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A review on the use of entomopathogenic fungi in the management of insect pests of field crops

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Abstract

Insect pest management has been dominated by the use of synthetic pesticides since its discovery. This has continued for decades until the publication of Rachel Carson's 'Silent spring' in 1962, which awoken the world on dangers pose by the synthetic chemicals. Since then, the search for alternative pest control products, which is safe and effective, has been prioritized. This review was aimed at bringing to the fore the entomopathogenic fungi (EPF) commercially available and the prospect of using them as an alternative to synthetic chemicals. It was reported that, more than 171 mycoinsecticides have been produced with at least 12 species from the over 800 fungi species identified as pathogenic to insects. Most of these products were developed based on *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosoroseus* propagules. They are currently available in countries of North and South America, Europe and Asia, with few in Africa and Middle East. Mycoinsecticides have been found effective in controlling insect pests of economic importance in agriculture; however, the successful marketing and utilization of these products have been rather slow, largely due to; high cost, low production efficiency, low performance under challenging environmental conditions and lack of awareness, however, mycoinsecticide is gradually becoming popular. Therefore, mycoinsecticides have the potentials to play a key role in integrated pest management (IPM) programme for effective and relatively safe insect pest management in field crops. To achieve this, vigorous research measures needs to be taken to improve on; their performance under challenging environmental conditions, the formulations that will increase persistence, longer shelf life and ease of application, pathogen virulence and spectrum of action.

Keywords: Entomopathogenic fungi, mycoinsecticide, insect, pest management, field crop, insecticide

1. Introduction

Entomopathogenic fungi (EPF) are fungal species that are pathogenic to insects. These fungal pathogens play a vital role in insect population dynamics making it the earliest insect pests control agents. Earliest farmers rely on the actions of predators, pathogens and host plant resistance for the control of insect pests until the discovery of insecticide. The first ground-breaking field trials with EPF started with a Russian microbiologist, Elie Metchnikoff in 1888, who later became a Nobel Prize winner and named *Metarhizium anisopliae* (more recently named *M. brunneum*) [1]. Metchnikoff mass produced fungal conidia on sterilized brewer's mash and combine cultures with sand granules for spreading on field crops. Though results were inconsistent, the work of Metchnikoff ignited curiosity around the world and led to programs in Europe and United States for experimentation with "friendly fungi" against insect pests [2]. Boverin, a *Beauveria bassiana*-based mycoinsecticide for the control of Colorado potato beetle and codling moth in the former USSR, was developed in 1965 [3]. The first formal and published proposal for microbial control came with John LeConte's suggested use of microsporidia to control grape phylloxera, *Daktulosphaira vitifoliae* (Fitch). The study presented visionary ideas for an effective and economic alternative method of pest control and paved the way for future scientists to study EPF.

Studies on EPF was quiet after the World War II when affordable synthetic chemical insecticides became available for insect pests control. Recent developments on EPF show that they can serve as an integral part of integrated pest management strategy. Many insect pathogenic fungi based bio-insecticides have been formulated and commercially manufactured [4]. The application of EPF in biological control is increasing largely because of greater environmental awareness, food safety concerns and the failure of conventional chemicals due to an increasing number of insecticide resistant species [5]. These 'ready to use' formulations are available in many developed and developing countries of Europe, Asian, Africa and the West. These microbial pesticides occupy around 1.3 per cent of the world's total pesticide

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market of which, 90 per cent of them are used as insecticides [6]. Several commercial formulations of EPF have been developed for crop pest management. Among the 171 products of EPF developed, products based on *B. bassiana* represent 33.9% of total products, *M. anisopliae* products were 33.9%, and *I. fumosorosea* and *B. brongniartii* products represented 5.8 and 4.1% respectively [7, 8]. In recent times, about 90 genera and almost above 700 species were considered as insect infecting fungi that represent about all the major classes of fungi [7, 6]. To date, over 750 fungi species are known to infect insects and mites [9].

