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ENTOMOPATHOGENIC FUNGI: REVOLUTIONARY ALLIES IN SUSTAINABLE PEST MANAGEMENT AND THE OVERCOMING OF CHALLENGES IN LARGE-SCALE FIELD APPLICATIONS

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ABSTRACT Entomopathogenic fungi (EPF) act as promising eco-friendly biological alternatives to traditional chemical pesticides because they deliver several beneficial features for sustainable pest control. The infection mechanisms that EPF use to target specific insect pests through adhesion, enzymatic degradation and immune system evasion have made them desirable for Integrated Pest Management (IPM) systems. The adoption of EPF remains restricted at large scale because of their slow operation speed paired with formulation complexity. EPF stability increased as well as their efficacy did through recent advances in genetic engineering and microencapsulation and biopolymer-based formulations which makes them functional under diverse environmental conditions. Future research needs to continue because it will address both environmental and economic obstacles and improve formulation methods while evaluating the program's sustained effects. The solution of environmental and economic challenges will enable EPF to match chemical pesticides while supporting sustainable agriculture and improving worldwide food security

Keywords: Fungi; Sustainable; Integrated; Pest; Biocontrol

INTRODUCTION Entomopathogenic fungi have gained traction among scientists as pesticide alternatives because they offer IPM as well as ecological and economic advantages for future pest control. Multiple problems have emerged because of excessive chemical pesticide use which causes pesticide resistance and environmental pollution and harms non-targeted species. EPF including Beauveria and Metarhizium species represent promising biopesticides because their biological mechanisms of spore retention and penetration and poison release eliminate insect target species (Gutiérrez Cárdenas et al., 2024). The fungi provide environmental benefits because they affect only target organisms but lead to biodiversity preservation at the same time. The endophytic behavior of EPF enables them to reside inside plant structures where they supply various sustainable advantages including accelerated plant development and increased uptake of nutrients alongside improved stress tolerance (El-Husseini, 2006). The inclusion of EPF into Integrated Pest Management strategies proves beneficial because they work well together with biopesticides and semiochemicals as well as biotechnology-based approaches to build pest management systems with decreased dependence on chemical pesticides (Priya et al.). The full utilization of EPF is limited by

their slower speed than chemical pesticides alongside the requirement for better colonization-formulation methods. EPF development and research must advance to overcome current hurdles if this method needs to establish itself fully in agricultural use. The beneficial characteristics of EPF regarding ecological compatibility together with reduced chemical residues establish their importance as main tools in sustainable agricultural practices which maintain long-term agricultural health and ecosystem health. EPF provide sustainable pest control alternatives along with essential roles in creating global sustainable pest management methods (El-Husseini, 2006).

Mechanisms of Action of Entomopathogenic Fungi

The infection mechanisms of Entomopathogenic fungi (EPF) including Metarhizium anisopliae and Beauveria bassiana make these microorganisms successful biocontrol agents because they possess unique infection mechanisms and avoid host immune systems while demonstrating host specificity (Karthi et al., 2024) as discussed in Figure 1. The first step of fungal infection occurs through fungal spore attachment to insect cuticles by means of adhesive proteins and exopolysaccharide mucilage according to (Shang et al., 2024) as presented in Figure 1. Following attachment the EPF release proteases together with chitinases

and lipases that target the insect cuticle to penetrate into the hemocoel which becomes their site of multiplication to eventually result in host death (Sarfaraz et al., 2023). The fungal enzymatic activity combines with secondary metabolites and toxic substances to inhibit host immune responses including impaired hemocyte functions and triggered cell death (Vilcinskas and Götz, 1999). Blastospores from the fungi lack immunogenic sugars which enhances their ability to evade the host immune system for nutrient acquisition while shown in Figure 1 (Butt et al., 2016). EPF achieve host specificity by identifying specific host surface molecules and adapting their fungal strains to environmental conditions in order to target particular pest species effectively yet avoid unwanted effects on non-target organisms shown in Fig 1 (Vivekanandhan et al., 2024). Scientific analysis displays M. anisopliae along with B. bassiana succeed in controlling pests such as Aedes aegypti and Drosophila species as per findings in (Moreira et al., 2024). The eco-friendly qualities of EPF derive from their ecological emergence points and their minimal hazard against non-target organisms and environmental impact makes them an environmentally-friendly alternative to chemical pesticides (Kidanu and Hagos, 2020). Ongoing research development will expand Field Pesticide's use in pest management while enhancing their virulence and environmental adaptability thus making them an appealing solution against conventional chemical pest control (Adegbola et al., 2024).

