J. Mycopathol. Res. **61(2)** : 253-257, 2023; ISSN : 0971-3719 (Print), 2583-6315 (Online) doi. 10.57023/JMycR.61.2.2023.253

In vitro evaluation of *Paecilomyces lilacinus* for biocontrol of *Helicoverpa armigera* (Hubner)

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Received : 23.02.2023 Accepted : 11.04.2023 Published : 26.06.2023

Entomopathogenic fungi play an important role in infecting insect naturally and kill them. This potential made the researchers to investigate on the effectiveness of fungi on against insec pest. In our experiment the main focus was on the fungal activity against *Helicoverpa armigera* which damage the crops and affect the economical condition of farmer. Five different spores concentration of isolate *Paecilomyces lilacinus* were used against adult insect *Helicoverpa armigera.* At higher concentration ($1x10^7$,$1x10^8$) the fungus elicited the highest mortality rate around 80% and 90% respectively after 20 days of post inoculation. Lethal concentration i.e LC_{50} and $LC_{90}$of infected adults were calculated.LC_{50} and LC_{90} values of isolate was calculated as $2.4x10^2$ spores/ml and $3.89x10^8$ spores/ml respectively. Results from experiment conclude that at higher spore concentration ($1x10^8$) maximum mortality was expressed. By this experiment it was also concluded that our isolate have the potential of biological control of insect pest *Helicoverpa armigera*.This isolates will be use in future as biocontrol agent in integerated pest management.

Keywords: Biological, Entomopathogenic ,Haemocytometer, pathogenic , lethal

INTRODUCTION

IMany researchers have already worked on the importance of entomopathogenic fungi as biological control agent against various insect pest affecting agricultural crops. For the proper isolation of entomopathogenic fungi various studies are performed either from cadaver insect or soil (Abdo *et al.* 2008, Bridgebrown *et al*. 2010, Glare *et al*. 2008, Vu *et al*. 2007). In recent studies, entomopathogenic fungi are used as main part of integeral pest management programme of biological control of insect pest. Entomopathogenic fungi (EPF) namely *Beauveria bassiana* Vuillemin, *Paecilomyces lilacinus* (Thom) Samson, *Metarhizium anisopliae* Sorkin, *Lecanicillium lecanii* (Zimm.) are studied as biological control agents (Ramle *et al.* 2004; Faria and Wright 2007).

The species related to Ascomycota belong to different genera *Metarhizium*, *Isaria*, *Beauveria*, *Cordyceps*, *Lecanicillium* and different species of *Pacilomyces* (Khan *et.al*., 2012, Tekaczuk *et al.*, 2015, Jaihan *et al*. 2016). *Paecilomyces* have multiple species and described as a natural control agent. Two species of *Paecilomyces* mainly *P. lilacinus* and *P. fumosorosus* are used as commercial biocontrol agent. They are described to infect insect pest by growth retardation either by feeding reduction and decrease reproduction rate or they also cause cell death by mycosis (Jessica *et al*. 2018).One of the main drawbacks of the EPF as biological control agent is its slower pathogenic activity as compared to synthetic insecticides.

Helicoverpa armigera (Hubner) is one of the most harmful agricultural pests. It is a widely distributed , polyphagous insect having host species ranging from economical important crops as chickpea ,cotton,maize, sorghum, tomato. These pests invade the fruit and flowers of these crops where larvae are hatched by feeding which cause agricultural damage of crops. *Helicoverpa* was selected for pest management by biological control

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not only due to wide range of host related to different families but also due to highly resistance to insecticides. Outbreak are observed on chickpea crop because of cultivation of other crops like cotton , pigeon pea, tomato, sorghum in neighbouring area (Patil *et al*.2007). Control of chickpea or Pod Borer or *Helicoverpaarmigera*is chief priority to sustain chickpea yields. Several insecticides (Pyrethroids, Organophosphates) are used for control of Pod Borer. Among varieties of insect pest *Helicoverpa armigera* ,causes around 50% of agricultural loss or damage in yield of crops such as pulses, maize, cotton , vegetables in India. In current studies , average loss of pulses suffered about 67% which is due to highly insecticide resistance of *Helicoverpa armigera*.