Naturally, pathogenic microorganisms have been regulators of insect pest population. These organisms are being used as a component of integrated pest management strategies in developed and developing countries. For many years, microbial control has been used as a component of IPM enjoying some level of success in Asia and South America [10]. In Africa for example, there is a long history of projects, which have sought microbial solutions to pest problems. The development of 'Green Muscle' for the control of locust was the first practical application of mycoinsecticide in insect pest management in African. The first company to be granted a licence was Biological Control Products of South Africa, which launched the product in 1998. Since then, many South and East African countries have been using it to control locusts and other grasshoppers.

Agriculture today is facing the biggest challenge of providing enough food to meet the pressing demands of the fast growing population of the world which is expected to reach 10.12 billion by 2100 [11]. In order to meet this demand, efforts have been geared towards improving crop varieties for higher quantity and quality of yields, shorter durations of life cycle and resistance to insect pests and diseases. Insect pests and diseases are responsible for reduction of crop yields by 20 to 40% annually [12]. A situation that is unacceptable if the world's vision of fighting hunger is to be met in near future. Insect pests control in vegetables is largely by the use of synthetic chemicals, which in many cases are used indiscriminately. Mycoinsecticide, which is a fungal-based formulation, is already making an impact as a component of IPM in field crops around the world. This review therefore, is aimed at bringing to the fore the fungi pathogenic to insects, commercially available mycoinsecticides and the prospect of using EPF as an alternative to synthetic chemicals.

2. Classification of entomopathogenic fungi

Entomopathogenic fungi belong to 12 classes within six phyla of the kingdom fungi [13]. Fungi that are pathogenic to arthropods are found in the divisions Ascomycota, Zygomycota, and Deuteromycota [14], as well as Oomycota and Chytridiomycota [5]. Many of the entomopathogenic fungi known so far belong to either the class Entomophthorales in the Zygomycota or the class Hyphomycetes in the Deuteromycota.

3. Mode of infection

Most EPF are soil borne fungal species and all have a similar mode of infection. When the fungal conidia encounter the host, they attach themselves to the cuticle through hydrophobic mechanisms and germinate to form germ tubes in favourable conditions [15]. During this process, the fungus produces a number of specialized infection structures that include penetration pegs and/or appressoria, which enable the

growing hyphae to penetrate into the host integument [16]. The germ tube penetrates the cuticle aided by the action of other enzymes such as, metalloid proteases and amino peptidases [17]. Once inside the insect, the fungi develop as hyphal bodies that disseminate through the haemocoel and invade diverse muscle tissues, fatty bodies, Malpighian tubes, mitochondria and haemocytes, leading to death of the insect 3 to 14 days after infection [15, 18].

Entomopathogenic fungi from hypomycetes group are opportunistic pathogens and usually cause insect mortality by nutritional deficiency, destruction of tissues and by the release of toxins. Cuticle degrading enzymes of entomofungal pathogens like chitinase, protease and lipase play an important role in the pathogenicity of these organisms on insects in the breakdown of insect cuticle for penetration of fungal germ tube into the insect body. The entry of entomopathogenic fungi through the insect cuticle occur by a combination of mechanical pressure and enzymatic degradation. Several mycotoxins like, Beauvericin, Beauverolides, Bassianolide (by *B. bassiana*, *V. lecanii*, *Paecilomyces spp.*) and Destruxins A, B, C, D, E, F (by *M. anisopliae*) are produced during pathogenesis and these acts like poisons to the insects. After the death of the insects, the fungus breaks open the integument and forms aerial mycelia and sporulation on the cadavers [19]. The fungi of entomophthorales group are obligate pathogens of insects and cause host death by tissue colonisation with little or no use of toxins [20].

4. Fungi as insect pathogens

Recently there has been a renewed interest in fungal pathogens of insect because of their potentials as biocontrol agents. In that regard, several fungi have been screened for virulence against insect and many have been found pathogenic to insects. More than 750 species of fungi, mostly from hyphomycetes and entomophthorales are pathogenic to insects; many of them offer great potential for pest management [9]. From these fungi, more than 171 products have been developed around the world based on at least 12 species [8, 21]. Presented in table 1 are some strains of fungi pathogenic to insect pests of some crops. Among the species already use in formulated mycoinsecticides are; *Beauveria bassiana*, *B. brongniartii*, *Metarhizium anisopliae*, *Lecanicillium spp.*, (previously *Verticillium lecanii*), *Hirsutella thompsonii*, *Cladospirium oxysporium* and *Isaria fumosorosea* (previously *Paecilomyces fumosoroseus*) (Table 1).

