the control (Fig. 8, Plate-10).

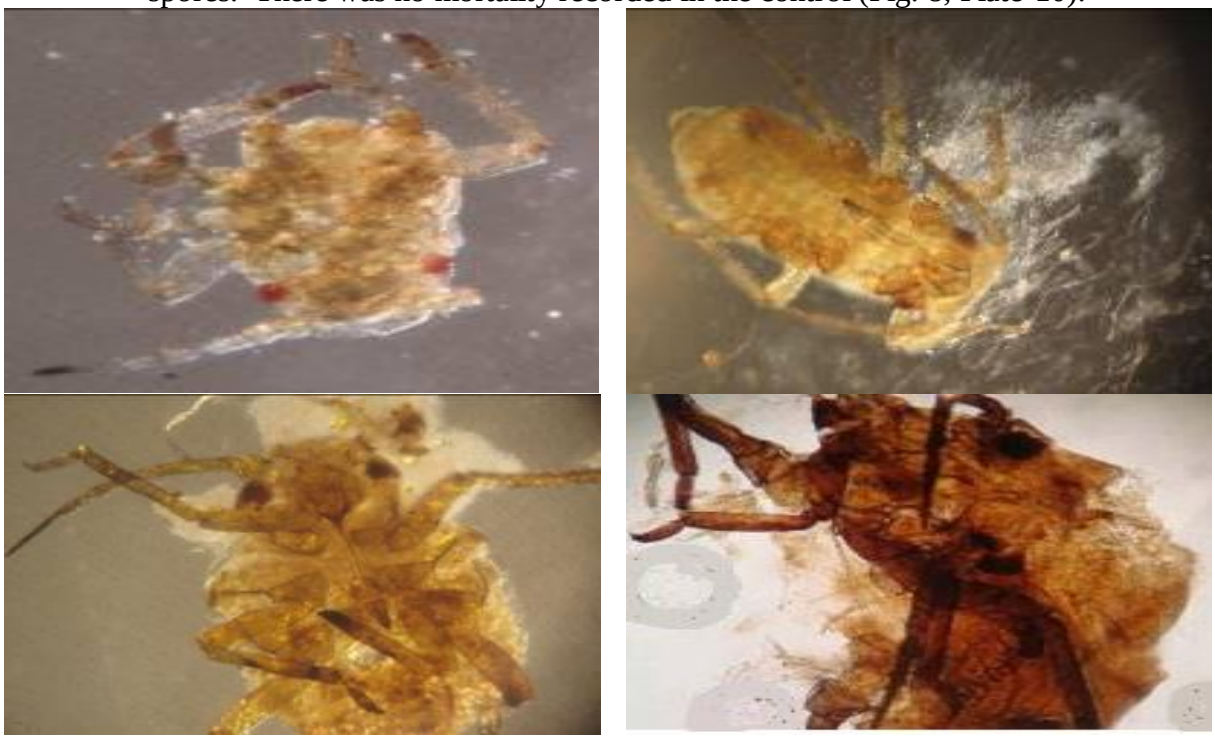
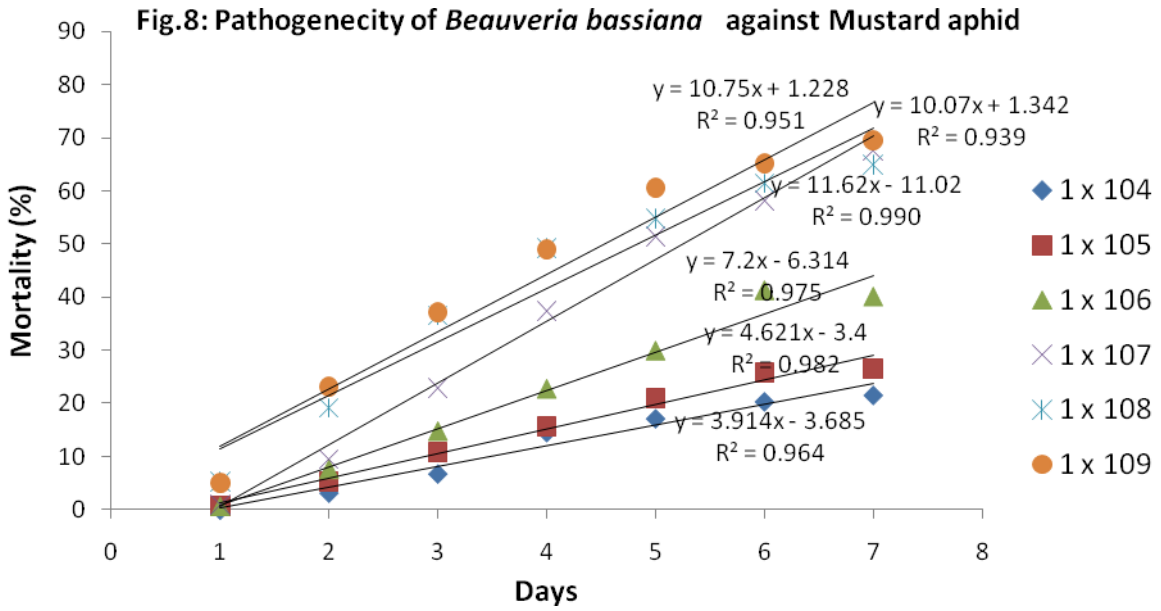


Plate 10: Dead larvae and adult Mustard aphid after treatment with *Beauveria bassiana* (after 2, 4, 7 and 8 days of post treatment)



12.5.1.8 **Pathogenicity of *Acremonium breve***

The fungal spore application of *A breve* on Mustard aphid showed effectiveness after 3rd day onwards. Higher mortality was observed at higher concentration of spores and recorded ≥60% mortality from the 5th day onwards. Highest mortality per cent of 77.1% was recorded in the treatment $1x10^9$ after 7 days which was followed by 72.8% in the treatment of $1x\ 10^9$ spore load after 6 days of post treatment. Similar response for higher pest mortality was also observed at the spore load $1x10^8$ and recorded 74.2% after 6 days of post treatment. Lowest pest mortality (25.7%) was recorded in the lower concentration ($1\ x\ 10^4$) of fungal spores. Pest mortality found to be insignificant in the control (Fig. 9, Plate-11).

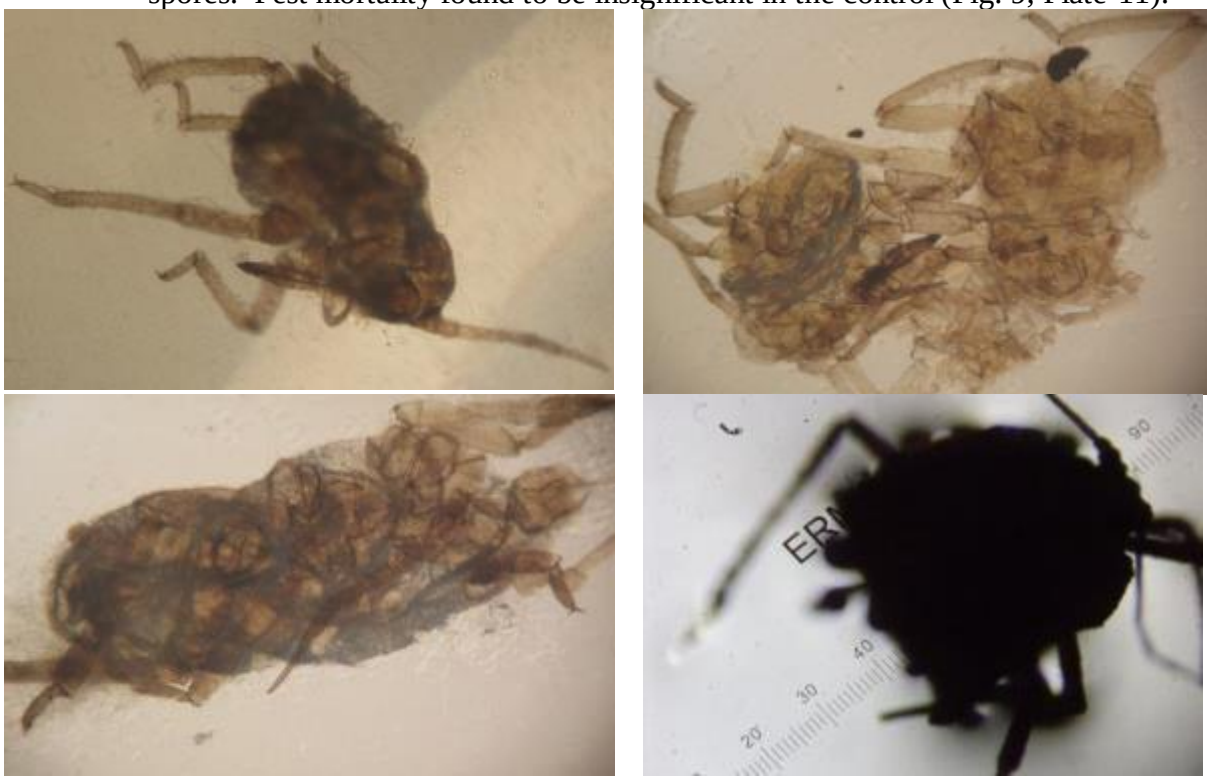
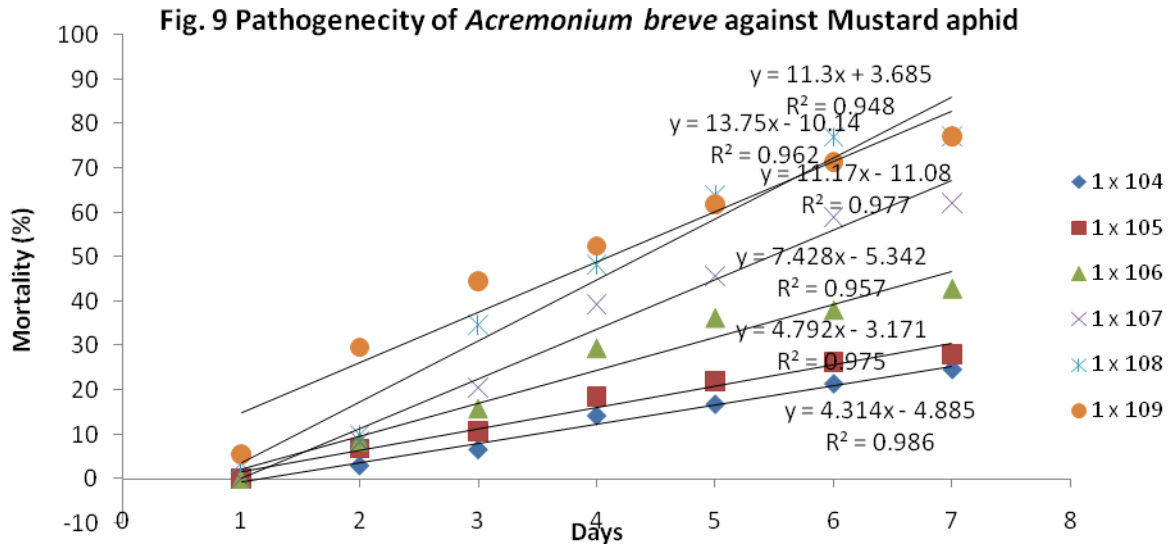


Plate 11: Dead larvae and adult Mustard aphid after treatment with *Acremonium breve* (after 2, 4, 7 and 8 days of post treatment)



12.5.1.9**Pathogenicity of *Nomuraea releyi***

The effect of entomopathogenic fungus *Nomuraea releyi* against Mustard aphid showed significant results from the 4th day and more than 60% mortality was obtained at 6th day at the spore load 1x10^{8} and above. The results showed that mortality was dose dependent which increased with enhancing the concentration while no mortality was recorded in the control (Fig.10, Plate-12).