MATERIALS AND METHODS

Isolation and maintenance of Entomopathogenic fungi

Around 100 soil samples were collected from the different location of Patna, Bihar region i.e.Naubatpur, Janipur, Arwal, Bihta. They were stored at 4°C until used. Soil samples were serially diluted up to 10^{-1} to 10^{-5} dilution in sterile distilled water. The freshly prepared mediums were used i.e Potato Dextrose Agar medium containing Yeast extract and chloramphenicol as an antibiotic (Norjmaa *et al*. 2019). The diluted soil suspensions were inoculated on agar plates under laminar air flow chamber. The plates were incubated at 27±1°C for 5-6 days to facilitate the proper colony growth and sporulation. The colonies were identified on the basis of morphological characteristics of colony. The isolated entomopathogenic fungi were maintained on PDA. The stock culture was maintained at 4°C until used. The sub- cultures were done after every 2 month and passage culture was used for the further pathogenecity test or experiments.

Mass production on solid substratum

The isolated entomopathogenic fungi were selected for the large scale production. For mass production of isolated fungus on solid substrate, firstly solid substrate was selected such as gram, chickpea or rice whey. Around 1kg of chickpea was soaked for overnight in 1000 m lof distilled water in conical flask. Next morning water was rinsed out.They were autoclaved at 121°C for 40 min. After cooling they were inoculated with 100ml of spore suspension. Then, the flask was shaken for few minutes and incubates at 25±2°C for 14 days. After 14 days of post incubation , a mycelia layer cover the outer surface of chickpea.

Rearing of H. armigera

The individual larvae were collected from the green gram field of Patna and Arwal district of Bihar region. The collections were done in the month of Jan 2022. The collected larvae were kept in sterile Polyvinyl plastic container containing natural diet castor leaves and also provided with semi synthetic prepared diet. The larvae were kept in container measuring 26x51x4 cm that was tightly covered with fine muslin cloth for providing proper aeration.After 5-6 days larvae were changed into pupal form. The artificially prepared diets were changed at regular interval to maintain the proper aseptic condition. The container was also allowed to clean everyday to avoid contamination. Newly emerged pupae were kept in separate container having soil surface. These reared insect were given temperature around 25±1°C,70-75% of relative humidity and also maintained the day/night photoperiod (16:8) for the emergence of adult insect.

The newly emerged adults were kept in rearing chamber in which they were fed with cotton soaked with 10% of honey solution which help them in ovi-position. In this present study, adult were used for test experiment to check the pathogenecity test.

Preparation of conidial suspension

The 15 days old culture were used .Spores were harvested by scratching with inoculating needle. These spores were transferred in 5 ml sterile between 80 (0.01%v/v). The conidial concentrations were counted using Neubauer Haemocytometer. After conidial count, five different conidialconcentration ($1x10^4$,$1x10^5$,$1x10^6$,$1x10^7$, $1x10^8$ conidia /ml)were prepared by diluting the spores in sterile distilled water(Klingen *et. al*. 2002a, Klingen *et.al* 2002b)

Bioassay

Each of 10 adults were kept in250ml of tap water having five different conidial concentration for 10 sec. In control normal tap water were used. After

dipping insect for 10 sec ,.adults were air dried by freely crawling in laminar air flow and then transferred in sterile container having fresh diets.

All the test insect were kept at 25 ±1°C in BOD incubator having 65% relative humidity. For each test, three replicate were prepared. They were monitored daily and dead adult were removed separately in Petri plates lined with moist filter paper to maintain the relative humidity. Mortality of adult and sporulation were recorded daily upto 15 days.

Statistical analysis

Mortality rate were recorded by the use of Abott's formula (1925). Mortality was calculated by using formula

$$\text{Mortality \%} = \frac{\text{Number of dead adult}}{\text{Number of live adult (sample size)}} \times 100$$

Lethal concentration (LC_{50} and LC_{90}) were calculated by Probit analysis with the use of Microsoft excel window software.

