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Entomopathogenic Fungi as Potent Agents of Biological Control

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***Abstract*— The various risk factors associated with the use of chemical insecticides such as development of resistance, associated resurgence in insects, accumulation of pesticidal residues in food chain, environmental pollution, health risks and high costs have led to development of alternative strategies of pest management, thus necessitating interest on the search for biological control agents that can control destructive pests of crops. Entomogenous fungi are potentially the most versatile biological control agents due to their wide host range. These fungi comprise a diverse group of over 100 genera with approximately 750 species, reported from different insects. Entomopathogenic fungi vary considerably in their mode of action and virulence. The present review gives a brief picture of environmental factors affecting pathogenicity as well as mode of virulence of entomopathogenic fungi with special mention to their virulence factors involved in pathogenesis**

***Index Terms*— Entomopathogenic, Virulence, Biological control, Pathogenicity**

I. INTRODUCTION

The increased use of conventional chemical pesticides over the years has contributed significantly to an increase in food production, but on the other hand, has resulted in unfavorable effects like resistance, pest resurgence, environmental pollution and risks to human health on the environment and non-target organisms. Henceforth, the necessity for sustainable crop production through eco-friendly pest management technique is being largely felt in recent times. The use of bio–control agents is considered as a suitable alternative to the use of chemical pesticides (Dhaliwal and Koul, 2007). These biological control agents like bacteria, viruses, protozoa, nematodes and most fungi exert considerable control of target populations.

Among micro-organisms, entomopathogenic fungi constitute the largest single group of insect pathogens. Entomogenous fungi are potentially the most versatile biological control agents. They belong to the orders Entomophthorales and Hypocreales (formerly called Hyphomycetes). Entomopathogenic fungi from other taxonomic groups are also known. Until now, over 700 species of fungi are known to infest insects (Wraight et al, 2007).

The advantages of EPFs over chemical pesticides are their significantly higher host specificity, the reduction of hazards and the inability of the insects to develop resistance as the EPFs simultaneously use several modes of actions and as a "living-pesticide" is subjected to adaptation too. The constraints include the slow efficacy in comparison to the chemicides and on-field exposure to various biotic and abiotic stresses. EPFs have significantly higher host specificity in comparison to the conventional biocontrol agents like bacteria, protozoa, nematodes, predatory insects and viruses. They are unique when compared to other microbes causing diseases in insects because they cause infection by growing through the insect cuticle and not required to be ingested, thus showing great potential for control of even sucking insect pests. An attractive feature of these fungi is that infectivity is by contact and the action is through penetration (Nadeau *et al*.,1996). Many of these offer a great potential in pest management. The most prominent fungal pathogens are *Metarhizium spp.*, *Beauveria spp.*, *Nomuraea rileyi*, *Verticillium lecanii* and *Hirsutella spp.* living in diverse habitats including fresh water, soil surfaces and aerospaces (Hajek and Ledger, 1994). They belong to Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina. Among 85 genera of entomopathogenic fungi only six species are commercially available for field application. However, comparatively few have been investigated as potential mycoinsecticides. Fungal pathogens particularly *B. bassiana*, *I. fumosorosea* and *M. anisopliae* are being evaluated against numerous agricultural and urban insect pests. Several species belonging to order IIsoptera (Hussain et al., 2010a ; Hussain et al., 2011), Lepidoptera (Hussain et al., 2009), Coleoptera (Ansari et al., 2006), Hemiptera (Leite et al., 2005) and Diptera (St. Leger et al., 1987) are susceptible to various fungal infections. This has led to a number of attempts to use entomopathogenic fungi for pest control with varying degrees of success.

II. ORIGIN

The origin of the entomopathogenic lifestyle may have arisen several times from a common saprophytic ancestor inhabiting soil and leaf litter (Spatafora and Blackwell, 1993). The biggest emission into different host groups occurred within Clavicipitaceae (Ascomycotina), and involved multiple inter-kingdom jumps between animals (e.g. insects), fungi and plants (Nikoh and Fukatsu, 2000; Artjariyasripong et al., 2001; Spatafora et al., 2007). Hyphomycete species exist as separate asexual (anamorph) and sexual (teleomorph) forms. Entire of the known genera of entomopathogenic Hyphomycetes, now proven teleomorphs in the Clavicipitales, and life cycle stages for Hyphomycetes may have become simplified in agricultural situations because of a superabundance of insect hosts (Evans 2003).