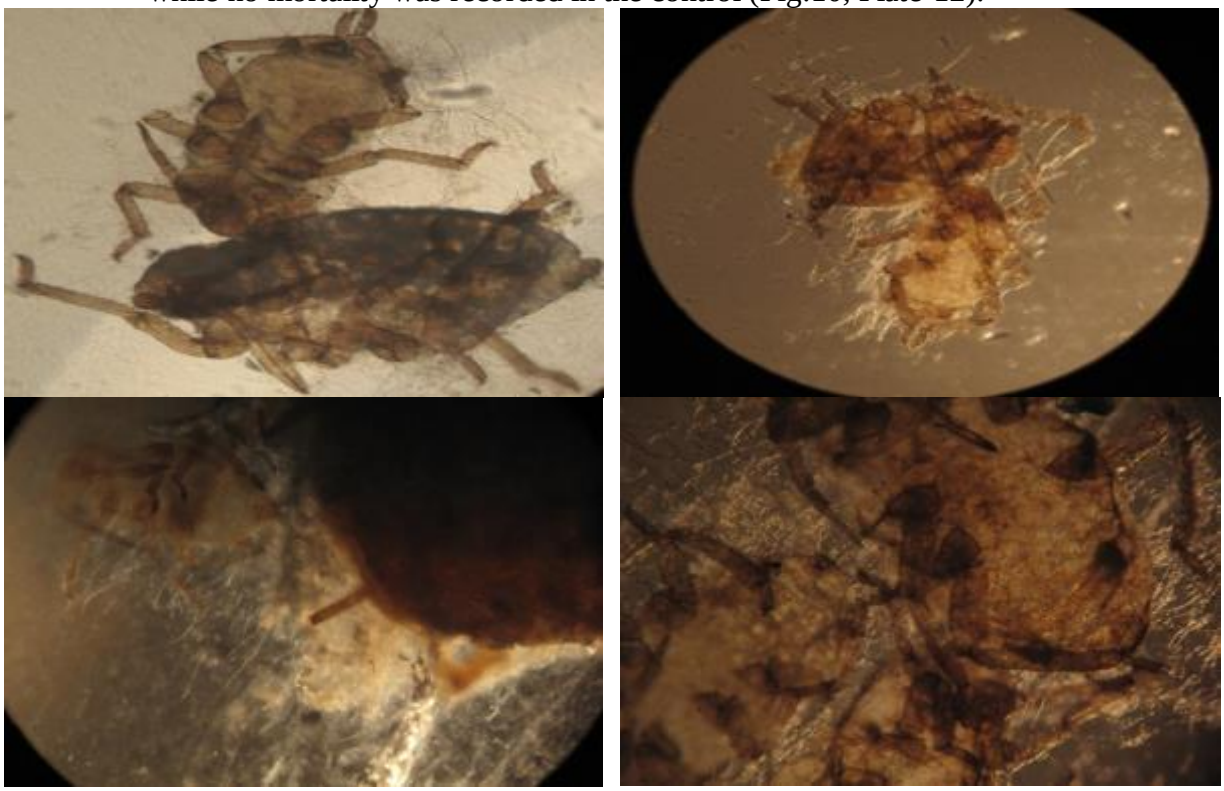
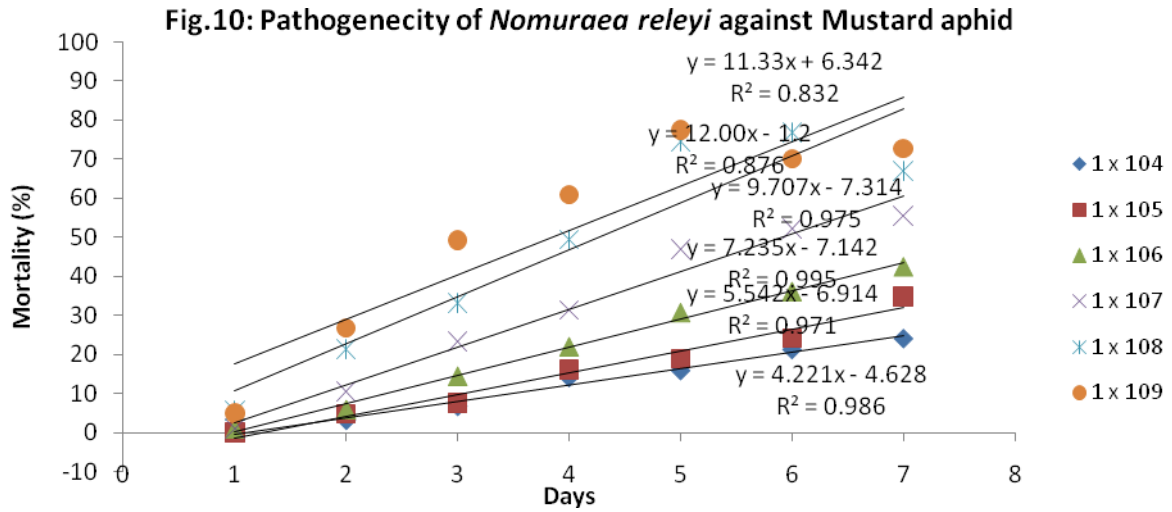


Plate 12: Infected and degraded Mustard aphid of different stages after treatment with *Nomuraea releyi* (after 3, 5, 7 and 8 days of post treatment)



12.5.1.10 **Pathogenicity of *Penicillium chrysogenum***

The application of *P. chrysogenum* on *Lipaphis erysinbi* showed significant result. Higher mortality was observed at higher concentration of spores and recorded ≥50% mortality from the 4th day onwards. Highest per cent mortality was observed in the treatment 1x10^{8} and 1x 10^{9} and recorded 64.2% and 74.2% mortality respectively after 7 days of post treatment. In contrast no mortality was observed in the control (Fig.11, Plate-13).

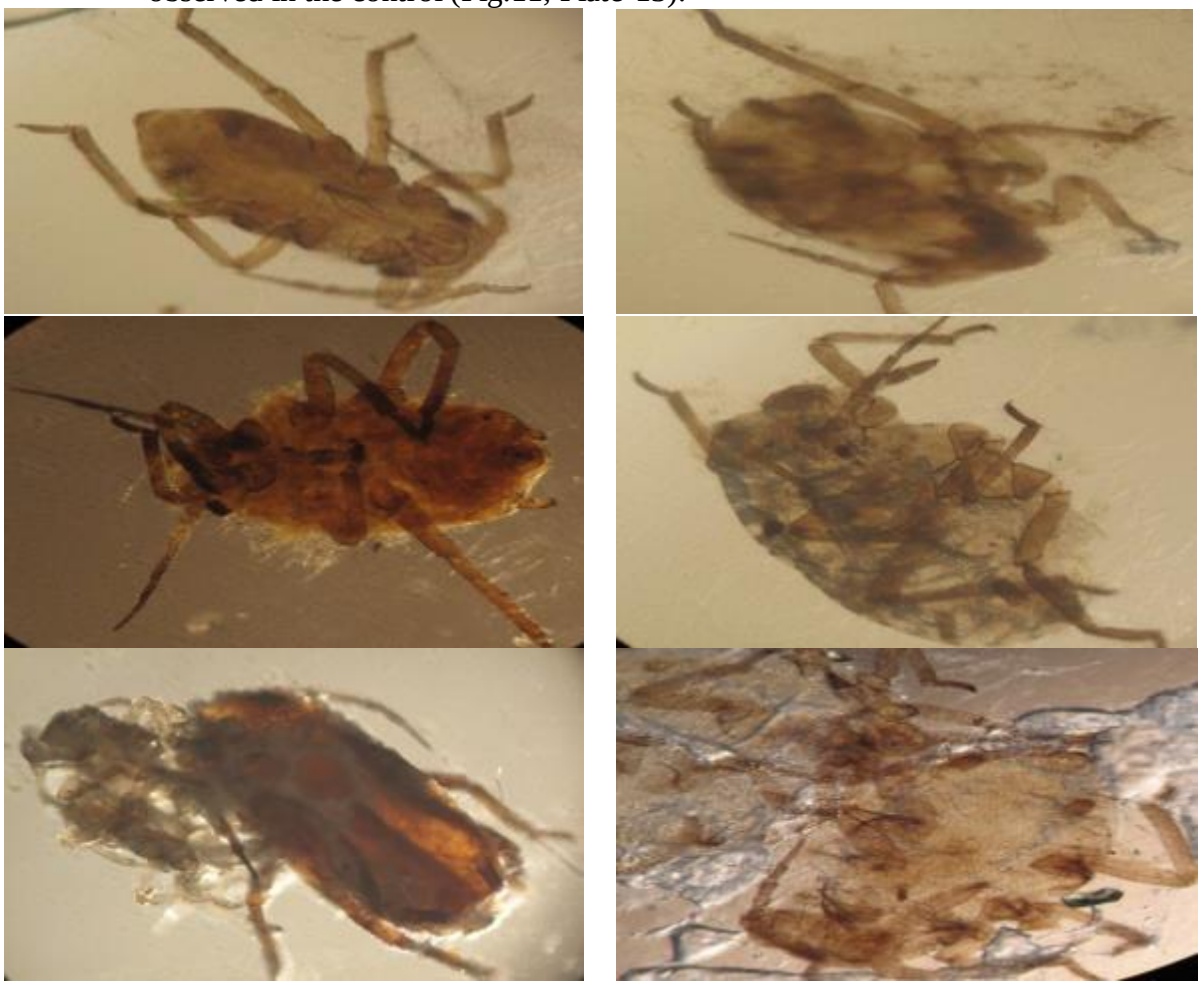
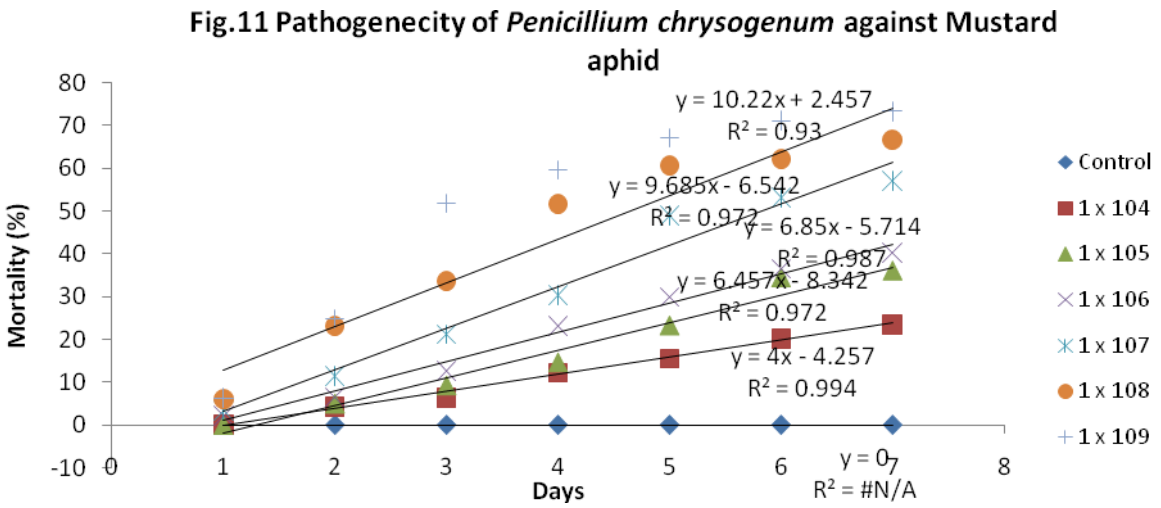


Plate 13: Different stages of infection and pest degradation after treatment with *Penicillium chrysogenum* (after 2, 4, 6 and 8 days of post treatment)



12.5.1.11 **Pathogenicity of *Aspergillus fumigates***

The effect of *Aspergillus fumigates* against Mustard aphid showed significant results from the 3rd day onwards and more than 50% mortality was obtained at 5th day but with the passage of time at 7th day 57.1 and 55.7% mortality was observed at 1x 10^9 to 1 x 10^8 concentrations respectively. The results showed that mortality was dose dependent and increased with higher spore concentration of *Aspergillus fumigates*. No mortality was recorded in the control (Fig.12, Plate-14).

