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Review Article

Safeness and effectiveness of entomopathogenic fungi for use as bioinsecticide: A mini review

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ABSTRACT: Usage of conventional synthetic insecticide to control insect pests has increased to improve crops yield and production to meet the global food demands of a growing population. However, it should not be neglected that synthetic insecticide causes negative impacts on humans, livestock as well as the environment. This review aims to provide data on entomopathogenic fungi species that can be developed into bioinsecticide to control insect pests, in order to reduce the usage of synthetic insecticide. The fungi are discussed based on two criteria, its effectiveness in controlling the targeted pests, and its safety level to humans, non-targeted organisms, and the environment. Relevant data and information on entomopathogenic fungi from various research tools including Google Scholar, NCBI, Science Direct and Researchgate were compiled into tables for comparison and analysed. Six entomopathogenic fungi namely *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii, Metarhizium (Nomuraea) rileyi, Paecilomyces fumosoroseus* and *Hirsutella thompsonii* are proposed as having the required criteria having potential to control targeted insects by means of producing various toxins or metabolites with insecticidal properties. Five out of the six species, *B. bassiana*, *M. anisopliae, V. lecanii* and *N. rileyi* are safe to humans, non-targeted organisms, and the environment. Furthermore, all these fungi can be mass produced to ensure their availability to be used as a biocontrol agents. However, future studies are required for further justification for harmful metabolites produced and their impact on environment.

KEY WORDS: Bioinsecticide, effectiveness, fungi, safety

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INTRODUCTION

Insecticides are used to kill pests that damages cultivated crops. Despite being effective in controlling pests and protecting crops, widespread use of synthetic insecticides became an issue because it affects non-targeted organism including animals, livestock, and humans (Aktar *et al*., 2009; Sarwar and Salman, 2015). Insecticides are delivered in different forms, for example sprays, dusts, and gel, which increases the risk to non-target organism (NPIC, 2019). In a reported study by Kamaruzaman *et al*. (2020), insecticides were responsible for 34% of the 11087-pesticide poisoning total cases and it was claimed that pesticide poisoning has become a common public health issue in Malaysia. Furthermore, insecticide resistance is another major problem of insecticide application. Failure to manage insecticide resistance in the past had led to consequences such as economic failure in agriculture, resurgence of insect-transmitted pathogens and damage to the ecosystem with increasing dosage or a more toxic insecticide used (Denholm and Devine, 2013). Biopesticides can be a safe alternative to reduce use of synthetic insecticides (Sharma *et al*., 2019).

Fungal-based biopesticide was found be effective in controlling pests without posing any threat to human or to the environment. Fungi as living organism can adapt to immune responses from the targeted pests and co-evolve with the target, preventing, the likelihood of the pest insect to develop resistance (Dara, 2017). Fungi can reproduce in large amounts within a relatively short period of time (McNeil, 2011). All these give fungi advantage to be developed into biopesticide. However, biopesticides only account for 5% shares of the global crop protection market and yet to gain the widespread usage on par with synthetic insecticides (Damalas and Koutroubas, 2018). Thus, it is important to identify types of entomopathogenic fungi to increase its acceptance to replace conventional pesticides. Hence, this review focuses on assessing different entomopathogenic fungus as potential biopesticide.

Safety level to non-targeted organism and the environment

Bioinsecticide must be as effective as chemical pesticide while at the same time it must be safe by means of not posing threats to humans, non-targeted organisms and to the environment. Based on the information obtained, six species namely *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii, Metarhizium (Nomuraea) rileyi, Paecilomyces fumosoroseus* and *Hirsutella thompsonii* were selected for further review. Safeety of each fungal species is summarized in Table 1.

Safeness of *B. bassiana* towards non-targeted species is supported by result on 100% survival on *Gallus domesticus* fed with the fungi, without any behavioural changes, which suggests there was no germination of fungi inside the digestive system of chicken (Haas-Costa *et al*., 2010). Rats as mammalian model when exposed to *B. bassiana via* injection, inhalation and feeding indicated that the fungi did not multiply inside the host system, and the rats showed no infection. Similarly, rats injected with the fungus in the intratracheal, intraperitoneal and peroral showed no sign of infection and survived (Zimmermann, 2007a). Despite such reports on the safety of *B. bassiana* on non-targeted organism including mammals, workers constantly exposed to high concentration of the *B. bassiana* spore in the air was reported to have allergic reactions. However, the report was inconclusive whether the causation agent due to spores of *B. bassiana* or it can be attributed to the presence of other microorganisms, protein, and polysaccharide antigens in air suspension (Zimmermann, 2007a). Furthermore, to date no study has shown that the fungus can cause pathological condition within the lung tissues.

