



ENTOMOPATHOGENIC FUNGI: BIO-RESOURCE; BOON POTENTIALITY WITH SPECIAL FOCAL POINT AS BIOPESTICIDE

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ABSTRACT

The aim of the present study is to contribute for understanding health effects of pesticides exposure on human. An entomopathogenic fungus is a fungus that can act as a parasite of insects and kills or seriously disables them. With its broad-spectrum occurrence and rich diversity all over the world, entomopathogenic fungi, perform a sustainable solution towards pest management technology. Entomopathogenic fungi have eco-friendly and diligence nature which are preferred to manage harmful insects at various stage of their life cycle. Entomopathogenic fungi are of different types according to their habitat as well as their mode of action and virulence efficiency. The escalating stipulation for decreasing chemical inputs in agriculture and increased resistance to insecticides has provided great thrust to the development of alternative forms of insect-pest control. Entomopathogenic fungi have wide range host specificity which can provide ecofriendly assistance in biological control of harmful insect pest in Agronomy.

KEY WORDS

Biopesticide, Bio-control agents, Entomopathogenic fungi, Natural alternate.

1 INTRODUCTION

In the present scenario, fungi play an important role to reduce global challenges like upgrading waste management, to enhance food quality and also improve stress condition of plants to grow in salt and drought state [1,2,3]. Fungi are highly diversified microorganism in the ecosystem. On the basis of their habitat, number of fungi like terrestrial fungi, endophytic fungi, mushrooms, entomopathogenic fungi, nematode trapping and coprophilous, marine and fresh water fungi represent the ecological diversity. By using fungi as biopesticide we can cover a wide range of applications and that can be used as controlling agents for harmful insects. *Hyphomycetes (Deuteromycetes)* is

one of the classes of fungi which are used as biopesticide in worst part of the world. Their conidial structure is different from other fungal group and also their host range [4,5]. Mushrooms have been utilized as sustenance supplement from times immemorial not just for their flavour, fragrance and nutritive qualities however likewise for their restorative properties. Wild mushroom holds an assortment of bioactive aggravates that have made it conceivable to be utilized as a looming hotspot for the change of pharmaceutical and nutraceuticals. These bioactive mixes have been utilized as resistant modulator, hostile to fibrotic, mitigating, against diabetic, against viral, cell reinforcement and antimicrobial operators [6]. There are different type of secondary metabolites, which are synthesized by

entomopathogenic fungi and have wide application like antibiotics, cytotoxins, attractor and repellent. Therefore, entomopathogenic fungi is an alternative mode of control of pathogenic insect in agriculture, they are natural, easy to formulate and rarely poisonous to plants and animal [2]. Entomopathogenic fungi produces enzymes which break down the insect's integument where Lipases are the first enzyme synthesized from the entomopathogenic fungi. In recent times cytochrome P450 subfamily, referred as CYP52XI and MrCYP52 has been identified in *Beauveria bassiana* and *Metarhizium robertsii*, respectively. These cytochromes break down long-chain of alkenes and fatty acids to become initial nutrients of fungi [6]. Cytochrome P450 monooxygenases (CYP) are involved in alkane and insect epicuticle degradation, and they showed that *Beauveria bassiana* differentially expressed CYP genes when grown on different hydrocarbons. The versatile CYP52 family (membrane bound class II) contains several enzymes with demonstrated activity towards alkenes or fatty acids. Although the genome of *M. robertsii* encodes 123 highly divergent CYP gene *M. robertsii* has only a single CYP52 (MAA_06634) compared to four in *M. Acridum* [8].

1.1 Pesticide

Pesticides consist of naturally occurring and synthetic substances which are used to manage damaging pests like insects, disease organisms and weeds, as well as several alternative living organisms like nematodes, arthropods apart from insects, and vertebrates that endangers our food offer, health, or comfort [5]. In specific, the term pesticide refers to chemical substances that biologically active and interfere with traditional biological processes of living organisms deemed to be pests, whether or not these harmful insects, weeds or toxic plants. Pesticides are wide employed in most areas of crop production to attenuate infestations by pests and therefore, defends crops from potential yield losses and reduction of product quality [9,10]. Their mode of action is by targeting systems or enzymes within the pests which can be identical and thus, they cause risks to human health and also the atmosphere. Pesticides have various useful effects like embody crop protection, preservation of food and materials and hindrance of vector-borne diseases [3]. Insecticide is a significant factor against insect pests in agriculture and essential role to crop protection. But due to over use of chemical pesticide it has a range of harmful effect on human and environment [6]. The term