Entomopathogenic fungi infect insects of almost all orders; most common are Hemiptera, Diptera, Coleoptera, Lepidoptera, Orthoptera and Hymenoptera [9]. In some insect orders, the nymphal and larval stages are more susceptible than the adult stages, while in others the reverse may be the case. Some fungi have restricted host ranges, e.g. *Aschersonia aleyrodis* infects only whiteflies and *Nomuraea rileyi* infects only lepidopteran larvae, while others like *B. bassiana* and *M. anisopliae* infect more than 700 species in several insect orders. However, most of the pathogenicity tested are against aphids, whiteflies, thrips, and few against lepidopteran and coleopteran pests. Although fungi pathogenic to scarab beetle in sugarcane and corn borer in maize have been documented. It is therefore evident, that many more of entomopathogenic fungi will be discovered for use in the future.

Table 1: Fungi pathogenic to insect pests of agricultural crops

Fungus	Target pest	Crop	Author (s)
<i>Beauveria bassiana</i> PDRL1187,	Mustard Ahpid, <i>Lipaphis erysimi</i> , <i>Aphis craccivora</i> Koch	Canola (<i>Brassica napus</i> L.)	[22]
<i>Beauveria bassiana</i> BB-01	<i>Schizaphis graminum</i> , <i>Rhopalosiphum padi</i> , <i>Brevicoryne brassicae</i> and <i>Lipaphis erysimi</i>	Laboratory	[23]
<i>Beauveria bassiana</i>	Whiteflies	Melon	[24]
<i>Beauveria bassiana</i>	<i>Myzus persicae</i>	Cabbage	[25]
<i>Verticillium lecanii</i> V17, PDRL922	Cabbage aphid, Mustard Ahpid (<i>Myzus persicae</i> , <i>Lipaphis erysimi</i>)	Cabbage, Canola (<i>Brassica napus</i> L.)	[22, 26]
<i>Verticillium lecanii</i>	<i>Myzus persicae</i> , <i>Aphis craccivora</i> Koch	Chili	[27]
<i>Paecilomyces fumosoroseus</i> n32,	Mustard aphids, Diamondback moth <i>Lipaphis erysimi</i> , <i>Plutella xylostella</i>	Cabbage, Canola (<i>Brassica napus</i> L.)	[22, 26, 28]
<i>Metarhizium anisopliae</i> L6, M440, PDRL711, PDRL526	Cabbage aphid, Mustard Ahpids, <i>Lipaphis erysimi</i> , <i>Aphis gossypii</i> , <i>Aphis craccivora</i> Koch	Cabbage, Canola (<i>Brassica napus</i> L.)	[22, 26]
<i>Paecilomyces lilacinus</i> PDRL812	Mustard Ahpids, <i>Lipaphis erysimi</i>	Canola (<i>Brassica napus</i> L.)	[22]
<i>Hirsutella thompsonii</i>	<i>Aphis craccivora</i> Koch	Cowpea	[29]
<i>Cladosporium oxysporium</i>	<i>Aphis craccivora</i> Koch	Cowpea	[29]

5. Mycoinsecticides

The use of microbial control agents particularly entomopathogenic fungi, have been investigated for the control of a wide range of orchard and field crop pests [30, 31, 32]. Many have been formulated into products that are commercially available for use in controlling insects of economic importance. Current commercial entomopathogenic products in the markets being used to control a number of insects worldwide include, "Cryptogram™" and Bb plus®, *Metarhizium* 50®, Biogreen® and Green Guard®, BIO 1020® and Green Muscle® [17]. These mycoinsecticides are used to control a number of insect pests in glasshouse and field crops. A list of commercially available mycoinsecticides, their target pests and crop are presented in the Table 2.

