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Entomopathogenic fungi: a potential bioinsecticide

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ABSTRACT

An effective/striking substitute process to reduce the use of chemical pesticides is Biological Control Agents (BCA). Biological pesticides are often touted as being safer and more sustainable than their chemical counterparts. But they also tend to be less effective and more costly, limiting their widespread use. Naturally occurred host (insect/pest/infectious material) related microbials will act as natural enemies and devastating the pest population with eco-safe manner on human health and environment. On these, insects related fungi i.e Entomopathogenic fungi havea significant role among all the biocontrol agents because of its mode of pathogenicity, broad host range, yet they only cover a small percentage of the total insecticide market. These fungi have been received a lot of curiosity due to their prospective as biocontrol agents against pests.When used in Integrated Pest Management systems, biopesticides' efficacy can be equal to or better than conventional products. In this review, Impact of entomopathogenic fungi, saleable availability, evolution, function, secretions (metabolites), effect on insects and their existing capacity as endophyte followed by its compatibility with pesticides was discussed elaborately. Bottle necks in the field of myco-insecticides also have also been discussed.

Keywords: Entomopathogenic fungi; bioinsecticide.

BACKGROUND

In India, more than 60% of the pesticides are used in the agriculture sector. Among the chemical pesticides, insecticides are used to a large extent of about 60% followed by fungicides and bactericides (20%) herbicides (17%) and other chemicals (3%). While the world average for herbicide use is about (45%) followed by insecticides (36%), fungicides (17%) and other chemicals (2%) (Wahab, 2003, 2005, 2009).Though in India only 12% of the total world usage of pesticides is used, almost 1/3rd pesticide poisoning cases are reported in India every year (Mathur, 1999, Aktar *et al*., 2009). Majority of chemical pesticides not only kill the pests but also kill many beneficial insects and non-target animals of ecosystem (Abhilash and Singh 2009).

Besides causing serious threat to environment, resistance, resurgence and persistency problems can also not be denied. These chemicals are also posing serious threat to human health (Maroni *et al*., 2006, Wahab, 2004).The use of agrochemicals, although decreasing the attack of insects and phytopathogenic microorganisms, still represents a high risk to field workers and consumers. Over the last 25 years, chemical pesticides have become less attractive for numerous reasons including increased cost, the development of pesticide- resistant insects and weeds, concerns raised about human health hazards, and deleterious effects upon non-target organisms.

Under such scenario, insect pest control strategy swing towards safer products which are called biopesticides. These products are developed with a strong emphasis on protecting the environment and consumers from harmful effects of poisonous chemical pesticides. As the microbial biocontrol agents express complex mode of action, current microbial agents *viz.* viruses, bacteria, nematodes, and fungi; used globally with higher advantage and success. However, fungal biocontrol agents for pest and disease management are the most important as compared to others due to easy delivery and broad spectrum activity (Wang and St. Leger, 2007; Butt, 2002; Qaziand Khachatourians, 2005; Thomas and Read,2007; Fan *et al.*, 2007). Entomopathogenic fungi have been found in many diverse habitats and associated with a broad range of insect hosts (Samson *et al.*, 1988). Seeking to use the potential of these organisms for pest control, commercial products have been developed with entomopathogenic fungi (McCoy and Couch 1988; Alves and Pereira, 1998).

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IMPACT OF ENTOMOPATHOGENIC FUNGI

Entomopathogenic fungi were among the first organisms to be used for the biological control of pests. These fungi subsume a heterogeneous group of over 100 genera with approximately 750 species, notified from different insects (Khachatourians and Sohail, 2008). Many of these are proved to be highly potential in pest management. The most considerable fungal species are *Metarhizium* spp., *Beauveria* spp., *Nomuraea rileyi*, *Verticillium lecanii* and *Hirsutella* spp. In 1883, Metchnikoff commenced mass culturing of fungus and carried out the first experiment with two beetle pests. *Metarhizium anisopliae* (Metchnikoff) Sorokin is the second most widely exploited entomopathogenic fungus in biocontrol trials. It is known to attack over 200 species of insects belonging to orders *Coleoptera, Dermoptera, Homoptera, Lepidoptera* and *Orthoptera* (Moore *et al*., 1996).