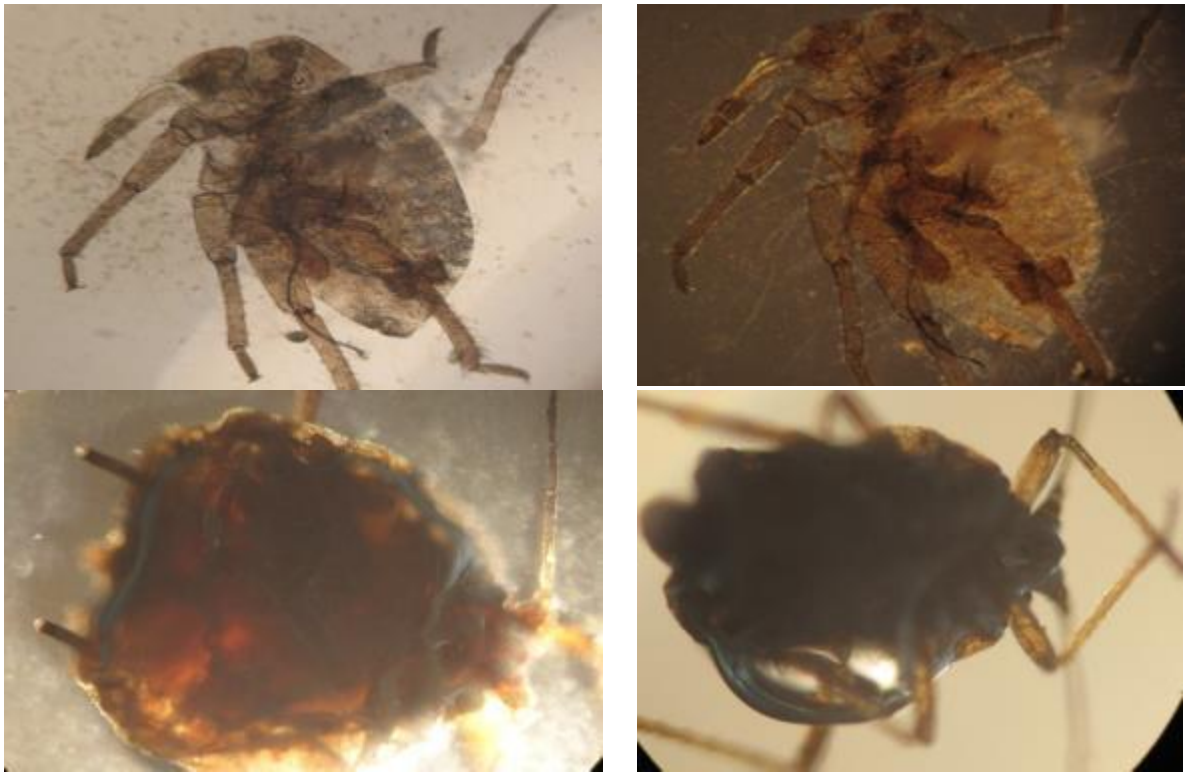
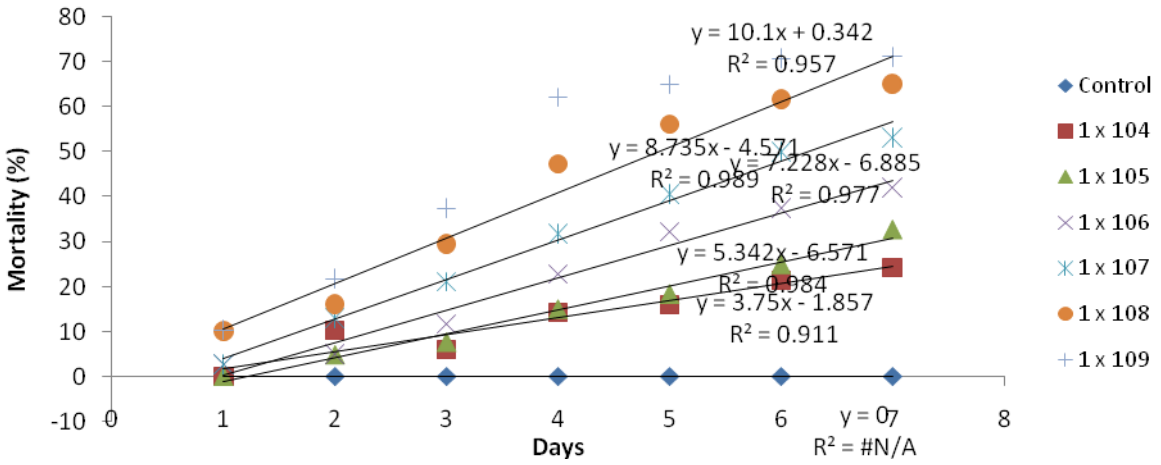


Plate 14: Dead Mustard aphids at different stages of infection after treatment with *Aspergillus fumigates*(after 2, 4, 6 and 9 days of post treatment)

Fig.12 Pathogenecity of *Aspergillus fumigates* against Mustard aphid

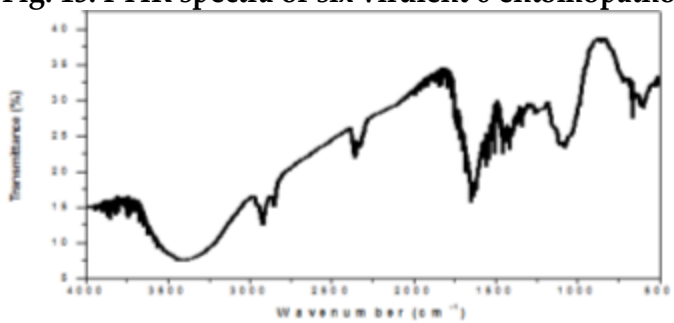


Fungal strains were evaluated for their potential use as biological control agents against the mustard aphid. The strains were screened for relative pathogenicity, and the median lethal concentration and time eliciting 50% mortality (LC50, LT50) was calculated. Calculated LC50 values for fungal isolates screened against mustard aphids with a dose response assay at different spore concentrations.

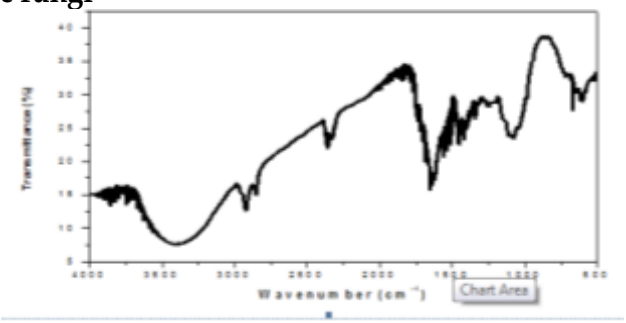
12.5.2 FTIR spectrum of selected fungal isolate extract

The FTIR spectrum of extracts for six fungal isolates such as *Penicillium pinophilum, Acremonium cellulolyticus, Aspergillus tamarii, Fusarium culmorum, Metarhizium anisopliae*and *Beauveria bassiana* are shown in figure 13. The FTIR spectrum was categorised based on published reference into fourzones such as i) 4,000 to 2,500 cm^{-1} single bonds, ii) 2,500 to 2,000 cm^{-1} triple bonds, iii) 2,000 to 1,500 cm^{-1} double bonds and iv) 1,500 to 400cm^{-1} fingerprint region single bonds. The zone 4,000 to 2,500 cm^{-1} single bonds is further sub classified into 3500-3300 cm^{-1} for N-H, 3100-3010 cm^{-1} for C-H and 3000-2500 cm^{-1} for O-H single bond. Likewise 2,500 to 2,000 cm^{-1} zone into C≡N 2240-2280 and C≡C 2100-2200; 2,000 to 1,500 cm^{-1}double bonds into C=O 1680-1750, C=N 1615-1700, C=C 1640-1680 and 1,500 to 400 fingerprint region single bonds into 1494-1357, 1182-995 and 680-530 cm^{-1}. In each wavenumber cm^{-1} sub zones the lowest transmittance (%) data was represented in the table 5, 6, 7.

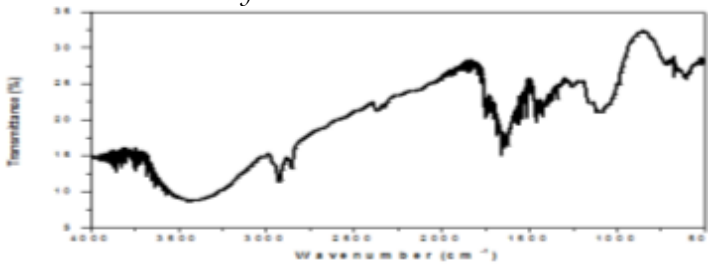
Fig. 13: FTIR spectra of six virulent 6 entomopathogenic fungi



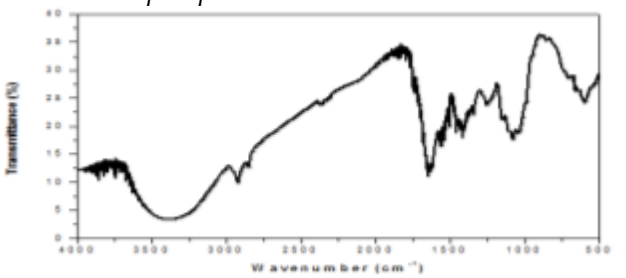
Acremonium cellulolyticus



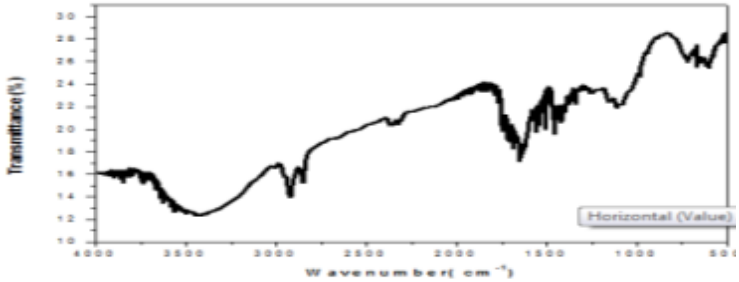
Penicillium pinophilum



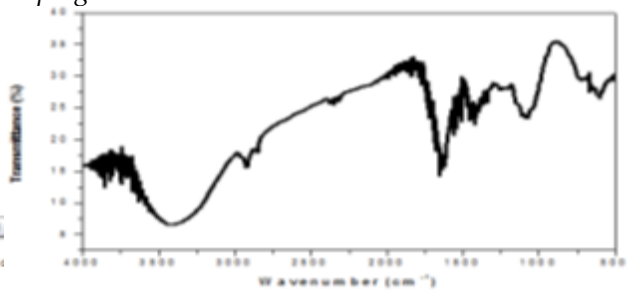
Fusarium culmorum



Aspergillus tamarii



Metarhizium anisopliae



Beauveria bassiana

Table 5: FTIR observed bands for 6 entomopathogenic fungi

Sl no	Strains	Lowest point of Transmittance % in wave number cm^{-1}									
		4,000 to 2,500, single bonds			2,500 to 2,000 triple bonds		2,000 to 1,500 double bonds			1,500 to 400 fingerprint region single bonds	
		N-H 3500-3300	C-H 3100-3010	O-H 3000-2500	C≡N 2240-2280	C≡C 2100-2200	C=O 1680-1750	C=N 1615-1700	C=C 1640-1680	1494-1357	1182-995	680-530
1	*Penicillium pinophilum*	7.52	13.70	12.60	27.38	28.35	19.89	15.84	15.84	22.53	23.40	27.60
2	*Acremonium cellulolyticus*	8.57	13.33	11.34	23.23	23.78	17.86	15.12	15.12	19.71	20.95	25.68
3	*Aspergillus tamarii*	3.37	9.31	9.88	26.20	27.33	17.15	11.08	11.08	18.07	17.60	24.17
4	*Fusarium culmorum*	10.85	14.41	10.91	20.64	20.93	14.69	14.72	14.72	16.99	18.63	24.65
5	*Metarhizium anisopliae*	12.28	15.72	13.95	21.48	21.78	18.25	17.14	17.12	19.58	21.92	25.42
6	*Beauveria*	6.45	14.37	15.56	27.37	28.00	18.22	14.0	14.21	22.4	23.27	26.52

bassiana	0	2

Classified Zone wise FTIR spectrum was blown up for the selected 6 fungal strains to locate specific wavenumber cm^{-1} with regard to lowest transmittance (%) shown in Fig 14a,b, 15 and tabulated in 5,6,7.

