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Review Article

Assessing the Safety and Efficiency of Entomopathogenic Fungi as Bioinsecticides: A Brief Review

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Abstract

To meet the rising food demands of an expanding global population, there has been a surge in the use of synthetic insecticides to control pests and enhance crop yield and production. Nonetheless, we cannot overlook the adverse effects of these synthetic insecticides on humans, livestock, and the environment. This review focuses on presenting information on specific species of entomopathogenic fungi that hold potential for development into bioinsecticides, offering an alternative to synthetic insecticides. The assessment of these fungi is based on their effectiveness in managing targeted pests and their safety concerning humans, non-targeted organisms, and the environment. We have compiled data and information on these fungi from various research sources like Google Scholar, NCBI, Science Direct, and ResearchGate for comparative analysis. Six entomopathogenic fungi, Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii, Nomuraea rileyi, Paecilomyces fumosoroseus formerly known as [Isaria fumosorosea], and Hirsutella thompsonii, are identified as promising candidates for pest control due to their ability to produce toxins or metabolites with insecticidal properties. B. bassiana, M. anisopliae, V. lecanii, and N. rileyi, out of the mentioned six species, are proven to be safe for humans, non-targeted organisms, and the environment. Moreover, all these fungi can be produced on a large scale, ensuring their availability for use as biocontrol agents. However, additional research is needed to fully understand the potential environmental impacts and the harmful metabolites they may produce.

Keywords: Entomopathogenic, Bioinsecticides, B. Bassiana, M. Anisopliae, V. Lecanni, N. Rileyi, P. Fumosoroseus, H. Thompsonii

1. Introduction

Insecticides play a crucial role in exterminating pests that inflict damage on cultivated crops. Although they are proficient at pest control and crop protection, the extensive usage of synthetic insecticides has raised concerns due to their impact on non-target organisms, encompassing animals, livestock, and humans [1, 2]. The delivery methods of insecticides, including sprays, dusts, and gels, further intensify the risk to these non-target organisms [3]. As evidenced in a study by, insecticides were implicated in 34% of the 11087 pesticide poisoning cases recorded, signifying that pesticide poisoning has evolved into a prominent public health issue in Malaysia. Additionally, insecticide resistance presents another significant challenge associated with insecticide usage [4]. In the past, inefficient management of insecticide resistance has led to dire outcomes such as agricultural economic setbacks, a resurgence of insect-transmitted pathogens, and ecological damage due to escalated dosages or use of more toxic insecticides [5]. In light of these issues, biopesticides emerge as a safer alternative, potentially diminishing the reliance on synthetic insecticides [6].

Biopesticides based on fungi have shown effectiveness in pest control without posing a risk to humans or the environment. Being living organisms, fungi can adapt to the immune responses of their targeted pests and co-evolve with them, thus decreasing the chance of these pests developing resistance [7]. Fungi's ability to reproduce rapidly in substantial quantities [8] makes them particularly suitable for biopesticide development. Despite these benefits, biopesticides currently constitute a mere 5% of the global crop protection market and have yet to achieve widespread usage equivalent to synthetic insecticides [8, 9]. Therefore, identifying varieties of entomopathogenic fungi to boost their acceptance as substitutes for conventional pesticides is vital. Accordingly, this review concentrates on evaluating various entomopathogenic fungi as potential candidates for biopesticides.

1.1. Assessing Harmlessness to Non-Target Organisms and Environmental Impact

The fundamental requirement for a bioinsecticide is that it should match the effectiveness of a chemical pesticide while ensuring it does not pose risks to humans, non-targeted organisms, and the environment. In our review, we've concentrated on six specific species: Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii, Metarhizium [Nomuraea] rileyi, Paecilomyces fumosoroseus, and Hirsutella thompsonii. Table 1 outlines the safety profiles of each of these fungal species.

