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Evaluation of antagonistic effect of entomopathogenic fungi *Paecilomyces lilacinus* against *Spodoptera litura* (Fab)

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Abstract
The present study is related to the *Spodoptera litura* (Fabricius), which is one the most important insect pest of the crops, vegetables, pulses, groundnut. An Entomopathogenic Fungi, *Paecilomyces lilacinus* was isolated from the soil. The media used for the isolation is PDA (Potato Dextrose Agar) in addition with Yeast extract and Chloramphenicol was used as antibiotic. The artificial rearing of *S. litura* was done in laboratory by providing artificial diet and natural diet. Adult stage was used for test experiment. Five different concentration ($1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, $1x\ 10^8$) were used for the test experiment. Isolated Fungus show the maximum pathogenicity/ mortality at the spore concentration of $1x\ 10^6$ spore ml^{-1}.

Keywords: Entomopathogenic, bioinsecticide, mortality, pathogenicity, haemocytometer, rearing

Introduction
The *Spodoptera litura* (fab) also known as tobacco caterpillar, is the most common polyphagous pest with causing huge losses to the crops in various countries like India, Pakistan and other neighboring countries. They are especially pest of Tobacco, Castor, Groundnut, Pulses and various vegetables. Entomopathogenic fungi were investigated as a biological control agent for their role for acting as natural enemies for this insect pest. Entomopathogenic fungi (EPF) particularly *Beauveria bassiana* (Bals) villumin, *Metarhizium anisopliae* (Metschn) Sorokin, *Paecilomyces lilacinus* (Thom. samsom),*Isaria fumosorosea* (wize) and *Lecanicillium lecanii* (Zimm) Zare and Gams, have been studied as biological biocontrol agent (Ramle *et al*. 2004, Faria and wraight 2007, Jaronski 2007, posadas and lecuona 2009) [22, 14, 16].
The exact number of Entomopathogenic genera and the species is indefinite but 700 belonging species and 90 genera were reported. It is previously proved that entomopathogenic fungi will be responsible for around 60% of insect related disease (Faria and Wright 2007) [14]. Entomopathogenic fungi can be used as an alternative with respect to chemical pesticides and are understood as non-harmful biological control agent which is related to human and environmental health (Faria and Wraight 2007) [14]. *Paecilomyces* species are easily available source to elaborate mycoinsecticide as affordable stable propagule, substrate such as blastophores or conidia can be very fastly and easily produced on large scale (Jackson, M.A Erhan, S. popraveskit. T, 2006). *Paecilomyces* has been demonstrate to control pest by reducing the insect growth by only way of reduce feeding (Hunter W.B Avery, P.B. Pick 2011) [17] (Kang B. R. Han, J.W Kim 2018) [18] reproduction (E.I Sharabasy H.M 2015) [19] or simply causing their death due to mycosis (Jessica J.J Peng, T.L Sajap, A.S Lu 2019).
In this study Ihsan and Ibrahim (2004), bioassay experiment revealed that *B. bassiana; M.anisopliae* and *P. fumosaroseus* were effective in killing adult female mite, polyphagotarosonems latus. The main objective of this experiment was to exhibit the pathogenicity of Entomopathogenic fungi *P. lilaninus* against insect pest *Spodoptera litura* (Fab), the major insect of Castor, groundnut, pulse etc.

Material and methods
This *in vitro* study was done at Plant Pathology and Microbiology laboratory, Department of

Botany, Patna University during the month of June 2021 to March 2022.

Isolation of Entomopathogenic fungi

Around 100 soil sample were collected from the Area of Naubatpur, Bihta, Maner region of Patna district. This soil sampling process was done in the month of July - Sept 2021. Soil sample were weight and serially diluted. The media used for the isolation process is Potato Dextrose Agar supplemented by Yeast extract, with chloramphenicol as antibiotic. The diluted soil were inoculated on prepared plates and incubated at 25±1 ^{0}C, relative humidity 65% for 7 days.

Morphological and Microscopic identification

An Entomopathogenic fungus *Paecilomyces lilacinus* was identified based on their morphological and microscopic characteristic. These characters include colony growth, colour, shape, and texture and growth rate. The colony characteristics were compare with other prior studies (Huarui wang *et al*. 2012) [24].