Table 6: Details of lowest Transmittance % of FTIR spectrum Blown up at 4000-400 wavenumber cm^{-1} for few entomopathogenic fungi

Fungal isolate	3650-3200 alcohol O-H		3200-2700 Alcohol C-H 3000-3100 SP2 C-H		2960-2850 SP3 C-H		2880-2950 SP3 C-H		2846-2872 SP3 C-H		1715 C=O	1654 C=C
	Wave number	Lowest Transmi-tance	Wave number	Lowest Transmi-tance	Wave number	Lowest Transmi-tance	Wave number	Lowest Transmi-tance	Wave number	Lowest Transmi-tance	Lowest Transmi-tance	Lowest Transmi-tance
Penicillum pinophilum	3420.9	7.528	3012.93	15.986	2956.03	15.198	2924.21	12.601	2853.8	15.052	24.841	15.842
Acremonium cellulolyticus	3447.9	8.575	3012.93	14.47	2955.07	13.784	2924.21	13.341	2853.8	13.221	21.445	15.126
Aspergillus tamarii	3384.25	3.373	3019.69	12.143	2950.25	11.93	2924.21	9.89	2853.8	12.653	24.841	11.081
Fusarium culmorum	3565.56	10.71	3011.97	14.936	2954.1	13.345	2925.17	10.919	2853.8	12.271	17.604	16.857
Metarhizium anisopliae	3446.94	12.282	3010.05	16.517	2954.1	15.77	2924.21	13.954	2853.8	15.232	19.098	17.355
Beauveria bassiana	3421.86	6.458	3019.69	17.154	2957	17.097	2923.24	15.562	2852.84	17.87	24.688	14.2166

Table 7: Details of lowest Transmittance % of FTIR spectrum Blown up at 3600-2500 wavenumber cm^{-1} for few entomopathogenic fungi

Sl No	Strains	Observed bands (Wavenumbers cm^{-1})		
		3655-3100	2966-2900	2874-2834
1	*Penicillium pinophilum*	7.52	12.60	15.05
2	*Acremonium cellulolyticus*	8.57	11.34	13.22
3	*Aspergillus tamarii*	3.37	9.88	12.65
4	*Fusarium culmorum*	10.71	10.91	12.27
5	*Metarhizium anisopliae*	12.28	13.95	15.23
6	*Beauveria bassiana*	6.45	15.56	17.87

Fig. 14 a,b : Details of FTIR spectrum Blown up at 3600-2500 wavenumber cm^{-1} for few entomopathogenic fungi

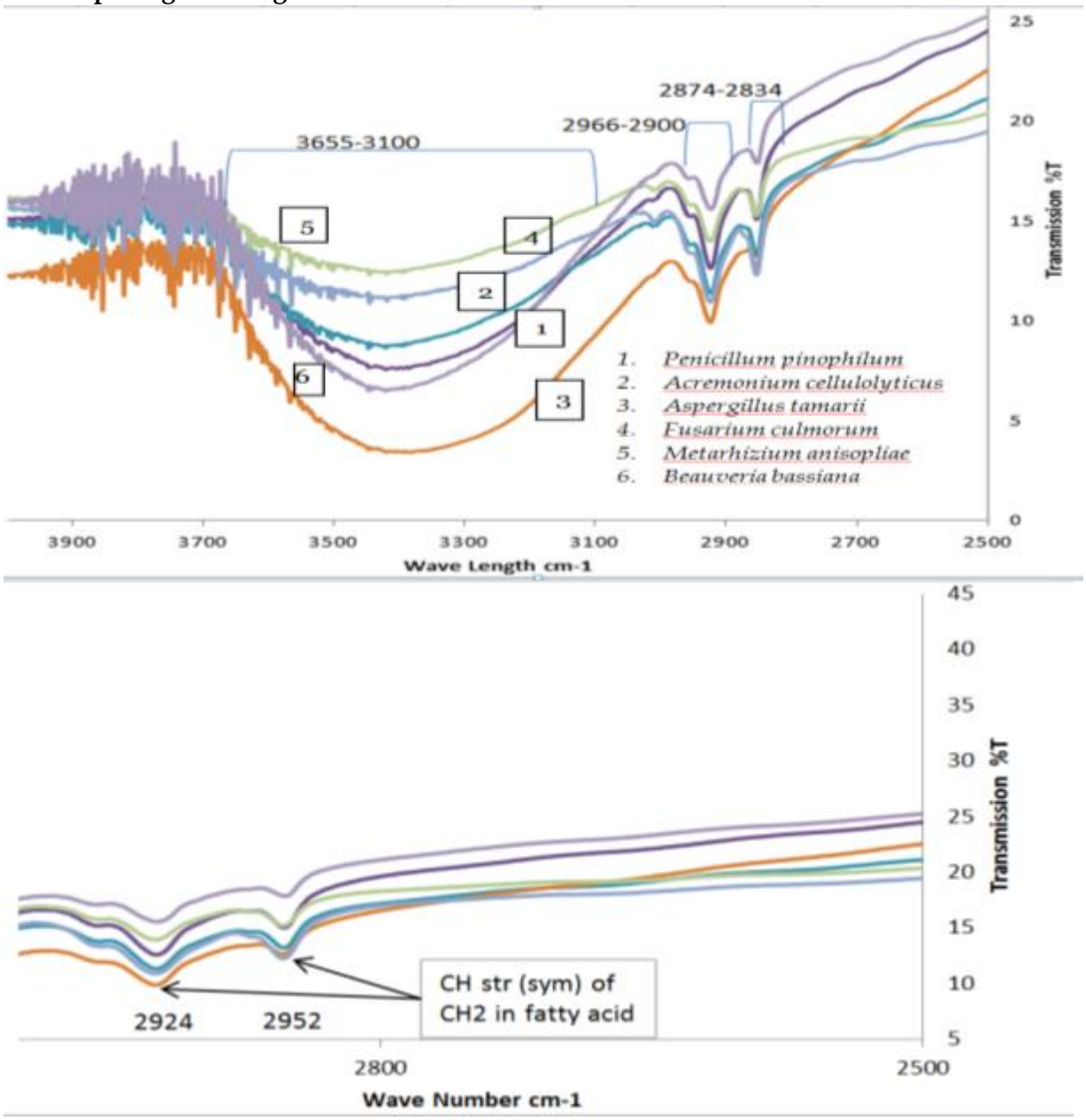
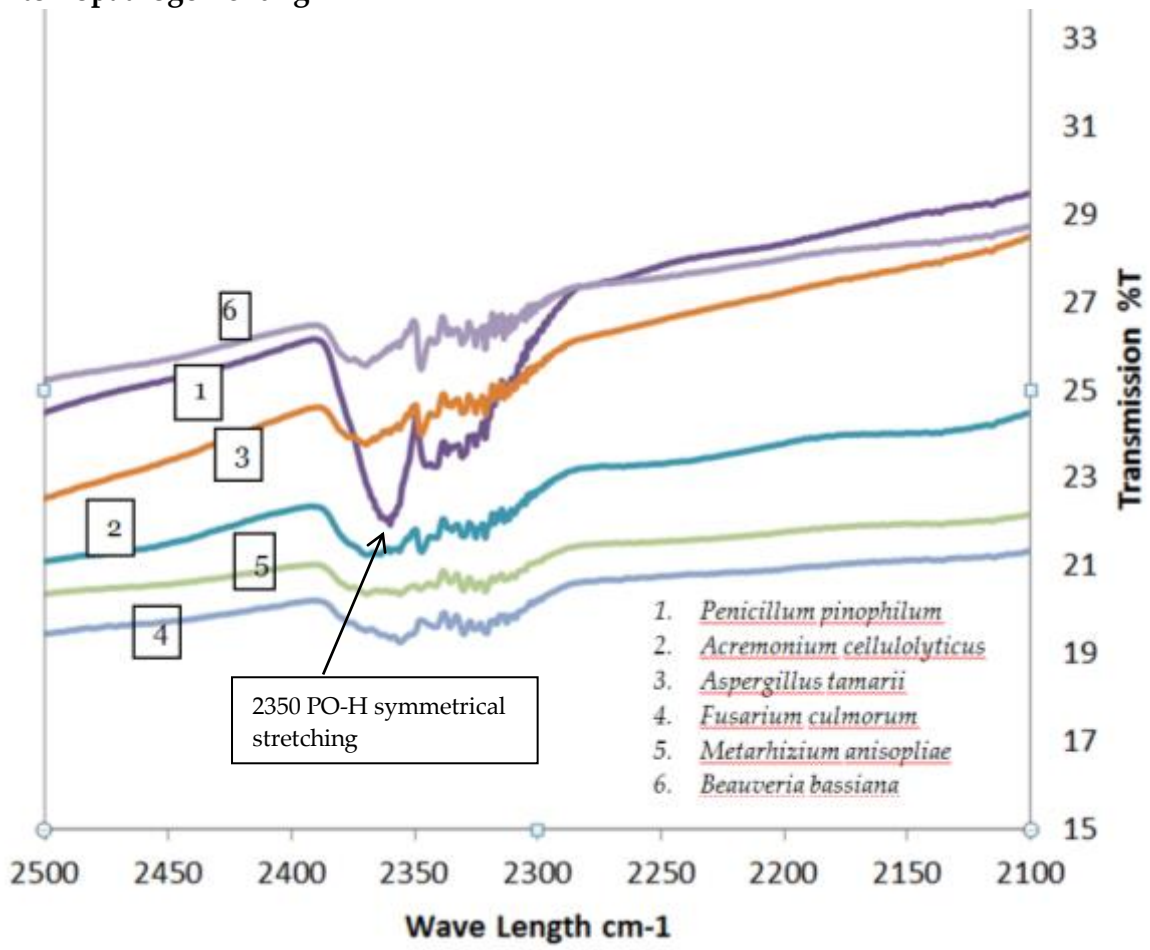


Fig.15 : Details of FTIR spectrum Blown up at 2500-2100 wavenumber cm^{-1} for few entomopathogenic fungi