The use of mycoinsecticides in pest management particularly in vegetable production has been reported in many countries

around the world [33]. Although, several species of entomopathogenic fungi are effective against many lepidopteran pest in a laboratory studies, their field efficacy has not been proven as their success largely depends on weather conditions (temperature and humidity). Twenty-eight products (all based on *Hirsutella thompsonii*) were exclusively developed as acaricides. South American companies and institutions developed approximately 43 per cent of all products [34]. The most common types of formulations were technical concentrates in the form of fungus-colonized substrates (26.3%), wettable powders (20.5%), and oil dispersions (15.2%). The remaining types include granules (2.9%), technical materials (2.9%), baits (1.8%), water dispersible granules (1.8%), oil miscible flowable concentrates (1.2%), ULV suspensions (0.6%), suspension concentrates (0.6%), and contact powders (0.6%).

Table 2: Mycoinsecticides use for the control of arthropod pests of agricultural crops

Name	Fungus	Target pest	Crop
Mycotrol/botani gard	<i>Beauveria bassiana</i>	Whiteflies/aphids/thrips	Glasshouse tomato/ornamentals
Naturalis	<i>B. bassiana</i>	Sucking insects	Cotton
BioBlast	<i>Metarhizium anisopliae</i>	Termites	Houses
Bio Magic	<i>M. anisopliae</i>	BPH	Rice
PFR-97	<i>Isaria fumosoroseus</i>	Whiteflies/thrips	Glasshouse crops
Vertalec	<i>Lecanicillium longisporum</i>	Aphids	Glasshouse crops
Mycotal	<i>Lecanicillium muscarium</i>	Whiteflies/thrips	Glasshouse crops
Green Muscle	<i>M. anisopliae</i>	locusts	Natural bushland
Betal	<i>B. bassiana</i>	Scarab beetle larvae	Sugarcane
Engerlingsplz	<i>Beauveria brongniartii</i>	Scarab beetle larvae	Pasture
Beauveria Schweizer	<i>B. brongniartii</i>	Scarab beetle larvae	Pasture
Ostrinol	<i>B. bassiana</i>	Corn borer	Maize
BioGreen	<i>M. flavoviride</i>	Red-headed cockchafer	Pasture/turf
Mycohit	<i>Hirsutella thompsonii</i>	Mites	Citrus

Source: [35, 8, 9, 13]

Mycoinsecticide form part of biopesticides produced and marketed for pest management. Although biopesticide represent only 3% of the global crop protection business, its growth is at the rate of 10% per year [36]. This growth is an indication that biopesticides will soon play an important role in insect pest management. Of the total global biopesticide market, mycoinsecticide is the second (27%) to *Bacillus thuringiensis* products [37]. Most of these mycoinsecticides are produce in America, Europe and Asia. At least 30 mycoinsecticides were registered in China; among them, *Beauveria bassiana* is the most popular species with up to 14 products for the control of locust, pine moth and diamond back moth [21]. *Metarhizium anisopliae* and *Paecilomyces lilacinus* with eight and seven products respectably registered

for application on grubs, corn borer, aphids and whiteflies. Some commercially available mycoinsecticides, their target pest(s), producer and country of production are presented in table 3. Most of the countries where mycoinsecticides are produced are in South and North American and European. India and South Africa are probably the leading producers of mycoinsecticides in Asia and Africa, respectively. However, mycoinsecticide products are available for use in pest management in many countries of Asia and Africa. Therefore, it is important to note that, many countries of Asia and Africa will soon join several countries of Europe and America in the use of mycoinsecticide as a promising alternative to the synthetic chemicals.

Table 3: Commercially available mycoinsecticides with their target pest, producer and country of production

