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Research Article

Metabolite profiling of Entomopathogenic fungi and its insecticidal efficacy against *Spodoptera litura* (Fabricius)

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ABSTRACT: *Spodoptera litura* is a serious pest that affects a wide range of crops. In an effort to find an alternative solution to synthetic insecticides, this study investigated the metabolite profile of the ethyl acetate extract of *Beauveria bassiana* and *Metarhizium anisopliae* and its insecticidal potential against the 3rd larval instar of *S. litura* in the laboratory setting. The crude extract of *B. bassiana* and *M. anisopliae* was prepared by the ethyl acetate solvent extraction method. The metabolite profile of *B. basssiana* and *M. anisopliae* was analysed using GC- MS analysis. The pathogenicity of the fungal metabolite was evaluated through larvicidal bioassays using five different concentrations (50, 100, 150, 200 and 250 µg/ml). The results showed that *B. bassiana* was most effective in killing the pest, with a mortality rate of 81%, whereas *M. anisopliae* exhibited 64% mortality after 120 hours. The results indicate that both fungal metabolites are effective against the third instar larvae of *S. litura* and could be considered for use in field applications.

KEYWORDS: Biocontrol, entomopathogenic fungi, metabolite extract, *S. litura*

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INTRODUCTION

Spodoptera litura (Fabricius) (Noctuidae; Lepidoptera) is a highly destructive and polyphagous lepidopteran pest and poses a significant threat to several crops (Singh *et al*., 2024). Chemical pesticides are widely used for the control of *S.litura*, which leads to several consequences. It's essential to find new compounds as alternatives to chemical pesticides. As a sustainable alternative, biological control agents like entomopathogenic fungi offer a promising strategy for insect pest management, mitigating the harmful impacts of chemical pesticides (Kumar *et al.,* 2023). Entomopathogenic fungi are a wide range of morphologically, phylogenetically, and ecologically diverse fungal species. Fungal entomopathogens have evolved mechanisms to evade the host's immune system. *B. bassiana* and *M. anisopliae* are widely distributed Entomopathogenic Fungus (EPF) that offer a promising approach to managing insect pests due to their broad host range and ease of mass production (Qu, S and Wang, S, 2018). Entomopathogenic fungi like *B. bassiana* and *M. anisopliae* have been widely studied among entomopathogenic fungi for their bioefficacy, leading to the development of several commercial products (de Faria and Wraight, 2007). The crucial factor for the pathogenicity of EPF is the production of secondary metabolites (Sarkar *et al*., 2024). Microbial toxins exhibit a more targeted mode of action, effectively controlling specific pests while reducing harm to beneficial species, such as natural enemies and pollinators (Karthi *et al.,* 2019). The metabolites produced by entomopathogenic fungi encompass a broad range of natural compounds, making them a valuable resource for developing natural biopesticides (Masi *et al.*, 2018). Metabolites derived from entomopathogenic fungi exhibit insecticidal and antifeedant activities against insect pests. This study investigates the potential of the metabolites of *B. bassiana* and *M. anisopliae* as a biopesticide, focusing on their metabolite profile and its larvicidal efficacy against third instar larvae *of litura*. The LC_{50} and LT_{50} were evaluated to assess the effectiveness of the metabolite extract of *B. bassiana* and *M. anisopliae* against the third instar larvae of *S. litura*.

MATERIALS AND METHODS

Rearing of *S. litura*

Spodoptera litura eggs were procured from the National Bureau of Agricultural Insect Resources (NBAIR), Bangalore. The neonate larvae were reared on fresh, surface-sterilised castor leaves (*Ricinus communis* L.) under laboratory conditions. Emerged adults were paired in a 1:1 sex ratio and fed a 10% honey solution fortified with vitamin E as adult diet. (Vengateswari *et al*., 2020).

Preparation of metabolite extract

The entomopathogenic fungi *B. bassiana* (7529) and *M. anisopliae* (7895) were obtained from ITCC, Indian Agricultural Research Institute, New Delhi. Both the fungus was cultured in Potato Dextrose Broth and incubated at 28-30°C for 14 days in darkness. The culture filtrate was then extracted with ethyl acetate, and the solvent was evaporated under reduced pressure using a rotary evaporator. The resulting crude extract was weighed and stored at 4°C for further use.