Current Use of Entomopathogenic Fungi in Pest Management

The market is increasingly accepting entomopathogenic fungi (EPF) as sustainable biocontrol agents for pest management by offering different application methods and integration within Integrated Pest Management frameworks. Three basic EPF application techniques consist of spraying along with seed treatments and adding them to the soil. Spraying provides a direct application of fungal spores to crops for effective pest control of surface-dwelling issues. The protection for growing plants starts from seed treatments that add fungal spores on seeds and soil incorporation brings fungi into the ground for pest management in soil (Sharma et al., 2023). The successful implementation of Metarhizium anisopliae and Beauveria bassiana in Kenyan agricultural sectors presented a real-life example of how both fungi controlled aphids in horticultural crops which spread across 132,980 hectares in 2019. The fungi operate by combining attacks to insect cuticles followed by tissue penetration which produces toxic metabolites that wipe out the host insects because of their wide pest-eliminating effectiveness (Mantzoukas et al., 2022). EPF align well with IPM systems since they affect few non-target organisms and enable use alongside other biological agents such as beneficial arthropods and entomopathogenic bacteria which enhances their performance in protected agricultural environments (Smagghe et al., 2023). EPF effectiveness faces two main barriers which include environmental influences and the necessity for enhanced formulation specifics. The technological advancement of biotechnology through genetic engineering and nanotechnology enables researchers to improve both virulence and stress resistance factors in EPF for better field-based performance (Mishra, 2023). EPF integration into IPM systems proves to be an environmentally beneficial approach to sustainable pest management that cuts down chemical pesticide usage in agricultural sectors (Shukla et al., 2023).

Challenges in Large-Scale Field Application

Entomopathogenic fungi (EPF) face three major obstacles when used at an industrial scale due to environmental elements together with stability issues and economic prerequisites. The survival of EPF is negatively impacted by environmental conditions that include UV radiation along with temperature and humidity because these factors lead to declining conidial numbers and inactive microorganisms as well as decreased virulence and infectivity potential (Quesada-Moraga et al., 2024). UV radiation together with high temperatures functions as destructive agents that cause fungal conidia to become pathogenically ineffective against pests such as Spodoptera littoralis (Mohamed et al., 2024). The combination of microencapsulation with biopolymers developed as a modern formulation approach offers improved UV protection and thermal defense of EPF which leads to better field performance (de Jesus Seabra et al., 2024). The selection of fungal strains with environmental adaptability in particular climatic areas remains crucial because it enables effective practice adoption and strain performance (Quesada-Moraga et al., 2024). The viability of EPF throughout storage and application represents a critical issue because stability of fungi affects their operational success. The use of oil-based and inverted emulsions serves to improve EPF infectivity alongside enhancing their storage durability (Mohamed et al., 2024). EPF prove cost-effective for long-term use because of sustainability and target-specific delivery mechanisms although designing and developing EPF initially involves high initial costs (Sharma et al., 2023). The research for economic improvement focuses on two main areas: process optimization and the development of economical application methods for farmers (Kidanu and Hagos, 2020). EPF integration into modern pest management systems with chemical pesticides enables pest managers to use their respective advantages (Adegbola et al., 2024).