Entomopathogenic fungi cause lethal infections of insects and can regulate their populations in nature by epizootics. Entomopathogenic fungi are often reported as causing high levels of epizootics in nature and are the most versatile biological control agents, and are environmentally safe. An attractive feature of these fungi is that the virulence caused by contact and the action is through penetration (Nadeau *et al*., 1996). Pathogenic fungi have a broad host range. 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, Federici and Maddox 1996). They are host specific with a low risk of attacking non-target organisms or beneficial insects. They are reported to infect a wide range of insects including lepidopteran larvae, aphids and thrips, which are of great concern in agriculture worldwide (Roberts and Humber, 1981).

To date, several mycopesticides have been developed, formulated and commercialized, used in several countries and recommended for economically important pests and insects (Table 1). Majorly entomopathogenic fungal based formulation, developed with *Beauveria bassiana, Metarhizium anisopliae* and *Verticillium lecanii* used to control a number of insect pests. *Paecilomyces* sp. can be used as a bio-nematicide to control nematodes by applying it to soil. The major reason for the small market share of these fungi as mycoinsecticides is its slow killing rate and an increase in market share is directly proportional to killing speed (St Leger and Wang, 2009). However, products have the advantage of a restricted host range, however,host specificity is also one of the limiting factors for their commercial use (Ownley *et al*.,2004).

ENTOMOPATHOGENIC FUNGI AND THEIR SECRETED ENZYMES

The traditional mode of infection of fungal entomopathogens such as *B. bassiana* involves spore deposition on the insect cuticle followed by formation of a germ tube, which through enzymatic and mechanical action penetrates the cuticle (Hajek and St. Leger, 1994). Once in the hemocoel, blasotospore growth causes tissue damage and nutrient depletion. Some entomopathogenic fungi are also known to produce metabolites, but their involvement in insect toxicosis is unclear (Gillespie and Claydon, 1989).

Interaction in the insect pathogenesis is mediated by mechanical force, enzymatic processes and perhaps certain metabolites (Khachatourians and Qazi, 2008). Insect cuticle mainly composed of chitin and protein. Enzymes *viz*., protease, peptidase, chitinases (both endo and exo-chitinases), lipases, phospholipases etc. produced by entamo pathogenic fungi like *Beauvaria* and their species viz., *bassiana*, *brongniartii*, (Charnley and St Leger 1991, Khachatourians (1991), Fang *et al* 2005); *M.anisoplae* (Khachatourians,1991,1996; St Legeretal.,1996, Nahar *et al*., 2004, Small and Bidochka (2005), Baratto *et al* 2003, 2006; *V.lecanii* (Sheng *et al*.,2006) are significant for degradation of the insect cuticle, saprophytic growth of the fungi, activation of the prophenoloxidase in the hemolymph, and they act as virulence factors.

Although the major bulk components of the insect cuticle are protein and chitin, the outer most epicuticular surface layer are made up of a complex mixture of non-polar lipids. Epicuticular lipids play a role in chemical communication events (Blomquist and Vogt, 2003),and in keeping the cuticular surface dry which affects insecticide and chemicals penetration (Hadley,1981; Blomquist *et al*., 1987; Juarez, 1994). They exhibit certain unique structural characteristics such as relatively high molecular mass and chemical stability, which is mainly due to specific physic chemical properties such as length and branching of the carbon chains (usually between 20 to more than 40 carbons), as well as the position and the kind of functional groups and double bonds.