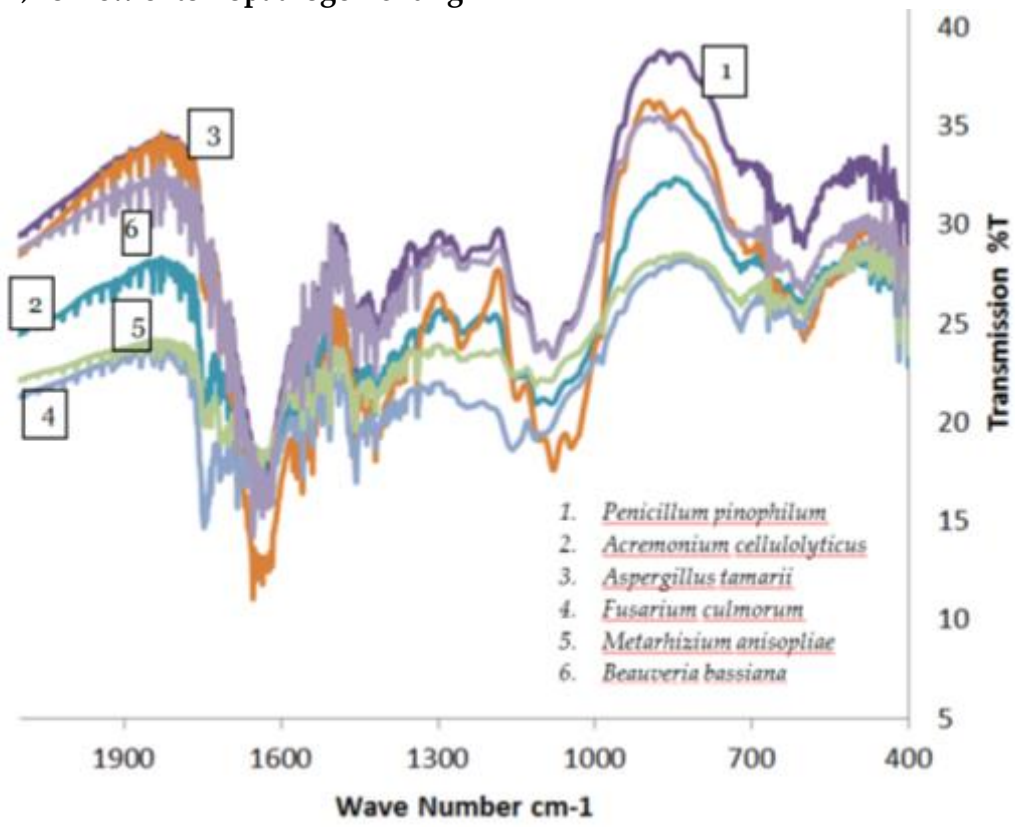


Classified Zone wise FTIR spectrum was blown up for the selected 6 fungal strains to locate specific wavenumber cm^{-1} with regard to find out the specific stretch. From the FTIR spectrum Blown up at 2500-2100 wavenumber cm^{-1} it was observed that there is presence of specific PO-H symmetrical stretching of *Penicillium pinophilum* strain at 2350 cm^{-1} which might have impact on virulence of the species (Fig. 15).

Table 8: Details of lowest Transmittance % of FTIR spectrum Blown up at 2500-2100 wavenumber cm^{-1} for few entomopathogenic fungi

Sl No	Strains	Observed bands (Wavenumbers cm^{-1})			
		2009-2015	2034-2039	2057-2064	2082-2087
1	*Penicillium pinophilum*	30.17	30.02	29.37	29.62
2	*Acremonium cellulolyticus*	25.31	25.16	24.94	24.63
3	*Aspergillus tamarii*	30.05	29.68	29.18	28.75
4	*Fusarium culmorum*	21.96	21.86	21.50	21.39
5	*Metarhizium anisopliae*	22.61	22.55	22.45	22.23
6	*Beauveria bassiana*	29.55	29.52	29.34	28.94

Fig.16 : Details of FTIR spectrum Blown up at Fingerprint region (1600-500 wavenumber cm^{-1}) for few entomopathogenic fungi



12.5.3 LC50 & LT50 study of entomopathogenic fungi

The LC50 values and associated 95% confidence limits of the LC50 were calculated for each isolate. From the bioassay it suggested that each bioassay the pattern of aphid mortality due to fungal pathogens were different. The conidial suspension of the fungal isolates was administered on adult mustard aphids under controlled conditions. The results indicated that the mustard aphids were susceptible to fungal isolates and the aphid populations were significantly reduced compared to control treatment. Data were analysed using the general linear regression procedure with probit analysis. The initial screening for pathogenicity of all the twelve isolates indicate that all of them are virulent, affecting significantly higher pest mortality by the seventh days of treatment, while the untreated controls had almost no mortality.

A smaller proportion of aphids died on each day although the first aphid death were recorded minimum average three days post-treatment which was almost similar in all the bioassay treatments. Seven days of post treatment the LC50 value of the fungus (*Penicillium pinophillium*) was obtained as 1.23 x 109 on 3rd day and 5.5 $x10^8$ spores/ml on 4th day of treatment (Table-9).

Table 9: LC50 for fungal entomopathogenic strains during laboratory bioassay

Fungal Isolate	LC_{50} Value (in 10^7 spore/ml) (95% confidence limit)						
	Day 1	Day 2	Day3	Day 4	Day 5	Day 6	Day 7
Acremonium cellulolyticus	$9.0x10^9$ ($5.7x10^8$-$1.24x10^9$)	$3.69x10^9$ ($3.08x10^8$-$7.70x10^9$)	$2.46x10^9$ ($9.0x10^8$-$4.03x10^9$)	$1.31x10^9$ ($7.9x10^8$-$1.83x10^9$	$9.0x10^8$ ($4.6x10^8$-$1.35x10^9$	$5.0x10^8$ ($2.4x10^8$-$7.6.x10^8$)	$8.2x10^8$ ($3.6x10^8$-$1.27x10^9$)
Penicillium pinophilum	$2.57x10^9$ ($8.3.20x10^8$ – $4.20x10^9$)	$1.6x10^9$ ($1.03x10^8$-$2.1x10^9$)	$1.23x10^9$ ($7.7x10^8$-$1.70x10^9$)	$5.5x10^8$ ($3.5x10^8$-$7.4x10^8$)	$3.3x10^8$ ($1.9x10^8$-$4.8x10^8$)	$2.0x10^8$ ($7.44x10^7$-$3.3x10^8$)	$0.87x10^7$ ($0.62x10^7$-$1.12x10^7$)
Fusarium culmorum	$1.11x10^9$ ($9.2x10^8$ – $1.29x10^9$)	$6.0x10^8$ ($4.8x10^8$-$7.2x10^8$)	$7.17x10^8$ ($5.67x10^8$-$8.68x10^8$)	$6.7x10^8$ ($4.8x10^8$-$8.5x10^8$)	$5.0x10^8$ ($3.3x10^8$-$6.6x10^8$)	$0.55x10^7$ ($0.37x10^7$-$0.73x10^7$)	$0.39x10^7$ ($0.25x10^7$-$0.53x10^7$)
Aspergillus tamarii	$1.38x10^9$ ($1.0x10^9$ – $1.69x10^9$)	$1.0x10^9$ ($8.1x10^8$-$1.27.x10^9$)	$8.0x10^8$ ($6.1x10^8$-$9.9x10^8$)	$3.9x10^8$ ($2.4x10^8$-$5.4.x10^8$)	$2.8x10^8$ ($1.4x10^8$-$4.2x10^8$)	$0.11x10^7$ ($0.08x10^7$-$0.14x10^7$)	$0.06x10^7$ ($0.05x10^7$-$0.07x10^7$
Aspergillus niger	$6.4x10^8$ ($5.0x10^8$ – $7.8x10^8$)	$3.9x10^8$ ($2.4x10^8$-$5.4x10^8$)	$2.6x10^8$ ($1.3x10^8$-$4.0x10^8$)	$3.6.x10^8$ ($2.1x10^8$-$5.0x10^8$)	$0.77x10^7$ ($0.66x10^7$ - $0.88x10^7$)	$0.44x10^7$ ($0.35x10^7$-$0.54x10^7$)	$0.03x10^7$ ($0.02x10^7$-$0.04x10^7$)
Metarhizium anisopliae	$1.03x10^9$ ($8.0.x10^8$-$1.25x10^9$)	$8.0x10^8$ ($5.7x10^8$-$1.03.x10^9$)	$8.8x10^8$ ($5.3.16x10^8$ $1.23x10^9$)	$6.4x10^8$ ($2.4x10^8$-$105.23x10^7$)	$4.9x10^8$ ($3.4.74x10^8$-$6.4x18^7$)	$1.71x10^7$ ($1.3.x10^8$-$9.92x10^7$)	$0.04x10^7$ ($0.03x10^7$-$0.05x10^7$)
Verticillium lecanii	$1.6xx10^9$ ($1.1x10^9$-$2.2x10^9$)	$1.2x10^9$ ($8.7x10^8$-$1.7x10^9$)	$1.0x10^9$ ($6.8x10^8$-$1.3x10^9$)	$1.0x10^9$ ($6.2x10^8$-$1.4x10^9$)	$5.1x10^8$ ($3.3x10^8$-$6.9x10^8$)	$1.1x10^8$ ($0.36x10^7$-$2.2x10^8$)	$5.2x10^8$ ($2.5x10^8$-$7.9x10^8$)
Beauveria bassiana	$1.6xx10^9$ ($1.1x10^9$-$2.2x10^9$)	$1.87.01x10^9$ ($1.02x10^9$-$2.7x10^9$)	$1.3x10^9$ ($8.0x10^8$-$1.8x10^9$)	$7.5x10^8$ ($5.0x10^8$-$1.0x10^9$)	$6.2x10^8$ ($4.0x10^8$-$8.4x10^8$)	$7.3x10^8$ ($3.4x10^8$-$1.1x10^9$)	$2.8x10^8$ ($1.5x10^8$-$4.0x10^8$)
Acremonium breve	$2.9x10^9$ ($6.6x10^8$ – $5.2x10^9$)	$1.5x10^9$ ($1.0x10^9$-$2.0x10^9$)	$1.0x10^9$ ($7.6x10^8$-$1.4x10^9$)	$9.2x10^8$ ($6.0x10^8$-$1.2x10^9$)	$5.8x10^8$ ($3.9x10^8$-$7.6x10^8$)	$3.8x10^8$ ($2.4x10^8$-$5.2x10^8$)	$1.7x10^8$ ($8.79x10^7$-$2.6x10^7$)
Nomuraea releyi	$2.0x10^9$ ($1.1x10^9$ – $3.0x10^9$)	$1.5x10^9$ ($1.0x10^9$-$9.7x10^8$)	$1.2x10^9$ ($8.3x10^8$-$1.7x10^9$)	$7.6x10^8$ ($5.4x10^8$-$9.8x10^8$	$6.6x10^8$ ($3.7x10^8$-$9.5x10^8$)	$2.8x10^8$ ($1.7x10^8$-$4.0x10^8$)	$2.8x10^8$ ($1.6x10^8$-$4.1x10^8$)
Penicillium chrysogenum	$2.3x10^9$ ($1.0x10^9$– $3.7x10^9$)	$1.8x10^9$ ($1.0x10^9$-$5.8x10^8$)	$1.1x10^9$ ($7.2x10^8$-$1.5x10^9$)	$9.3x10^8$ ($5.6x10^8$-$1.3x10^9$)	$8.2x10^8$ ($3.6x10^8$-$1.2x10^9$)	$4.2x10^8$ ($2.7x10^8$-$5.7x10^8$)	$2.6x10^8$ ($1.6x10^8$-$3.6x10^8$)
Aspergillus fumigates	$2.8x10^9$ ($7.5x10^8$ – $4.9x10^9$)	$1.9x10^9$ ($9.5x10^8$-$3.0x10^9$)	$9.8x10^8$ ($6.2x10^8$-$1.3x10^9$)	$5.5x10^8$ ($3.5x10^8$-$7.4x10^8$)	$3.3x10^8$ ($1.9x10^8$-$4.8x10^8$)	$4.1x10^8$ ($2.7x10^8$-$5.5x10^8$)	$4.0x10^8$ ($1.8x10^8$-$6.2x10^8$)