pesticide includes a wide range of compounds including, fungicides, molluscicides, herbicides, rodenticides, insecticides nematocides, plant growth regulators and others. Ideally, a pesticide must be lethal to the targeted pests but not to non-target species including plants and animals [9]. For sustainable agriculture and strengthening of the environment and human health, the importance of using secure pesticides has assumed global importance subsequent to the 'Earth Summit' in 1992 [10,11].

1.2 Chemical Pesticide

Varieties and utilization of chemical pesticides universally have been increasing clearly as increased human population and crop production. Miss use of chemical pesticide becoming more and more serious, which has resulted in heavy environmental pollution and health risk of human [12]. Chemical pesticide is generally undertaken to target specific organisms, pesticide applications often cause harm to non-target species. Due to differences in synthetic composition, approach of action, and appliance techniques, the exchange of one pesticide for addition may after effect in altered furnishings on non-target populations [13,14]. Chemical pesticides and agrochemicals, in general became an important for common agronomics systems during the last century, acceptance for a apparent access in crop yields and element production, chemicals activated on crops, as for example toxaphene applied in cotton crops in Nicaragua, remained in soils year after year and were carried by surface runoff to watersheds and coastal lagoons where residues contaminated aquatic biota [15]. The growing demand for reducing chemical inputs in agriculture and increased resistance to insecticides have provided great impetus to the development of alternative forms of insect-pest control. Myco-biocontrol offers an adorable alternative way to control the insect pest. Myco-biocontrol agents by itself occurring organisms which are perceived to control the chemical pesticide and their hazardous effect [16].

1.3 Bio-Pesticide

Bio-pesticides are naturally occurring substances that are control harmful pests by bio-specialised mechanisms. Bio-pesticides are living organisms or their products (Bio-chemicals, microbial products) or by-products (semiochemicals) that can be used for the management of pests which are harmful to our crops and they consist of less threat to the environment and to human health. The most commonly used bio-pesticides are living organisms, which are pathogenic

for the pest of interest. This include bio-fungicides (*Trichoderma*), bioherbicides (*Phytophthora*) and bioinsecticides (*Bacillus thuringiensis*) [17]. In worldwide bio-pesticides cover up about 1% of the total plant protection products, but in past two decades their number and growth rate have been showing an increasing trend in the past about 175 bio-pesticides active ingredients and 700 products have been registered [6,18,19].

2 FUNGI IN AGRICULTURE

To protect the plant from the harmful insects or pathogenic microbes or for obtain high yield traditionally we use chemical pesticide, insecticide, and fertilizer which are very harmful for human being or as well as for environment because they contain different type of methylated or ethylated substance and aromatic group that shows very dangerous effect on the environment [20]. (Table no.1 is depicted here).

Entomopathogenic fungi (EPF)

The word entomogenous is made up of two Greek words, “*entomon*” that means insects and “*genous*” meaning arising in. Therefore, the etymological meaning of entomogenous microorganism is “microorganisms which arise in insects.” The entomogenous microorganisms is directly involve in natural or microbial control of insect pests and is related to human welfare which has attracted the attention of microbiologist, molecular biologists, and entomologists in the recent years [33]. Entomopathogenic fungi are classified as fungi that infect, invade and eventually kill the insects. Entomopathogenic fungi have been investigated for use against a broad range of insect pests. Diverse toxic metabolites have been described in several fungal biological control agents isolated from *Beauveria*, *Metarhizium sp.* and *Paecilomyces sp.* Some of the fungal metabolites have also been found to display antibacterial and antifungal activity. Production of oosporein, beauvencin, bassianolide, and cyclosporine are commonly observed as secondary metabolites from entomopathogenic fungi. Secondary metabolites of *Beauveria bassiana* are reported to have insecticidal, antifungal and antibacterial properties against human pathogens [3]. Entomopathogenic fungi are accepted as ecofriendly biological control agents of insects. Basically, the entomopathogenic fungi have wide range of pathogenic activity that depends on the ability of its enzymatic activity, which consist of lipases,

proteases and chitinases. These enzymes break down the insect’s integument where Lipases are the first enzyme synthesized from the entomopathogenic fungi. Recently cytochrome P450 subfamily, referred as CYP52XI and MrCYP52 has been identified in *Beauveria bassiana* and *Metarhizium robertsii*, respectively. These cytochromes break down long-chain of alkenes and fatty acids to become initial nutrients [7].