The insect epicuticle contains lipo-proteins, fats, and waxy layers which would be barriers to entomopathogenic fungi as they exhibit anti-fungal activities (Khachatourians, 1996). Lord *et al* (2002) showed a role for the lipoxygenase pathway through eicosanoid-mediated cellular immune response to the *B. bassiana*. James *et al* (2003) demonstrated that conidial germination of *B. bassiana* and *P. fumosoroseus* are affected by cuticular lipids and silver leaf whitefly (*B. argentifolii*). The white fly nymphs produce thick coating of long chain wax esters affecting spore germination. Hence forth, lipids represent major chemical constituents of the insect cuticle, enzymes capable of hydrolyzing these compounds, such as phospholipases, could be expected to be involved in the cuticle disruption processes that occur during host invasion, the action of phospholipases can result in the destabilization of

Table:1. Entomopathogenic fungi developed for control of insects. List organized by products (trade name) and their sources country wise.

Beauveria bassiana			
Country	**Trade name**	**Manufacturer**	**Source (s)**
France	Ostrinil	Natural Plant Protection (NPP)	Wraight et al. (2001),
India	BioGuard Rich	Plantrich Chemicals & Biofertilizers Ltd.	www.indiamart.com/biofertilizers
	Bio-Power	T.Stanes & Company Limited.	www.tstanes.com
	Racer*	Agri Life	www.somphyto.com
	Daman	International panacea Ltd.	www.iplbiotech.com
	Beavera	Jai Biotech Industries	www.jaibiotech.com
	Brigade	Kan Biosys Pvt Ltd.	www.kanbiosys.com
	Bio-Be-Ba	Microplex – Hosted by Nagrjuna Agro Chemicals	http://nagarjunaagrochemicals.com/Biopesticides.php
	Baba	Multiplex Bio Tech Pvt. Ltd.	www.multiplexbiotech.com
	Mycojaal	Pest Control (india) Pvt. Ltd.	www.pestcontrolindia.com
	Metabeave	R.B. Herbal Agro.	www.rbhpomo.com
	Jas Beesi	Shri Ram Solvent Extractions	www.neemplus.com
	BBC	Sri Biotech Laoratories India ltd.	www.sribio.com
	Toxin	Varsha Bio Science & Technology	www.varshabioscience.com
Spain	Trichobass-L & Trichobass-P	Trichodex S.A.	www.amcchemical.com
South Africa	Bb Plus & Bb Weevil	Biological Control Products SA (Pty) Ltd.	www.biocontrol.co.za
USA	Balence	Jabb of the Carolinas Inc.,	www.epa.gov/oppbppd1/biopesticides/ingredients.
	BotaniGard & Mycotrol	Laverlam International Corporation, USA	www.laverlamintl.com
	CornGard	Mycotech Corp., USA	Hajek et al. (2001), Shah and Goettel (1999)
	Naturalis L	Troy Biosciences Inc., USA	www.troybiosciences.com
	Organigard	Emerald BioAgriculture Corp.,	www.epa.gov/oppbppd1/biopesticides/ingredients.
Colombia	Agronova	Live Systems Technology S.A., Colombia	http://lstsa.com
Paecilomyces sp.			
India	Paci Hit Rich	Plantrich Chemicals & Biofertilizers Ltd.	www.amcchemical.com
	Mysis	Varsha Bioscience & Technology	www.varshabioscience.com
	Nematox	Sri Biotech Laboratories India ltd.	www.sribio.com
	Nematonashak	R.B. Herbal Agro	www.rbhpomo.com
	Niyantran	Jai biotech Industries	www.jaibiotech.com
	Nematofree	International panacea Ltd.	www.iplbiotech.com
USA	MelCon WG	Certis	http://certisusa.com/
***Lecanicillium muscarium* (formerly *Verticillium lecanii*):**			
UK,	Mycotal	Koppert Biological Systems,Netherlands (previously: Tate and Lyle, UK)	www.koppert.nl/
Russia	Verticillin	Biodron, Russia	Shternshis (2004),
Spain	Trichovert	Trichodex S.A., Spain	www.amcchemical.com
India	Bio-Catch	T.Stanes & Company Limited, India	www.biocontrol.co.za
	Biovert Rich	Plantrich Chemicals & Biofertilizers Ltd, India	www.amcchemical.com
	Mealikil	Agri Life, India	www.indiamart.com/biofertilizers
	Vertimust	Jai Bioteh Industries	www.jaibiotech.com
	Biogade-V	Kan Biosys Pvt. Ltd.	www.kanbiosys.com
	Vertifire-L	International panacea Ltd	www.iplbiotech.com
	Cropfit	Microplex – Hosted by Nagrjuna Agro Chemicals	http://nagarjunaagrochemicals.com/Biopesticides.php
	Varsha	Multiplex Bio Tech Pvt. Ltd.	www.multiplexbiotech.com
	Biosar	R.B. Herbal Agro	www.rbhpomo.com
	Jasverti	Shri Ram Solvent Extraction Pvt. Ltd	www.neemplus.com
	Spider	Sri Biotech Laboratories India ltd.	www.sribio.com
	Shock	Varsha Bioscience & Technology	www.varshabioscience.com
Brazil	Vertinat*	Natural Rural, Brazil	www.naturalrural.com.br
Colombia	Ago Biocontrol Verticillium 50	Ago Biocontrol, Colombia	Shah and Goettel (1999)
Metarhizium anisopliae			
Spain	Trichomet*	Trichodex S.A., Spain	www.amcchemical.com
India	Bio-Magic*	T.Stanes & Company Limited, India	www.biocontrol.co.za
	Biomet Rich	Plantrich Chemicals & Biofertilizers Ltd, India	www.amcchemical.com
	Pacer	Agri Life, India	www.indiamart.com/biofertilizers
	kalichakra	International Panacea ltd	www.iplbiotech.com
	Cropmet	Microplex – Hosted by Nagrjuna Agro Chemicals	http://nagarjunaagrochemicals.com/Biopesticides.php
	Metrocid	Sri Biotech Laboratories India ltd.	www.sribio.com
	Metaz	Jai Biotech	www.jaibiotech.com
	Metarhizium	Multiplex Biotech Pvt Ltd.	www.multiplexbiotech.com
	Jasmeta	Shri Ram Solvent extraction Pvt ltd	www.neemplus.com
	Biostorm	Varsha Bioscience & Technology	www.varshabioscience.com
Australia	BioCane & Chafer Guard	Becker Underwood Inc., USA— Australian division (previously: Bio-Care Technology Pty Ltd)	www.beckerunderwood.com
USA	Taenure	Novozymes Biologicals Inc., USA (previously: Earth BioSciences; (Taensa Co., USA)	www.epa.gov/oppbppd1/biopesticides/ingredients

