Journal of Environment and Earth Science
ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online)
Vol.10, No.3, 2020

Research and Application of Entomopathogenic Fungi as Pest Management Option: A Review

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Abstract
Entomopathogenic fungi are myco-biocontrol, potentially the most versatile biological control agents with a wide host range and are an environmentally sound and effective means of reducing insect-pests. 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 and are a widespread component of most terrestrial ecosystems. Entomopathogenic fungi are a major component of integrated pest management techniques as biological control agents against insect pests and other arthropods in horticulture, forestry and agriculture and are found in the divisions of Zygomycota, Ascomycota, Deuteromycota, Chytridiomycota and Oomycota, which were previously classified within fungi. Insect control using entomopathogenic fungi is achieved when sufficient infective propagules, conidia contact a susceptible host and conditions are suitable for a lethal mycosis to develop. A wide range of fungi occur in the soil environment and they have various ecological functions. Most of these fungi, along with a range of bacteria, can grow on artificial media *in vitro*. Several methods have been used to describe the variation within a species of entomopathogenic and mycoparasitic fungi including morphological characteristics of spores and colonies, extracellular protein profiles, pathogenecity, growth and nutrient requirements. Furthermore, immune taxonomic and chemotaxonomic methods have been used, though only with limited success. Taxonomic procedures are becoming more and more complex and it is generally accepted that some forms of molecular identification techniques are needed in addition to the traditional morphological characteristics formally used to classify fungal species. During the last four decades, over 80 companies worldwide have developed 171 mycoinsecticides and myco-acaricides. Use of mycoinsecticdes 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.
Keywords: Eco-friendliness, fungi**,** insect pest**,** myco-acaricides, mycoinsecticdes
DOI: 10.7176/JEES/10-3-03
Publication date: March 31st 2020

1. Introduction

The various risk associated with the continuous use of chemical insecticides mainly development of resistance, resurgence in insects, accumulation of pesticide residues in food chain, environmental pollution, health risks have led to development of alternative strategies of pest management. The necessity for sustainable crop production through eco-friendly pest management technique is being largely felt in recent times. Thus, the exploitation of bio-control agents is considered as a suitable alternative to the use of chemical pesticides (Dhaliwal and Koul, 2007). Among the various bio-control agents, entomopathogenic fungi are being a major component of an integrated approach that can provide significant and selective insect control. A group of fungi that kill an insect by attacking and infecting its insect host is called entomopathogenic fungi (Singkaravanit *et al*., 2010). Because of their wide host range they are potentially the most versatile biological control agents. These fungi comprise a diverse group of over 100 genera with approximately 750 species, reported from different insects.

Myco-biocontrol is an environmentally sound and effective means of reducing insect-pests with its effects through the use of natural enemies. It is the exploitation of fungi in biological processes to lesser the insect density with the aim to reduce crop damage by insect pests. The effectiveness of myco-biocontrol agents depend on the susceptibility of the insect and also virulence of the fungus. Virulence of the fungus depends on the selection of the stable strain with specific efficacy for the target hosts. All groups of insects may be affected and over 700 species of fungi have been recorded as pathogens. Unlike other potential biocontrol agents, fungi do not have to be ingested to infect their hosts but invade directly through the cuticle and so they can be used for control of all insects including sucking insects. Thus, entomopathogenic fungi are a major component of integrated pest management techniques as biological control agents against insect pests and other arthropods in horticulture, forestry and agriculture (Inglis *et al*., 2000).

Entomopathogenic fungi are among the first organisms to be used for the biological control of pests. These entomopathogens, due to their eco-friendliness and bio-persistence, are preferred to kill insects at various stages of its life cycle (Gul *et al*., 2014). Entomopathogens such as *Metarhizium anisopliae* and *Beauveria bassiana* are well characterized because of their pathogenicity to several insects of different orders. *B. bassiana* and *M. anisopliae* are among the first entomopathogenic fungi being successfully used for the myco-biocontrol of insect pests. Therefore the objective of this review was to overview fungi entomopathogenic research and its application

to insects' management in agriculture.