Varieties of entomopathogenic fungi such as *Beauveria bassiana*, *Nomuraea rileyi*, *Metarhizium anisopliae*, *Paecilomyces fumosoroseus* and *Paecilomyces farinosus* directly infected to numerous insect species [8]. These fungi have been considered as a source of bio-pesticide agents and more recently they have also been considered to be a rich source of natural bioactive compounds. However, the abundance and biodiversity of these fungi is poorly known, especially in Central India [34]. Myco-biocontrol is an environmentally sound and effective means of reducing or extenuating insect-pests and its effects through the use of natural enemies. Myco-biocontrol is the use of fungi in biological processes to lower the insect density with the aim of reducing disease-producing activity and consequently crop damage [35]. All groups of insects may be affected and over 700 species of fungi have been recorded as entomopathogens. Some of these fungi have restricted to host ranges, like *Aschersonia aleyro* disinfects only scale insects and whiteflies, while other fungal species have a wide host range, with individual isolates being more specific to target pests. Some species are facultative generalist pathogens, such as *Aspergillus* and *Fusarium*. However, most species are obligate pathogens, often quite specific and rarely found. Entomopathogens such as *M. anisopliae* and *B. Bassiana* are well characterized in respect to pathogenicity to several insects and have been used as myco-biocontrol agents for biological control of agriculture pests worldwide. About 11 companies offer at least 16 products based on the entomopathogenic fungi *B. bassiana* at Columbia. These products are not only used in coffee crop but also in other crops such as bean, cabbage, corn, potato, and tomato. They are used to treat haematophagous insect pests and vectors of diseases like mosquitoes and flies. Under natural conditions, fungi are the frequent and often important natural mortality factor in insect populations. Unlike other potential biocontrol agents, fungi do not have to be ingested to infect their hosts but invade directly

through the cuticle and so can, potentially, be used for control of all insects [36].

Beauveria species

Originally known as *Tritirachium shiotae*, the fungus was renamed after the Italian lawyer and scientist Agostino Bassi, who first implicated it as the causative agent of a white (later yellowish or occasionally reddish) muscardine disease in domestic silkworms. *B. bassiana* is a potential candidate for biological control and it is essential to assess genetic variation among isolates and to determine their distribution, abundance and potential for genetic exchange between and within populations. Thus, biodiversity of *B. bassiana* on various insect hosts and in different geographical regions of the world is a major concern [37,38,39]. Entomopathogenic fungi attack insects, important agents for biocontrol and a part of integrated pest management reported that some proteins extracted from *B. bassiana* isolates gave significant mortalities against *Spodoptera litura* [40]. Collections of *B. bassiana* from Central India have revealed significant variation between isolates. Conidial morphology did not show any considerable difference in the measurements between all the isolates. Cultural characteristics have been used to separate some populations of *B. bassiana*. It is noted that adequate and accurate separation of strains of *B. bassiana* becomes increasingly important for their exploitation in pest control. The unique fingerprinting for individual genotypes will enable monitoring of the environmental impact of a particular isolate following its use as a mycoinsecticide. *B. bassiana* fungi requisite superior spore concentration and shorter revelation period to kill 50 percent of the worker population [39]. Entomopathogenic fungi *Beauveria* cause infection to their host insects. The infection occurs of the subsequent steps: attachment of the spore to the insect cuticle, spore germination on cuticle, penetration through the cuticle, overcoming the host immune response, proliferation within the host, saprophytic outgrowth from the dead host and production of new conidia [41,42]. The enzyme chitinase is mostly used as biopesticide because it breakdown chitin that is a chain homopolymer of N-acetylglucosamine (GlcNAc) connected by β -1, 4 glucosidic linkages. This enzyme synthesised by *Beauveria bassiana* which is one of the most insect pathogenic fungus and uses globally for insect pest control [43]. Survivability of fungal conidia is longest at lesser temperature (0-20°C) and with relative humidity levels (0-53% RH), but due to having heat

sensitivity character, their conidia are unable to tolerate higher temperature (30-40°C) [44].