membranes, cell lysis and the release of lipid second messengers (Ghannoum, 2000).

Although the secretions (secondary metabolites) of entomopathogenic fungi undoubtedly play an important role during pathogenesis, it is highly unlikely that all of these metabolites would in fact be produced to kill the host, or to suppress its immune system (Demain and and Fang, 2000, Molnár and Farkas 2010).

ENTOMOPATHOGENIC FUNGI AS ENDOPHYTES

Fungal endophytes-mediated plant defense as a novel biological control mechanism against several insects (Funk et al., 1983; Bush et al., 1997; Clay and Schardl, 2002). Most reports on the effects of endophytes on insect herbivores have concentrated on turf and agronomic grasses infected with endophytic clavicipitalean fungi which systemically infect mostly grasses (Clay, 1989; Breen, 1994). *B.bassiana* has been reported as an endophyte in maize (Vakili, 1990; Bing and Lewis, 1991, Cherry *et al*., 2004; Wagner and Lewis, 2000; Arnold and Lewis, 2005), potato, (Jones, 1994), tomato (Leckie, 2002; Ownley *et al*., 2004), and other commercial crops like cocoa (Evans *et al*., 2003), date palm (Go´mez-Vidal *et al*., 2006), and in coffee (Posada *et al*., 2007). Fuller-Schaefer *et al.* (2005) have reported on the colonization of sugarbeet roots by the fungal entomopathogens *B. bassiana* and *M.anisopliae* (Metschn.) Sorokin. Other entomopathogenic fungi have also been reported as endophytes: *V.lecanii* in an Araceae (Petrini, 1981); *Paecilomyces* sp. in rice (Tian *et al*., 2004); and mangroves (Ananda and Sridhar, 2002).