2. Host range of Entomopathogenic Fungi

Entomopathogenic fungi cause lethal infections of insects and can regulate their populations in nature by epizootics. Today, about 35 genera with more than 400 species of entomopathogenic fungi are known. Pathogenic fungi have a broad host range. About 1800 associations between fungi and different insects were recorded. A host range is the set of species that allow survival and reproduction of a pathogen. The ecological host range is the current, yet evolving, set of species with which a parasite naturally forms symbioses, resulting in viable parasite offspring (Onstad and McManus, 1996). Physiological host range is based solely on laboratory observations of infection. Species identified as hosts in the laboratory may not be hosts in the field (Federici and Maddox, 1996). An association between pathogen and an insect exists when the host is naturally infected in field or in the laboratory by the pathogen and the infectious propagule is produced. When infection has been attempted but not observed, then no associations exist.

3. Classification of Entomopathogenic Fungi

Entomopathogenic fungi are found in the divisions Zygomycota, Ascomycota and Deuteromycota (Samson *et al.,* 1988), as well as Chytridiomycota and Oomycota, which were previously classified within Fungi. Many of the genera of entomopathogenic fungi currently under research belong either to the class Entomophthorales in the Zygomycota or to the class Hyphomycetes in the Deuteromycota. It is important to mention that fungal infections occur in other arthropods as well as insects and/or species that are not pests of cultivated crops. For example, *Gibellula* species infect spiders and several species of *Cordyceps* and *Erynia* infect ants.

4. Geographical and Ecological Distribution of EPF

Entomopathogenic fungi are an important and widespread component of most terrestrial ecosystems. It seems they are not only in places where there are neither victims' insects nor other arthropods. Of course spread of individual species of entomopathogenic fungi are different. Entomopathogenic fungi have been also recorded in north of the Arctic Circle. They have been *Tolypocladium cylindrosporum, B. bassiana* and *M. anisopliae* in Norway (Klingen *et al.,* 2002), and *B. bassiana*, *M. anisopliae* and *Isaria farinosa* (*Paecilomyces farinosus)* in Finland (Vänninen, 1995). What more, entomopathogenic fungi have been reported also from Arctic Greenland (Eilenberg *et al*., 2007) and Antarctica. In the latter location including endemic Antarctic species *Paecilomyces antarctica* isolated from the Antarctic springtail *Cryptopygus antarcticus* in the peninsular Antarctic (Bridge *et al*., 2005). Forest Ecosystems, more than just trees also cosmopolitan fungi belonging to the genus *Beauveria, Lecanicillium*, *Conidiobolus* and *Neozygites* have been found on Antarctic sites, but without their arthropod hosts (Bridge *et al.*, 2005). Studies of Quesada-Moraga showed that altitude has no influence on presence of entomopathogenic fungi in range up to 1608 m, what more altitude was found to be predictive for the occurrence of *B. bassiana* (Quesada-Moraga *et al*., 2007). However, studies made on wider range of altitudes (up to > 5200 m) made showed great importance of this factor on the species diversity of insect-associated fungi (Sun and Liu, 2008). While other species of *Hypocreales* such as *Beauveria*, *Metarhizium* and *Isaria* were the dominant fungi found on soil insects (Samson *et al*., 1988). Despite the fact that both *B. bassiana* and *M. anisopliae* are common everywhere there is known that *B. bassiana* seems to be very sensitive to the disturbance effects of cultivation and thus restricted to natural habitats. Entomopathogenic fungi are commonly found in soil and leaf litter of worldwide forests, however in temperate forests the diversity of entomopathogenic fungi are relatively low in comparison with tropical habitats (Aung *et al*., 2008). However, compared to agricultural areas the diversity of entomopathogenic fungi in the temperate forests is quite high (Sosnowska *et al*., 2004).