Metarhizium species

Metarhizium anisopliae, initially known under the name *Entomophthora anisopliae* was first described near Odessa in Ukrain from infected larvae of the wheat cockchafer *Anisopliae austriaca* in 1879 and later on, *Cleonus punctiventis* by Metchnikoff. It was later renamed as *M. anisopliae* by Sorokin in 1888. *Metarhizium species* is also one of the biopesticide on alarming harmful effects from insect and is explored for mycobiococontrol of abominable insect pests. A complete bioactivity of *M. anisopliae* has been activated on teak skeletonizer, *Eutectona machaeralis* and found *M. anisopliae* to be a potential myco biocontrol agent of teak pest has tested spore production of *M. anisopliae* by solid state fermentation [45]. *M. anisopliae* fungi begin their infective stage after the attached spores are retained on the integument surface of where germinative tube forms, fungi starting to secrete enzymes such as proteases, chitinases, quitobiases, Upases and lipoxygenases. Given enzymes act on the surface and degrade the insect's cuticle and help in the process of penetration by mechanical pressure that is initiated by the apresorium, a specialized structure formed in the germinative tube, formerly in the interior the insect, spores of fungi build up as hyphal bodies that spread through the hemocoel and enter by force diverse muscle tissues, fatty bodies, malpighian tubes, mitochondria and haemocytes, leading to death of the insect 3 to 14 days after infection. Once the insect dies and many of the nutrients are exhausted, fungi start mycelia growth and invade all the organs of the host. Finally, hyphae penetrate the cuticle from the interior of the insect and emerge at the surface, where they initiate spore formation under appropriate environmental conditions [46,47].

Paecilomyces species

Paecilomyces fumosoroseus is one of the broadly spreads entomopathogenic fungus. *Paecilomyces fumosoroseus* exemplify enormous insecticidal activity in opposition to different insect pest in which whiteflies and *Bemesia tabaci* are the most imperative ones. *Paecilomyce* sp. Produce a disease name as "Yellow Muscardine disease". There are so many studies which indicated that the entomopathogenic fungus, *Paecilomyces fumosoroseus* is mostly precious microbial insecticide as well as it is considered to be an efficient resource of active antibacterial compounds.

Entomopathogenic fungi are productively being used as biological control agents against variety of insect pests but its antibacterial potential is still not much explored [48]. The corpuscle of this fungus acts by infecting, parasitizing and killing eggs, juveniles and adolescent adults of a lot of phytophagous nematode species. When the corpuscle of Bio-Nematon comes in acquaintance with altered stages of the nematodes, they germinate and spread throughout the nematode eventually paralysing the nematode which causes death of the nematode [29,36].

Nomuraea species

Among the several alive entomogenous fungi, *Nomuraea rileyi* is a broad-based breed infecting abounding noctuids such as *Helicoverpa armigera*, *Spodoptera litura*, *Tricoplusia Anticarsia gammatalis*, *Pseudoplusia* includes and has a abeyant for ability into mycoinsecticide [49,50]. A recent study on the pathogenesis of *N. rileyi* against *S. litura* showed that the infection process starts with adhesion of conidia on the insect cuticle [51]. The germ tube penetrates through the cuticle, causes lysis of endocuticle followed by development of hyphal bodies in the hemocoel which convert to invasive mycelia and causes death of the host. At the end of the infection cycle, mycelia emerge from the cuticle and produces conidiophores. Also *N. rileyi* secretes a proteinaceous substance inhibiting larval moult and metamorphosis [52]. Entomopathogenic fungi also possess added advantage over other microbial control agents as they are capable of attacking all developmental stages of insects including pupal stages [53,54]. *N. rileyi* against *S. litura* showed that the infection process starts with adhesion of conidia on the insect cuticle. The germ tube penetrates through the cuticle, causes lysis of endocuticle followed by development of hyphal bodies in the hemocoel which convert to invasive mycelia and causes death of the host. At the end of the infection cycle, mycelia emerge from the cuticle and produces conidiophores [15].

