Annals of Plant and Soil Research 26(4): 675-682 (2024)
https://doi.org/10.47815/apsr.2024.10416

Assessing the efficacy of *Penicillium citrinum* IBA5VYT as biocontrol agents against *Spodoptera litura*: A Promising approach for pest management

ANAMIKA SHARMA[1]* AND PRAGYA KULKARNI[2]

[1-2]Department of Microbiology, Government V.Y.T. PG Autonomous College, Durg - 491001, Chhattisgarh, India

Received, July, 2024; Revised accepted, October, 2024

ABSTRACT

The tobacco cutworm or cotton leafworm, Spodoptera litura is considered an important pest in the Asian tropics and other temperate and tropical environments which cause loss of yields and productivity of more than 350 host species. The use of fungal strains as biological control agents to complement chemical techniques has achieved great success. This study assesses the use of indigenous entomopathogenic fungus (IBA5 & IBC6) in Kabirdam district of Chhattisgarh, India as a potential biopesticide to improve integrated pest management practices in the region. The mortality rate of IBA5 (Penicillium spp.) was 96.66% in the third stage and 80% in the fourth stage, while the efficacy of IBC6 (Fusarium spp.) was relatively lower, with 73.33% and 50% mortality, respectively. The incubation period was 10 days. Phylogenetic analysis identified IBA5 as belonging to Penicillium citrinum IBA5VYT, and these results were observed at a spore concentration of 1 × 10^7 spores/ml. Further investigation of the effectiveness of P. citrinum IBA5VYT against 3rd and 4th instar larvae of S. litura resulted in LC50 values of 4.5×10^4 spores/ml and 4.2×10^5 spores/ml, LC90 values of 1.6×10^6 spore/ml and 2.1×10^7 spores/ml, and LT50 values 121 hours and 134 hours. These results highlight the forte and effectiveness of P. citrinum against different larval stages of tobacco caterpillars and highlight the robustness and effectiveness of P. citrinum IBA5VYT as an entomopathogenic fungus to address environmental problems.

Keywords - *Penicillium citrinum*, *Fusarium sp.*, *Spodoptera litura*, Biocontrol, Pest management.

INTRODUCTION

Spodoptera litura is considered a polyphagous pest. According to Shu *et al.* 2018, literature reveals that larvae exhibit a broad dietary range, consuming between 120 and up to 180 plant species. Additionally, information indicates the existence of 389 host species for this insect, spanning across 40 different plant families. In the Asia-Pacific region, *S. litura* poses a significant threat to open field crops, causing substantial losses in cotton, maize, groundnut, soybean, tobacco, and various vegetables of economic importance. Furthermore, it emerges as a major concern in protected cultivation environments such as glasshouses and greenhouses, where it inflicts significant damage on crops like eggplants, sweet peppers, and tomatoes (Bragard *et al.,* 2018).

Sustainable pest management offers a comprehensive approach that includes environment friendly, biological, mechanical, and regulatory control measures. These methods aim to manage forest and crop pests with reduced reliance on concentrated chemical pesticides which cause harmful effect on soil fertility as well as beneficial microbial biomass of soil. (Dara, 2019; Goswami *et al.,* 2020). Biological control, a key component of this approach, utilizes substances with precise biological effects to regulate agricultural pests. This can involve the application of products containing biocontrol agents sourced from natural origins such as animals, plants, microorganisms, or specific minerals, along with their genes or metabolites (Gamarra *et al.,* 2023). As the use of natural and organic product like biofertilizer, organic plant growth promoters increase nowadays (Singh *et al.,* 2015) biological control methods are also explored for pet control. Classical biological control typically entails the introduction of natural enemies to combat exotic hosts that lack their complete array of natural adversaries in a particular area (Shah *et al.,* 2014). Within the realm of biocontrol, microbial agents such as entomopathogenic bacteria, fungi, nematodes, and viruses are frequently employed for pest management across diverse cropping systems (Navon *et al.,* 2002).

Several entomopathogens, highlighted by Zhou *et al.* 2009 demonstrate significant efficacy