Table 1: Safeness of different entomopathogenic fungi having bioinsecticide potential

Fungal species	Safeness	References
Beauveria bassiana	Safe to vertebrates, non-targeted organisms, and environment; Can cause allergic reaction	(Zimmermann, 2007a)
Metarhizium anisopliae	Safe with minimal risk to vertebrates, humans and environment; can cause allergic reaction	(Zimmermann, 2007b)
Verticillium lecanii	Safe to mammals, environment, plants and non-targeted organisms	(European Food Safety Authority, 2010)
Nomuraea rileyi	Safe to avian species, beneficial invertebrates, mammals and environmentally friendly	(Ignoffo et al., 1976; Rai et al., 2014)
Paecilomyces fumosoroseus	Safe to non-targeted organisms, mammals, vertebrates and environment	(Zimmermann, 2008)
Hirsutella thompsonii	Safe to vertebrates and mammals. Effect on the environment and non-targeted organisms is not known	(McCoy and Heimpel, 1980)

The safety of B. bassiana for non-target species is substantiated by a study which showed 100% survival of Gallus domesticus [chickens] after being fed with these fungi. This finding, along with no observed behavioural changes, suggests no fungal germination within the chicken's digestive system [10]. Similarly, when rats were exposed to B. bassiana through injection, inhalation, and feeding, the fungi didn't proliferate within the host system, and the rats didn't exhibit signs of infection. Even when injected intratracheally, intraperitoneally, or orally, rats exhibited no infection signs and survived [11]. Nevertheless, there have been reports of allergic reactions in workers continually exposed to high concentrations of airborne B. bassiana spores. However, it remains unclear whether these reactions were caused by the B. bassiana spores themselves or were attributable to other airborne microorganisms, proteins, or polysaccharide antigens [11]. Furthermore, up until now, no study has indicated that this fungus can induce pathological conditions within lung tissues.

Metarhizium anisopliae has been reported to not be toxic or have any harmful effects on animals. A number of experiments carried out on rats, white mice, and guinea pigs involving injection, inhalation, and feeding demonstrated that the animals did not exhibit toxic or pathogenic reactions. None of the animals perished, and there were no changes in their body weight, behaviour post-experiment, or any tissue abnormalities [11]. However, there have been six reported incidents in Brazil of humans exposed to airborne spore suspensions of M. anisopliae experiencing dermal hyperallergic reactions and asthmatic symptoms [11]. Despite this solitary case, the US Environmental Protection Agency [EPA] conducted environmental risk assessments and determined that M. anisopliae does not have detrimental effects on birds, mammals, terrestrial and aquatic plant species [12]. Furthermore, the EPA has categorised M. anisopliae as not posing any threats to humans through ingestion, inhalation, or direct contact with the fungus.

Verticillium lecanii, now known as Lecanicillium lecanii, is classified as non-toxic, non-pathogenic, and non-infective, as per the European Food Safety Authority, based on results from acute toxicity tests conducted on rats. This fungus has been reported to be non-toxic, non-infective, and non-pathogenic to birds, aquatic organisms, bees, and earthworms. Moreover, there have been no recorded instances of L. lecanii colonizing plants. Therefore, due to its narrow "natural" host range, L. lecanii is considered to be safe for non-target organisms [13].

Reports suggest that Nomuraea rileyi can't proliferate when the host temperature surpasses 35°C and it gets easily neu-

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tralized by the acidity of human gastric juice [14]. Have highlighted that the host specificity and environmental friendliness of N. rileyi promote its application in the management of insect pests [15].

Paecilomyces fumosoroseus is reported to be non-hazardous and non-pathogenic towards amphibians, birds, and mammals, given the host infectivity is 1x106 colony-forming units [CFU]/animal [16]. A fungus only demonstrates toxicity or pathogenicity when the infectivity exceeds 1x108 CFU/animal. Nonetheless, acute dermatitis has been observed in rats inhaling spores of P. fumosoroseus with an infectivity rate higher than 1x109 conidia/animal. Despite this, P. fumosoroseus has been approved as biocontrol agents for greenhouses and interior scapes. Thus, further investigation is warranted to verify the potential risk prior to its usage as a bioinsecticide.