Fungus	Brand name	Target pest	Producer	Country
<i>Beauveria bassiana</i>	Mycotrol WP	Whiteflies/Aphids/Thrips	Emerald BioAgriculture Corp. (Previously Mycotech Corp.)	USA
	Myco-Jaal	Diamonback moth	Pest Control India (Pvt) Ltd.	India
	Conidia	Coffee berry borer	AgrEvo	Germany
	Naturalis L	Whiteflies/thrips/aphids/white grub	Troy Biosciences Inc.	USA
<i>Metarhizium flavoviride</i>	Biogreen	Scarab larvae	Bio-Care Technology	Australia
<i>Metarhizium anisopliae</i>	Bioblast	Termites	Ecoscience	USA
	Metaquino	Spittle bugs		Brazil
	DeepGreen	White grub	Live System Technology S.A.	Colombia
<i>Isaria fumosoroseus</i>	PFR-97	Whitefly	Eco-tek	USA
	Pae-Sin	Whitely	Agrobionsa	Mexico
<i>Metarhizium anisopliae</i> var. <i>acidum</i>	Green Muscle	Locust, Grasshoppers	Biological Control Products SA (Pty) Ltd (under licence from CABI,UK)	South Africa
	Green Muscle	Locusts	-	China
<i>Lagenidium giganteum</i>	Laginex	Mosquitoes	Agra Quest	USA
<i>Baeuvaria brongiartii</i>	Betel	Scarab beetle larvae	NPP(calioppe)	France
<i>Nomuraea rileyi</i>	Numoraea 50	Lepidoptera	Ago Biocontrol	Colombia
<i>Hirsutella thompsonii</i>	Mycohit	Acari	Plantrich Chemicals & Biofertilizer Ltd	India
<i>Conidiobolus thromboides</i>	Vektor 25SL	Aphids/Thrips/Whiteflies	Mycolab	South Africa
<i>Lecanicillium longisporum</i>	Vertalec	Aphids	Kopper Biological System.	Netherlands
<i>L. muscarium</i>	Mycotal	Whiteflies/Thrips	Kopper Biological System.	Netherlands
<i>B. bassiana</i> + <i>M. anisopliae</i> + <i>I.fumosoroseus</i>	Tri-Sin	Psyllid	Agrobiologicos del Noroeste S.A. de C.V. (Agrobionsa)	Mexico

Source: [35, 38, 8, 39, 18]

6. Mycoinsecticides in Africa

Research and development into utilization of mycoinsecticides in pest management in Africa has been slow compared to many other countries in some parts of the world. Research into the potentials of entomopathogenic fungi as pest control agents started in the 19th century with work of Agostina Basi, which geared efforts towards identification, formulation and application of these fungi in pest management. The first attempt to control a pest with a fungal agent was carried out in Russia (former USSR) in 1888 with the fungus *Metarhizium anisopliae* (Metsch.) [8]. In 1965, Boverin, a *Baeuvaria bassiana*-based mycoinsecticide was developed in former USSR for control of the Colorado beetle and Codling moth. Since then, several mycoinsecticides have been developed and registered in many countries of the American continents, Europe, Asia and Australia. Later in Africa, a research programme code named LUBILOSA was launched in 1989 to develop mycoinsecticide for the control of locusts and other grasshoppers [40]. The product named 'Green Muscle' was formulated based on the propagules of *Metarhizium anisopliae* var. *acidum* and registered in South African by Biological Control Products SA (Pty) Ltd, under the licence of CABI, UK. It has also been registered in East and South African countries including Mozambique, Namibia, Tanzania, Sudan and Zambia for the control of locusts. Other mycoinsecticides use in South Africa include Bb Plus and Bb weevil based on *Baeuvaria bassiana* propagules for the control of aphids and weevils respectively. *M. anisopliae* (var. *acidum*) has been found effective against the brown locust, *Locustana pardalina* in Africa, *Locusta migratoria* in Madagascar and the Australian plague locust *Chortoicetes terminifera* and *L. migratoria* in Australia. With variable success, *M. flavoviride* has also been tested against the tree locust *Anacridium melanorhodon* in Sudan, the rice grasshopper *Hieroglyphus daganensis* in Benin, Mali and Senegal and the desert locust, *Schistocerca gregaria* in Mauritania [9]. Accordingly, Kenyan Standing Technical

Committee of Imports and Exports (KSTCIE) has approved mycoinsecticide products based on *B. bassiana* and *M. anisopliae* propagules for importation and use in Kenya [41]. However, as at 2010 in Kenya, Bio-power and Botanigard all based on *B. bassiana* GHA were also registered for use [37]. In Nigeria, for example, several synthetic pesticides have been registered for production and use but not a single mycoinsecticide has been registered for use.