GC-MS analysis

The crude metabolite extract of *B. bassiana* and *M. anisopliae* was analysed by GC-MS using a Perkin Elmer Clarus 500 system equipped with an HP-5 column (30 m × 0.2 mm × 0.25 µm). Helium was used as the carrier gas at a flow velocity of 32 cm/s. Turbomass 5.2 software and the NIST library were used for data analysis and compound identification (Lu *et al*., 2025).

Bioassay

The efficacy of metabolite extracts of *B. bassiana* and *M. anisopliae* was analysed against 3rd instars of *S. litura* by the leaf disc method (Surface application). Three groups of experimental setups with 25 larvae were subjected to the treatment. The stored crude fungal metabolite extract was reconstituted with water and applied at various concentrations (50, 100, 150, 200 and 250 µg/ml). 1ml of each concentration was uniformly sprayed over the leaf discs and dried before the assay. Evaporated solvent (ethyl acetate) was used as a negative control after complete drying. The experiment was replicated thrice. Dead larvae were counted at 24 h intervals for five days, and the cumulative percentage of mortality was calculated. Based on the percentage mortality against five different concentrations, LC_{50} and LT_{50} were calculated, and the data were tabulated.

Statistical analysis

The Median lethal concentration (LC_{50}) and Median lethal time (LT_{50}) were assessed by Probit Analysis (Finney, 1971). Statistical analyses were carried out using GraphPad Prism 5 software.

RESULTS AND DISCUSSION

GC-MS analysis

Gas chromatography and mass spectrometry analysis of compounds was carried out in the ethyl acetate extract