Innovations and Advancements in Overcoming Challenges

Multiple innovative techniques help improve EPF usage at large scales in pest control by solving concerns about fungal stability and operational effectiveness and monetary feasibility. The biotechnology technique which utilizes CRISPR-Cas9 plays a significant role in boosting the aggressiveness and longevity of EPF. Scientific researchers use CRISPR-Cas9 technology to disable Beauveria bassiana genes that result in higher oosporein production and improved fungal virulence through host immune system evasion according to (Mascarin et al., 2024a). The genetic modification of Beauveria bassiana through endochitinase overexpression generated faster pathogen-induced mortalities and lowered lethal dose requirements simultaneously without harmful pleiotropic consequences (Mascarin et al., 2024b). Consumer interests have been attracted by formulation improvements which include microencapsulation technology. These sodium alginate/maltodextrin microparticles formed

through spray-drying encase B. bassiana conidia to enhance pathogenicity and viability while protecting them from thermal stress making them viable for field-based application (de Jesus Seabra et al., 2024, Murtaza et al., 2024, Li et al., 2024). The use of chitosan and starch-based biopolymers improves spore survival while delivering prolonged effectiveness yet they present obstacles noted in Table 1 (Friuli et al., 2023). Researchers are using bioreactors combined with microbial

additives to enhance the production process and application of EPF as part of sustainable ecological pest reduction efforts (Joshi et al., 2020) as shown in Table 1. These new inventions resolve biological and economic obstacles of EPF while creating solid groundwork for their adoption into integrated pest management systems which reduce chemical pesticide usage (Sharma et al., 2023, Murtaza et al., 2024, Li et al., 2024).

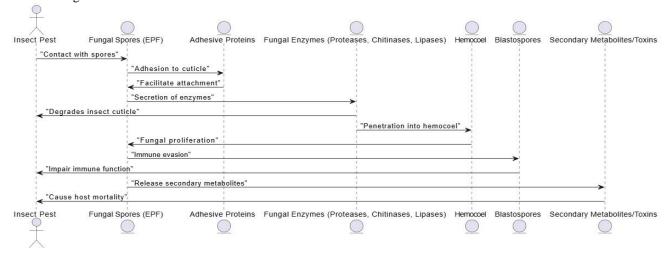


Figure 1: Mechanism of Action of Entomopathogenic Fungi (EPF) in Insect Hosts

Table 1: Innovations and Advancements in Overcoming Challenges in the Application of Entomopathogenic Fungi (EPF) for Pest Control

Innovation/Advancement	Description	Benefits	Challenges	Examples
Genetic Engineering (CRISPR-Cas9)	Using genetic tools like CRISPR- Cas9 to enhance virulence and resilience of EPF by modifying specific genes.	- Increased fungal virulence - Enhanced host immune evasion - Faster mortality rates in pests	- Genetic modifications may lead to unintended effects - Regulatory hurdles	- Beauveria bassiana engineered for enhanced production of oosporein, improving virulence (Mascarin et al., 2024).
Microencapsulation	Encapsulating EPF conidia in biopolymer microparticles, such as sodium alginate/maltodextrin, to improve stability and viability under stress.	- Improved viability under stress - Enhanced spore persistence in field conditions	- Production costs of microparticles - Stability in long-term storage	- Beauveria bassiana conidia encapsulated in biopolymers for thermal stress protection (Seabra et al., 2024).
Biopolymer-based Formulations	Using biodegradable polymers (chitosan, starch) to improve EPF formulation for better spore delivery and persistence in the field.	Better delivery of spores - Enhanced spore protection from environmental stress	- High production costs - Stability issues in extreme conditions	- Chitosan-starch formulations for <i>B. bassiana</i> to improve fungal viability and persistence (Friuli et al., 2023).
Bioreactors for Large- Scale Production	Utilizing bioreactors to scale up EPF production more efficiently for field applications.	- Increased fungal production - Reduced time for production - Scalable for large-scale use	- Bioreactor maintenance costs - Scaling challenges for consistency in fungal quality	- Use of bioreactors in EPF production to optimize fungal output and consistency in the field (Voloşciuc & Batco, 2024).
Microbial Additives	Incorporating microbial additives (such as other biocontrol agents) to enhance the efficacy of EPF by supporting their growth or action against pests.	- Synergistic effects in pest control - Enhanced fungal activity against a broad spectrum of pests	- Compatibility issues between different microbial species - Cost of additives	- Combination of <i>B. bassiana</i> with entomopathogenic bacteria for enhanced pest control (Sharma et al., 2023).
Formulation Optimization for Storage	Developing specialized formulations to optimize the storage and shelf-life of EPF products, ensuring their efficacy remains high during storage and application.	- Longer shelf-life - Improved field application efficacy	- Balancing formulation for cost-effectiveness and stability - Potential loss of virulence over time	- Oil-based and inverted emulsions for improving the infectivity and storage stability of <i>Metarhizium</i> (Mohamed et al., 2024).