It was observed from the LT50 values for all isolates that it decreases with the increasing concentrations of fungal spore load. The calculated LT50 values decreased as the concentration of spore increased for all the fungal isolates tested. However, the pest mortality due to the isolate *Penicillium pinophilum* at concentrations 1 x 10^5 and 1 x 10^6 spores/ml were recorded to be almost similar whereas the mortality of mustard aphids due to other isolates was found to be less at the smaller concentration of fungal bioagents. Mustard aphids treated with higher concentration (1 x 10^8 spores/ml) began to die to fungal infection two days after inoculation compared to other treatments in which the pest mortality began after 3 to 4 days (Table - 10). The fungal isolate was found as the most effective of the studied fungal isolates where mustard aphids began to die due to fungal infection on the second day onwards of the bioassay treatment and recorded 90% aphid mortality on 2nd day and with the increasing time of post treatment *i.e.,* after 2nd day onwards 100% mortality was observed at the spore load 1x10^8. The LT50 value of the fungus *P. pinophilum* at a concentration of 10^8 spores/ml was obtained as 4.46 days for mustard aphid. Similarly the LT50 value of the fungus *Acremonium cellulolyticus* was recorded as 5.23 days. The LT50 decreased as the conidial concentration increased. It was observed that *P. pinophilum* strains were more virulent with lower LT50 values than the other entomopathogenic fungal strains studied. The estimated LC50 and LT50 indicated that *P. pinophilum* was more virulent followed by *A. cellulolyticus* to control mustard aphids. The median lethal concentrations (LC50) of all the isolates were also determined. The *P. pinophilum* strains had low LC50 values compared to other fungal strains studied. The calculated LT50 values for the isolates suggest that in general *P. pinophilum* strains elicited quicker mortality (1-3 days) compared with other fungal strains studied. Results of the probit-transformed mortality data obtained from applying known conidial suspensions have been used for determination of LC50 values for each fungal isolate. The LC50 values ranged from 28 to 100 conidia per mustard aphid. The LC50 values reflect the relative virulence of each isolate. *P. pinophilum* strains had lower LC50 values compared with other fungal strains. The differences observed in fungus-induced mortality to mustard aphid with different conidial doses illustrate the general trend for the isolates. At the lower concentrations the *P. pinophilum* strains elicited 66.6% mortality, while other strain elicited lower mortality. The dosage mortality test illustrates the stronger correlation between dose and time; the higher the concentration the less the time required to reach the LT50 (Table-10). The *P. pinophilum* strains caused more than 80% mortality at the lowest concentration used. All the isolates showed the strongest positive correlation between mortality and dose. Higher level of pest mortality recorded with higher dose of fungal pathogen tested. The *P. pinophilum* strains had achieved 50% mortality in less time compared with other mycogenic entomopathogens studied. Promising results have been obtained when entomopathogenic fungi *P. pinophilum, A. cellulolyticus, e*tc. were applied in the mustard aphid infested crop fields. The entomopathogenic fungal strains able to control the mustard aphid population in the infested area significantly for about 1 month compared with control. Mean Percentage mortality after 7 days and median lethal time (LT50) calculation for mustard aphids exposed for 7days to 14 days old fungal cultures.

Table 10: LT50 for fungal entomopathogenic strains during laboratory bioassay

Fungal Isolate	LT_{50} Value (in 10^7 spore/ml) (95% confidence limit)						
	Day 1	Day 2	Day3	Day 4	Day 5	Day 6	Day 7
Acremonium cellulolyticus	7.16 –(4.36 - 4.5)	8.74 (7.36 - 10.13)	7.45 - (6.59- 8.32)	6.26 - (5.75- 6.78)	6.50 - (5.70- 7.29)	5.23 - (4.72- 5.74)	5.32 - (4.77- 5.87)
Penicillium pinophilum	7.16 – (4.36- 4.5)	8.65 – (7.26- 10.04)	7.41 – (6.58- 8.25)	6.64 – (6.03- 7.25)	5.10 –(4.74 - 5.45)	4.46 –(4.04- 4.87)	4.28 – (3.89 - 4.68)
Fusarium culmorum	7.16 – (436.60- 450.91)	8.33 - (7.19- 9.47)	7.46 - (6.60- 8.32)	4.94 – (4.59- 5.29)	4.59 – (4.20- 4.98)	1.94 – (1.71 - 2.17)	1.30 – (1.11- 1.50)
Aspergillus tamarii	7.16 – (436.60- 450.91)	8.16 - (7.10 - 9.21)	7.62 - (6.78 - 8.47)	6.62 – (6.00 - 7.23)	5.77 - (5.20- 6.33)	4.10 – (3.61- 4.58)	2.97 - (2.26- 3.69)
Aspergillus niger	7.16 – (436.60- 450.91)	8.48 - (7.31- 9.65)	5.61 – (5.22 - 5.99)	4.05 –(3.73 - 4.37)	1.34 – (1.09- 1.58)	0.98 - (23.74 - 25.71)	0.97 – (41.35- 43.30)
Metarhizium anisopliae	7.16 – (436.60- 450.91)	7.22 – (6.29 - 8.16)	5.79 – (5.23- 6.35)	5.18 - (4.64- 5.73)	4.08 – (3.56 - 4.59)	3.04 – (2.30- 3.77)	1.04 – (0.15- 2.23)
Verticillium lecanii	7.16 – (455.14- 469.46)	9.32 - (7.55- 11.09)	7.52 - (6.63 - 8.40)	5.82 - (5.31 - 6.34)	5.59 - (4.87- 6.31)	5.69 - (4.65 - 6.72)	4.57 – (3.74 - 5.39)
Beauveria bassiana	7.16 – (436.60 - 450.91)	9.58 – (7.58- 11.58)	8.56 - (7.15- 9.98)	7.06 – (6.24- 7.88)	5.12 – (4.73- 5.50)	4.88 – (4.42- 5.35)	4.30 – (3.91 - 4.68)
Acremonium breve	7.16 – (436.60- 450.91)	8.47 - (7.25 - 9.69)	8.09 – (6.94- 9.24)	6.83 – (6.11- 7.55)	5.29 – (4.93- 5.64)	4.32 –(3.99- 4.64)	3.91 – (3.56- 4.26)
Nomuraea releyi	7.16 – (436.60 - 450.91)	8.64 - (7.30- 9.98)	7.88 – (6.84 - 8.92)	7.30 - (6.45- 8.15)	5.70 – (5.26- 6.14)	4.73 – (4.35- 5.11)	4.35 – (3.94- 4.76)
Penicillium chrysogenum	7.16 – (4.36- 4.50)	9.59 - (7.61- 1.1)	7.44 - (6.65 - 8.24)	7.34 – (6.44- 8.23)	6.23 – (5.60- 6.87)	4.74 – (4.32 - 5.15)	4.04 – (3.67 - 4.40)
Aspergillus fumigates	7.16 –(4.3- 4.5)	8.27 – (7.12- 9.42)	6.97 – (6.24 - 7.71)	6.29 - (5.64- 6.94)	5.81 - (5.18 - 6.44)	5.12 – (4.56- 5.68)	4.73 – (4.19 5.27)

Table 11: Total colony forming units (CFU values) of the fungal isolates studied

Sl No	Fungal Isolate	CFU/mL
1	*Acremonium cellulolyticus*	1.12×10^3
2	*Penicillium pinophilum*	1.32×10^3
3	*Fusarium culmorum*	1.08×10^2
4	*Aspergillus tamarii*	1.26×10^2
5	*Aspergillus niger*	1.00×10^2
6	*Metarhizium anisopliae*	0.83×10^3
7	*Verticillium lecanii*	0.67×10^2
8	*Beauveria bassiana*	0.95×10^2
9	*Acremonium breve*	0.83×10^2
10	*Nomuraea releyi*	0.77×10^2
11	*Penicillium chrysogenum*	0.92×10^2
12	*Aspergillus fumigates*	1.06×10^2

The total colony forming units (CFU) of the isolated entomopathogenic fungal strains was calculated by following the formula:

CFU/mL = cfu/ml = (no. of colonies x dilution factor) / volume of culture plate.

Among the studied isolates *Penicillium pinophilum* shows higher total CFU (1.32×10^3) followed by *Aspergillus tamarii* (1.26×10^2), *Acremonium cellulolyticus* (1.12×10^3), *Fusarium culmorum* (1.08×10^2) and *Aspergillus fumigates* (1.06×10^2). The lowest total CFU was recorded for the fungal isolate *Verticillium lecanii* (0.67×10^2) (Table-11).

12.6 Pathogenicity study of fungal strains against Lady Bird beetle (predator) and Honey bee (pollinator).

The virulence heatmap of 12 fungal strains against target pest (Mustard aphid), predators (lady Bird Beetle) and pollinator (Honey bee) was analysed and represented in red to green scale in Table 12. The heatmap color gradient toward green represent positive indicator interms of incubation days, higher virulence against target pest (mustard aphid), low virulence against predator (Lady Bird Beetle) and pollinator (honey bee). The heatmap clearly indicates two most efficient fungal strains *Penicillium pinophylum* followed by *Acremonium cellulyticus* as bioagent with least incubation period (3 says) against target pest (mustard aphid) without any harmful effect on predators and pollinators.

Table 12: Virulence heatmap of 12 fungal strains against mustard aphid pest and friendly insect NA sequence

Sl No	Strains	Virulence (subject mortality %) heatmap in laboratory bioassays @ 1 x 10^8spore/ml after 3 & 7 days					
		Mustard aphid		Lady bird beetle		Honey bee	
		3 days	7 days	3 days	7 days	3 days	7 days
1.	*Acremonium breve*	35	77	0	66	60	60
2.	*Acremonium cellulolyticus*	62	100	0	0	0	0
3.	*Aspergillus fumigates*	29	65	68	68	50	50
4.	*Aspergillus niger*	43	67	100	100	0	30
5.	*Aspergillus tamarii*	35	56	0	0	0	0
6.	*Beauveria bassiana*	37	65	0	30	10	30
7.	*Fusarium culmorum*	45	73	0	30	0	20
8.	*Metarhizium anisopliae*	35	69	0	10	0	20
9.	*Nomuraea releyi*	33	67	0	0	0	0
10.	*Penicillium chrysogenum*	34	52	0	40	0	40
11.	*Penicillum pinophilum*	100	100	0	0	0	0
12.	*Verticillium lecanii*	34	61	0	10	0	0

Table 13: Effectiveness of Fungal bioagents on predator (Lady bird beetle).

Treatment	Mortality % (days after treatment)						
	1 day	2 days	3 days	4 days	5 days	6 days	7 days
Acremonium cellulolyticus	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
Penicillium pinophilum	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
Fusarium culmorum	0±0.00	0±0.00	0±0.00	0±0.00	30±1.00	30±0.60	0±0.00
Aspergillus niger	30±0.54	60±1.70	100±0.00	0±0.00	0±0.00	0±0.00	0±0.00
Metarhizium anisopliae	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	30±1.60	30±1.40
Acremonium breve	0±0.00	0±0.00	0±0.00	0±0.00	30±0.50	60±0.20	0±0.00
Aspergillus fumigates	0±0.00	30±0.20	70±0.30	0±0.00	0±0.00	0±0.00	0±0.00

As the predatory Ladybird beetles mainly feeds on aphids and play an important role in integrated pest management so the success of IPM programme with the application of mycopathogens depends on the optimal use of selective biocontrol agents that are less harmful to the natural predator. To avoid the harming effect of entomopathogens for mustard aphid management the present study evaluate the pathogenicity of the potent fungal isolates screened for mustard aphid also validate against the predator (Ladybird beetle). The findings indicate the susceptibility of Ladybird beetle to some selective entomopathogenic fungi which are also pathogenic for mustard aphid. Among the fungal pathogens studied *Aspergillus fumigates* was the most pathogenic with 70% mortality of Ladybird beetle after 3 days of treatment (Table – 13). It was followed by *Acremonium breve* with 60% mortality of beetle after 6 days of treatment. Among the fungal entomopathogen studied *Penicillium pinophilum* and *Acremonium cellulolyticus* showed no effect on the predator up till 7 days after treatment (Plate- 15-vi-ix).

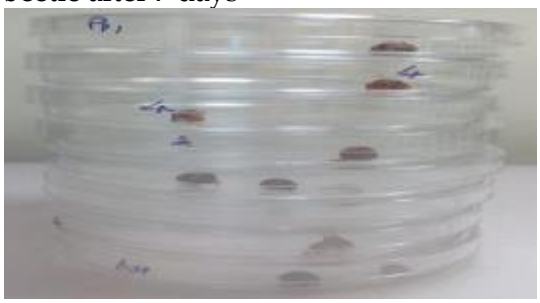
Thus these two entomopathogenic fungal isolates may be utilized for biocontrol of Mustard aphids.