7. Future prospect of using mycoinsecticide as an alternative to synthetic pesticide

Entomopathogenic fungi are naturally occurring microorganisms that play an important role in insect population dynamics in a natural ecosystem. Their activities play a significant role in the stability of insect population dynamics in natural ecosystem. The understanding of this role lead to the research for possible utilization of pathogenic fungi in insect pest management. The first ground-breaking field trials with EPF started with a Russian microbiologist, Elie Metchnikoff in 1888, who later became a Nobel Prize winner and named *M. anisopliae* (more recently named *M. brunneum*) [1]. Since then, researchers around the world have identified more than 750 fungi species that are pathogenic to insects [9] of different orders. From these fungi, more than 171 mycoinsecticides have been produced using propagules from at least 12 fungi species. These mycoinsecticides are used to control various insect pests of economic importance in different countries around the world.

Currently, the largest single microbial control program using fungi involves the use of *M. anisopliae* for control of spittlebugs (Cercopidae) in South American sugarcane and pastures [42]. The application of *B. bassiana* for the control of pine moth *Dendrolimus spp.* in China probably represents one of the largest uses of a biocontrol agent over one million hectares of pine forest [2]. *B. bassiana* strain Bb-147 is registered on maize in Europe for the control of the European corn borer, *Ostrinia nubilalis* and the Asiatic corn borer,

Ostrinia furnacalis. The strain GHA is registered in the US for the control of the whitefly, thrips, aphids and mealybugs and strain ATCC 74040 is registered against many soft-bodied insects of the orders Homoptera, Heteroptera and Coleoptera [9]. Although biopesticide represent only 3% of the global crop protection business, its growth is at the rate of 10% per year [36]. Of the total global biopesticide market, mycoinsecticide is second (27%) to *Bacillus thuringiensis* products [37]. This growth is an indication that mycoinsecticides will soon play an important role in insect pest management. [4] Stated that, 'use of insect pathogenic fungus is unavoidable as it is an integral part of integrated pest management programs in many ecological zones'. Although mycoinsecticides have their limitations, which lead to withdrawal of some products (Boverin from former USSR, Mycar, Taerain and CornGaurd from the USA, Bio 1020 from Germany, etc.) from the market, their potentials as biocontrol agents remain promising.

8. Benefits of using entomopathogenic fungi

- Their residues have no known adverse effects on the environment.
- Entomopathogenic fungi are little or non-toxic to non-target organisms.
- They have narrow area of toxic action, mostly specific to a single group or few species.
- They can be used in combination with synthetic chemical insecticides.
- They are self-perpetuating under ideal environmental conditions.
- Reduce chemical insecticide use.
- Protects biodiversity in managed ecosystem.
- Potential development of pest resistance to mycoinsecticide is less common or may develop more slowly due to unique mode of action.

9. Limitations of entomopathogenic fungi

- They need specific environmental conditions to germinate and cause infection.
- Can be very costly to produce for commercial use.
- They have short shelf life
- The pest must be present before the pathogen can be usefully applied thus making preventive treatment difficult.
- Lack of persistence and low rate of infection under challenging environmental conditions.
- Often slow acting and require high application rate and thorough spray coverage.

10. Conclusion

Since the establishment of the fact that fungi pathogenic to insects can be key components in the fight against insect pests in agriculture, several large scale researches have been undertaken by governments, institutions, organizations and individuals to explore their potentials. To date, a number of mycoinsecticides have been developed and are being used against many insect pests of economic importance in a number of countries. Nonetheless, more fungi, which are pathogenic to insects are still being discovered, a situation which presents brighter future for the use of entomopathogenic fungi in insect pest management. However, use of mycoinsecticides in pest management is generally moving at a slow pace even in the developed countries where production of mycoinsecticides began more than five decades ago. In spite of this, mycoinsecticides are gradually becoming

popular. With the 10%, annual increase in global biopesticide market [36], which mycoinsecticide accounts for 27% [37] of it, use of mycoinsecticides in insect pest management will soon increase dramatically. Nevertheless, it is still far behind synthetic chemicals in efficacy and popularity. While acknowledging limitations, one can still argue that, use of mycoinsecticides is likely to rise if research is focus on; improving its performance under challenging environmental conditions, formulations that will increase persistence, longer shelf life, ease of application, pathogen virulence and wider spectrum of action.

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