Metarhizium anisopliae is reported to be neither toxic nor has any adverse effect in animals. Numerous experiments conducted on rats, white mice and guinea pigs through injection, inhalation and feeding showed that there were no toxic and pathogenic reactions, none of the experimental animals died, no increment nor decrement in animals' body weight, animals behaved normally post-experiment, and there were no abnormalities in the tissues (Zimmermann, 2007a). However, there were six reported cases of *M. anisopliae* causing dermal hyperallergic reaction and asthmatic symptoms in human exposed to environmental spores' suspension in the air in Brazil (Zimmermann, 2007a). Despite this sole reported case so far, the US Environmental Protection Agency (EPA) has performed environmental risk assessments and indicated that *M. anisopliae* do not have negative impacts on birds, mammals, terrestrial and aquatic plant species (US EPA, 2011). The EPA also listed *M. anisopliae* as posing no threat to human in terms of ingestion, inhalation, or direct contact with the fungus.

Verticillium lecanii is classified as non-toxic, non-pathogenic and noninfective according to the European Food Safety Authority (2010), based on results obtained from acute toxicity tests done on rats. Presently the fungus is named as *Lecanicillium lecanii* and is reported to be non-toxic, non-infective and non-pathogenic to birds, aquatic organisms, bees, and earthworms. No colonization of *L. lecanii* on plants were ever recorded. Thus, *L. lecanii* is considered safe to non-targeted organisms due to its narrow "natural" host range.

Metarhizium (Nomuraea) rileyi was reported to be unable grow when the host temperature exceeded 35°C and easily deactivated by the acidity of the human gastric juice (Ignoffo *et al*. 1979). Rai *et al*. (2014) reported that the host specificity of *N. rileyi* and its ecofriendly nature encourage its use in insect pest management.

Paecilomyces fumosoroseus was reported to be non-toxic and non-pathogenic towards amphibians, birds and mammals since the host infectivity was $1x10^6$ colony forming units (CFU)/animal (Zimmermann, 2008). A fungus exhibits toxicity or pathogenicity only when the infectivity exceeded

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)
Metarhizium (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)

1x10^8 CFU/animal. However, acute dermatitis developed in rats inhaling spores of *P. fumosoroseus* with infectivity >1x10^9 conidia/animal. Despite this, *P. fumosoroseus* was approved as biocontrol agents in greenhouses and interiorscapes. Therefore, further study is required to confirm the potential risk before using it as bioinsectiside.

Several tests including acute oral toxicity, eye irritation, primary skin irritation, acute dermal toxicity and acute inhalation toxicity in rats, albino rabbits and guinea pigs exposed to *H. thompsonii* were evaluated (McCoy and Heimpel, 1980). The tests results suggested that whole culture broth, mycelia and conidia of *H. thompsonii* is non-toxic and non-pathogenic to mammals, regardless of whether it is administered individually or given in combination. The dosage used in the experiments were higher than what is available in the normal environment where human live. However, there is lack of information regarding the effect of *H. thompsonii* on the environment and in fact studies regard to these matters could hardly be found. This is the main reason for classifying *H. thompsonii* as less considerable fungi to be used as a biocontrol agent. This is because to commercialize it into the market for use in the agriculture field, its safeness regarding the environment (Usta, 2013), human and non-targeted organisms are important to be known.

Based on the criteria of possible adverse effects of the selected fungi on human and other non-targeted organism, *B. bassiana*, *M. anisopliae, V. lecanii,* and *N. rileyi* can be regarded as safe to be developed as biopesticide. However, in the case of *P. fumosoroseus* and *H. thompsonii,* more information regarding its potential risk and its safety towards the environment is needed.

Effectiveness against target pests

Table 2 summarises the effectiveness of each of the selected entomopathogenic fungi on target pests. Some of them share similar insect target while others have an entirely different target. Each fungus can eliminate targeted insects by causing diseases or by releasing toxins and metabolites. Proteases, chitinases and lipases are enzymes common in entomopathogenic fungi that help fungus to penetrate cuticle of insects to carry out the remaining steps in the infection process, starting with destroying host defence mechanism, followed by fungus proliferation in host insect and lastly the saprophytic stage of fungus growing out from the insect's body and producing conidia on the exoskeleton body (Zimmermann, 2007a).