5. Biology and lifecycle of Hypocreales and Entomophthorales

The life cycles of *Hypocreales* and *Entomophthorales* are slightly different. Nevertheless, the survival and spread in the environment of both groups is dependent on the infection of the host that invariably leads to its death. The life cycle of entomopathogenic fungus consists of a parasitic phase (from host infection to its death) and a saprophytic phase (after host death). In contrast to other entomopathogens (bacteria and viruses), which enters the insects with food, entomopathogenic fungi infect their host through the external cuticle. The process of infection involves: adhesion of the spore on the insect cuticle, penetration of the cuticle by the germ tube, development of the fungus inside the insect body and colonization of the hemocoel by fungal hyphae. The spores of the entomopathogenic fungi are usually covered with a layer of mucus composed of proteins and glucans, which facilitates their attachment to the insect cuticle. Germinating spores of several entomopathogenic fungi produce specialized structures called appressoria. The appressorium is responsible for attachment of germinating spore to the epicuticular surface. The process of penetration of the insect cuticle is a result of mechanical pressure and enzymatic activity of the germ tube. The major role in the penetration plays the secretion of sequential lipases, proteases and chitinases. Inside the insect body most entomopathogenic fungi grow as yeast-like propagules

(blastospores), hyphal bodies or protoplasts lacking a cell wall. These structures are spread through the hemocoel. Death of an insect is usually a result of mechanical damage caused by growing mycelia inside the insect (mummification), or toxins produced and released by the pathogen. *Beauveria*, *Metarhizium*, *and Tolypocladium* are known that secrete a whole range of toxins. Some of them like destruxin, bavericin, and efrapeptins are fully described chemically, and is known their action and contribution in the process of pathogenesis (Roberts, 1981; Hajek and St. Leger, 1994). For *Entomophthorales* there are limited data about the release of toxins (Boguś and Scheller, 2002). In this case, death is the result of the total colonization of host tissues by the fungus. Forest Ecosystems More than Just Trees After host death; the fungus can colonize the cadaver within 2-3 days forms aerial hyphae and then sporulates. Whereas *Hypocreales* produce only asexual spores, species of *Entomophthorales* produce two types of spores: asexual (primary conidia) and sexual (zygo or azygospores) called resting spores. Conidia of *Hypocreales* and primary conidia of *Entomophthorales* are produced externally on the surface of an insect after its colonization and death. *Entomophthorales* and *Hypocreales* differ in the way dispersal of spores. The first of these are actively discharged from cadavers by hydrostatic pressure, while the latter are spread by wind. If primary conidium from cadavers does not land on a new host, it germinates and forms secondary conidia (some species can also produce tertiary and quaternary conidia). The majority of *Entomophthorales* produce resting spores (internally within cadavers). Cadavers containing resting spores (azygospores) initially attach to the branches of trees, and then fall to the ground and then azygospores are leached into the soil. Under favorable conditions, azygospores begin to germinate to form germ conidia and infect new hosts. Resting spores allow entomophthoralen species to survive unfavorable periods or the temporary lack of hosts. In this way many species of *Entomophthorales* synchronize their development with the development of insects. Hypocrealen fungi can also survive in the environment (if do not land on a new host), as mummified cadavers or as conidia in soil (Hajek and St. Leger, 1994; Hajek and Shimazu, 1996).