*Correspondence E-mail: sharma.anamika7914@gmail.com

against *S. litura*. Examples include *Apanteles colemani* Forster (Braconidae: Hymenoptera) as a parasite of larvae (Ramaiah and Maheswari 2018), *Aspergillus flavus* Link (Trichomaceae: Eurotiales) as a pathogen (Kaur *et al.,* 2020) , *Bacillus thuringiensis* Berliner (Bacillaceae: Bacillales) as a pathogen , *Bracon brevicornis Fabricius* (Braconidae: Hymenoptera) as a larvae parasite (Ghosh *et al.,* 2020), *Cotesia glomerata Linnaeus* (Braconidae: Hymenoptera) as a larvae parasite (Ahuja *et al.,* 2012), *Granulosis virus* as a pathogen (Gupta *et al.,* 2015), *Metarhizium anisopliae* Sorokin (Clavicipitaceae: Hypocreales) as a pathogen (Sarwar, 2015), *Telonomus remus Nixon* (Platygastridae: Hymenoptera) as an egg (Shivankar *et al.,* 2008), and *Trichogramma chillonis* as an egg parasite (Mani & Krishnamoorthy 1986). *Nucleopolyhydroviruses* demonstrated high pathogenicity (37.65–96.82%) in the early larval stage, with mortality rates increasing over time and decreasing with larval age (Sajid *et al.,* 2021). Entomopathogenic fungi (EPF) are particularly promising as eco-friendly biocontrol agents due to their rapid reproduction, specific activity against target pests, minimal impact on beneficial organisms, and ability to survive in the absence of hosts (Mudgal *et al.,* 2013). While various EPF species like *Beauveria, Metarhizium, Aspergillus, Fusarium, Penicillium,* and *Paecilomyces* are naturally present in agricultural soils, their natural spore levels are often insufficient for effective pest control. Therefore, applying EPF for pest management requires products with adequate spore numbers (Singh *et al.,* 2020).

P. citrinum and certain *Fusarium sp.*, initially known as phytopathogens (Dara *et al.,* 2016), have also been explored for their entomopathogenic properties, exhibiting potential as biocontrol agents and growth promoters in plants (Waqas *et al.,* 2015; Damasceno *et al.,* 2019). Overall, these biocontrol strategies offer sustainable alternatives to conventional pesticide use, providing effective pest management while minimizing environmental impacts. In this paper indigenous entomopathogenic fungi *P. citrinum* and certain *Fusarium sp.* was explored for their biocontrol activity under laboratory condition against *S. litura,* and molecular identification of potential isolates was carried out.

MATERIALS AND METHODS

The study sites

The present study was conducted from insect infested areas of Kabirdham district of Chhattisgarh. The region located at 21.32' and 22.28' north latitude and 80.48' and 81.48' east longitude, covering an area of 4,447.5 km2, the district boasts a crop area of 241,458 hectares, with agricultural yields reaching 184,662 hectares. During the Rabi and Kharif seasons, crops such as Rice, Sugarcane, Gram, Maize, Soybeans, and seasonal vegetables are cultivated in this area. (https://agriportal.cg.nic.in/PDF/AakasmikYojna/CHH3-KABIRDHAM-10.08.12.pdf)

Isolation of Fungal Culture

Soil samples were collected randomly from different locations (Lohara village vegetable farm, Lalpur village rice crop field, and Pandaria village sugarcane and maize crop field etc.) in Kabirdham district, Chhattisgarh, India, at a depth of 5-10 cm in sterilized polythene bags. These samples were then brought to the laboratory for analysis. Indigenous entomopathogenic fungi were isolated using selective media following established protocols (Goettel & Inglis, 1997) and inoculated using a serial dilution method. The petri plates were incubated at 25°C for 4-7 days, during which fungal growth was regularly monitored. Morphological characterization was conducted by observing macroscopic and microscopic features such as colony colour, hyphal structure, and spore-bearing structures using lactophenol cotton blue stain under higher magnification of a microscope.

Insect Culture

Second instar larvae and egg patches of *S. litura* were obtained from both field and laboratory sources at Indira Gandhi Krishi Vishwavidyalaya, Raipur, India. Eggs were used for in vitro rearing, while larvae were used for bioassays.

Spore Suspension Preparation

Spore suspensions of the fungal isolates were prepared from 20-day-old cultures. Mycelial discs were extracted and eluted in sterile water with 0.01% Tween 80. The resulting spore

suspensions were filtered and adjusted using Neubauer's improved hemacytometer to various concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 spores/ml) for use in bioassays (Sarwar, 2017).

Mortality Bioassay

Bioassays were conducted to determine concentration-dependent and time-dependent mortality effects. *S. litura* larvae of the third and fourth instars were exposed to different spore concentrations for a brief period (5-10 second) and then provided with fresh castor leaves in controlled environments. 0.01% Tween 80 was added to sterile water to treat the controls. Mortality rates were monitored over 10 days, with mortality percentages were calculated using Abbott's formula. Additionally, temporal mortality experiments were conducted using single concentrations for specific larval instars (1×10^7 spores/ml for 3rd instar larvae and 1×10^8 spores/ml for 4th instar larvae). (Abbott, 1925)

The formula is:

$$M = (T - C) / (T - C + U)$$

Where:

M = Mortality percentage

T = Total number of insects in the treatment group

C = Number of insects in the control group that died (natural mortality)

U = Number of insects in the treatment group that died

The formula is used to correct for natural mortality in the control group and provides a more accurate estimate of the mortality caused by the treatment or exposure. (Abbott, 1925)

Statistical Analysis

(LC50, LC90, and LT50 value)

Probit analysis was employed to determine the lethal concentration (LC50, LC90) and lethal time (LT50) required to kill 50% and 90% of the population, using SPSS software with a significance level set at $p < 0.05$ and 95 % CI.