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III. ENVIRONMENTAL INFLUENCE ON NATURAL PATHOGENICITY

Environmental factors which influence the virulence of entomopathogens must be considered for the successful development of the fungus as a biocontrol agent. A wide range of factors such as water, ions, fatty acids, nutrients on the cuticle surface and the physiological state of the host, influence spore germination and behavior (Hassan et al., 1989). Environmental conditions, especially temperature and humidity, have long been recognized to play a significant role in the incidence of epizootics of insect pathogens (Benz 1987). For example, Daoust and Pereira (1986) demonstrated that temperature and humidity affect both survival and germination rates in *B. bassiana.*

Of all the ecofactors that influence epizootics of a mycopathogen, high humidity is most critical for sporulation, germination and invasion of the host (>90% RH) (Getzin, 1961). Pathogenesis occurs at much lower ambient values (Ramoska, 1982) probably because of high humidity in the microclimate at the insect cuticle. However, the external sporulation never occurs on the killed insect, if the relative humidity is too low. Milner *et al.* (2002) studied the effect of relative humidities (RH) from 90 to 100 percent on germination of a termite-active isolate of *M. anisopliae* (isolate F125 and FI610) using a liquid germinating medium. Germination was found to be delayed at water activities equivalent to 99, 98 and 96 per cent RH and was completely inhibited at 94, 92 and 90 percent. Although much higher humidity is required for conidial germination, *B. bassiana* infections have been shown to occur at ambient humidities as low as 50%, probably due to micro environmental conditions on the insect cuticle (Ramoska 1984, Marcandier and Khachatourians 1987b).

The optimum temperature for the development of the fungus is not necessarily the same for development of the disease. However, temperature has a profound influence on the host insect and hence it must be taken into consideration, since very short periods between moults resulting from a high temperature may reduce, for example, the duration of the instar to an extent that penetration of the fungus through the integument is impeded. The rapidity of mycelial development and therefore, the rapidity of the evolution of infection depends on temperature. Generally, optimum values fall between 20°C and 30°C (for example, 23°C for *Beauveria brongiartii*, 24°C for *Entomophthora obscura*, 25°C for *Beauveria bassiana* and *Nomuraea rileyi* and 27°C-28°C for *Metarhizium anisopliae*) with limits between 5° and 35°C. Temperatures lower than the optima distinctly retard the development of mycosis without necessarily affecting the total mortality (Ferron, 1978). If temperature affects germination percentage in conidia, then it also affects the concentration of infective units in the field. For example, Inglis et al. (1997), found that the incidence of *B. bassiana* infections decreased when acridids actively increased their body temperature by habitat selection(sunning).

IV. MODE OF ENTRY AND VIRULENCE OF ENTOMOPATHOGENIC FUNGI

Pathogenesis is the process of chain of events in the disease development in a host upon infection. Unlike bacteria and viruses, fungal pathogenesis in insects occurs via a series of systematically integrated events progressing upon spore attachment to germination, penetration, growth and proliferation within the body of the host, interaction with insect defense mechanism and finally re-emergence on the cadavers (Nadeau *et al.*, 1996; Thomas *et al.*, 1996).

The cuticle is the first barrier to infection by fungi. Hence, rapid and direct penetration of the cuticle is important for virulence (Pekrul and Grula, 1979). The insect procutide is primarily chitin fibrils embedded in a protein matrix and penetration involves both mechanical and enzymatic components (Charnley and St. Leger, 1989). Penetration is a stage of infection where specificity may be determined since, many pathogens are virulent after being injected into the haemolymph of an otherwise nonsusceptible host. In terrestrial environment, fungal conidial germination proceeds with the formation of germtube (Boucias & Pendland, 1991) or appressorium (Zacharuk, 1970a),which forms a thin penetration peg that breaches the insect cuticle via mechanical (turgor pressure) or enzymatic means (proteases) (Zacharuk, 1970b). In *M. anisopliae*, appressorium formation, hydrophobins, and the expression of cuticle-degrading proteases are triggered by low nutrient levels (St. Leger et al, 1992), demonstrating that the fungus senses environmental conditions or host cues at the initiation of infection.