Rearing of *Spodoptera litura*

The egg mass were collected from the Naubatpur, Bihta region of Patna district. The collected eggs were brought to the lab and carefully transferred to the surface sterilize container containing fresh and healthy castor leaves. They were also reared on artificially prepared diet. The ingredient containing artificially prepared diet consist of

1. Chickpea Flour (200g)
2. Yeast Powder(20g)
3. Ascorbic Acid (3.5)
4. Methyl- p- hydroxyl (2g)
5. Ascorbic acid (1g)
6. Formaldehyde sol.(1.5ml)
7. Agar (10g)
8. 500ml distilled water.

The ingredients were firstly dissolve in boil agar solution, then mix properly. The prepared mixer was pour into plastic container and kept it for 2-3 hour for solidification. The diets were kept for overnight and next morning they were used as diet for larvae to maintain the septic condition. The leaves and diet were change every day and container were surface sterilize. The containers were covered with muslin cloth to avoid the escape of larvae from the container.

The rearing processes were maintain under control condition around 25±1 ^{0}C, 65% relative humidity with 8:16 hour of photoperiod of light and dark ratio. After the 5th larvae instars were obtained, the collected larvae were undergo pupation. The processes of pupation were occur in soil. Under appropriate condition pupae were transformed in adult male or female *Spodoptera litura*. The adults were kept in chamber in which they were feed with 10% honey and water solution. Honey solutions were dip into cotton to feed the adult *Spodoptera litura*. Male and female were identified on the basis of their patches present on their body.

Conidial concentration preparation

From seven days old culture plate the spores were scrap with the help of inoculation loop under aseptic condition. The spores taken were collected in test tube/ vial containing 5ml of sterilized 0.5% tween 20. Spores suspensions were homogenize with the help of vortex homogenizer. After that spore suspension were filter into flask with the help of cheese cloth. The cell concentrations were determined using hemocytometer (Klingen. *et al*. 2002) [23]. Five different concentration (1x10^4, 1x105, 1x10^6, 1x10^7, 1x 10^8, control) of conidial cell were prepared for the purpose of testing pathogenicity.

5. Bioassay and Pathogenicity detection

The adult stage of S. *Litura* was used in pathogenicity test. The adult stage of *S. Litura* were immersed in six different spore suspensions. For each concentration 10 adult were placed in clean petri plates containing artificial diet. For each test, 10 adult were placed in glass funnel containing Whatman filter paper then 20ml of spore suspension were poured in the funnel. For control, sterile distilled water was used. After dipping the adults in spore suspension for 10 sec, the adults were left to crawl for 1min for getting dry. Then transferred to the petri dish with filter paper to reduce the humidity. The treated adults were incubate at 25 to 27 ^{0}C, 65% relative humidity. After 5 days of post immersion, dead larvae were taken out and allowed to sporulate in separate Petri plates in which thick mycelial layer were formed on the body surface. This can be done in three replicate. Mortality of the adults suspended in conidial suspensions were checked daily for the next 20 days of inoculation. Mortality were calculated by the given formula;

$$\text{Mortality percent} = \frac{\text{Number of dead adult}}{\text{Number of live adult}} \text{x } 100$$

Result

Morphology of *Paecilomyces lilacinus*

The isolated Entomopathogenic fungi was identified as *Paecilomyces lilacinus*. The colony of fungus on Petri plate were white, fluffy, round in shape, dense, floccose, centrally bulged and reverse is yellowish brown in colour. The colonies were fast growing. The microscopic view were shown in the vegetative hyphae are separated by walled, septate 1-2 micrometer wide. Conidiophores from each separate hypae are arising were forming tuft of conidia. Conidia produced from each hypae and phialide neck were form divergent base.

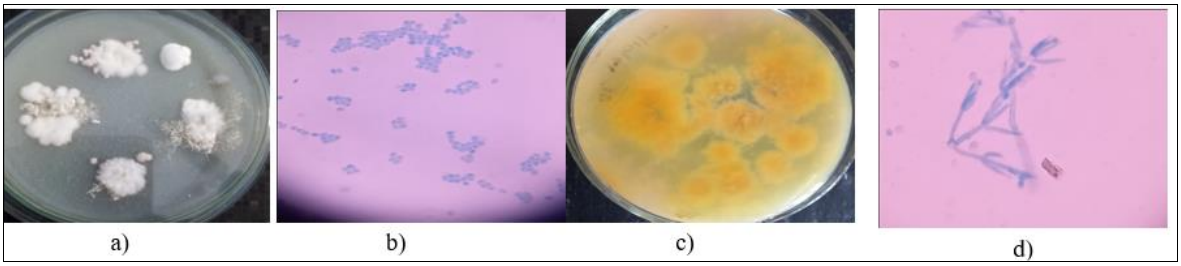

Fig 1: a) Paecilomyces culture plate b) Microscopic view of spores of *Paecilomyces lilacinus* c) Reverse of culture plate d) Microscopic view of pure culture