Cordyceps species

Cordyceps, the name given to the fungi on insects, has been identified and used as a medication in China for over 300 years and was initially recorded in Ben-Cao-Bei Yao by Wang Ang in 1694. In China, this fungus is usually alleged 'Dong Chong Xia Cao' which agency 'Worm in winter and grass in summer' and the name about refers to *Cordyceps sinensis* [52,55]. This insect parasitizing fungus lives primarily on the head of the larva of one

particular species of moth, *Hepialus armoricanus* Oberthur (Lepidoptera), but is occasionally found growing on other moth species. *Cordyceps* was first introduced to Western society during the 17th century. In 1878 Saccardo, an Italian scholar, called *Cordyceps* acquired from China clearly as *Cordyceps sinensis* (Berk.) Sacc. [56,57]. The Bio-metabolite cordycepin was aboriginal abandoned from the brewed borsch of the alleviative augment *Cordyceps militaris*. This is an entomopathogenic fungus that grows parasitically on lepidopteron larvae and insect pupae. The genus *Cordyceps* is well known in traditional Chinese medicine and exhibits a variety of clinical health effects including immunomodulatory, anticancer, antioxidant, anti-inflammatory and anti-microbial activities. Fungi produce various kinds of fungal metabolites, which are insecticide. The accessory metabolites yield the insect to its final activity date and ultimately insect dies out. Fungal mycelium emerges out through the cuticle and advance to the accumulation of fruiting physique beneath acceptable ecological conditions [58].

Verticillium species

Verticillium lecanii, an important entomopathogenic fungus, acts as a promising biological control agent for various insect pests. *Verticillium* section Prostrata was reclassified in the genera *Lecanicillium*, *Pochonia*, *Haptocillium* and *Simplicillium* based on morphological observations and molecular analysis. *V. lecanii* as a successful mycopesticide work against *Trialeurodes vaporariorum* in South Korean greenhouses. These fungi harm to nymphs and adult and staccato the leaf inside the body by a filamentous mycelium. In 1970s, *Verticillium lecanii* was developed to ascendancy whitefly and several aphids species, including the aphids (*Myzus persicae*) for use in the greenhouse [27]. The parasitism of *V. lecanii* appears to occupy the following events: adherence of conidia to the host cuticle through a thin mucilaginous matrix; germination of the conidia and production of mycelium that colonizes the surface of the cuticle; penetration of germ tubes into the aphid cuticle 24 h after application of the pathogen; extensive lateral development of hyphae accompanied by pronounced degradation of the cuticular layers by 72 h. Labeling with the WGA/ovomuroid-gold complex showed that penetration and colonization of the cuticle by the fungus resulted from localized enzymatic hydrolysis, likely through the synergistic action of chitinases and mechanical pressure; production of blastospores and massive invasion of aphid internal

tissues; assimilation of nutrients and accumulation of lipids by fungal cells; and production of conidiophores and release of the fungus from aphid cadavers [59,60].

GENERAL MODE OF PATHOGENESIS AS BIOPESTISIDE

Successful infectivity depends first and foremost on the adherence and penetration capability of a fungus to the host integuments [61]. Conidia start to sprout under appropriate circumstances. Penetration of the cuticle is proficient by the germ tube itself or by the formation of an appressorium that attaches to the cuticle and gives rise to a narrow penetration tube. Penetration is both as mechanical and an enzymatic process with the intake of nutrients contained in the cuticle [56]. Most terrestrial pathogens are known to penetrate directly, rarely via wounds, sense organs or spiracles. The penetration process is considered to be a combination of enzymatic and mechanical forces. The exact mechanism for entry is usually peculiar to the species. A range of cuticle-degrading enzymes is produced during penetration into the host [61]. The insects die due to the cell collapse caused by depletion of nutrients and poisoning by toxic metabolites produced by the fungus, in this process fungus penetrate then spread fungal spores afterwards infection. The appearance of host were completely change due to gradually spreading of mycelium, host were completely covered by mycelium which in acceptable after develops an abundant amount of conidia [41,62].