Molecular Characterization

Genomic DNA was extracted from the samples, and ribosomal RNA (rRNA) internal transcribed spacer (ITS) sequencing was performed to identify *Penicillium* isolates. PCR amplification of the ITS1-5.8S-ITS2 rDNA region was carried out using specific primers (ITS4 and ITS5), and the PCR products were analyzed through electrophoresis on agarose gel. The resulting sequences were compared to sequences in the GenBank database for identification.

RESULTS AND DISCUSSION

Isolation of fungal culture & Identification

Soil samples were collected biannually over a three-year period for the isolation of EPF, specifically IBC6 (*Fusarium sp.*) and IBA5 (*P. citrinum*). *Fusarium sp*. colonies on potato dextrose agar (PDA) exhibited white coloration with aerial mycelia, possessing a cottony or somewhat ropey texture (Figure 1a). Abundant uni- or bicellular ovoid to ellipsoid microconidia were observed (Figure 1b), alongside canoe-shaped macroconidia containing three to five septa, featuring a long apical cell and a foot-shaped basal cell (Figure 1c). Chlamydospores were predominantly single-celled (Figure 1d). On PDA medium, *P. citrinum* IBA5VYT colonies developed a basal felt with floccose aerial mycelium, generating conidiophores. These colonies were characterized as short, floccose, flat, round, with an olive-green top and white periphery (Figure 2 a,b). Conidiophores were solitary, possessing a smooth, thin wall and irregular penicillus bearing conidia and phialides. Conidia were produced in distorted chains, spherical to subspherical (Figure 2c). The morphological features of the isolates aligned with previous characterizations and identifications of *P. citrinum* as reported by Nguyen *et al.* (2023).

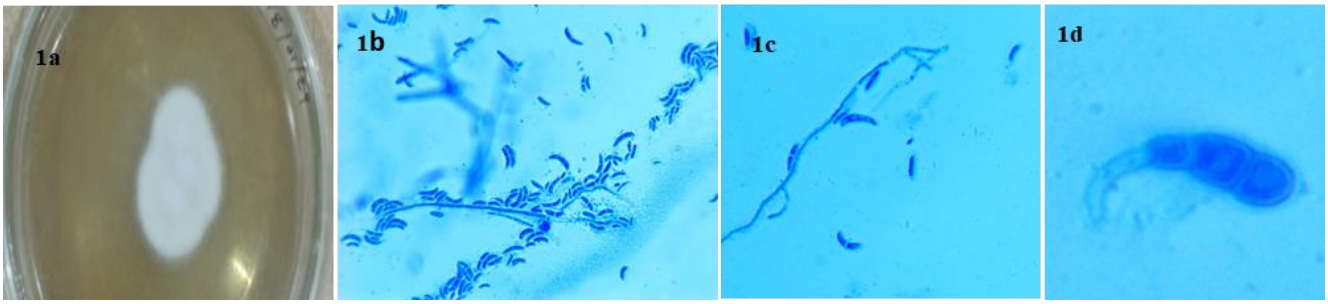

Figure 1: *Fusarium sp.* macroscopic and microscopic picture (1a) colony on PDA plate (1b) Bicellular microconidia (1c) Macroconidia (1d) Chlamydospores

Figure 2**:** *P. citrinum* IBA5VYT macroscopic and microscopic picture (2a) colony on PDA plate (2b) Back pigmentation (2c) isolate with septate hyphae, branching conidiophores and conidia

.**Mortality bioassay**

For mortality bioassays, five concentrations ranging from 1 × 10^4 to 1 × 10^8 spores/ml were tested for both isolates to determine their minimal inhibitory concentration (MIC). *P. citrinum* IBA5VYT exhibited an MIC of 1×10^7spores/ml for third instar larvae, and 1 × 10^8 spores/ml for fourth instar larvae, while *Fusarium sp*. showed an MIC of 1 × 10^8 spores/ml for both larval stages (shown in **Figure3, graph1** and **Figure4, graph2**)