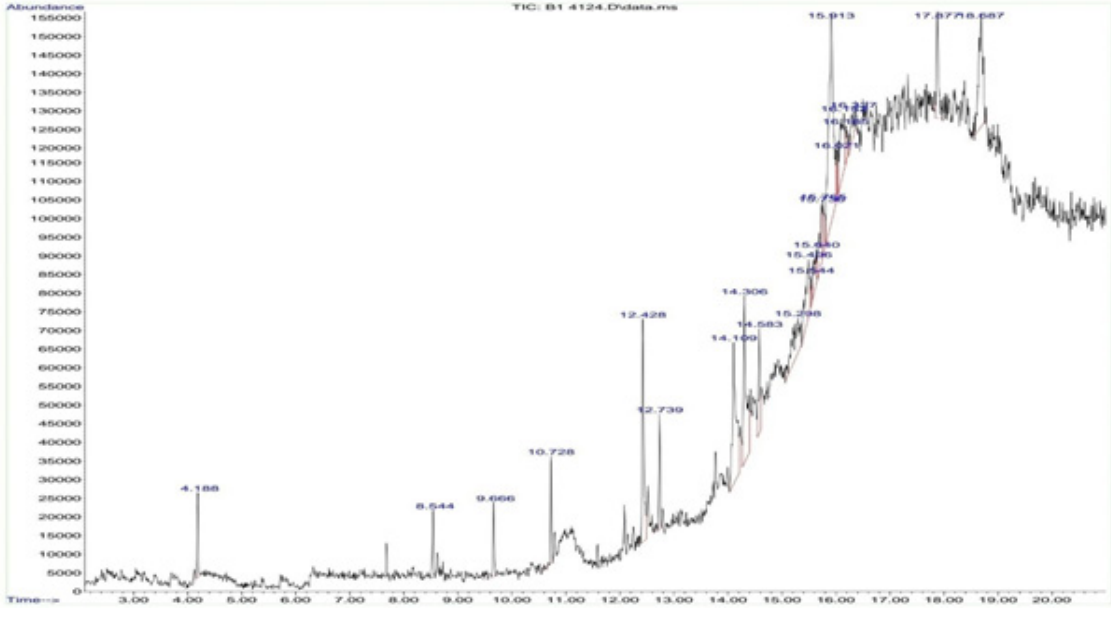


Figure 1. GC MS profile of *Beauveria bassiana.*

of the metabolite extract of *B. bassiana* (Figure 1, Table 1) and *M. anisopliae* (Figure 2, Table 2). The metabolite profile of the crude extract of *B. bassiana* revealed the presence of Naphthalene. n-Hexadecanoic acid, Tridecanoic acid, 1-Octadecene, 2-Hexadecanol, Octadecanoic acid, Tetracosanoic acid, Lauricanhydride, Dodecanoic acid, 1,2,3-propanetriylester, Ginsenol, 4-Quinolinecarboxylic acid, Di-n-decylsulfone, Chloroacetic acid, 3-pentadecylester. Among these compounds, Naphthalene is shows promise as a natural pest control agent in agriculture (Daisy *et al*., 2002). According to Vivekanandhan (2018), *B. bassiana* extracts showed effective larvicidal and pupicidal activity against *Cx. quinquefasciatus*, with N-hexadecanoic acid identified as a potential bioinsecticide. Previous studies by Abdullah R. R. H (2019) and Abdullah (2024) reported that Trideconoic acid, 1-octadecane, and octadecanoic acid had insecticidal activity. According to Abd El Azeem (2023), 2 Hexadecanol and Dodecanoic acid exhibit various lethal effects against insect pests.

In this study, the metabolite extract of *M. anisopliae* showed the presence of Isobenzofuranone. GC-MS profile *M. anisopliae* metabolite extract composed of Naphthalene, Azulene, 1(3H)-Isobenzofuranone, 1-Nonadecene, n-Nonadecanol-1, Trichloroacetic acid, pentadecyl ester, 3-(Prop-2-enoyloxy) tetradecane, 2-Hexadecanol, Trifluoroacetic acid pentadecylester, Tetradecanoic acid, n-Hexadecanoic acid, 1-Heneicosanol, N-Methyl-1-adamantaneacetamide, Oleic Acid, Octadecanoic acid, Silicic acid, (trimethylsilyl) ester. Huang *et al.,* (2012) stated that derivatives of Isobenzofuranone exhibit a range of biological activities, including antioxidant and cytotoxic effects. Metabolites like n-Hexadecanoic acid and tetradecanoic acid produced by certain entomopathogenic fungi exhibit significant larvicidal and pupicidal activity against insect pests. These compounds induce tissue damage, causing significant mortality in larvae and pupae, and show promise as eco-friendly alternatives to synthetic insecticides, as evidenced by studies done by Abdullah, Reda, R., (2019); Ragavendran, C., *et al.* (2017). Octadecanoic acid, commonly known as stearic acid, is a saturated fatty acid found in the crude extracts of certain fungal species, including *Penicillium* species. These extracts have shown effective larvicidal activity against pests like *S. litura* (Bihal, R., *et al*., 2023). According to San Juan-Maldonado *et al*., (2024), Oleic acid synthesised by the entomopathogenic fungus *M. rileyi* has been shown to enhance its biocontrol effectiveness. The fungus utilises lipases to degrade the insect cuticle, with oleic acid playing a role in the infection process, enhancing its pathogenicity.

