International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 15-06-2022, Accepted: 30-06-2022, Published: 17-07-2022
Volume 7, Issue 7, 2022, Page No. 29-32

Potential of Metarhizium as a microbial bio-control agent with an emphasis on its application and utility: A mini-review

Bhabesh Deka[1], Azariah Babu[1*], Archana Borah[2]
[1] Department of Entomology, North Bengal Regional Research and Development Centre, Nagrakata, West Bengal, India
[2] Department of Zoology, B Borooah College, Guwahati, Assam, India

Abstract
Metarhizium is an entomopathogenic fungus that is a significant biocontrol agent for agricultural crops. Initially, the *Metarhizium* species were identified mostly on the basis of conidial morphological traits. However, genomic profiling has made it possible to classify this fungus appropriately. This genus has 30 species, with a few species having a varied host range. To be an effective biocontrol agent, this fungus must penetrate the protein-chitin-rich insect cuticle and nourish host tissues via the secretion of a large number of degradative enzymes. *Metarhizium* has been used as a microbial biocontrol agent against insect pests that devastate economically significant agricultural crops, as well as for the control of human disease vectors. By identifying important genes that differentiate closely related species, genomic studies can contribute in the successful commercialization of biopesticides. Human and other animal biosafety research use cryo and intragastric experiments in mammalian models. *Metarhizium* offers considerable potential as a non-toxic, eco-friendly alternative to chemical pesticides in integrated pest control of crop pests.

Keywords: fungi, biological control, entomopathogen

Introduction
Metarhizium anisopliae sensu lato *(*s.l.) Sorokn *(=M. anisopliae)*, popularly known as "green muscardine fungus", is a Sordariomycetes fungus of the order Hypocreales that has revealed potential efficiency against a broad range of insect pests in various crops [1] *(*Fig 1). Metarhizium can be anamorphic *(*no synnemata) or teleomorphic *(*identical to Metarcordyceps) and each truncate or elongate branch contains one or more phialides. Conidia can be yellow, green, or brown [2]. This genus, *Metarhizium* comprises the most-studied entomopathogenic fungi at the molecular and biochemical levels.

Habitat associations
Metarhizium is considered as a large and diversified genus that includes more than 30 species with a few that have a diverse host range and habitats. Temperature has a variable effect on the growth and survival of *Metarhizium* spores and hyphae. The germination and proliferation of *Metarhizium* was found to be optimum at temperature ranges 10 and 40°C and beyond which results in thermal death at 50°C [3]. Even though, research investigations found no correlation between heat and cold tolerance and latitude, as cold-active isolates were discovered in northern locations, but none below 43.5° latitude, while certain tropical isolates could flourish at temperatures over 35°C. *Metarhizium* community composition is linked to both forest and agricultural ecosystems and their abundance is connected to plant diversity [4].

Fig 1: *Metarhizium anisopliae*

Mechanism of infection

When fungal hyphae penetrate an insect's or mite's cuticle, they induce the synthesis of phenoloxidases and the activation of hemocytes, which create bioactive chemicals that induce phagocytosis, encapsulation, or nodulation. Pathogen Recognition Receptors (PRRs), β-glucan-binding proteins, and peptidoglycans interact with fungal PAMPs such as mannans and fungal β-glucans to activate defensive responses [5]. When hyphae enter the host, they are replaced by yeast-like blastospores. The fungus continues to consume nutrients as blastospores multiply in the hemocoel of the host. *Metarhizium* produces acid trehalase, which hydrolyzes the primary sugar present in insect hemolymph [6]. Some *Metarhizium* strains produce secondary metabolites (destruxins) in addition to primary metabolites, which aid pathogenesis and cause flaccid paralysis by modifying the cellular structure of the middle intestine and malpighian tubules, as well as muscular tissues blocking H+ channels [7]. Destruxin A may have the ability to work as immune modulators, lowering insect immunological responses, but it is not sufficient to kill the insect. As immunomodulators, Destruxins may be able to reduce the host insect's immune response to their presence [8]. This process continues until the insect is entirely coated in mycelia, which occurs as the cellular mass grows. Conidia and structures that protrude from the insect's corpse are formed when the fungus consumes the insect's internal contents [5].

Biological microbial control agent development

Since 1960, many mycoacaricides and mycoinsecticides have been developed. However, *B. brongniartii* and *Isaria fumosorosea* are active components in 171 distinct formulations [9]. For easy handling, application, and enhanced effectiveness of mycoinsecticides, live entomopathogenic fungal propagules are combined with an inert component, agent, or adjuvant [10]. Aqueous suspensions, humectant powders, soluble powders, water-soluble and dispersible granules, and other formulations are also available for use. Fungi propagules are affected by the application method, type of formulation, and environmental conditions. Both laboratory and field research have demonstrated *M. anisopliae's* utility and potential in controlling arthropods responsible for the transmission of diseases like malaria and dengue fever [11]. It was reported to be effective against spider mites attacking tea plants, as well as termites, ticks, and cockroac1hes [12, 13, 14].