An array of tests was conducted on H. thompsonii, which included acute oral toxicity, eye irritation, primary skin irritation, acute dermal toxicity, and acute inhalation toxicity. These tests involved subjects such as rats, albino rabbits, and guinea pigs [17]. The results suggested that the whole culture broth, mycelia, and conidia of H. thompsonii are not toxic or pathogenic to mammals, irrespective of whether they are administered individually or in combination. The dosages used in the experiments exceeded the normal environmental exposure levels for humans. However, there is a shortage of information concerning the impact of H. thompsonii on the environment, and related studies are scarce. This deficit is the primary reason H. thompsonii is considered less viable for use as a biocontrol agent. To commercialize it for agricultural use, knowledge about its safety with respect to the environment [18], humans, and non-targeted organisms is essential. Upon considering the potential adverse effects of the chosen fungi on humans and other non-target organisms, B. bassiana, M. anisopliae, V. lecanii, and N. rileyi can be deemed safe for development as biopesticides. However, in the case of P. fumosoroseus and H. thompsonii, more data regarding their potential risks and their safety towards the environment is needed.

1.2. Effectiveness Against Target Pests

Table 2 provides a comprehensive overview of the efficacy of selected entomopathogenic fungi against specific target pests. While some fungi target similar insect species, others have distinct preferences. The mechanisms employed by these fungi to eliminate targeted insects involve inducing diseases or releasing toxins and metabolites. Key enzymes such as proteases, chitinases, and lipases are commonly found in entomopathogenic fungi, facilitating the penetration of the insect's cuticle and enabling the subsequent stages of infection. These stages include neutralizing the host's defense mechanisms, allowing the fungus to proliferate within the host insect, and eventually entering the saprophytic stage, where the fungus grows outward from the insect's body and produces conidia on the exoskeleton [11].

Beauveria bassiana, a well-known entomopathogenic fungus, produces a diverse array of toxins and metabolites, each with unique properties. One of its prominent toxins is beauvericin, which exhibits insecticidal, antibiotic, cytotoxic, and ionophoric effects. In laboratory experiments, it was observed that exposing lepidopteran Spodoptera to 1µM of beauvericin led to a 10% decrease in viable insect cells [11].

Another toxin produced by B. bassiana is bassiacridin, which was found to cause 50% mortality in nymphs of Locusta migratoria when exposed to a concentration of 3.3µg toxin per unit of body weight. Oxalic acid is another essential component produced by B. bassiana, and it serves to solubilize specific cuticular proteins in insects, including the fungus itself. Interestingly, there exists a positive synergistic relationship between oxalic acid and the growth of B. bassiana conidia [11]. Furthermore, B. bassiana also synthesizes bassianolide, a compound with ionophoric and antibiotic properties [11]. Bassianolide demonstrated oral toxicity to larvae of Bombyx mori and caused atonic reactions upon exposure, as reported by [19]. These findings highlight the diverse and potent effects of the various toxins and metabolites produced by B. bassiana, showcasing its potential as a powerful tool for insect pest management.

Lecanicillium lecanii, another entomopathogenic fungus, is known to produce several biologically active compounds, including bassianolide and dipicolinic acid, as well as two previously unreported compounds with molecular structures C25H38O3 and C25H38O4, all possessing insecticidal properties. In laboratory settings, L. lecanii has demonstrated a remarkable capacity for dipicolinic acid production, yielding as much as 320mg/L in vitro. Dipicolinic acid has been found to target blowflies, specifically Calliphora erythocephala, as reported by [19]. Apart from dipicolinic acid, L. lecanii synthesizes bassianolide, a compound with insecticidal and ionophoric properties, known for its oral toxicity to larvae of certain insect species, including Bombyx mori. Additionally, [19] revealed the existence of two novel compounds with molecular structures C25H38O3 and C25H38O4, possessing insecticidal properties. These compounds represent exciting discoveries that could have potential implications in pest management and warrant further investigation. The diverse range of secondary metabolites produced by L. lecanii showcases its ability to effectively target and control various insect pests, making it a promising candidate for biocontrol strategies in agriculture and beyond.