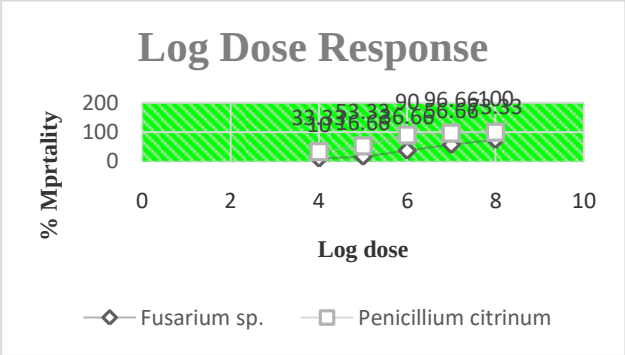

Figure 3, Graph 1: Dose response of 3rd instar larvae of *S.litura* against *Fusarium sp*. and *P. citrinum* IBA5VYT

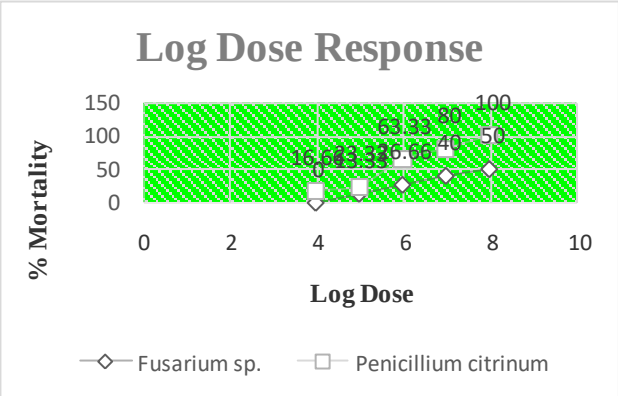

Figure 4, Graph 2: Dose response of 4th instar larvae of *S.litura* against *Fusarium sp*. and *P. citrinum* IBA5VYT

Regarding efficacy, *P. citrinum* IBA5VYT caused notable mortality rates of 96.66% in third instar larvae and 80% in fourth instar larvae, whereas *Fusarium sp.* exhibited lower efficacy with mortality rates of 73.33% and 50%, respectively, after 10 days of incubation. These results are consistent with findings by Nguyen *et al.* (2023), who reported *P. citrinum* pathogenicity against third instar larvae at a concentration of 1 × 10^8 spores/ml after 7 days of treatment (**Figure5, graph3 and Figure 6 graph4**). Additionally, *P. citrinum* strains CTD-24 and *B. bassiana* ZK-5 showed significant effects on egg mortality and reduced early third instar feeding efficacy of *S. frugiperda* larvae at higher concentration levels. These potent fungal isolates hold promise for the development of integrated biopesticides to manage FAW populations, as noted by Idrees *et al.* 2023 and Hernandez *et al.* 2019**.**

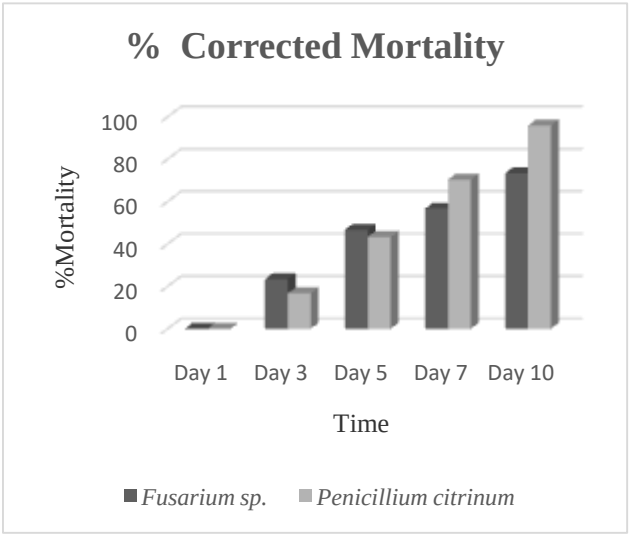

Figure 5, Graph 3 - Corrected % mortality in 3rd instar larvae after exposure of both isolates.

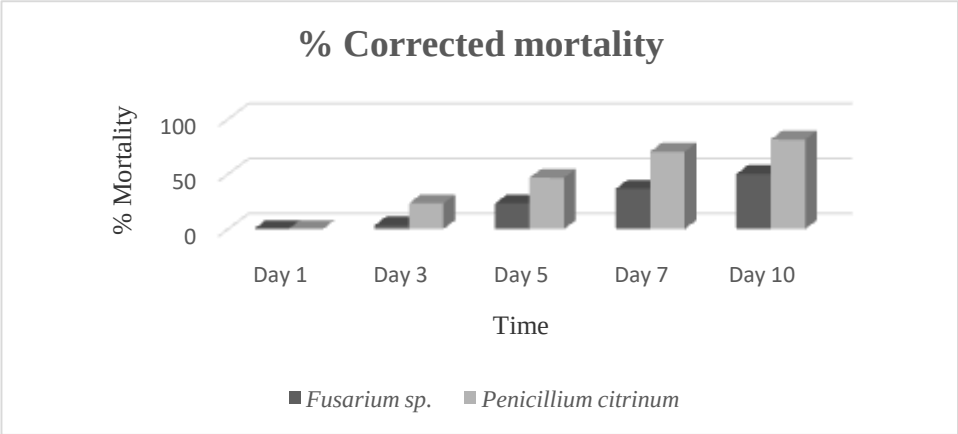

Figure 6, Graph 4- Corrected % mortality in 4th instar larvae after exposure of both isolates