RESULTS AND DISCUSSION

Morphological identification of fungal isolate

The Entomopathogenic fungi isolated from the soil dilution method were identified on the basis of their morphological characteristic both macroscopic view of fungal plates and microscopic identification at 100x oil immersion . The isolated colonies werewhitish, fluffy, round shape, dense and reverse of the plate is yellow to brown in colour. The vegetative hyphae were divided in 1-2 micrometer wide septate. Conidia were arising from tuft of linear conidiophores. Conidia from each hyphae were forming divergent base.

The virulence were checked at the regular interval of 5 days. The whole evaluation was done up to 20 days. By the 5th days of post inoculation, a mycelial layer was formed on the outer surface of the adult insect. At $1x10^4$conidial concentration, the insect will shown the least mortality. The maximum mortality were shown at $1x10^8$conidia /ml . Further the LC_{50} and LC_{90} calculation were done by Probit Analysis using regression analysis in Microsoft excel .The lethal concentration at which 50% and 90% of insect were calculated as $2.4x10^2$ spores/ ml and $3.89x10^8$spores/ml respectively.

Bioassay of isolated entomopathogenic fungi *Paecilomyces lilacinus* against the adult stage of selected insect pest *Helicoverpa armigera* proved that *Paecilomyces lilacinus* infect the insect on dose dependent manner the isolates with high spore concentration leds to maximum virulence against the adult of *H. armigera*. The LC_{50} and LC_{90} values are also dose dependent. Previously it was reported that the mortality of *S. littoralis* against spore concentration around 10^{-9} spore ml^{-1} shows the largest pathogenic effects. *P. fumosoroseus* was found to produced appressoria in nymph integument on second instar stage.It was comparing with other report as no apressoria formation in nymph integument on *Plutellaxylostella* (Altre and Vandenberg , 2001). EPF has been used as one of the biological control agent to remove insect pest of economically important crops (Coates *et.al* 2002; Mcguire*etal.* 2005). The effects of isolated fungal isolates were effective against all of the developmental stages of insect pest (Anand *et al.* 2008).

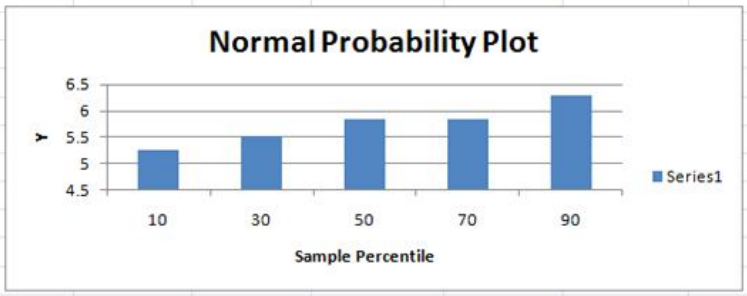
Table.1: Pathogenecity of *Paecilomyces*isolates against *H.armigera* for 20 days post inoculation

	Mortality percent and log_{10} calculation							
Concentration (µg/ml)	**Log_{10}**	**5th**	**10th**	**15th**	**20th**	**Total**	**%Death**	**Probit**
$1x10^4$	4	2	2	1	1	6	60	5.25
$1x\ 10^5$	5	3	2	1	2	8	80	5.84
$1x\ 10^6$	6	3	1	2	1	7	70	5.52
$1x10^7$	7	4	2	1	1	8	80	5.84
$1x\ 10^8$	8	5	2	1	1	9	90	6.28

Table. 2 : Probablity Calculation by Regression Analysis

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.84104755
R Square	0.707360981
Adjusted R Square	0.609814642
Standard Error	0.241909074
Observations	5



ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.42436	0.42436	7.251537936	0.07423334
Residual	3	0.17556	0.05852		
Total	4	0.59992			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	4.51	0.471567599	9.563846231	0.00242515	3.009261438	6.010738562	3.009261438	6.010738562
X Variable 1	0.206	0.076498366	2.692867976	0.07423334	-0.037451942	0.449451942	-0.037451942	0.449451942