Bioassay treatment of Ladybird beetle with 10* fungal spore load(after 24 h

Bioassay treatment of Ladybird beetle after 3 days of treatment

Control treatment with alive ladybird beetle after 7 days

Alive ladybird beetle after 5 days bioassay treatment with *Penicillium pinophyllium*.



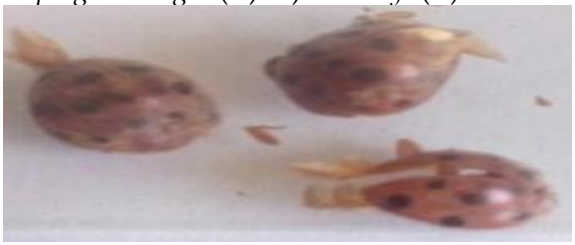
Alive ladybird beetle after 7 days of bioassay with *Penicillium pinophyllium*.

Alive ladybird beetles after 7 days of bioassay treatment with *Acremonium cellulolyticus*

Alive ladybird beetle after 7 days of bioassay treatment with *P. pinophyllium*

Ladybird beetle i) treated with *A. brave* (L) ii) *Aspergillus niger* (C) iii) *N/ releyi* (R)after 7 days

Ladybird beetle after treatment with *Acremonium breave* (dead) after 6 days



Degradation initiates after 10 days of bioassay of Ladybird beetle with *Aspergillus fumigates*

Plate 15: Lady bird beetle after bioassay treatment (different stages of infection) with screened fungal isolates

Table 14: Effectiveness of selected fungal bioagents on pollinator (honey bees, *Apis cerana*).

Treatment	Mortality % (days after treatment)						
	1 day	2 days	3 days	4 days	5 days	6 days	7 days
Acremonium cellulolyticus	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
Penicillium pinophilum	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
Fusarium culmorum	0±0.00	0±0.00	0±0.00	0±0.00	10±0.30	10±0.30	20±0.70
Aspergillus niger	0±0.00	0±0.00	0±0.00	10±0.30	10±1.00	20±0.80	30±0.20
Metarhizium anisopliae	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
Acremonium breve	10±0.01	20±0.10	60±0.30	0±0.00	0±0.00	0±0.00	0±0.00
Aspergillus fumigates	10±0.50	20±0.10	50±0.30	0±0.00	0±0.00	0±0.00	0±0.00

The present study on entomopathogenic fungal isolates screened with virulence for mustard aphids were also validated for pathogenicity against pollinator, Honey bee (*Apis cerana*). Bioassay consisted of exposing group of bees to the laboratory condition for each selected fungus shown >70% mortality in the laboratory bioassay against Mustard aphids within 4 days of treatment. Susceptibility to fungi and degree of virulence varied among the fungal isolates studied. Among the fungal isolates studied the *Acremonium breve* was found to highly active against honey bees and caused higher (60%) mortality within 3 days of treatment which was followed by *Aspergillus fumigates* with 50% mortality of bees within 3 days of treatment respectively (Table - 14, Plate - 16). The entomopathogenic fungi *Aspergillus niger* and *Fusarium culmorum* were found to be moderately active and caused moderate impact on the pollinator honey bees. *Acremonium cellulolyticus*, *Penicillium pinophilum* and *Metarhizium anisopliae*showed no negative impact on the honey bee (*Apis cerana*) up to 7 days of treatment. From the study it was established that the highly virulent fungal pathogens screened against mustard aphid with lower pathogenic impact on the pollinator may be incorporated for integrated pest management of mustard aphids.

Bee hive installation at Khetri Experimental Plot

Field maintenance of Bees hives

Collection of worker bees for Bioassay

Lab bioassay treatment of honey bees

Plate 16: Pathogenicity study of screened fungal isolates against pollinator, Honey bee (*Apis cerana*).

The laboratory bioassay study of the selected pathogenic isolates (based on >70% mortality against Mustard aphids) against predator and pollinator species showed that though few entomopathogenic fungal species were highly pathogenic to Mustard aphid, however, few of them were with no negative effect on the studied predator and pollinators. On the other hand few fungal strains viz. *Acremonium breve*, *A.spergillus fumigates* and *As. niger* were found to be pathogenic for Mustard aphid as well as other tested predators and pollinators. From the laboratory bioassay study *Acremonium cellulolyticus* and *Penicillium pinophilum* both were found to be comparatively safe to predator and pollinators. As both the strains were screened as highly virulent fungal pathogens against mustard aphid with lower pathogenic impact on the pollinator and predators so these two strains were finally selected for standardization and development of Bioformulation.

12.7 Bioformulation of the virulent fungal isolates

12.7.1 Granule formulation

The highly sporulated *P. pinophyllium* and *A. cellulolyticus* fungal culture were taken and conidial suspension of 10^8conidia/ml concentration was prepared by diluting with sterile distilled water and adjusted by using Haemocytometer. Carboxymethyl cellulose (0.5% prepared in distilled water), and white flour gum

(1.5%) were used as adhesives for the formulation. Carrier organic substrate Rice bran (75micron size) was mixed with the adhesive (CMC and white flour gum) and 2% mannitol(Table-15). The moisture content adjusted to 25 per cent by adding distilled water. The pH of the mixture was adjusted to 7 by adding required amount of CaCO3. Then the mixture was distributed into poly begs (100g each) an autoclaved at 121°C for 30 minutes. After proper cooling the sterilized polybags were placed under laminar air flow hood and subsequently, the adjusted fungal conidial suspension of *P. pinophyllium* and *A. cellulolyticus* of 10^8onidia/ml concentration(10ml/100g) was transferred separately to the 100 g sterilized mixture by using sterile needle and thoroughly mixed. The punctured holes were sealed with cello tape and maintained at room temperature at low humid condition.

Table 15 : Granule formulation of fungal bioagents for Mustard aphid control

Sl no	Recipe	Quantity	Proportion %
1.	Rice bran (75 micron) in gm	5000	62.42
2.	Sucrose gm	500	6.24
3.	CMC gm	50	0.62
4.	CaCO3 gm (pH adjustment)	10	0.12
5.	Manitol	150	2.00
6.	White flour gum	100	1.50
7.	H2O ml	2000	24.96
8.	pH 7		
9.	Inoculum ml (10^6 spore /ml)	200	2.49
	Total	8010	

12.7.2 Liquid formulation

The screened virulent fungal bioagents (*P. pinophyllium* and *A. cellulolyticus*) were cultured in a liquid Potato Sucrose broth medium and incubated (at 28°C, 180 rpm) for seven days followed by allowing static sporulation at 28°C without shaking for another 7 days. After that fungal conidia were harvested from the highly sporulated mycelial mate and suspended in pre-sterilized 10% sucrose water and finally adjusting at 10^{12}conidia/ml. The resulting spore suspensions were then mixed with 2% humic acid and 0.1% Glycerol (biosurfactant) for formulation. For field experiment 200ml of liquid formulation was added to 100 litres of water (Table 16).

Table 16: Liquid formulation of fungal bioagents for Mustard aphid control

Sl no	Recipe	Quantity	Proportion %
1.	Pre sterile distilled water ml	1000	63.69
2.	Sucrose gm	100	6.37
3.	Humic acidml	20	0.64
4.	Glycerol	20	0.12
5	pH	7	
6.	Inoculum ml (10^{12} spore /ml)	200	2.55
	Total	1347	

12.7.3 Viable population of fungal isolates

It was observed from the viability study that starting from the inoculation time, conidia concentration decreased from initial levels applied for all the concentrations evaluated for both liquid and organic granule formulations of *Penicillium pinophilum* and *Acremonium cellulolyticus* isolates. Except the treatment B of liquid Acremonium *cellulolyticus* the fungal c.f.u. counts were found to decrease on 2nd month and also further declined on 3rd month (Table- 17). Though the c.f.u. declined however the concentration was still effective for field control of mustard aphids. Based on these results, the storage time for proper application of the bioformulation was set to 3 months. Depending on the spore density in soil for an effective long term control of mustard aphids the amount of formulation may be reduced.

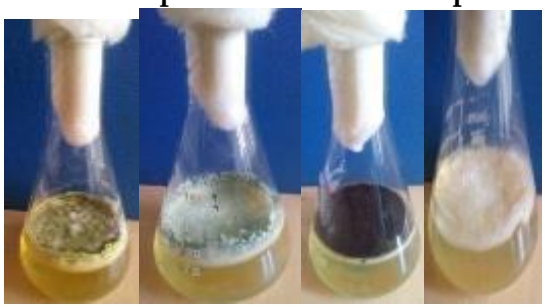
Table 17: Viability assessment of fungal isolates in different bioformulation during storage

Formulation	**Spore load of fungal bio-agents during storage (CFU/ml)**		
	1 month	2 months	3 months
Fungal spore suspension (*Penicillium pinophilum*)	1.70 x 10^3	1.63 x 10^3	2.00 x 10^3
Fungal spore suspension (*Acremonium cellulolyticus*)	1.53 x 10^3	1.72 x 10^3	1.30 x 10^3
Organic granules (*Penicillium pinophilum*)	2.16 x 10^3	2.03 x 10^3	1.56 x 10^3
Organic granules (*Acremonium cellulolyticus*)	1.80 x 10^3	1.56 x 10^3	1.5 0x 10^3

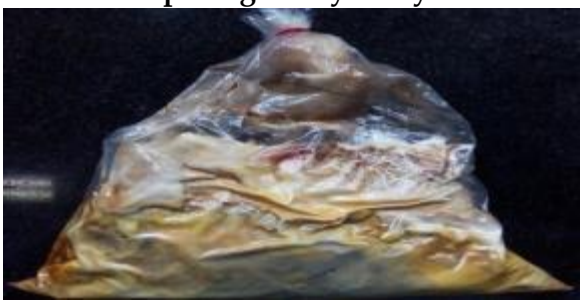
12.8 Field evaluation of Bioformulation on mustard aphid.

Bioformulations of laboratory screened highly virulent fungal isolates were developed and tested for their efficacy against mustard aphid at field condition (Plate 17). The bioformulation treatment dose for field application was @ 2.5g/l (for organic granule formulation) and 50ml/l (for liquid formulation) used for spraying. High volume knapsack sprayer with a spray volume of 500 Litre / hectare was used at 30 and 50 days after sowing for the first and second spray. Both the Organic granule formulation and liquid formulation effectively able to reduce the incidence of mustard aphids on mustard crop in comparison to control treatments i.e. without any fungal spore suspension (Plate 18 b, c, d).

Plate 17: Population of Mustard aphid maintained for pathogenicity study



(a) Potent Fungal isolates in liquid broth



(b) Organic granule Bio-formulation

(c)Liquid formulation of *P. pinophilum*

(d) Organic granule Bio-formulation of *P. pinophilum*

(e)Field treated with Bioformulation of *Penicillium pinophilum*

(f) Mustard aphids Load before bio-formulation treatment

Plate 18: Bio-formulation preparation (both liquid and organic granule) and their field evaluation

Table 18: Effectiveness of Bioformulation against Mustard aphids in mustard crop field trial.

Block	Treatment	Avg. no of live aphid after treatment recorded different days after application						
		0 Day	3 Days	6 Days	10 Days	15 Days	20 Days	25 Days
A	Spore Suspension *P. pinophilum* (liquid)	3.56±0.55	0.27±0.08	0.24±0.04	0.17±0.01	0.55±0.04	0.83±0.02	0.41±0.00
B	Spore Suspension *A. cellulolyticus* (liquid)	5.04±0.06	0.66±0.02	0.25±0.02	0.49±0.01	0.85±0.02	0.9±0.04	0.63±0.02
C	Organic granule *P. pinophilum*	3.2±0.09	0.34±0.01	0.28±0.02	0.17±0.01	0.72±0.02	0.79±0.01	0.46±0.02
D	Organic granule *A. cellulolyticus*	2.32±0.11	1.0±0.10	0.18±0.00	0.22±0.00	0.55±0.01	0.62±0.00	0.51±0.00
E	Control	2.14±0.05	2.02±0.02	1.62±0.03	1.86±0.02	0.97±0.01	1.28±0.07	1.56±0.10

Pre-treatment observation showed that the Mustard aphid was found to be predominant pest in the experimental plots (Plate-18 f). From the table it was very clear that incidence of aphid infestation was reduced greatly after application of both fungal pathogens. Among the four treatments *Penicillium pinophilum* formulation abled to highly reduce the mustard aphid population as compared to other fungal pathogens studied. The entomopathogenic treatment of both liquid formulation and organic granule formulation of *Penicillium pinophilum* showed significant reduction of mustard aphid in field condition (Table - 18). At the beginning and after application of entomopathogenic bioformulation treatment the mustard aphid population was counted. Except control the pest population decreased significantly in all the treatments evaluated.