Behavior in the environment

The potential of biological microbial control agents relies on the ability of beneficial fungi to spread from infected hosts to healthy ones through horizontal transmission. Conidia survivability may increase the efficiency during field application. However, its reduction leads to a reduced rate of survival of fungi in the environment [15]. Conidia persistence and migration are influenced by factors such as the type of soil, plants, animals, and tillage, which also affect the fungal prevalence and persistence [16].

Strain improvement

The effectiveness of *Metarhizium* strains as biocontrol agents has been influenced by both biotic and abiotic factors. *Metarhizium* could be genetically modified to have the desired persistence or virulence characteristics. The pathogenicity of *M. anisopliae* was improved by the enhanced production of insect cuticle-degrading protease (Pr1A). According to St. Leger *et al.* [17], the transgenic strain significantly lowered the target insect's survival time (LT50). When produced in hemolymph, the scorpion toxin AaIT boosted fungal pathogenicity 22-fold against *Manduca sexta* and 9-fold against adult *Aedes aegypti* [18]. Wang *et al.* [19] highlighted that the esterase gene (MestI) from *Metarhizium robertsii* was successfully transferred to *Metarhizium acridum*, a group of fungal isolates that are known to be virulent and specific to grasshoppers (Acrididea). *Metarhizium* recombinant strains are designed to kill malarial parasites in mosquitos [20]. Changes in culture or growth conditions could make *Metarhizium* spp. pathogenic. *M. robertsii* demonstrated higher virulence to mealworm beetle *Tenebrio molitor* in the presence of anoxia or nutritional constraint [21].

Bio-safety of *Metarhizium spp.*

The bio-safety of the entomopathogenic fungus *M. anisopliae* has been evaluated and accomplished that, it was not harmful to vertebrates or the environment [15]. Hence, the application of chemical insecticides for the management of pests and diseases in agroecosystems is harmful to the environment and human health, it is critical to evaluate the impacts of biopesticides before recommending them for field application [22]. Pesticide registration has been made compulsory by several governments and international organizations, and a series of bio-safety evaluations and data generation for the WHO bio-insecticide registration proposal have been conducted since 1981, which includes bio-efficacy, residue analysis, product analysis, effect on non-target organisms and environement and toxicity etc. [23]. For bio-pesticide registration, toxicological information, eco-toxicological information, and stability studies have become mandatory nowadays. Hence, progressive research is needed in these aspects as we would be using living micro-organisms as biological control agents, their impact on non-target species, and human health must all be considered.

Conclusions

In this mini-review, we have focused our attention, on highlighting the role of *Metarhizium*, as a potential entomopathogen, which has been proved by several authors. This microorganism could be formulated and made available for its incorporation as an effective alternative to chemical pesticides, for the management of crop pests, owing to its higher effectiveness against target pests, low environmental impact, and high mammalian safety. The application of the formulation of *Metarhizium*, as a potential biological control agent is still in its early stage in many countries, which demands the necessity to have many *Metarhizium* strains for their potential use in various agroecosystems as a microbial biocontrol agent.

Conflict of Interest

The authors declare that they do not have competing interests.

Funding

This study was performed as a part of in-house project of the center. It was not financially funded by any external funding agency.

Acknowledgements

The authors are thankful to the Director, Tea Research Association, Tocklai Tea Research Institute, Jorhat, Assam, India and the Principal, B. Borooah College, Guwahati, Assam, India for proving all necessary facilities and support for the successful completion of study.

References:

1. Anitha S, Mahendran P, Selvakumar S, Janarthanan P, Raghunath M, Megala R, Vinitha Ebziba C, Vidya SL, Sagadevan P. Bio-efficacy of entomopathogenic fungus *Metarhizium anisopliae(*METSCH) against the tea mosquito bug, *Helopeltis theivora(*Waterhouse) and the red spider mite, *Oligonychus coffeae(*Nietner) infecting tea in south India. International Journal of Entomology Research,2019:4(3):49-54.
2. Kepler RM, Humber RA, Bischoff JF, Rehner SA. Clarification of generic and species boundaries for *Metarhizium* and related fungi through multigene phylogenetics. Mycologia,2014:106(4):811-829. doi:10.3852/13-319
3. Bidochka MJ, Kamp AM, Lavendar MT, Dekoning J, De Croos JNA. Habitat association in two genetic groups of the insect-pathogenic fungus *Metarhizium anisopliae*: Uncovering cryptic Species? Applied and Environmental Microbiology,2001:67(3):1335-1342. doi:10.1128/AEM.67.3.1335
4. Masoudi A, Koprowski JL, Bhattarai UR, Wang D. Elevational distibution and mor- phological attributes of the entomopathogenic fungi from forests of the Qinling Mountains in China. Applied Microbiology and Biotechnology,2018:102(3):1483-1499.
5. Butt TM, Coates CJ, Dubovskiy IM, Ratcliffe NA. Entomopathogenic fungi: new insights into host-pathogen interactions. Advances in Genetics,2016:94:307-364. doi:10.1016/bs. adgen.2016.01.006
6. Schrank A, Vainstein MH. *Metarhizium anisopliae* enzymes and toxins. Toxicon. 2010; 56*(*7), 1267-1274. doi: 10.1016/j.toxicon,2010:03(008)
7. Dumas C, Robert P, Pais M, Vey A, Quiot JM. Insecticidal and cytotoxic effects of natural and hemisynthetic destruxins. Comparative Biochemistry Physiology,1994:108(2):195-2003. doi:10.1016/1367-8280(94)90031-0
8. Ríos-Moreno A, Garrido-Jurado I, Resquín-Romero G, Arroyo-Manzanares N, Arce L, Quesada-Moraga E. Destruxin A production by *Metarhizium brunneum strains* during transient endophytic colonisation of *Solanum tuberosum*. Biocontrol Science and Technology,2016:26(11):1574-1585. doi:10.1080/09583157.2016.1223274
9. Faria MRD, Wraight SP. Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. Biological Control. 2007; 43(3), 237-256. doi: 10.1016/j.biocontrol,2007:08:(001)
10. García de León S, Mier T. Visión general de la producción y aplicación de bioplaguicidas en méxico. Sociedades Rurales, Producción Y Medio Ambiente,2010:10(20):37-63.
11. Bukhari T, Takken W, Koenraadt CJM. Development of *Metarhizium anisopliae* and *Beauveria bassiana* formulations for control of malaria mosquito larvae. Parasites and Vectors,2011:4(23):1-14.
12. Alonso-Díaz MA, García L, Galindo-Velasco E, Lezama-Gutierrez R, Angel-Sahagún CA, Rodríguez-Vivas RI, Fragoso-Sánchez H. Evaluation of *Metarhizium anisopliae(*Hyphomycetes) for the control of *Boophilus microplus(*Acari: Ixodidae) on naturally infested cattle in the Mexican tropics. Veterinary Parasitology,2007: 147(3-4):336-340. doi: 10.1016/j.vetpar. 2007.03.030
13. Deka B, Babu A, Peter AJ, Kumhar KC, Sarkar S, Rajbongshi H, Dey P, Amalraj ELD and Talluri VR. Potential of the entomopathogenic fungus, *Metarhizium anisopliae s.l.* in controlling live-wood eating termite, *Microtermes obesi (*Holmgren) *(*Blattodea: Termitidae) infesting tea crop. Egyptian Journal of Biological Pest Control,2021:31:132. https://doi.org/10.1186/s41938-021-00477-4.
14. Deka B, Babu A, Pandey AK, Kumhar KC, Rajbongshi H, Dey P, Peter AJ, Amalraj ELD, Talluri VR. Potential of the entomopathogenic fungus, *Metarhizium anisopliae s.l.* for control of red spider mite, *Oligonychus coffeae* Nietner on tea crop. International Journal of Acarology,2022:48(2):121-129, DOI: 10.1080/01647954.2022.2041089.
15. Zimmermann G. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Science and Technology,2007:17(9):879-920. doi:10.1080/09583150701593963

16. Lacey LA, Grzywacz D, Shapiro-Ilan DI, Frutos R, Brownbridge M, Goettel MS. Insect pathogens as biological control agents: Back to the future. Journal of Invertebrate Pathology,2015:132:1-41. doi: 10.1016/j.jip.2015.07.009
17. St. Leger, R, Joshi L, Bidochka MJ, Roberts DW. Construction of an improved mycoinsecticide over expressing a toxic protease. Proceedings of the National Academy of Sciences of the United States of America, 1996:93(13):6349-6354. doi:10.1073/pnas.93.13.6349
18. Wang C, St. Leger RJ. _bookmark10A scorpion neurotoxin increases the potency of a fungal insecticide. Nature Biotechnology,2007:25(12):1455-1456. doi:10.1038/nbt1357
19. Wang S, Fang W, Wang C, St. Leger RJ. Insertion of an esterase gene into a specific locust pathogen *(Metarhizium acridum*) enables it to infect caterpillars. PLoS Pathogens, 2011, 7(6) doi: 10.1371/journal.ppat.1002097
20. Fang W, Vega-Rodríguez J, Ghosh AK, Jacobs-Lorena M, Kang A, St. Leger RJ. Development of transgenic fungi that kill human malaria parasites in mosquitoes. Science,2011:331(6020):1074-1077. doi:10.1126/science.1199115
21. Oliveira AS, Rangel DE. Transient anoxia during *Metarhizium robertsii* growth increases conidial virulence to *Tenebrio molitor*. Journal of Invertebrate Pathology,2018:153:130-133.
22. Toriello C. Bioseguridad de *Metarhizium anisopliae (*Metschnikoff) Sorokin *(*Hyphomycete). Vedalia,2003: 9:107-113.
23. Siegel JP. Testing the pathogenicity and infectivity of entomopathogens to mammals. In L. Lacey *(*Ed.), Manual of Techniques in insect pathology,1997,325-336. San Diego, CA: Academic Press.