CONCLUSION

Multiple innovative techniques help improve EPF usage at large scales in pest control by solving concerns about fungal stability

and operational effectiveness and monetary feasibility. The biotechnology technique which utilizes CRISPR-Cas9 plays a significant role in boosting the aggressiveness and longevity of EPF. Scientific researchers use CRISPR-Cas9 technology to disable Beauveria bassiana genes that result in higher oosporein production and improved fungal virulence through host immune system evasion. The genetic modification of Beauveria bassiana through endochitinase overexpression generated faster pathogen-induced mortalities and lowered lethal dose requirements simultaneously without harmful pleiotropic consequences. Researchers have found advancements through microencapsulation technology to improve beyond genetic modification methods. These sodium alginate/maltodextrin microparticles formed through spray-drying encase B. bassiana conidia to enhance pathogenicity and viability while protecting them from thermal stress making them viable for field-based application. These innovative approaches resolve biological together with economic hurdles of EPF thus establishing a basis for broader usage in integrated pest management tools instead of chemical pesticides.

REFERENCES

- Adegbola, R., Adegbola, A. & Sanni, L. 2024. Entomopathogenic Fungi As Myco-Biocontrol Agent Of Stored Grain Insect Pests. Nigerian Journal of Agriculture and Agricultural Technology, 4, 59-73.
- Butt, T., Coates, C., Dubovskiy, I. & Ratcliffe, N. 2016. Entomopathogenic fungi: new insights into host–pathogen interactions. Advances in genetics, 94, 307-364.
- De Jesus Seabra, M. G., Santos, T. S., De Souza Varize, C., Souto, E. B., Severino, P. & Da Costa Mendonça, M. 2024. New Beauveria bassiana aerial conidia-based bioinsecticide obtained by spray-dried microencapsulation of the entomopathogenic fungi in biopolymers for crop protection. Egyptian Journal of Biological Pest Control, 34, 58.
- El-Husseini, M. M. 2006. Microbial Control of Insect Pests: is it an effective and environmentally safe alternative. Arab Journal of Plant Protection, 24, 162-169.
- Friuli, M., Pellegrino, R., Lamanna, L., Nitti, P., Madaghiele, M. & Demitri, C. 2023. Materials engineering to help pest control: A narrative overview of biopolymer-based entomopathogenic fungi formulations. Journal of Fungi, 9, 918.
- Gutiérrez Cárdenas, O. G., Cortez Madrigal, H. & Garzón, A. 2024. Use and impact of endophytic entomopathogenic fungi: Their potential in the context of agricultural sustainability. Biotecnia, 26.
- Joshi, M. J., Prithiv Raj, V., Solanki, C. B. & Birari Vaishali, V. 2020. Role of biotechnology in insect-pests management. Agric. Food e-Newsletter, 2, 574-576.
- Karthi, S., Vasantha-Srinivasan, P., Senthil-Nathan, S., Han, Y.
 S., Shivakumar, M. S., Murali-Baskaran, R. K.,
 Kalaivani, K., Radhakrishnan, N., Park, K. B. &
 Malafaia, G. 2024. Entomopathogenic Fungi Promising
 Biocontrol agents for managing lepidopteran pests:

- Review of current knowledge. Biocatalysis and Agricultural Biotechnology, 103146.
- Kidanu, S. & Hagos, L. 2020. Research and application of entomopathogenic fungi as pest management option: a review. Journal of Environment and Earth Science, 10, 31-39.
- Li, X., Yang, X., Yang, L. E., Muhu-Din Ahmed, H. G., Yao, C., Yang, J., Wang, L., Yang, T., Pu, X. & Zeng, Y. 2024. Evolution and association analysis of SSIIIa in rice landraces of Yunnan Province. Biologia, 1-9.
- Mantzoukas, S., Kitsiou, F., Natsiopoulos, D. & Eliopoulos, P. A. 2022. Entomopathogenic fungi: interactions and applications. Encyclopedia, 2, 646-656.
- Mascarin, G. M., Shrestha, S., De Carvalho Barros Cortes, M. V., Ramirez, J. L., Dunlap, C. A. & Coleman, J. J. 2024a. CRISPR-Cas9-mediated enhancement of Beauveria bassiana virulence with overproduction of oosporein. Fungal Biology and Biotechnology, 11, 21.
- Mascarin, G. M., Shrestha, S., Dunlap, C. A., Ramirez, J. L. & Coleman, J. J. 2024b. Enhanced virulence through genetic engineering of Beauveria bassiana blastospores by overexpression of a cuticle-degrading endochitinase.
- Mishra, M. 2023. The Potential Application of Entomopathogenic Fungi (EF) in Insect Pest Management. Microbial Biocontrol: Molecular Perspective in Plant Disease Management. Springer.
- Mohamed, H. F., Sweilem, M. A.-E., El-Sharkawy, R. M. & El-Khawaga, O. E. A. A. 2024. Impact of Temperature and Ultra-Violet Radiation on the effectiveness of Beauveria bassiana inverted emulsion formulation on Spodoptera littoralis. Benha Journal of Applied Sciences, 9, 21-27.
- Moreira, H. V. S., Bitencourt, R. D. O. B., Da Rocha, I. U., Ribeiro, M. L., Bittencourt, V. R. E. P., Gôlo, P. S. & Da Costa Angelo, I. 2024. Entomopathogenic fungi against the dengue vector Aedes aegypti: laboratory and semifield assessment. CONTRIBUCIONES A LAS CIENCIAS SOCIALES, 17, e7559-e7559.
- Murtaza, G., Usman, M., Ahmed, Z., Hyder, S., Alwahibi, M. S., Rizwana, H., Iqbal, J., Ali, B., Iqbal, R. & Ahmad, S. 2024. Improving wheat physio-biochemical attributes in ciprofloxacin-polluted saline soil using nZVI-modified biochar. Ecotoxicology and Environmental Safety, 286, 117202.
- Priya, V. A., Suganthy, M., Sowmiya, A., Preetha, G., Janaki, P., Parameswari, E. & Krishnan, R. Green guardians: Harnessing biopesticides for sustainable vegetable pest management. Plant Science Today.
- Quesada-Moraga, E., González-Mas, N., Yousef-Yousef, M., Garrido-Jurado, I. & Fernández-Bravo, M. 2024. Key role of environmental competence in successful use of entomopathogenic fungi in microbial pest control. Journal of Pest Science, 97, 1-15.
- Sarfaraz, S., Abdul Qayyum, M., Alam, S., Ullah, B., Sharif, U., Ullah, S., Faiq, M. & Pasha, A. N. 2023. Biological management of Bactrocera zonata through an effective delivery system of mycoproteins of Beauveria bassiana

- integrated with synthetic attractant baits. MYCOPATH, 21
- Shang, J., Hong, S. & Wang, C. 2024. Fights on the surface prior to fungal invasion of insects. Plos Pathogens, 20, e1011994.
- Sharma, A., Sharma, S. & Yadav, P. K. 2023. Entomopathogenic fungi and their relevance in sustainable agriculture: A review. Cogent Food & Agriculture, 9, 2180857.
- Shukla, P., Bankar, D. R., Kumar, A., Aman, A. S., Mishra, P. K., Raghuvanshi, H. R. & Gayithri, M. 2023. Advancements in the use of entomopathogenic microbes for pest and disease management-A review. International Journal of Environment and Climate Change, 13, 945-953
- Smagghe, F., Spooner-Hart, R., Chen, Z.-H. & Donovan-Mak, M. 2023. Biological control of arthropod pests in protected cropping by employing entomopathogens: Efficiency, production and safety. Biological Control, 105337.
- Vilcinskas, A. & Götz, P. 1999. Parasitic fungi and their interactions with the insect immune system. Advances in parasitology, 43, 267-313.
- Vivekanandhan, P., Swathy, K., Sarayut, P. & Patcharin, K. 2024. Classification, biology and entomopathogenic fungi-based management and their mode of action against Drosophila species (Diptera: Drosophilidae): A review. Frontiers in Microbiology, 15, 1443651.