Statistical Analysis (LC50, LC90, and LT50 value)

Furthermore, probit analysis was conducted using SPSS software at a 95% confidence level to determine the lethal concentrations (LC) and lethal times (LT) of *P. citrinum* IBA5VYT against third and fourth instar larvae. The analysis revealed that, LC50 (the concentration required to kill 50% of the test population); 4.5 × 10^4 spores/ml (third instar) and 4.2 × 10^5 spores/ml (fourth instar). LC90 (the concentration required to kill 90% of the test population); 1.6 × 10^6 spores/ml (third instar) and 2.1 × 10^7 spores/ml (fourth instar). LT50 (the time required to kill 50% of the test population); 121 hours (third instar) and 134 hours (fourth instar). These findings are consistent with previous research by Arunthirumeni *et al.* (2023), who investigated the larvicidal activity of *Penicillium sp.* ethyl acetate extract on *Spodoptera litura*. Their study reported significant larval mortality after 48 hours of exposure, with LC50 and LC90 values of 72.205 mg/ml and 282.783 mg/ml, respectively. The present study's results corroborate these findings, highlighting the potential of *P. citrinum* IBA5VYT as a biopesticide agent against *S. litura* larvae.

Table 1: *P. citricum* LC50, LC90 value for *S. litura* in vitro mortality

Treatment	Larvae stages	LC50	LC90	χ^2	Reg. Equation
P. citricum	Third instar	4.5× 10^4	1.6× 10^6	.477	Y=.820x+(-3.819)
	Fourth instar	4.2× 10^5	2.1× 10^7	1.251	Y=.754x+(-4.245)

Table 2: *P. citricum* LT50 value for *S. litura* in vitro mortality

Treatment	Larvae stages	LT50	χ^2	Reg. Equation
P. citricum	Third instar	121 hrs	.120	Y=5x+(-10.420)
	Fourth instar	134 hrs	.291	Y=2.962x+(-6.302)

3.4 Molecular characterization- for confirmation of potential isolate**.**

Aligned Sequence Data of Sample – 6_I (599 bp)

CTTCCTCCGCTTATTGATATGCTTAAGTTCAG
CGGGTATCCCTACCTGATCCGAGGTCAACCT
GAGTATAATTCAAACGTGTTGGACTGTGGCA
CTGCGCTCGCGCGTCTCGGCCGGGCCTACT
AGAGCGGGTGACGAAGCCCCATACGCTCGA
GGACCGGACGCGGTGCCGCCGCTGCCTTTC
GGGCCCGTCCCCCCGGCGGGGGGGACGGG
GCCCAACACACAAGCCGGGCTTGAGGGCAG
CAATGACGCTCGGACAGGCATGCCCTCCGG
AATACCAGAGGGCGCAATGTGCGTTCAAAGA
CTCGATGATTCACTGAATTCTGCAATTCACAT
TAGTTATCGCATTTCGCTGCGTTCTTCATCGA
TGCCGGAACCAAGAGATCCGTTGTTGAAAGT
TTTAACTAATTTCGTTATAGGTCTCAGACTGC
AACTTCAGACAGCGTTCAGGGGGGCCGTCG
GCGGGCGCGGGGCCCGCCGAGGCAACATA
GGTTCGGGCAACACGGGTGGGGAGGCTGG
GCCCCGAGGGGACCCGCACTCGGTAATGAT
CCTTCCGCAGGTTCACCTACGGAAACCTTGT
TACGACT TTTACTTCCATC

The Microbe was identified as ***Penicillium citrinum*** **IBA5VYT accession number PP721327** as it showed highest similarity of 96.97% with *Penicillium citrinum* strain Xia16 small subunit ribosomal RNA, internal transcribed spacer with accession no. OR346130.1 (Fig.7)