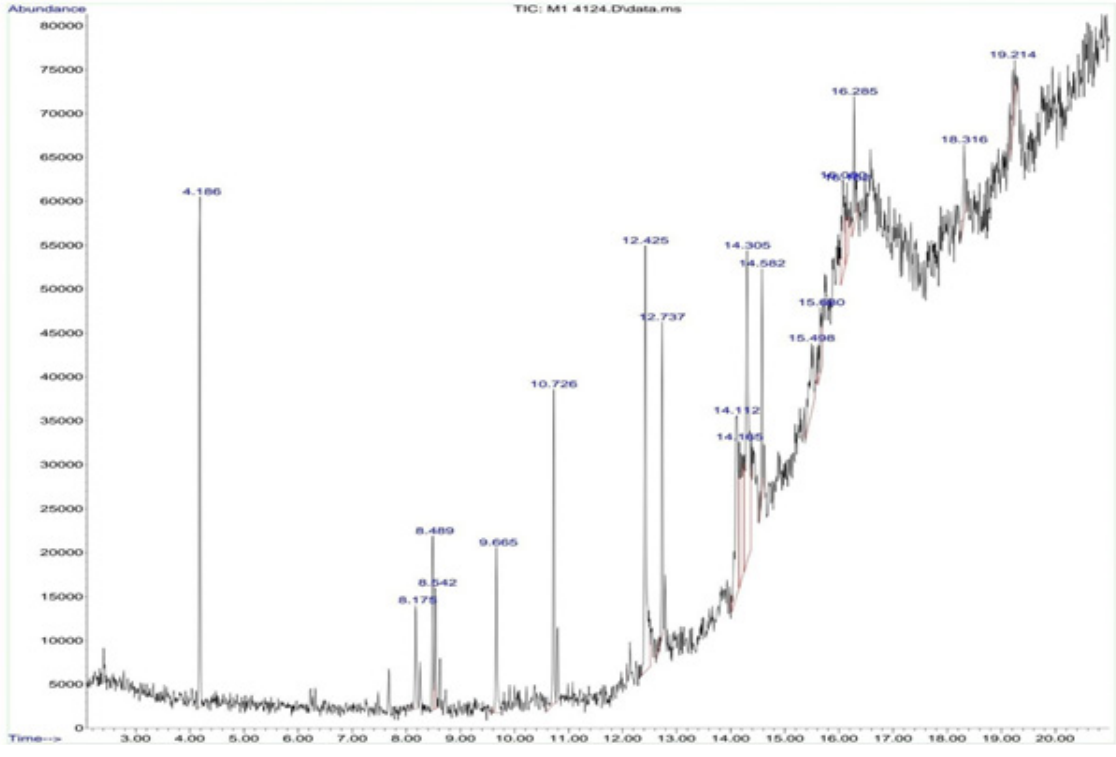


Figure 2. GC-MS profile of *Metarhizium anisopliae.*

Table 1. GC-MS analysis of crude metabolite extract of *B. bassiana*

Sr. No.	Chemical compound	R_t (min)
1	Naphthalene	4.20
2	n-Hexadecanoic acid	12.40
3	Tridecanoic acid	12.43
4	1-Octadecene	12.74
5	2-Hexadecanol	14.11
6	Octadecanoic acid	14.30
7	Tetracosanoic acid	14.00
8	Lauricanhydride	14.59
9	Dodecanoic acid, 1,2,3-propanetriylester	15.27
10	Ginsenol	15.54
11	4-Quinolinecarboxylic acid	15.64
12	Di-n-decylsulfone	15.91
13	Chloroacetic acid,3-pentadecyl Ester	14.59

Table 2. GC-MS analysis of crude metabolite extract of *M. anisopliae*

Sr. No.	Chemical compound	R_t (min)
1	Naphthalene	4.20
2	Azulene	4.19
3	3-Methyl-4-methylamino-1,2,4-triazole-5-thiol	8.20
4	1(3H)-Isobenzofuranone	8.49
5	1-Nonadecene	8.50
6	n-Nonadecanol-1	8.55
7	Trichloroaceticacid, pentadecyl Ester	10.73
8	3-(Prop-2-enoyloxy) tetradecane	9.67
9	2-Hexadecanol	9.70
10	Trifluoroacetic acid, pentadecyl Ester	10.70
11	Tetradecanoic acid	12.50
12	n-Hexadecanoic acid	12.40
13	1-Heneicosanol	12.70
14	N-Methyl-1-adamantaneacetamide	15.50
15	Oleic Acid	14.10
16	Octadecanoic acid	14.30
17	Silicic acid, diethyl Bis (trimethylsilyl) ester	18.30

Mortality analysis, LC_{50} and LT_{50}

The efficacy of metabolite extract of *B. bassiana* and *M. anisopliae* was evaluated against the 3rd instar larvae of *S. litura* in the laboratory using five different concentrations (50, 100, 150, 200 and 250 µg/ml), which were diluted with water. Results showed that the metabolite extract was effective against 3rd instar larvae of *S. litura*. Larval mortality increased dose-dependently, with higher concentrations inducing greater mortality. The metabolite extract of *B. bassiana* exhibited the highest mortality of 81%, and *M. anisopliae* resulted in 64% mortality after 120 hours of treatment at a concentration of 250 µg/ml (Figure 3). The metabolite extract of *B. bassiana* is more effective against the third instars of *S. litura* than the metabolite extract of *M. anisopliae*, which might be due to the presence of more insecticidal compounds. The larvae subjected to the treatment showed morphological deformities during larval development of the larvae, including pupal deformation and reduced emergence of adults as sublethal effects. The findings of the current study align with Sowmya *et al*., 2024, where *M. anisopliae* culture filtrate extracted with ethyl acetate and acetone showed significant insecticidal activity against *S. litura* (91.2% mortality at 100 µg) and *A. crassivora* (100% mortality at 100 µg). According to Kaur *et al.*, 2016, the ethyl acetate extract of the fungus *A. niger* affects the survival and development of *S. litura*, causing significant larval mortality, retarded development, and reduced emergence and egg hatchability. These adverse effects intensified with increased metabolite concentrations. The fungal metabolites also exhibit antifeedant properties, leading to reduced food consumption. Thakur *et al*., 2013, reported that ethyl acetate extracts of the endophytic fungus *Cladosporium uredinicola* showed significant insecticidal potential against *S. litura*.