The production of cuticle-degrading enzymes, chitinases, lipases and proteases, has been recognized since long as an important determinant of the infection process in various fungi, facilitating penetration as well as providing nourishment for further development (Hussain et al, 2010b). Among the proteases found in entomopathogenic fungi, the spore bound Pr1 has been well characterized and its role in cuticle invasion has been established (Hussain et al, 2010b; St. Leger, 1994). Ultra structural studies of *M. anisopliae* penetration sites on *Manduca sexta* larvae have shown high levels of Pr1 coincident with hydrolysis of cuticular proteins (St. Leger et al, 1989). Furthermore, it has also been reported that successive *in vivo* passage enhanced the capacity of the fungus to cause infection (Daoust et al, 1982; Hussain et al, 2010b), which ultimately increased the activity of spore bound Pr1 (Shah et al, 2007). On the death of the insect host, the fungus appears from the dead host and sporulation occurs on the outside of the cadaver. Sporulation can occur internally when ambient humidity precludes external sporulation.

V. ENTOMOPATHOGENIC FUNGI - A NEW PARADIGM

Despite the publication of approximately 7,000 papers on topics related to entomopathogenic fungi since 1983 (Vega et al, 2009), there is still limited success in solving agricultural problems with entomopathogenic fungi. Few proposed research areas mentioned below should lead to a new paradigm for entomopathogenic fungi that should refocus our efforts and hopefully lead to exciting new findings that will bring success to the field. Various unexpected roles have been reported for fungal entomopathogens, including their presence as fungal endophytes, plant disease antagonists,

rhizosphere colonizers and plant growth promoting fungi (Vega et al., 2009).

A. Standardized Mass Production technology for fungal biocontrol agents

Recently, *M. anisopliae* was shown to be capable of producing sclerotia in liquid culture fermentation (Jaronski and Jackson, 2008). The ability of *M. anisopliae* to form sclerotia may be important for rhizosphere competence following a pattern seen in phytopathogenic fungi. The use of sclerotial preparations for granular application of biocontrol agents like *M. anisopliae* in soil and the use of conidia or blastospores in foliar applications for phylloplane insects are examples of how the ecology of the fungus-insect interaction directs the production and use of appropriate infective propagules. Likewise, the use of endophytic entomopathogenic fungi for insect control will require an understanding of the ecological factors that enhance the ability of the fungus to become endophytic. Awareness of these ecological factors will aid in the development of production and formulation technologies that deliver optimally infective fungal propagules.

B. Formulation of fungal propagules

The formulation of propagules of fungal entomopathogenic fungi for use in biocontrol has been guided by the need to improve product shelf-life, biocontrol efficacy, and/or the physical characteristics of the product for application (Wraight et al., 2001). Progress in understanding the mechanisms of pathogenicity is now being made, particularly in the area of cuticular penetration where the key enzyme is probably an endoprotease (St. Leger et al., 1986). To increase the speed of insect kill, it may be possible in the future to insert toxin genes from *Bacillus thuringiensis* into fungi. However, improvement in strains achieved by genetic manipulation could be more useful. Future research should concentrate on the development of formulation and the targeting of the pests of economically valuable crops. By achieving this aim, agriculture will benefit from prolonged pest control, reduced risk of resistance and a high degree of safety to non-target organisms without disturbing the agro-ecosystem and the quality of environment.

VI. FUTURE RESEARCH

Entomopathogenic fungi being component of an integrated approach can provide significant and selective insect control. In the near future, we expect to see synergistic combinations of microbial control agents with other technologies (in combination with semiochemicals, soft chemical pesticides, other natural enemies, resistant plants, chemigation, remote sensing, etc.) that will enhance the effectiveness and sustainability of integrated control strategies.

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