6. Entomopathogenic Fungi and the Environment

An improved understanding of the ecology of indigenous populations of these beneficial organisms is a prerequisite for the evaluation of their contributions to pest control and for predicting the impact of agricultural practices on their populations. The anamorphic entomopathogenic fungi *B. bassiana* (Balsamo) Vuillemin and *M. anisopliae* (Metschnikoff) Sorokin are natural enemies of a wide range of insects and arachnids and both fungi have a cosmopolitan distribution (Rehner, 2005). Due to natural occurrence of EPF, it is thought that they are generally environmentally friendly with low to no mammalian and residual toxicity. As a result they have been developed as microbial insecticides for controlling many major arthropod pests in agriculture, forestry and urban settings in several countries, including the United States (Goettel *et al*., 2005). In air samples, *B. bassiana* was isolated among a large array of airborne fungi (Airaudi and Marchisio, 1996) and deposition from the air could be one likely source of the newly documented occurrence of *B. bassiana* on phylloplanes of hedgerow plants (Meyling and Eilenberg, 2006). However, localized transmission onto plant parts by rain splash has also been shown (Bruck and Lewis, 2002) but rainfall also removed fungus inoculum that had been applied to foliage (Inglis *et al*., 2001). In the soil environment the hypocrealen entomopathogenic fungi can persist, but extensive proliferation and dispersal are limited. Population build up relies on the conversion of host cadaver resources into infective conidia that are released from cadavers over time following sporulation (Gottwald and Tedders, 1982). The number of conidia released per host is dependent both on fungus species, host species, and host size. For example, *B. bassiana* released 10–200 times more conidia than *M. anisopliae* from adult pecan weevils (Gottwald and Tedders, 1982). Entomopathogenic fungi are dispersed by living infected hosts which migrate and die in another place than where they became infected (Hajek, 1997). This implies that *B. bassiana* is able to travel over long distances as infections in hosts, which can later lead to new infections and establishment far away from the original site of the fungus. The potential of arthropods to disperse and vector entomopathogenic fungi by their activity has been demonstrated in different terrestrial ecosystems. In the soil, collembolans dispersed conidia of *B. bassiana* and *M. anisopliae* which were not pathogenic to them (Dromph and Vestergaard, 2002), both by carrying conidia on the cuticle and by ingesting conidia which, after passage through the digestive tract, could remain viable (Dromph, 2001).

7. Entomopathogenic Fungi as Biopesticides

The significance of fungi in regulating insect populations was noted early in recorded history by the ancient Chinese (Roberts & Humber, 1981) due to the frequency of natural epizootics and the conspicuous symptoms that are associated with fungus-induced mortality (Steinhaus, 1964; McCoy *et al*., 1988). EPF, like other natural enemies of insects, can be employed in classical biological control, augmentation or conservation. The safety of EPF for humans, for the environment and for non target organisms makes for a safer alternative for IPM than is the use of chemical insecticides (Goettel and Hajek, 2000). Although fungal pathogens have much in common with viruses, bacteria and other insect pathogenic microbes, they are unique in several different ways (Ferron, 1978). The most significant difference lies in their mode of infection. Whereas most entomopathogens infect their hosts through the gut following ingestion, fungi typically penetrate the insect cuticle, thus becoming the only major

pathogens that are known to infect insects with sucking mouthparts in the orders Hemiptera and Homoptera (Roberts and Humber, 1981). Most EPF are best used to control insect populations below a specific economic threshold, with some crop damage being regarded as acceptable, rather than for the total eradication of a pest. Despite there being an estimated 700 species of EPF in approximately 90 genera (Roberts & Humber, 1981), most of the commercially produced fungi are species of *Beauveria*, *Metarhizium*, *Lecanicillium* and *Isaria*, which are all relatively easy to mass produce. Fungal pathogens, particularly *B. bassiana,* Vuillemin, *Isaria fumosorosea* Wize and *M. anisopliae*, are currently being evaluated for use against agricultural and urban insect pests. Several species belonging to the orders Isoptera (Hussain *et al*. 2010, 2011), Lepidoptera and Diptera (Goble, 2009), Coleoptera (Ansari *et al*., 2006), Hemiptera (Leite *et al*., 2005) are susceptible to various fungal infections. This has led to a number of attempts to use EPF for pest control, with varying degrees of success.