Table 3. Log-probit analysis and LC_{50} of *M. anisopliae* and *B. bassiana* crude extracts

Fungal extract	LC_{50}	R^2	P value	Intercept	Slope
Beauveria bassiana	91.2	0.90	0.01	1.47	1.80
Metarhizium anisopliae	154.17	0.93	0.006	1.17	1.75

Table 4. Log-probit analysis and LT_{50} of *M. anisopliae* and *B. bassiana* crude extracts

Fungal extract	LT_{50}	R^2	P value	Intercept	Slope
Beauveria bassiana	43.45	0.90	0.01	2.05	1.81
Metarhizium anisopliae	74.13	0.93	0.006	1.73	1.74

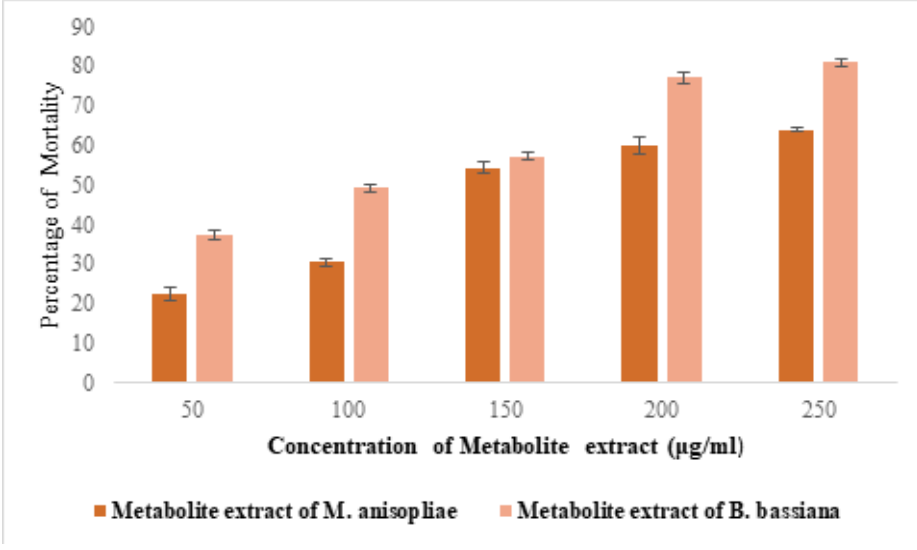


Figure 3. Effect of metabolite extracts of *B. bassiana* and *M. anisopliae* against third instar larvae of *S. litura* after 120 hours of treatment.

The estimated lethal concentration (LC_{50}) required for killing 50% of the larvae of *S. litura* treated with the metabolite extract of *B. bassiana and M. anisopliae* is 91.2 and 154.2 over 120 hrs of treatment respectively (Table 3). The (LT_{50}) time required for killing 50% of the larvae treated with the metabolite extract of *B. bassiana and M. anisopliae* at a concentration of 250 µg/ml is 43.5 hrs and 74.1 hrs, respectively, under laboratory conditions (Table 4). Results showed that the mortality resulting from the crude metabolite extract of *B. bassiana* and *M. anisopliae* was statistically significant ($P \leq 0.05$). Fungal metabolites exert toxic effects on insect pests, impairing their feeding, development, and physiological functions. The efficacy of *B. bassiana* and *M. anisopliae* against *S. litura* observed in our study supports the results reported by Bakr *et al.,* (2025).