Table 19: Healthy and infested mustard plant population after treatment with mycogenic bioformulation against mustard aphid infestation at different field condition

Treatments	Type of formulation	Aphid at early flowering stage		Late flowering & early fruiting stage		At late fruiting stages	
		No of healthy plants/m2	No of infested plant/m2	No of healthy plants/m2	No of infested plant/m2	No of healthy plants/m2	No of infested plant/m2
Control	Water	22.2±1.02	6.2±1.02	21.4±1.07	8.2±1.11	23.0±0.89	7.6±4.00
Penicillium pinophilum	*Organic granular*	30.4±1.93	0.6±0.24	30.0±0.63	1.6±0.24	29.0±0.77	1.2±0.37
Acremonium cellulolyticus	*Organic granular*	26.0±1.89	2.4±0.24	28.0±1.58	1.8±0.20	30.0±0.63	1.8±0.37
Penicillium pinophilum	*Liquid*	28.0±1.54	4.6±0.5099	20.6±1.32	3.4±0.66	27.0±0.72	2.6±0.24
Acremonium cellulolyticus	*Liquid*	25.0±1.22	4.0±0.5477	20.0±1.00	5.6±0.47	25.6±0.92	5.2±1.02

Number of healthy plant, infested plants and per cent infestation of mustard plant showed significant differences with two different mycogenic formulations. The results on reduction of numbers of mustard aphid infested plants/m^2 at different flowering and fruiting stages are presented in the Table-19. In the control plot higher number of infested plants observed at late flowering and early fruiting stages. After treatment with mycogenic bioformulation the highest percent reduction of aphid infested plants/m^2 was recorded at early flowering stages on the *P. pinophilum* (0.6±0.24) treated plots, which was followed by late fruiting (1.2±0.37) and early fruiting stages (1.6±0.24). The higher population of mustard aphid infested plants/m^2 was recorded on the late flowering and early fruiting control plot (8.2±1.11) which was followed by liquid formulation of *A. cellulolyticus* (5.6±0.47) treated plot. Higher reduction of infested plants/m^2 was also recorded at late flowering (1.8±0.20) and at late fruiting (1.8±0.37) stages in the plots treated with granular *Acremonium cellulolyticus* bio formulation treatment. In comparison to the liquid formulation the granular bioformulation showed higher efficacy for Mustard aphid management in field condition.

Table 20: Field evaluation of different fungal bioagents for controlling aphid infestation with growth and yield of mustard crop

Treatment	**Type of formulation**	**No of siliqua/plant**	**Siliqua length(cm)**	**Weight of 1000 seeds**	**Seed yield(t/ha)**
Control	Water	94±1.975	3.00±0.54	2.80±0.37	1.60±0.40
Penicillium pinophilum	Organic granular	114±13.08	3.62±0.91	3.34±0.67	2.30±0.20
Acremonium cellulolyticus	Organic granular	102±5.55	3.14±0.40	3.26±0.50	2.20±0.44
Penicillium pinophilum	*Liquid*	91±1.300	3.40±0.44	3.57±0.37	1.89±0.31
Acremonium cellulolyticus	*Liquid*	80±2.040	3.02±0.40	2.86±0.96	2.2±0.37

The highest number of siliqua was recorded in *Penicillium pinophilum* (114) organic granular treated plot which was followed by *Acremonium cellulolyticus* (102). *Penicillium pinophilum* was significantly different with control and other fungal strain studied. Maximum siliqua length (3.62 cm) was recorded in the granular formulation of *Penicillium pinophilum* treated plots followed by liquid *f*ormulation of same bioagents(3.40 cm). The lowest siliqua length was found in control (3 cm) plots. Treatments with *Penicillium pinophilum* and *Acremonium cellulolyticus* were also showed difference in seed test weight of mustard. It was recorded to be highest in the plot treated with liquid formulation of *Penicillium pinophilum* (3.57 g) which was followed by the treatment with granular formulation of the same species (3.34 g). In case of *Acremonium cellulolyticus* treated plot the higher seed yield (3.26 g) was recorded in granular treated plots. Treatments viz. *Acremonium cellulolyticus* and *Penicillium pinophilum* are significantly different with control. In control, lowest yield was recorded (1.6 t/ha) (Table 20).

13. Conclusions summarizing the achievements and indication of scope for future work:

Twelve fungal isolates of 8 genera such as *Acremonium breve*, *Acremonium cellulolyticus*, *Aspergillus fumigates*, *Aspergillus niger*, *Aspergillus tamarii*, *Beauveria bassiana*, *Fusarium culmorum*, *Metarhizium anisopliae*, *Nomuraea releyi*, *Penicillium chrysogenum*, *Penicillum pinophilum* and *Verticillium lecanii* were isolated from mycosed cadavers of mustard aphids, soil sample on agar plate and insect baiting methods. The strains were identified using ITS 1-4 region DNA sequence alignment tools.

Anlaysis of the virulence heatmap of 12 fungal strains against target pest (Mustard aphid), predators (lady Bird Beetle) and pollinator (honey bee) were carried out in laboratory bioassays @ 1 x 10^8 CFU for 3 & 7 days. The heatmap revealed two most efficient fungal strains *Penicillium pinophylum* (within 3 days) followed by *Acremonium cellulyticus* as bioagent with least incubation period (within 7days) against target pest (mustard aphid) without any harmful effect on predators and pollinators. The FTIR spectrum of extracts for the fungal isolates such as *Penicillium pinophilum*, *Acremonium cellulolyticus*, *Aspergillus tamarii*, *Fusarium culmorum*, *Metarhizium anisopliae* , *Beauveria bassiana* were analysed. The FTIR spectrum was categorised based on published reference into four zones such as i) 4,000 to 2,500 cm^{-1} single bonds, ii) 2,500 to 2,000 cm^{-1} triple bonds, iii) 2,000 to 1,500 cm^{-1} double bonds and iv) 1,500 to 400 cm^{-1} fingerprint region single bonds. The zone 4,000 to 2,500 cm^{-1} single bonds are further sub classified into 3500-3300 cm^{-1} for N-H, 3100-3010 cm^{-1} for C-H and 3000-2500 cm^{-1} for O-H single bond. Likewise 2,500 to 2,000 cm^{-1} zone into C≡N 2240-2280 and C≡C 2100-2200; 2,000 to 1,500 cm^{-1} double bonds into C=O 1680-1750, C=N 1615-1700, C=C 1640-1680 and 1,500 to 400 fingerprint region single bonds into 1494-1357, 1182-995 and 680-530 cm^{-1}. Classified Zone wise FTIR spectrum was blown up for the selected 6 fungal strains to locate specific wavenumber cm^{-1} with regard to find out the specific stretch. From the FTIR spectrum Blown up at 2500-2100 wavenumber cm^{-1} it was observed that there is presence of specific PO-H symmetrical stretching of *Penicillium pinophilum* strain at 2350 cm^{-1} which might have impact on virulence of the species. Bioformulations of *Penicillium pinophyllium* and *Acremonium cellulolyticus* were developed and tested for their efficacy against mustard aphid at laboratory and field condition. The formulated bioagents effectively reduced the incidence of aphids on mustard. It was also observed that the bioformulation mixtures attracted the natural enemy population like lady bird beetle and pollinators like honey bees at field treated conditions. In addition to this, a significant increase in mustard seed yield compared with the control. The fungal isolates effectively reduced the incidence of aphid infestation on mustard crop and showed the possibility of controlling the sucking pest aphid using the bioformulation.

Presence of unique PO-H *symmetrical* stretching of *Penicillium pinophilum* strain at FTIR 2350 cm^{-1} might have specific role on fungal pathogenicity so; there is scope for further detail study to correlate the pathogenicity of fungal isolates with FTIR spectra.

14. New Observation:

Anlaysis of the virulence heatmap of 12 fungal strains against target pest (Mustard aphid), predators (lady Bird Beetle) and pollinator (honey bee) were carried out in laboratory bioassays @ 1 x 10^8 CFU for 3 & 7 days. The heatmap revealed two most efficient fungal strains *Penicillium pinophylum* (within 3 days) followed by *Acremonium cellulyticus* as bioagent with least incubation *period* (within 7days) against target pest (mustard aphid) without any harmful effect on predators and pollinators. FTIR spectral study reveal the presence of unique PO-H symmetrical stretching of *Penicillium pinophilum* strain at 2350 cm^{-1} which might have impact on higher virulence of the species.

15. Innovation:

The heatmap revealed two most efficient fungal strains *Penicillium pinophylum* (within 3 days) followed by *Acremonium cellulyticus* as *bioagent* with least incubation period (within 7dsays) against target pest (mustard aphid) without any harmful effect on predators and pollinators.
The *formulated* bioagent *Penicillium pinophylum* effectively reduced the incidence of aphids on mustard crop.

16. **Application Potential**:

- Development of new Bio-formulation with effective bioagents for the control of Mustard aphid.
- Screening of highly virulent entomopathogenic fungi against Mustard aphid with biocontrol potentiality
- Low impact on natural predator and pollinator.

4. S&T benefits accrued: Nil

i. List of Research publications

S No	Authors	Title of paper	Name of the Journal	Volume	Pages	Year

ii. Manpower trained on the project:

a) Research Scientists or Research Associates : One

b) No. of Ph.D. produced : Nil

c) Other Technical Personnel trained:

iii. Patents taken, if any : Preparing for Application of Patent

5. Financial Position:

No	Financial Position/ Budget Head	Funds Sanctioned	Expenditure	% of Total cost
I	Salaries/ Manpower costs	780000	1034280	46.985
II	Equipment	200000	200000	9.086
III	Supplies & Materials	600000	600000	27.257
IV	Contingencies	100000	100000	4.543
V	Travel	90000	90000	4.089
VI	Overhead Expenses	177000	177000	8.041
VII	Others, if any			
	Total	1947000	2201280	100%

6. Procurement/ Usage of Equipment:

S No	Name of Equipment	Make/Model	Cost (FE/ Rs)	Date of Installation	Utilisation Rate (%)	Remarks regarding maintenance/ breakdown
1	Laminar Air Flow	90x60x60 cm, Acco Model no. AC 071.02	80150	09.11.2013	100%	Working
2	Autoclave	NSW 227	119850	28.04.2014	100%	Working

b) Plans for utilising the equipment facilities in future

Name and Signature with Date

a._____
(Principal Investigator)

b._____
(Co-Investigator)

Biotechnological research of the TERI Northeastern Regional Centre, Guwahati is traced back to a micropropagation project in the year 1999. Since then the centre has consistently strengthened its facility and effort towards biotechnological research. Subsequently, the Biotechnology Area was created in 2009 to tap the potential of rich bioresources of the north-eastern region of India through application of biotechnology. This area is engaged in development of micropropagation protocol for production of quality planting materials of economically important plant species and improvement of indigenous Malbhog cultivar of banana through mutation. It is also engaged in understanding molecular genetics involved in oleoresin formation in *Aquilaria malaccensis* through cDNA amplified fragment length polymorphism (AFLP) tools, and transcriptomics for fruit ripening process of *Hippophae* using NextGen platform. It has also expanded its research effort into exploration of potential algal strains for biofuel production. The area is presently pursuing research in the field of micropropagation, algal biofuel, field trial in various agro-climate zones, and crop improvement through mutation induction.

The Energy and Resources Institute
www. teriin.org