ENTOMOPATHOGENIC FUNGI AND THEIR COMPATIBILITY WITH PESTICIDES

The effect of pesticides applied in the field on the occurrence and abundance of entomopathogenic fungi in soils is difficult to evaluate, because fungi are influenced directly or indirectly by numerous biotic and abiotic factors in the environment (Roberts and Campbell, 1977). Mietkiewski *et al.* (1997) have suggested that pesticides may have a direct impact on the natural occurrence, infectivity, and population dynamics of fungal entomopathogens, as also have direct impact on other macro- and micro-organisms in soils which affect the entomopathogenic fungi indirectly. Certain key biotic and abiotic factors influencing the joint action of fungi as insecticides include type of formulations, carrier materials, emulsifying agents, dosage, soil types and condition of host plants. Any suppression of these fungi may be detrimental to their capacity as natural enemies. Previous investigators have demonstrated that pesticides used under field conditions are unlikely either to kill all the entomopathogenic fungi present in the treated area or to limit their recolonization. It seems likely, therefore, that pesticides have the potential to be used in conjunction with mycoinsecticides in integrated pest management systems.

Fungal entomopathogens can be used as an important component in integrated pest management either alone or in combination with reduced amounts of insecticides. Myco- insecticides containing viable conidia and sub lethal doses of insecticides have been reported to be more effective (Anderson *et al.,* 1989). The resultant enhanced action in combination between pathogens and pesticides has been referred to as inter specific economic synergism (Benz, 1971). Fargues (1975) demonstrated that the effects of the components in these combinations are additive. Many experiments have been carried out aiming to detect pesticides side effects on entomopathogenic fungi (Clark *et al*., 1982; Gardner and Storey, 1995; Neves *et al*., 2001; Olmert and Kennth, 1974). *In vitro* studies indicate inhibition of *B. bassiana* by many pesticides (Ramarajah *et al*., 1967; Olmert and Kenneth 1974). Neves *et al.* (2001) pointed out the importance of conidial germination in compatibility studies. Todorove *et al.* (1998) reinforced the importance of pesticides influence on conidial germination. Integrated pest management (IPM) programs the compatibility between entomopathogenic fungi and pesticide used in fields as a major concern (Todorova *et al*., 1998). De Olivera and Neves (2004) evaluated compatibility of *B. bassiana* whit 12 acaricides formulation and showed that the formulations more compatible with *B. bassiana* were Avermectin and the pyrethroids.

Conidial survival can be affected due to agrochemicals, environmental factors or by bio-pesticide and/or chemical products used to protect plants (Anderson and Roberts, 1983). The impact of pesticides on the processes of germination, fungal growth and sporulation vary depending on the fungal species and strain (Vanninen and Hokkanen, 1988; Anderson *et al.,* 1989). Majority of work on joint action of microbials-synthetic insecticides indicate that fungal sporulation is synergized at subnormal insecticide concentrations (Todorova *et al.,* 1998; Ambethgar *et al.,* 2009). The most important issues that need to be addressed while considering insecticide resistant management through co-application of insecticide-fungus combinations include: (i) resurgence of less important insect pests, (ii) effect on non-target organisms, and (iii) speed of action on target species. Many researchers have examined the factors which influence the synergism (Hassan and Charnley, 1989; Boucias *et al.,* 1996; Kaakeh *et al.,*1997; Quintela and McCoy, 1997, 1998a).Chemical insecticides, botanicals, insect growth regulators and mineral oils at sub-normal doses have been combined with entomo fungi for obtaining enhanced control of certain insect pests.