8. Mode of Action of EPF

All fungi have the same basic mode of action. Insect control by entomopathogenic fungi is achieved when sufficient infective propagules (generally conidia) contact a susceptible host and conditions are suitable for a lethal mycosis to develop. Fungi have been applied for soil pest control by direct incorporation of conidia, mycelial pellets, microslerotia or inert or nutrient-based granules containing fungal propagules (conidia or mycelia), whereas foliar-feeding pests have typically been targeted by sprays of formulated conidia (Jaronski, 2010). Fungal isolate virulence toward different arthropod hosts varies. Virulence generally decreases with repeated sub-culture on artificial media, and can often be regained through host passage (Nahar *et al*., 2008). Virulent isolates generally express an abundance of spore-bound proteases, efficiently produce and release exoenzymes during cuticular penetration, and generate toxins as the fungus colonizes the host (Vey *et al*., 2001). Selecting superior strains exhibiting these characteristics, or manipulating isolates to promote these traits, has been seen as a way of overcoming what is often considered a significant impediment to their wider use, i.e., fungi kill their hosts too slowly. Fungal virulence can also be improved through directed genetic manipulation whereby specific genes are inserted into the fungal genome to promote expression of toxins that increase the virulence of the parent organisms, e.g., insertion of scorpion toxin genes into *M. anisopliae* and *B. bassiana* (Wang and Leger 2007). Entomophthoralean fungi actively eject spores when conditions are favorable (high humidity) that can rapidly infect a susceptible insect, even when these conditions only prevail for short periods (Steinkraus, 2006). This trait gives these pathogens great epizootic potential, and in many groups of insects, they are among the most important natural mortality factors. In contrast, spores of the hypocrealean fungi *Beauveria* and *Metarhizium spp*. tend to be dispersed passively, via wind currents or rain splash, although transmission can also occur when susceptible insects contact infected individuals, or conidia can be distributed on the bodies of other arthropods (Vega *et al.*, 2007). Both hypocrealean and entomphthoralean fungi can survive repeated intervals of low humidity, recommencing development (infection) when favorable conditions return. This can result in spectacular epizootics such as those observed in whitefly infestations on cotton when the canopy closes and creates a humid microclimate that favors host infection and spread of the disease within the population (Lacey *et al*., 1996). These fungi can, though, infect insects even under conditions of low ambient humidity; attachment of the small conidia at infection sites within inter-segmental folds or under elytra where humidity levels are high may account for this, and the localized microclimate that exists around an insect or at the insect–leaf interface may have a more significant impact on the infection process than ambient conditions (Inglis *et al*., 2001).

9. Isolation and characterization of entomopathogenic fungi

Several methods have been used to describe the variation within a species of entomopathogenic and mycoparasitic fungi. These include morphological characteristics of spores and colonies, extracellular protein profiles, pathogenicity and growth or nutrient requirements (Samson 1981). Furthermore, immunotaxonomic and chemotaxonomic methods have been used, though only with limited success (Bidochka *et al*. 1994). Obviously, taxonomic procedures are becoming more and more complex and it is generally accepted that some forms of molecular identification techniques are needed in addition to the traditional morphological characteristics formally used to classify fungal species (Bridge and Arora 1998). Different molecular techniques were used for various applications and on different entomopathogenic and mycoparasitic fungi (Castle *et al*. 1998). The RAPD (random amplified polymorphic DNA) technique was described in 1990 (Williams *et al*. 1990). It is a modification of PCR (polymerase chain reaction) and allows revealing polymorphism within completely unknown samples without the need of probe hybridization or DNA sequencing. Only one short oligonucleotide primer (6–12 bases) is used for the reaction, and the sequence of primers is fully arbitrary. The product of a reaction is a spectrum of DNA fragments differing from each other in length and nucleotide sequence. The total number of products and the length of each depend on the template DNA and primer used and is specific for a particular combination. (Williams *et al*. 1990). Hyphomycetes are distinguished by the morphology of their conidia and conidiogenous cells and by the identity of their hosts. However, it is almost impossible to distinguish individual isolates using only morphological characters because of limited distinctive characteristics (Samsinakova *et al*. 1983). Moreover, neither standard

laboratory bioassays nor interactions with their natural hosts offer sufficient information to identify fungi on the subspecies level (Osborne and Landa 1992).