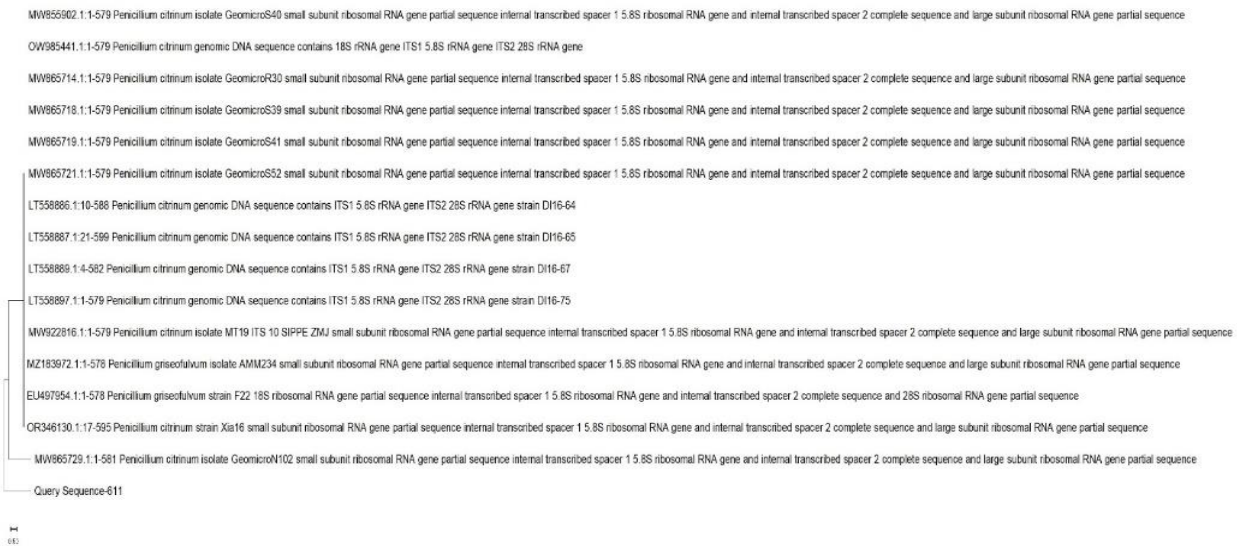

Figure 7: Phylogenetic tree of P. citrinum

Several studies have confirmed significant mortality rates of common entomopathogenic fungi against *S. litura*, although the efficacy of *P. citrinum* IBA5VYT reported in this study exceeds typical concentrations. For example, *Nomuraea Riley* exhibited 100%, 93.4 %, 86.2 and 74.3 %mortality on fourth and fifth instar larvae at conidia concentrations above 10^{10}, $10^{9,}$ $10^{8,}$ 10^{7} with mortality rates of 88%, 89%, and 77% at lower concentrations, respectively, according to Namasivayam *et al.,* 2015. Additionally, Fitriana *et al.,* 2021 reported *S. litura* mortality rates of 74.07% with *Beauveria bassiana* and 25-60% with *Aspergillus oryzae* for third instar larvae.

Penicillium citrinum, widely studied as a filamentous fungus, has gathered attention not only in biotechnology and pharmaceuticals but also for its potential as a plant growth promoter. Some strains of *P. citrinum* have been explored for their biocontrol properties against plant pathogens and insects, secreting bioactive compounds like indole-3-acetic acid (IAA), siderophores, cellulases, and chitinases. These compounds play crucial roles in plant-microbe interactions, including hormone regulation, nutrient uptake facilitation, and defence against pathogens. Researchers are investigating the formulation of *Penicillium citrinum*-based biostimulants for agricultural use (Patel *et al.,* 2019; Kaur & Saxena 2023; David *et al.,* 2023).

CONCLUSION

The investigation into the utilization of indigenous Entomopathogenic Fungi (EPF) in Kabirdham district reveals promising prospects for enhancing integrated pest management (IPM) practices. By harnessing local resources and biodiversity, this research offers a potential alternative to synthetic pesticides, promoting environmental conservation and sustainability. Further research is necessary at the localized level to comprehend the pest invasion dynamics in the region. Future research prospects include the efficient formulation of *Penicillium citrinuum* IBASVYT spores with minimal concentrations of chemical pesticides and their field trials for IPM. Additionally, genetic manipulation may be explored if required. This research contributes to local agricultural sustainability and aligns with global efforts towards eco-friendly pest control methods. Moving forward, further research and implementation of these biopesticides could significantly benefit farmers by improving soil health, fertility, and the entire ecosystem, ultimately contributing to a more sustainable agricultural practice.

ACKNOWLEDGEMENT

The authors are thankful to department of Microbiology, Govt. V.Y.T. PG Autonomous College, Durg for providing necessary research facilities, Indira Gandhi Krishi Vishwavidyalaya Raipur for insect sample and also thankful to Cytogene research and development centre, Lucknow for molecular identification.

AUTHOR CONTRIBUTIONS

Anamika Sharma and Pragya Kulkarni conceived the research. Anamika Sharma conducted the experiments, analyzed the data and wrote the manuscript. Pragya Kulkarni reviewed and edited the manuscript. Both the authors read and approved the manuscript.

REFERENCES

Abbott, W.S. (1925) A method of computing the effectiveness of an insecticide. *J. econ. Entomol*, *18*(2), 265-267.