BOTTLENECKS

However, there is still a lack of knowledge about the fundamental ecology of these fungi in both managed and natural ecosystems, but such information is necessary both for risk assessments prior to release of biocontrol agents in the environment as well as it was to understand the distribution of the fungi and their impact on host populations.

There is a continual need to source new organisms for use in biocontrol strategies. And this review raises some fundamental question – why do so many entomopathogenic fungi kill a large proportion of their hosts? It has long been considered that a well adapted parasite is one which is only moderately pathogenic or not pathogenic at all, because its interest is to preserve its living environment. Hosts and pathogens are engaged in an evolutionary arms race to develop new pathogen defenses, and new methods of overcoming those defenses, respectively.

The conventional premise adopted by parasitologists and some medical microbiologists is that evolutionary adaptation of parasites to their hosts, and the evolutionary arms race, pushes the relationship towards commensalism. Hence highly virulent pathogens, like many of our entomopathogenic fungi, are thought to be in new associations with their hosts.

Conidial survival can be effected by interaction with agrochemicals, environmental factor (Benz, 1987) or by bio-pesticide and/or chemical product used to protect plants (Anderson & Roberts, 1983; loria *et al*., 1983; Alves and Lecuona, 1998).

Conidial germination is very important step in pest management with fungi, because the beginning of epizootics is conditioned to the capacity of these structures to germinate on the host. The entomopathogenic fungus success, however, depends on conidial viability (Batista Filho *et al*., 1998; De Olivera and Neves, 2004).

Development of efficient production systems that provide inoculum that is 'fit for purpose' (e.g. environmentally robust) is also critical. Novel delivery systems also need to be explored; conventional pesticide application techniques, i.e. sprays, may ultimately not be the most effective method of delivering inoculum to a target pest.

For other beneficial microbes (e.g. fungi), mass production is limited to solid substrates; these tend to require less specialized equipment but are more labor intensive. Although liquid fermentation of these organisms is possible, there are issues associated with the relative infectivity of the resulting biomass, its ecological competence, and preservation for enhanced shelf life (Vega *et al*. 2003; Leland *et al*. 2005a, b). Biomass should not be the sole criterion for the definition of production processes; conditions must also ensure that virulent and stable

Advances in formulation technologies now permit stabilization of environmentally sensitive microbes, and have applications to a diverse variety of beneficial organisms. Novel formulation techniques would permit the cost-effective utilization of fungi (Brownbridge, 2006, Dunlap *et al*. 2007).

Use strategies must be rigorously tested and refined to devise robust protocols that not only provide consistent levels of control, but can be readily implemented at the farm level. Technology transfer to farmers is vital yet frequently overlooked as a crucial step in the development process. Education and outreach programmes and access to personnel with expertise in microbial control and IPM will ensure correct use of products to achieve maximum efficacy, and to modify farmers' expectations; it is important that the end-users, as well as those involved in their sale and marketing, understand the differences between conventional and biological controls and can revise expectations and farming practices accordingly.

To work effectively, biological controls must be used within a compatible programme, and by growers committed to their successful implementation. In all instances; access to a viable market is essential to commercial success. The experience of the Sri Biotech Laboratories India ltd – India, which produced commercial volumes of a water-dispersible granule (EPN) and (EPF) formulation from liquid culture from the last 2 decades, proved how difficult it was for such products to compete with chemical controls in low-value crops; by targeting high-value and organic crops, though, they became a commercially viable option. Increasing acreage under organicproduction combined with other high-value export crops where pesticide residuesare undesirable, should promote market opportunities for microbial pestmanagement products.

CONCLUSIONS

This knowledge should facilitate the choice of chemicals compatible with or less harmful to naturally occurring or artificially inoculated beneficial fungi. If entomopathogenic fungi are to be incorporated into a pest management program, it is necessary to determine the effects of pesticides on it. Concerning the effect of the products presently used on vegetative growth and sporulation, a significant reduction was found in relation to the control treatment. By combining performance and safety, biopesticides perform efficaciously while providing the flexibility of minimum application restrictions, superior residue and resistance management potential, and human and environmental safety benefits.

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