9.1. Isolation of Entomopathogenic Fungi (EPF)

Enthomopathogenic fungi can be isolated by different methods from different areas for their important application of insect management

9.1.1. Methods for isolation of EPF from soil sample

There are methods to isolate EPF from soil that using Selective media and Insect bait method are the major one.

9.1.1.1. Selective media

A wide range of fungi occur in the soil environment and they have various ecological functions. Most of these fungi, along with a range of bacteria, can grow on artificial media *in vitro*. These abilities have long been exploited to isolate microorganisms from soil samples and specific media have been developed to select for certain groups of microorganisms. Some media for the selective isolation of entomopathogenic fungi have also been developed. Bacteria can be inhibited by the application of broad-spectrum antibiotics such as chloramphenicol, tetracycline or streptomycin (Goettel and Inglis, 1997). The main remaining obstacle in using this isolation method is that the hypocrealean entomopathogenic fungi grow relatively slowly in comparison to the ubiquitous opportunistic saprotrophic fungi found in the soil environment. Thus the contents of the media need to include substances that prevent these fungi from overgrowing the species of interest. Generally, the species *Metarhizium anisopliae*, *B. bassiana* and *B. brongniartii* have been investigated the most. Goettel and Inglis (1997) provide a list of suitable selective media for *Beauveria* and *Metarhizium* (Goettel and Inglis, 1997). The suggested medium for isolation of *Metarhizium* spp. is often called Veens semiselective medium (Hu and St Leger, 2002). The medium contains the antibiotics chloramphenicol as well as the fungicides dodine and cyclohexamide (Goettel and Inglis, 1997). In different laboratories modifications have usually been made to optimize isolation results based on experience. For example, Hu and St. Leger (2002) used Veens medium to isolate *M. anispoliae*, but omitted cyclohexamide to study the occurrences of other fungi than *M. anisopliae*.

9.1.1.2. Insect bait method

The use of selective media exploits the saprotrophic abilities of hypocrealean entomopathogenic fungi. However, to exploit the ability of the fungi to infect host, the insect bait method can be used. This method was originally developed to isolate entomopathogenic nematodes from soil samples, but fungi were sometimes additionally isolated (Zimmermann, 1986). Thus Zimmermann (1986) suggested that this method could also be a standard isolation method for entomopathogenic fungi. For the method to be feasible insects, which are easily reared and are susceptible to the fungi, must be used. The traditional bait insect is the highly susceptible larvae of the wax moth, *Galleria mellonella*, (Lepidoptera: Pyralidae) but also mealworm larvae, *Tenebrio molitor* (Coleoptera: Tenebrionidae), are suitable. Few studies has evaluated the use of several bait insects from different taxa. Klingen *et al.* (2002) found that dipteran larvae isolated fungi differently than *G. mellonella*. More specifically, larvae of *Delia floralis* (family Anthomyiidae) isolated *Tolypocladium cylindrosporum* more frequently than did *G. mellonella* (Klingen *et al.*, 2002). Thus the use of insect baits can also be considered to be a selective isolation method. However, the "*Galleria* bait method" appears to be more sensitive than traditional plating on media (Keller *et al.*, 2003) and is therefore useful for isolation and identification of the spectrum of entomopathogenic fungi indigenously present in soils.