Ahuja, D.B., Ahuja, U.R., Srinivas, P., Singh, R. V., Malik, M., Sharma, P. & Bamawale, O.M. (2012) Development of farmer-led integrated management of major pests of cauliflower cultivated in rainy season in India. *Journal of Agricultural Science*, 4(2), 79.

Arunthirumeni, M., Vinitha, G., & Shivakumar, M. S. (2023) Antifeedant and larvicidal activity of bioactive compounds isolated from entomopathogenic fungi Penicillium sp. for the control of agricultural and medically important insect pest (Spodoptera litura and Culex quinquefasciatus). *Parasitology International*, 92, 102688.

Damasceno, C.L., Sá, J.O.D., Silva, R.M.D., Carmo, C.O.D., Haddad, L.S.A.M., Soares, A.C.F., & Duarte, E.A.A. (2019) Penicillium citrinum as a potential biocontrol agent for sisal bole rot disease. *Journal of Agricultural Science*, *11*(10), 206.

Dara, S.K. (2019) The new integrated pest management paradigm for the modern age. *Journal of Integrated Pest Management*, *10*(1), 12.

Dara, S.K., Dara, S.S., Dara, S.S. & Anderson, T. (2016) First report of three entomopathogenic fungi offering protection against the plant pathogen, Fusarium oxysporum f. sp. vasinfectum. *EJ. Entomol. Biol.*

David, O.M., Olawusi, A.C., Oluwole, O.A., Adeola, P.O., & Odeyemi, A.T. (2023) Isolation, Molecular Characterization and Application of Aspergillus niger and Penicillium chrysogenum with Biofertilizer Potentials to Enhance Rice Growth. *Tropical Journal of Natural Product Research*, ***7***(4).

EFSA Panel on Plant Health (PLH), Bragard, C., Dehnen-Schmutz, K., Di Serio, F., Gonthier, P., Jacques, M.A., and MacLeod, A. (2020) Pest categorisation of Spodoptera eridania. *EFSA Journal*, *18*(1), e05932.

Fitriana, Y., Suharjo, R., Swibawa, I.G., Semenguk, B., Pasaribu, L.T., Hartaman, M., and Solikhin, S. (2021) Aspergillus oryzae and Beauveria bassiana as entomopathogenic fungi of Spodoptera litura Fabricius (Lepidoptera: Noctuidae) infesting corn in Lampung, Indonesia. *Egyptian Journal of Biological Pest Control*, ***31***, 1-12.

Gamarra, H., Sporleder, M., Supanta, L., Rodríguez, A., Kroschel, J., & Kreuze, J. (2023) Development of Encarsia tabacivora (Viggiani) (Hymenoptera: Aphelinidae) in nymphs of Bemisia tabaci (Gennadius) MEAN 1 (Hemiptera: Aleyrodidae). *bioRxiv*, 2023-01.

Ghosh, E., Varshney, R., & Venkatesan, R. (2020) Performance of Bracon brevicornis (Wesmael) on two Spodoptera species and application as potential biocontrol agent against fall armyworm. bioRx

Goettel, M.S., & Inglis, G.D. (1997) Fungi: hyphomycetes. In *Manual of techniques in insect pathology* (pp. 213-249). Academic press.

Goswami, M.R., & Tripathi, S. (2020) Impact of insecticides on soil microbial biomass and its activity inegg plant (Solanum melongena L.) under field condition. *Annals of Plant and Soil Research*, ***22***(1), 25-31.

Gupta, M., Tara, J., Sharma, S., & Bala, A. (2015) Biology and morphometry of Spodoptera litura Fabricus, a seriousdefoliator of mango (Mangifera indica) in Jammu Region (J&K). Munis Entomology & Zoology, **10**, 215-221.

Hernandez-Trejo, A., Estrada-Drouaillet, B., López-Santillán, J.A., Rios-Velasco, C., Varela-Fuentes, S.E., Rodríguez-Herrera, R., & Osorio-Hernández, E. (2019) In vitro evaluation of native entomopathogenic fungi and neem (Azadiractha indica) extracts on Spodoptera frugiperda. *Phyton*, *88*(1), 47.

https://agriportal.cg.nic.in/PDF/AakasmikYojna/CHH3-KABIRDHAM-10.08.12.pdf

Idrees, A., Afzal, A., Qadir, Z.A., and Li, J. (2023) Virulence of entomopathogenic fungi against fall armyworm, Spodoptera frugiperda (Lepidoptera: Noctuidae) under laboratory conditions. *Frontiers in Physiology*, *14*, 1107434.