The different stages of *S. Litura*

The rearing of *S. Litura* was carried on castor leaves as well as artificial diet. The leaves and artificial diet were change daily from fresh one and freshly prepared diet. The different stages of *S. litura* were undergo development in appropriate temperature (25±1 ^{0}C) and relative humidity (65%) with (8:16) hour of photoperiod. The total life cycle period of *S. litura* were 21 to 35 days in which different stages will formed? Following are the different stages of *S. litura* are:

a) Egg: Eggs were taken from field in which they were present at lower side of the leaves.

b) Larva: The egg after 3-4 days were hatch to form larva. There are 1-5 instars larvae were formed.

c) Pre pupal stage: In this stage larvae were not fed leaves. They enter into the soil.

d) Pupa: Pupa was light brownish red colour and forming a hard coat around the surface.

e) Adult: After 10-12 days of pupation, adult were emergent from pupa which were further fed with cotton soaked with10% honey solution.

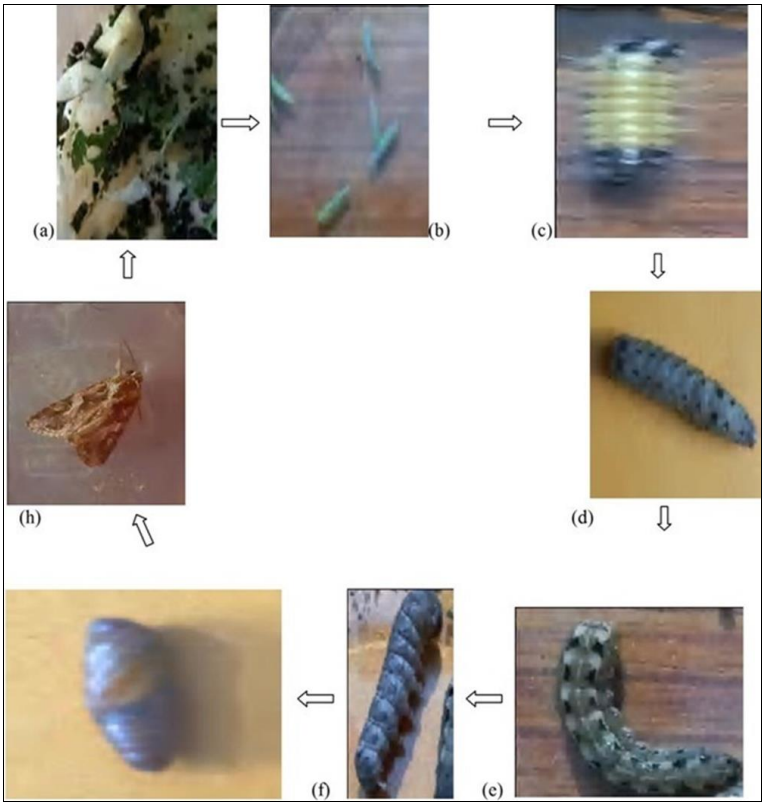

Fig 2: Different developmental stages of *S. Litura*, a) Egg b) Larva c) 1st instar, d) 2nd instar, e) 3rd instar f) 4th instar g) Adult stage

Pathogenicity of *P. Lilacinus* against *S. Litura*

The adult stages of *S. Litura* were shown the mortality of adult. The layer of mycelia forming on the body surface after 5- 20 days of treatment. A white mycelia layer was formed on the surface of *S. Litura*. After 5 days of treatment 1x10^4 was showed the least mortality and the conc. 1x10^6 was showed the maximum mortality after 20 days of treatment.