CONCLUSION

This study focuses on identifying the chemical profile and determining the effectiveness of metabolite extracts of *B. bassiana* and *M. anisopliae* against third-instar larvae of *S. litura*. Results revealed the presence of bioactive compounds with insecticidal properties in the metabolite extracts, as substantiated by larvicidal bioassay assessments. The study highlights the potential of entomopathogenic fungal metabolites as effective biopesticides, supporting large-scale production for plant protection. The data will also benefit researchers developing biocontrol strategies against insecticide-resistant pests. However, further research, including field trials, is necessary to fully determine its efficacy against *S. litura.*

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REFERENCES

Abd El Azeem, E. M., Ghareeb, E. M., Hussien, R. H. M. 2023. Effectiveness of ethyl acetate extract from *Aspergillus flavipes* AUMC 11390 culture filtrate on biological and physiological performance of the spiny bollworm, *Earias insulana,* (Boisd.) (Lepidoptera: Nolidae). *Beni-Suef Univ J Basic Appl Sci*, **12**: 67. https://doi.org/10.1186/s43088-023-00390-9

Abdullah, R. R. H. 2019. Insecticidal activity of secondary metabolites of locally isolated fungal strains against some cotton insect pests. *J Plant Prot Pathol Mansoura Univ,* 10(12): 647–653. https://doi.org/10.21608/jppp.2019.79456

Abdullah, R. R. H., Abd El-Wahab, A. H. and Abd El-Salam, S. A. 2024. Insecticidal activity and possible modes of action of secondary metabolites of some fungal strains and wild plants as natural pesticides against *Spodoptera frugiperda*. *Beni-Suef Univ J Basic Appl Sci*, 13: 9. https://doi.org/10.1186/s43088-024-00467-z

Abdullah, Reda, R. 2019. Insecticidal activity of secondary metabolites of locally isolated fungal strains against some cotton insect pests. *J Plant Prot Pathol*, **10**(12): 647-653. https://doi.org/10.21608/jppp.2019.79456

Bakr, W. H., Ghaly, M. F., Tantawy, W. G. 2025. Detection of some secondary metabolites of *Beauveria bassiana* and the potential effects on *Spodoptera littoralis*. *Chem Biol Technol Agric*, **12**: 59. https://doi.org/10.1186/s40538-025-00774-2

Bihal, R., Al-Khayri, J. M., Banu, A. N., Kudesia, N., Ahmed, F. K., Sarkar, R., Arora, A., Abd-Elsalam, K. A. 2023. Entomopathogenic fungi: an eco-friendly synthesis of sustainable nanoparticles and their nanopesticide properties. *Microorganisms*, **11**(6): 1617. https://doi.org/10.3390/microorganisms11061617 PMid: 37375119 PMCid: PMC10302739.

Daisy, B. H., Strobel, G. A., Castillo, U., Ezra, D., Sears, J., Weaver, D. K., Runyon, J. B. 2002. Naphthalene, an insect repellent, is produced by *Muscodor vitigenus*, a novel endophytic fungus. *Microbiology*, **148**(11): 3737-41. https://doi.org/10.1099/00221287-148-11-3737 PMid: 12427963.

de Faria, M. R. and Wraight, S. P. 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biol Control*, **43**(3): 237-256. https://doi.org/10.1016/j.biocontrol.2007.08.001

Finney, D. L. Probit analysis. Cambridge University Press, London, 1971.

Huang, X. Z., Zhu, Y., Guan, X. L., Tian, K., Guo, J. M., Wang, H. B., Fu, G. M. 2012. A novel antioxidant isobenzofuranone derivative from fungus *Cephalosporium* sp. AL031. *Molecules*, **17**(4): 4219-4224. https://doi.org/10.3390/molecules17044219 PMid: 22481542 PMCid: PMC6268973.

Karthi, S., Senthil-Nathan, S., Kalaivani, K., Vasantha-Srinivasan, P., Chellappandian, M., Thanigaivel, A., Ponsankar, A., Sivanesh, H., Stanley-Raja, V., Chanthini, K. M., Shyam-Sundar, N. 2019. Comparative efficacy of two mycotoxins against *Spodoptera litura* Fab. and their non-target activity against *Eudrilus eugeniae* Kinb. *Ecotoxicol Environ Saf*, **15**(183): 109474. https://doi.org/10.1016/j.ecoenv.2019.109474 PMid: 31394378 PMCid: PMC9512179.

Kaur, T., Kaur, J., Kaur, A. and Kaur, S. 2016. Larvicidal and growth inhibitory effects of endophytic *Aspergillus niger* on a polyphagous pest, *Spodoptera litura*. *Phytoparasitica*, **44**(4): 465-476. https://doi.org/10.1007/s12600-016-0541-2

Kumar, A., Suroshe, S. S., Saini, G. K., Singh, J. 2023. Efficacy of genetically transformed *Metarhizium anisopliae* against *Spodoptera litura* and *Aphis craccivora*. *Saudi J Biol Sci*, **30**(1): 103493. https://doi.org/10.1016/j.sjbs.2022.103493 PMid: 36466218 PMCid: PMC9712132.