Metarhizium anisopliae, another entomopathogenic fungus, has been found to produce a potent insecticidal compound known as destruxin [20]. When destruxin is applied through direct spraying on the insect Empoasca vitis or its surrounding habitat, it leads to the formation of pores in cellular membranes. This action acts as mitochondrial ATPase inhibitors, resulting in morphological changes in the insect and ultimately leading to its death. In addition to its effect on Empoasca vitis, destruxin has also been reported to reduce feeding in the larvae of Plutella xylostella and Phaedon co-

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chlearia, further demonstrating its efficacy as an insecticidal agent. When examined on cell lines of the silkworm Bombyx mori, destruxins were found to induce cytopathological effects, including cell contraction, granulation, and ultimately causing the cells to stop dividing [20]. Another metabolite identified in Metarhizium anisopliae is anisoplin [21]. Anisoplin functions by cleaving the sarcin/ricin loop of ribosomal RNA, which interferes with protein production in insect cells, further contributing to the fungus's insecticidal properties.

These findings underscore the diverse range of insecticidal compounds produced by Metarhizium anisopliae, making it a promising candidate for biological pest control and potentially offering sustainable alternatives to conventional chemical insecticides.

Table 2: Effectiveness of entomopathogenic fungi against targeted insect pests

Fungal species	Targeted pest	Disease	Toxins and metabolites	References
Beauveria bassiana	Aphids, thrips, whiteflies, mealybugs, caterpillars and beetles	white muscardine disease that leads to death	Proteases, chitinases, lipases, beauvericin, bassianin, bassianolide, beauverolides, tenellin, oxalic acid and Bassiacridin	(Moshman et al., 2018; Zimmermann, 2007a)
Metarhizium anisopliae	Orthoptera, Dermaptera, Hemiptera, Diptera, Hymenoptera, Lepidoptera and Coleoptera	green muscardine disease that leads to death	proteases, chitinases, lipases, destruxins, cytochalasins C, cytochalasins D, anisoplin and swainsonine	(Aw and Hue, 2017; Olombrada et al., 2016; Zimmermann, 2007b
Lecanicillium lecanii (V. lecanii)	greenhouse aphids, whiteflies, and thrips	high virulence level and epizootic efficiency that kills insects	C25H38O3, C25H38O4, bassianolide and dipicolinic acid	(Claydon and Grove, 1982; Goettel and Glare, 2010; Gillespie and Claydon, 1989; Hasan et al., 2011)
Metarhizium (Nomuraea) rileyi	Insect species of Lepidoptera and Coleoptera	epizootic death	phenylacetic acid, 1-Phenylbuten-2,3-diol, cycle (Pro-Val), cycle (Pro-Leu) and cycle (Pro-Phe)	(Ignoffo et al., 1979; Marcinkevicius et al., 2017; Sandhu et al., 2012)
Paecilomyces fumosoroseus	Whiteflies and nematodes	yellow muscardine disease and pathogenesis	Beauvericin, pyridine-2,6-dicarboxylic acid, beauverolides and dipicolinic acid	(Iannacone and Gomez, 2008; Sandhu et al., 2012; Zimmermann, 2008)
Hirsutella thompsonii	Aphis craccivora and mites	larvae death and delay in pupa stage	HtA and α-sarcin	(Maina et al., 2018; Reddy et al., 2020)