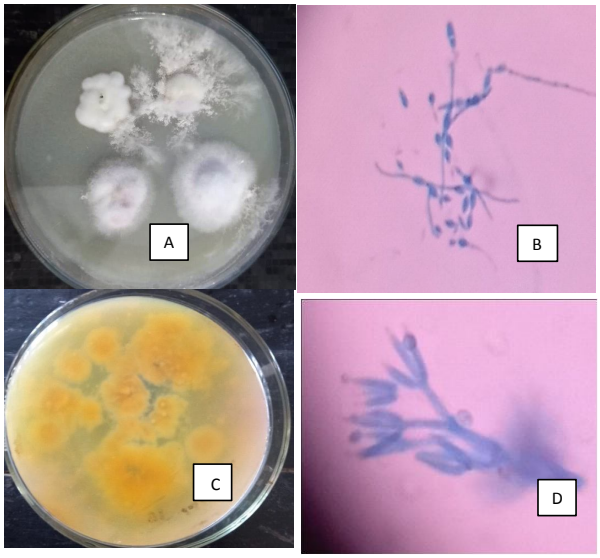


Fig. 1: Mycelial growth of *Paecilomyces lilacinus* on PDAY culture plate, front view (A), reverse view (C); Microscopic view of conidia of *P. lilacinus* (C) and (D) (100X)

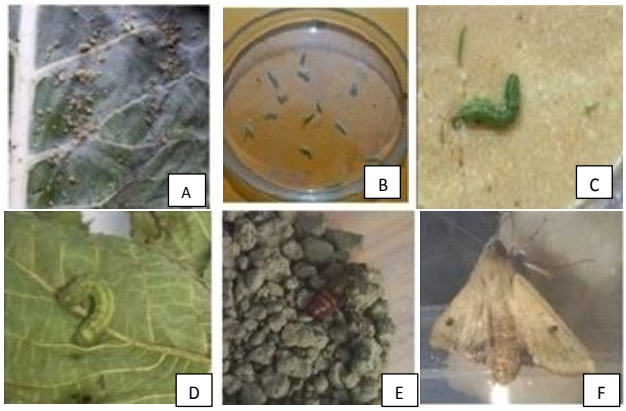


Fig. 2: (A)Eggs masses of *Helicoverpa armigera* (B-D) Larval stages of *H. armigera* (E)Pupal stage in soil containing chamber (F)Adult emergence in rearing chamber

Fig. 3:(A) View of unaffected adult *of H. armigera* (B)View of adult showing pathogenecity against isolated *Paecilomyces lilacinus*(C) Posterior view of affected insect *H.armigera*

Morality per cent and Log_{10} value indicate that the virulence of isolated EPF are very effective against *H. armigera* . LC_{50} and LC_{90} values also give the high virulent property of isolated entomopathogenic fungi. From this study we suggest that our isolates *Paecilomyces lilacinus* are recommended to develop as an biological agent against *H. armigera*.

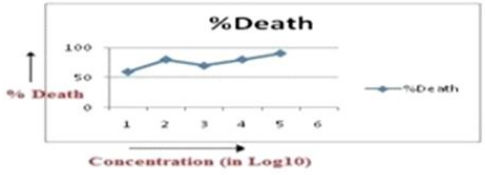


Fig .4: Mortality % of *H. armigera* against Log_{10} Concentration of *P. lilacinus*

CONCLUSION

Based on pathogenecity test against the adult stage of *H. armigera*, it was concluded that mortality

rate was totally dependent on dose concentration. The lethal concentration (LC_{50} and LC_{90}) was varying at different spore concentration in which higher the concentration maximum around 90% of mortality was done. Prior studies related to mortality rate and lethal rate was compared with present result. It is reported that in *Ephestia kuehniella* (Lepidoptera; Pyralidae) there was 100% mortality showed after 10 days of treatment having 10^8 conidia /ml of *Beauveria bassiana*. Kaplan –Muer Survival analysis (Wilcoxon –test) indicate the difference between the dose and concentration. In our study, the effect of entomopathogenic Fungi is directly proportional to conidial concentration. The dose depending on the mortality per cent agree with most of the entomopathogenic fungi.

ACKNOWLEDGEMENT

Authors would like to thank the Head, Department of Botany, Patna University, Patna for giving the facilities of laboratory and infrastructure of Plant Pathology and Microbiology Laboratory, Department of Botany, for completing this research work.

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