Percent mortality of adult *S. Litura* was calculated using the formula:

$$\text{Mortality Percent} = \frac{\text{Number of dead adult}}{\text{Number of live adults (sample size)}} \text{X } 100$$

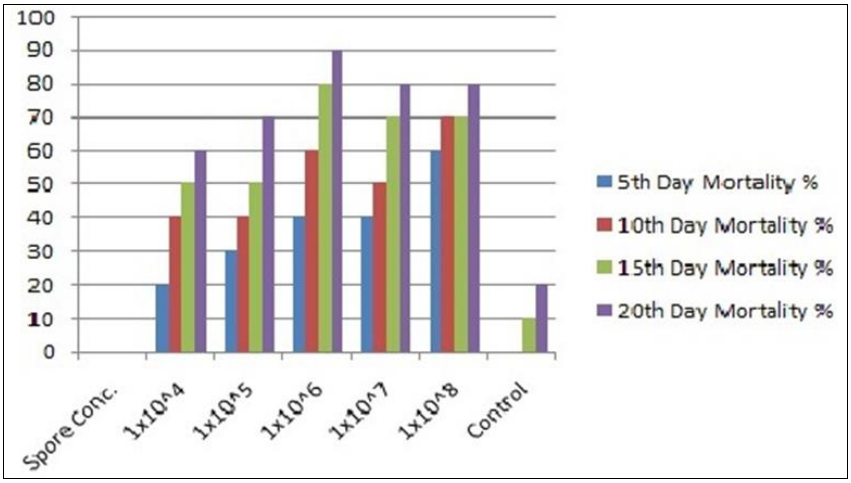

Fig 3: Showing Graph plotted between spore concentration and mortality % wrt. To observation period (No. of days)

	Mortality %				Sample Size
Spore Conc.	**5th Day**	**10th Day**	**15th Day**	**20th Day**	**Total Adults**
$1x10^4$	20	40	50	60	10
$1x10^5$	30	40	50	70	10
$1x10^6$	40	60	80	90	10
$1x10^7$	40	50	70	80	10
$1x10^8$	60	70	70	80	10
Control	0	0	10	20	10

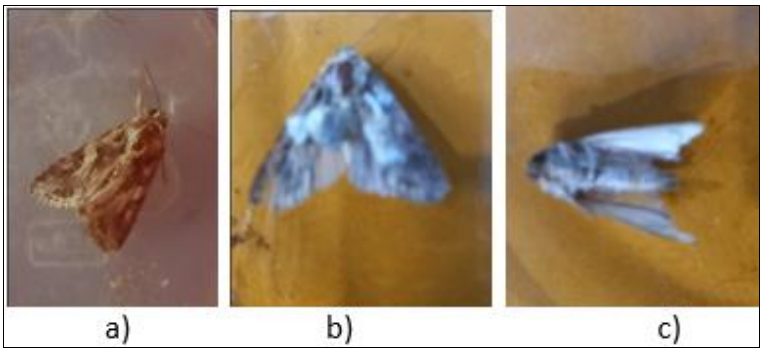

Fig 4: a) Normal adult *S. Litura* b) Affected *S. Litura* having mycelial growth c) Posterior view of *S. Litura*

Discussion and Conclusion

The most common and studied species of genus *Paecilomyces* are *Paecilomyces variotti* and *Paecilomyces fumosoroseus* (Michelin *et al*. 2008, 2010) [20]. Samson (1974) [21] monographed the genus *Paecilomyces* based on morphological characteristics. The isolated entomopathogenic fungus in the study showed that the maximum pathogenicity at the concentration around $1x10^6$ (90%). The treated *S. Litura* adult was more effective at $1x10^6$ concentrations after 15-20 days of post inoculation. At $1x10^7$ and $1x10^8$ spore concentration, the graph plotted was showed the equal mortality rate (80%). The mortality rate was compared from the isolate *Metarhizium anisopliae* FT83 that elicit 100% mortality against larval stages of *S. Exigua* and *Paecilomyces fumosoroseus* FG340 was also responsible for the 100% mortality/ pathogenicity after 6 days of post inoculation at $1x10^4$ conidia/ml) (Han JH, Kim *et al.* 2014). The result from this study suggest that the isolated fungus have the high potential to biocontrol the *S. Litura* at the conidial concentration of 1X 10^6 conidia/ml. Thus, the isolated entomopathogenic fungi will be considered as an ecofriendly and natural agent in control of insect that damage the agricultural crop.

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