Nomueaea rileyi, a species of fungus, has been found to produce a series of metabolites with potent insecticidal properties against various pests, including Spodoptera frugiperda Smith [Lepidoptera], Ceratitis capitata Wiedemann [Diptera], and Tribolium castaneum Herbst [Coleoptera] [22]. The identified metabolites are phenylacetic acid, 1-Phenylbuten-2,3-diol, cycle [Pro-Val], cycle [Pro-Leu], and cycle [Pro-Phe]. All five of these metabolites have demonstrated antifeedant properties, effectively reducing the feeding activity of the targeted insects. Among them, cycle [Pro-Val] exhibited the highest antifeedant activity, recording a remarkable Feeding Election Index [FEI] value of 86.02, followed closely by cycle [Pro-Phe] with a FEI of 73.47. Furthermore, all five metabolites were found to act as repellents against Tribolium castaneum. Phenylacetic acid showed the most potent repellent effect, with a Repellency Index [RI] of 42%, followed by cycle [Pro-Val] with an RI of 41%. These findings highlight the significant insecticidal potential of the metabolites produced by Nomueaea rileyi, particularly in their ability to deter feeding and repel pests. The discovery of these natural compounds opens up exciting possibilities for the development of eco-friendly pest control strategies in agriculture and pest management applications.

Paecilomyces fumosoroseus, like B. bassiana and V. lecanii mentioned earlier, produces the insecticidal compounds

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beauvericin and dipicolinic acid. These compounds exhibit similar insecticidal properties and effects in terms of their ability to target and control pests. However, compared to B. bassiana and V. lecanii, there is limited available information on other toxins or metabolites produced by P. fumosoroseus [16].

Due to the scarcity of information on additional metabolites, it is believed that the pathogenicity of P. fumosoroseus may be even higher than that of B. bassiana and V. lecanii. The presence of unidentified compounds, in addition to beauvericin and dipicolinic acid, could potentially enhance the fungus's effectiveness as an insecticidal agent. While the full extent of P. fumosoroseus insecticidal arsenal remains to be fully elucidated, its known production of beauvericin and dipicolinic acid already positions it as a promising candidate for pest management strategies. Further research into its metabolites and toxins may uncover additional potent compounds, paving the way for more targeted and efficient biocontrol approaches in the future.

Hirsutella thompsonii is known to produce two highly toxic ribotoxins, HtA and α-sarcin, both of which demonstrate strong insecticidal properties against Galleria mellonella larvae. When injected into the larvae, HtA and α-sarcin caused their death and also led to a delay in pupation. However, HtA proved to be more effective than α-sarcin, as it required a lower quantity to achieve similar lethal effects [23].

In summary, each of the six fungi discussed in this context exhibits various metabolites and toxins with the potential to effectively target and eliminate specific insect pests. These bioactive compounds make them promising candidates for use as bioinsecticides in commercial applications, offering environmentally friendly alternatives to synthetic insecticides. With their diverse mechanisms of action and insecticidal properties, these fungi hold significant promise in sustainable pest management and have the potential to replace the reliance on harmful chemical pesticides [24-31].

2. Conclusion

In this mini review, we explored the potential of several fungal species, namely Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii, and Metarhizium [Nomuraea] rileyi, as bioinsecticides for controlling insect pests. These fungi possess the necessary criteria to be considered as viable options for bioinsecticide use. Notably, all five fungal species have been deemed safe for humans, non-target organisms, and the environment, making them environmentally friendly alternatives to chemical pesticides. One of the key strengths of these fungi lies in their ability to produce toxins and metabolites with strong insecticidal properties, enabling them to effectively combat specific target insect pests. Through their unique mechanisms of action, they can significantly contribute to pest management strategies in agriculture and other fields.

However, it is essential to highlight that two fungal species, P. fumosoroseus and H. thompsonii, currently have limited available information concerning their safety levels and potential risks. Further empirical studies are required to thoroughly assess their safety and efficacy as bioinsecticides. This review primarily focuses on evaluating the safety and effectiveness of the fungi discussed as potential bioinsecticides. Nevertheless, to solidify and refine these findings, additional in-depth research and empirical studies on the selected fungal species are imperative. Only through rigorous investigation and testing can we fully harness the potential of these fungi as safe and efficient tools in the battle against insect pests.

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