Lu, J., Liu, J., Li, H., Sun, Y., Ma, Y. and Li, Y. 2025. Analysis of toxic components in secondary metabolites of entomopathogenic fungi *Clonostachys rosea* (Hipocreales: Bionectriaceae) from *Cephalcia chuxiongica* (Hymenoptera: Pamphiliidae). *Microorganisms*, **13**(10): 2289. https://doi.org/10.3390/microorganisms13102289 PMid: 41156749 PMCid: PMC12565781.

Masi, M., Nocera, P., Reveglia, P., Cimmino, A., Evidente, A. 2018. Fungal metabolite antagonists of plant pests and human pathogens: structure-activity relationship studies. *Molecules*, **23**(4): 834. https://doi.org/10.3390/molecules23040834 PMid: 29621148 PMCid: PMC6017029.

Qu, S., and Wang, S. 2018. Interaction of entomopathogenic fungi with the host immune system. *Dev Comp Immunol*, **83**: 96-103. https://doi.org/10.1016/j.dci.2018.01.010 PMid: 29355579.

Ragavendran, C., Dubey, N. K., and Natarajan, D. 2017. *Beauveria bassiana* (Clavicipitaceae): a potent fungal agent for controlling mosquito vectors *of Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *RSC Advances*, **7**(7): 3838-3851. https://doi.org/10.1039/C6RA25859J

San Juan-Maldonado, N., Rodrí, S., Angel-Cuapio, A., Vá, N., and Barranco-Florido, J. E. 2024. Secondary metabolites of entomopathogenic fungi, biological alternative for the control of agricultural pests and disease: present and perspectives. *Adv Enzyme Res*, **12**(1): 1-15. https://doi.org/10.4236/aer.2024.121001

Sarkar, I., Ghorui, M., Chowdhury, S., Burla, S. 2024. The role of entomopathogenic fungal metabolites in pest management. Entomopathogenic Fungi: Prospects and Challenges, **26**: 381-408. https://doi.org/10.1007/978-981-97-5991-0_15

Singh, A., Kumar, S., Yadav, M. 2024. Tailored midgut gene expression in *Spodoptera litura* (Lepidoptera: Noctuidae) feeding on *Zea mays* indicates a tug of war. *Arthropod-Plant Interact*, **18**: 547–567. https://doi.org/10.1007/s11829-024-10048-7

Sowmya, R., Namasivayam, S. K. R., Sowmya, D. and Sivasuriyan, K. S. 2024. Pesticidal activity of extra cellular metabolites extracted from the green muscardian fungi *Metarhizium anisopliae*. *Environ Qual Manag*, **34**(1): 22235. https://doi.org/10.1002/tqem.22235

Thakur, A., Singh, V., Kaur, A. and Kaur, S. 2013. Insecticidal potential of an endophytic fungus, *Cladosporium uredinicola*, against *Spodoptera litura*. *Phytoparasitica*, **41**(4): 373-382. https://doi.org/10.1007/s12600-013-0298-9

Vengateswari, G., Arunthirumeni, M., Shivakumar, M. S. 2020. Effect of food plants on *Spodoptera litura* (Lepidoptera: Noctuidae) larvae immune and antioxidant properties in response to *Bacillus thuringiensis* infection. *Toxicol Rep*, **8**(7): 1428-1437. https://doi.org/10.1016/j.toxrep.2020.10.005 PMid: 33134089 PMCid: PMC7585150.

Vivekanandhan, P., Kavitha, T., Karthi, S., Senthil-Nathan, S., Shivakumar, M. S. 2018. Toxicity of *Beauveria bassiana*-28 mycelial extracts on larvae of *Culex quinquefasciatus* mosquito (Diptera: Culicidae). *Int J Environ Public Health*, **15**(3): 440. https://doi.org/10.3390/ijerph15030440 PMid: 29510502 PMCid: PMC5876985.

AUTHOR CONTRIBUTION

GR: Designed the study, analysed and interpreted the data. Wrote the original draft of the manuscript. SR: Validated the data, reviewed and edited the manuscript. Both authors have read and agreed to the published version of the manuscript.