ADVANTAGES OF ENTOMOPATHOGENIC FUNGI AS BIO-CONTROL AGENTS

There are wide advantages of using fungi as biocontrol agents, fungi have high degree host specificity for pest control enable to control dangerous insect pests without affecting beneficial insect predators and non-harmful parasites. They have no effects on mammals lead in decrease of pollution of the environmental hazards normally encountered with insecticide applications. The less chance of developing resistance to insect and prolonged pest control. A high potential for further development by biotechnological research. High persistence in the environment provides long-term effects on pest suppression [59]. They are self-generating within or on the surface of the plant and can efficiently provide constant protection as the plant grows. The broad host variety of fungi means one can often do well to control of numerous pests with the same produce [58]. Southern blot and hybridization of

undigested fungal DNA of wild type and four transformants, probed with α -tubulin sequence of pSV50, showed hybridization at high M_r region of genomic DNA in four transformants, whereas in wild type genomic DNA, no homology of the sequence was observed. Digested genomic DNA, of four transformants gave a complex hybridization pattern. The efficiency of transformation by linear and circular pSV50 cosmid was 8 and 10 transformants per μ g DNA per ml viable protoplast by electroporation, respectively, and 4 and 6 by the protoplast PEG method, respectively [63]. A unique and supportive free database has been constructed regarding entomopathogenic fungi *Beauveria bassiana* available in WWW.bbepf.in/, that is user friendly and easy to access the analysed data. This database provides scientific platform to grab sufficient relative or required database to explore specific details [64].

CONCLUSION

The effects of pesticides on soil micro-organisms are less invasive when Biopesticides are used. The diversity and distribution of entomopathogenic fungi occurring in Central India could play an important role in regulating the forest and agricultural pest populations. Morphological characters are limited in their ability to predict the species therefore; molecular technique-based analysis will be valuable in future population studies, which will further help in developing a long-term management and conservation program required to protect the crop species. The selection of fungal entomopathogens for further development as commercial mycoinsecticide is possibly the most critical steps in the process.

Entomopathogenic fungi being element of an integrated approach will give important and selective insect management. In future we have a tendency to expect to ascertain synergistic combos of microbic management agents with alternative technologies that may enhance the effectiveness and property of integrated pest management methods. A brief description about the fungi in biopesticide has been illustrated. Furthermore, mechanism of synthesis and its diverse applications has been discussed. Fungi have an upper edge over other biological systems due to its wide diversity, easy to culture methods, time and cost-effectiveness as well as eco-friendly approach for eco-friendly pesticide synthesis. Mycopenicins are relatively great

development; the future lies in the optimization of biochemical reactions for producing biopesticide with improved composition, size, shape and monodispersity. Genetic engineering technique can be employed to improve the enzyme properties in near future. Additional research is needed to clarify phylogenetic relationships among all the species of indigenous entomopathogenic fungi.

Table 1 List of commercially available products derived from entomopathogenic fungi.

S.No.	Fungus	Product	Reference
1	<i>Beauveria bassiana</i>	Bio-power, Beevicide, Brocaril, Ostrinil, Boverol, Naturalis, Mycontrol-WP, Naturalis-O and BotaniGard	21,22
2	<i>Metarhizium anisopliae</i>	Bio-green, Biopath, Biologic Bio 1020, Meta-Sin	21,22,25
3	<i>Beauveria brongniartii</i>	Betel, Engerlingspilz, Melocont-Pilzgerste	21,24,26
4	<i>Verticillium lecanii</i>	Mycotal, Verelac, Biomite, Biomite	21,22,27,28
5	<i>Paecilomyces fumosoroseus</i>	Pfr21	21,22
6	<i>Paecilomyces lilacinus</i>	Pelicide	21,29
7	<i>Aspergillus. Flavus</i>	Used for Bioremediation	21,30
8	<i>Trichoderma sp.</i> and <i>Paecilomyces sp.</i>	Trypae Mix	21,32
9	<i>Hirsutella thompsonii</i>	Mycar	21,31
10	<i>Nomuraea rileyi</i>	Nomuri	21,15

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