Beauveria bassiana expresses a variety of toxins and metabolites. Amongst them being beauvericin which has insecticidal, antibiotic, cytotoxic and ionophoric properties. 1µM of beauvericin was shown to kill lepidopteran *Spodoptera frugiperda* by decreasing the insects' viable cells by 10% (Zimmermann, 2007a). Bassiacridin caused 50% death in nymphs of *Locusta migratoria* when exposed to the toxin at 3.3µg toxin/body weight. Oxalic acid is known to solubilize specific cuticular proteins in insects including *B. bassiana* and there is a positive synergistic relationship between oxalic acid and the growth of conidia of *B. bassiana* (Zimmermann, 2007a). Bassianolide with ionophoric and antibiotic properties (Zimmermann, 2007a) was shown to induce oral toxicity to larvae of *Bombyx mori* and causes atonic reaction (Gillespie and Claydon, 1989).

Similarly, *Lecanicillium lecanii* produces bassianolide and another secondary metabolite, dipicolinic acid. The fungus was reported to produce a high yield (320mg/L) of dipicolinic acid *in vitro* which has been shown to target blowflies *Calliphora erythocephala* (Gillespie and Claydon, 1989). Aside from these two metabolites, Gillespie and Claydon (1989) identified two new unreported compounds with molecular structure $C_{25}H_{38}O_3$ and $C_{25}H_{38}O_4$ that also have insecticidal properties.

Metarhizium anisopliae was found to produce destruxin that has insecticidal properties (Zimmermann, 2007b). Spraying of destruxin directly on *Empoasca vitis* or the surrounding habitat was reported to kill the insect *via* formation of pores in cellular membranes, as mitochondrial ATPase inhibitors, leading to morphological changes. Destruxin was also reported to reduce feeding of larvae of *Plutella xylostella* and *Phaedon cochlearia.* Cytopathological effects of destruxins on cell lines of *Bombyx mori* include contraction, granulation and eventually stop dividing (Zimmermann, 2007b). Another metabolite identified in the fungus was anisoplin (Olombrada *et al*., 2016). Anisoplin cleaves the sarcin/ricin loop of ribosomal RNA and interferes with the protein production in insect cells.

In *Nomueaea rileyi* a series of metabolites with insecticidal properties against *Spodoptera frugiperda* Smith (Lepidoptera), *Ceratitis capitata* Wiedemann (Diptera) and *Tribolium castaneum* Herbst (Coleoptera) were reported to be phenylacetic acid, 1-Phenylbuten-2,3-diol, cycle (Pro-Val), cycle (Pro-Leu) and cycle (Pro-Phe) (Marcinkevicius *et al*., 2017). All five metabolites possess antifeedant property with maximum antifeedant activity recorded in cycle (Pro-Val), [Feeding election index (FEI) = 86.02] followed by cycle (Pro-Phe) (FEI = 73.47). In addition, all five metabolites act as repellent agent against *T. castaneum*, with phenylacetic acid showing greatest repellent effect [Repellency index (RI) = 42%) followed by cycle (Pro-Val) (RI = 41%).

Paecilomyces fumosoroseus can beauvericin and dipicolinic acid with similar insecticidal properties and effects as mentioned earlier in *B. bassiana* and *V. lecanii,*

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	$C_{25}H_{38}O_3$, $C_{25}H_{38}O_4$, 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 ɑsarcin	(Maina *et al*., 2018; Reddy *et al*., 2020)

respectively. Information available regarding other toxins or metabolites produced by *P. fumosoroseus* is limited (Zimmermann, 2008). Hence, it is believed the pathogenicity of *P. fumosoroseus* can be higher due to the presence of other metabolites besides beauvericin and dipicolinic acid.

Hirsutella thompsonii expresses ribotoxins HtA and ɑ-sarcin, both are highly toxic against *Galleria mellonella* larvae. Injection of HtA and ɑ-sarcin caused larvae death and delay in pupation. However, HtA was found to be more effective as the quantity of HtA needed to cause similar death effects is lower compared to ɑ-sarcin (Reddy *et al*., 2020).

In summary, all six fungi have various metabolites/ toxins to potentially kill targeted insect pests and can be commercially used as bioinsecticides to replace synthetic insecticides.

CONCLUSION

In this mini review, *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii* and *Metarhizium (Nomuraea) rileyi* were found to possess the required criteria for use as bioinsecticide in the control insect pests. Five of the fungal species were found safe to humans, non-target organisms, and the environment. Each of the fungal species produce toxins and metabolites with insecticidal properties that can effectively control target insect pests. The fungal species with the least potential are *P. fumosoroseus* and *H. thompsonii,* due to limited information available on its safely level and potential risk. Although this review allows for the identification of fungi as bioinsecticide, more empirical studies on the selected species need to be conducted to further test and refine the findings. This review is primarily concerned with the safeness and effectiveness of fungus.

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