10. Current markets of EPF

During the last four decades, over 80 companies worldwide have developed 171 mycoinsecticides and mycoacaricides. This contrasts sharply with the situation less than three decades ago, when only one commercial mycoinsecticide was available (Ignoffo and Anderson, 1979). Although most products are based on specific types of propagules, the end product may contain small or even substantial amounts of other propagule types. Products based on aerial conidia may contain hyphae, and vice-versa, and mycoinsecticides produced through liquid fermentation may present a mix of submerged conidia, blastospores and hyphae (Leite *et al*., 2003). The exact propagule composition of biopesticide products is rarely stated by manufacturers, and, in some cases, the specific propagule comprising the active ingredient is not indicated. For many of these products, the active ingredient is quantified in terms of colony forming units. Based on the available information, a significant proportion of products (25.7%, most of these classified as technical concentrates) contain both asexual spores and hyphae. However, 67.5% of all products are described as being based exclusively on asexual spores, with aerial conidia being the most common among all products (41.2%). Only 4.1% of listed products are claimed to contain only blastospores, whereas this kind of propagule is also present in two other products, one including submerged conidia and one including submerged conidia and hyphae. No products have been reported as containing only submerged conidia, and those based exclusively on hyphae account for only 2.3% of all products. The type of asexual spore could not be determined for 22.2% of products.

10.1. Current markets of EPF in Latin America

The use of biological control agents as an integral component of biologically-based pest management strategies has had increased awareness during the last decades. Microbial pesticides have been successfully promoted to farmers in many countries of South America, mainly in systems where not chemical pesticides are available or when pest/disease resistance has made chemical alternatives increasingly expensive and or unreliable. Although farmers in general show a high level of satisfaction with the microbial pesticides they also recognize technical shortcomings with the current generation of biopesticides that will require further technical development to overcome. Faster and reliable action, good storage characteristics and technologies to apply are the main constrains considered. Brazil, Chile and Colombia have 17, 36 and 48 biocontrol products registered in 2010, respectively. However, regulation is critical for the development of biological control; there are differences in time and expenses involved in registration where there is a regulatory system. In some countries there is no regulation for biopesticides, in others as in Brazil and Chile the legislation is the same for chemical pesticides, although in Brazil, a decree establishing the criteria for registration of BCAs for organic agriculture was approved in July 2009, and in others as in Colombia, since 1994 there is a specific regulation, which was updated in 2004. Thus, the low quality of some products and the regulatory and bureaucratic problems related to registration process are responsible for the increased number of illegal biocontrol products in South America (Cotes, 2010).

10.2. Current markets of EPF in Europe

Europe is the largest market in the world for beneficial insects and the second largest market for microbial biopesticides. In 2000, the market was around 97 millions dollars (including pheromones), with beneficial organisms accounting for 55 %, microbial biopesticides for 26%. In 2004, the European market was estimated to reach 110 million dollars. 27 biological control agents have been approved at EU level in 2005. Only 6 biofungicides were registered at a European level. There are also 18 old micro-organisms (11 bioinsecticides and 7 fungicides). Among the 27 micro-organisms, bacteria (34 %) and fungi (54 %) account for the majority of registered agents, followed by viruses (12 %). Fifteen other biological control agents (giving 50 biopesticides products) have been approved by national regulatory authorities and are being sold in those countries.

10.3. Current markets of EPF in Africa

In Africa, a research programme code named LUBILOSA was launched in 1989 to develop mycoinsecticide for the control of locusts and other grasshoppers (Thomas, 1999). The product named ‘Green Muscle’ was formulated based on the propagules of *M. anisopliae* var. acridum 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 *Baeuveria bassiana* propagules for the control of aphids and weevils respectively. *M. anisopliae* (var. acridum) 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 (Ramanujam, 2014). 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 (Songa, 2003). However, as at 2010 in Kenya, Bio-power and Botanigard all based on *B. bassiana* GHA were also registered for use (Kabaluk *et al.,* 2010). In Nigeria, for example, several synthetic pesticides have been registered for production and use but not a single mycoinsecticide has been registered for use.

11. 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 mycoinsecticdes 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 mycoinsecticdes began more than five decades ago. In spite of this, mycoinsecticides are gradually becoming market, which mycoinsecticide accounts for 27% 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 mycoinsecticdes 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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