Kaur, M., Chadha, P., Kaur, S., Kaur, A., and Kaur, R. (2020) Schizophyllum commune induced oxidative stress and immunosuppressive activity in Spodoptera litura. BMC microbiology, **20**(1), 1-10

Kaur, R., & Saxena, S. (2023) Penicillium citrinum, a drought-tolerant endophytic fungus isolated from wheat (*Triticum aestivum L.*) leaves with plant growth-promoting abilities. *Current Microbiology*, ***80***(5), 184.

Mani, M., & Krishnamoorthy, A. (1986) Susceptibility of Telenomus remus Nixon, an exotic parasitoid of Spodoptera litura (F.), to some pesticides. International Journal of Pest Management, **32**(1), 4

Mudgal S, De Toni A, Tostivint C, Hokkanen H, Chandler D. (2013) Scientific support, literature review and data collection and analysis for risk assessment on microbial organisms used as active substance in plant protection products –lot 1 environmental risk

Namasivayam, S.K.R., & Bharani, A.R.S. (2015) Biocontrol potential of entomopathogenic fungi *Nomuraea rileyi* (F.) Samson against major groundnut defoliator Spodoptera litura (Fab.) Lepidoptera; Noctuidae. *Adv Plants Agric Res*, ***2***(5), 221-225.

Natikar, P.K., & Balikai, R.A. (2015) Tobacco caterpillar, Spodoptera litura (Fabricius): Toxicity, ovicidal action, oviposition deterrent activity, ovipositional preference and its management. Biochem. Cell. Arch, **15**(2), 383-389

Navon, A., & Ascher, K. R. S. (Eds.). (2000) *Bioassays of entomopathogenic microbes and nematodes*. Cabi.

Nguyen, H.C., Lin, K. H., Nguyen, T.P., Le, H.S., Ngo, K. N., Pham, D.C., and Barrow, C.J. (2023) Isolation and Cultivation of Penicillium citrinum for Biological Control of Spodoptera litura and Plutella xylostella. *Fermentation*, ***9***(5), 438.

Patel, D., Patel, S., Tekwani, S., Patel, M., Menon, S., Vadakan, S., and Goswami, D. (2019) Assessing mycoparasytic activity exhibited by phyto-friendly-fungi (PFF) in combating phytopathogenic fungi by producing various glucanases. In *Biotechnology and Biological Sciences* (pp. 259-263). CRC Press.

Ramaiah M, Maheswari TU. Biology studies of tobacco caterpillar, Spodoptera litura Fabricius. J Entomol. Zool. Stud 2018; **6**(5):2284-2289.

Revathi, K., Chandrasekaran, R., Thanigaivel, A., Arunachalam Kirubakaran, S., and Senthil-Nathan, S. (2014) Biocontrol efficacy of protoplast fusants between Bacillus thuringiensis and Bacillus subtilis against Spodoptera litura Fabr. Archives of Phytopathology and Plant Protection, **47**(11), 1365-1375.

Sajid, Z., Ramzan, M., Shafiq, M.M., Usman, M., Murtaza, G., & Pareek, V. (2021) A Review on Nucleopolyhydroviruses (NPV) as Biological Control of Army Worm, Spodoptera litura.

Sarwar, M. (2017) Characteristics of armyworm Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) occurrence and control on cotton. *Scholars Journal of Research in Agriculture and Biology*. 2(1), 33-38.

Shah, S.M.M., *et al.* (2014) "Rearing of Bactrocera zonata (Diptera: Tephritidae) for parasitoids production and managing techniques for fruit flies in mango orchards." *International Journal of Tropical Insect Science* **34**.S1: S108-S113.

Shivankar, S.B., Magar, S.B., Shinde, V.D., Yadav, R.G., and Patil, A.S. (2008) Field bio-efficacy of chemical, botanical and bio-pesticides against Spodoptera litura Fab. in sugarbeet. Annals of Plant Protection Sciences, **16**(2), 312-315.

Shu, B., Zhang, J., Cui, G., Sun, R., Yi, X., & Zhong, G. (2018) Azadirachtin affects the growth of Spodoptera litura Fabricius by inducing apoptosis in larval midgut. *Frontiers in physiology*, ***9***, 137.

Singh, A.K., Beer, K., & Pal, A.K. (2015) Effect of vermicompost and bio-fertilizers on strawberry growth, flowering and yield. *Annals of Plant and Soil Research*, ***17***(2), 196-99.

Singh, S., Singh, A.P., Chaurasiya, D.K., Mukherjee, S., and Mondal, G. (2020) Entomopathogenic fungi as biocontrol agents in agriculture. *Res. Today*, *2*, 264-266.

Waqas, M., Khan, A.L., Hamayun, M., Shahzad, R., Kang, S.M., Kim, J.G., and Lee, I.J. (2015).

Zhou, Z. (2009) A review on control of tobacco caterpillar, Spodoptera litura. Chinese Bulletin of Entomology, **46**(3), 354-361.