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RESEARCH ARTICLE

BEAUVERIA BASSIANA: A POTENTIAL BIOCONTROL AGENT AGAINST PLANT-PARASITIC NEMATODES

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ABSTRACT

Plant-parasitic nematodes are the most damaging pest in agriculture throughout the world. Biological control offers a striking alternative to the use of chemical pesticides. Nematophagous fungi are one of the biocontrol agents for suppression of plant-parasitic nematodes. Among the nematophagous fungi, *Beauveria bassiana* has shown some promise of the use of fungi. This review updates about the recent progress in biocontrol of plant-parasitic nematodes through the utilization of *Beauveria bassiana* and their possible mechanism of action to further enhance our understanding about the biological control of plant-parasitic nematodes.

INTRODUCTION

Plant-parasitic nematodes are the most damaging pest of many vegetable and field crops. Several non-chemical methods including use of organic amendments, resistance varieties, soil solarization and biological control showed promising role to suppress the population of nematodes in different cropping systems. Biological control can be evident to involve the use of biological agents for reducing the damage caused by nematode population. Nematophagous fungi are natural antagonists of nematode parasites, and these offer an ecophysiological source of novel biocontrol strategies. Many researchers have recommended fungi being a potential biological control agent mainly due to their high reproductive capabilities, target specific activity, short generation time, and having resting stage. Some nematophagous fungi have shown promising results as a biocontrol agent against the destructive plant-parasitic nematodes (Jatala 1986; Stirling, 1991). Approximately 700 species of nematophagous fungi have been described thus far (Yu *et al.*, 2014).

Status of *Beauveria* spp: A broad range of *Beauveria* spp. has been isolated from a variety of insect worldwide which are of medicinal or agricultural importance. This ubiquitous fungus has long been known to be, the most common causative agent of disease associated with dead and moribund insects in nature

(McLeod, 1954) and has been scrutinized worldwide as a microbial control agent of hypogeous species (Ferron, 1981). *Beauveria bassiana* (Bals.-Criv.) Vuill. is a filamentous fungus, belongs to a class of insect pathogenic deuteromycete and also known as imperfect fungus. This fungus grows naturally in soils and acts as a pathogen on various insect pests such as stem borers, beetles, aphids, mites, termites, white flies, mealy bugs, thrips etc., causing white muscardine disease (Sandhu *et al.*, 1993; Sandhu *et al.*, 2001; Sandhu and Vikrant 2004 ; Thakur *et al.* 2005; Jain *et al.*, 2008; Biswas *et al.*, 2012). *B. bassiana* is the anamorph (asexually reproducing form) of *Cordyceps bassiana*. The latter teleomorph (the sexually reproducing form) has been collected only in eastern Asia (Li *et al.*, 2001). Rehner and Buckley (2005) have shown that *B. bassiana* consists of many distinct lineages that should be recognized as distinct phylogenetic species. *B. bassiana* is composed of many genetically distinct variants associated with geographical location and host which differs substantially in their ability to produce pathogenesis. Implementation of PCR-based tools for characterization of organisms has greatly advanced the understanding of the phylogenies and species in entomopathogenic fungi, especially in *B. bassiana*. A number of unspecific DNA-based methods have been used specially in *Beauveria* (Glare 2008). Random amplified polymorphic DNA (RAPD) has been used in many studies. UP-PCR has been used to separate sympatric isolates of *Beauveria* in Denmark and was used to place isolates in genetic groups (Meyling and Eilenberg 2006). Thakur *et al.* (2005) studied forty-eight isolates of indigenous strains of *B. bassiana* collected from

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Central India employing protease zymography and RAPD analysis. High genetic and biochemical diversities were indicated with a clear group of strains from Lepidopteran and Coleopteran insect hosts. Different strategies have also been used for the analysis-RFLPs, AFLPs (Bidochka 2001; de Muro *et al.*, 2003; de Muro, 2005; Inglis, 2008). *B. bassiana* have been characterized using AFLP, inter-simple-sequence repeats (ISSR), simple sequence repeats (SSRs), or microsatellites. Recent development of microsatellite markers (Rehner and Buckley 2003; Enkerli *et al.*, 2005) has surely provided an insight in the population ecology of *B. bassiana*. *B. bassiana* has been linked to plants as an endophytic fungus (Arnold and Lewis, 2005). EST (expressed sequence tag) analysis of entomopathogenic fungus *Beauveria* (*Cordyceps*) *bassiana* has been studied using cDNA libraries (Cho *et al.*, 2006).

Nematicidal activity of *Beauveria Bassiana*: *Beauveria bassiana* is a well known biopesticide for insects, but few studies have done on plant-parasitic nematodes. Anke *et al.*, (1995) and Mayer (1995) reported that beauvericin (BEA) produced by *B. bassiana* had weak nematicidal activity against *M. incognita*. Chen *et al.* (1996, 2008) found that *B. bassiana* showed little parasitism of nematode eggs but reduced hatch of *Heterodera glycines*. Junxianke, a fermentation product using a fungal isolate Snef 907 (*B. bassiana*), is lethal to *Ditylenchus destructor*, *Heterodera glycines* and *Meloidogyne incognita* (Liu *et al.*, 2007). Liu *et al.* (2008) evaluated culture filtrate of isolate *B. bassiana* against eggs and juveniles of *M. hapla* *in vitro* and glass-house conditions. *In vitro* study showed that *B. bassiana* parasitized (100%) of the eggs, the egg hatching rate was (36%) and juvenile mortality rate was (18.1%) of *M. hapla*. They found the filtrate of *B. bassiana* successfully inhibited the invasion of *M. hapla* juveniles compared with the chemical pesticide. The nematode population densities and subsequent gall formation and egg-mass production by *M. hapla* were found to be decreased by the filtrates. BEA shows nematicidal activities against the pine wood nematode *Bursaphelenchus xylophilus* and the free-living nematode *Caenorhabditis elegans* (Shimada *et al.*, 2010). Zhao *et al.*, (2013) further demonstrated that the culture filtrate of different isolates of *B. bassiana* had different levels of activities against the same nematode, and the same culture filtrate had selective toxicity against different nematodes (Kepenekci *et al.*, 2017).

Mode of action of *Beauveria bassiana*: Attachment of a fungal spore to the cuticle surface of a susceptible host represents the initial event in the establishment of mycosis. It was observed that dry spores of *B. bassiana* possess an outer layer composed of interwoven fascicles of hydrophobic rodlets. This rodlet layer appears to be special to the conidial stage and has not been reported on the vegetative cells. The adhesion of dry spores to the cuticle was suggested to be due to nonspecific hydrophobic forces imposed by the rodlets (Boucias *et al.*, 1988). Some of these moieties like lectins, a kind of carbohydrate binding glycoproteins, have also been detected on the conidial surface of *B. bassiana*. It was also observed that lectins could be involved in binding between conidia and the host cuticle. The exact mechanisms responsible for the interaction between fungal spores and the cuticle remain to be determined (Latge *et al.*, 1988). When the pathogen reaches and adheres to the host surface, it proceeds with rapid germination and growth which are profoundly influenced by the availability of water, nutrients, oxygen as well as pH, and temperature, and by the effects of toxic host-surface compound.

Fungi with a broad host range germinate in culture in response to a wide range of nonspecific carbon and nitrogen sources (Sandhu, 1995). *B. bassiana* conidia germinate on the host surface and differentiate an infection structure termed appressorium. The appressorium represents an adaptation for concentrating physical and chemical energy over a very small area so that access may be achieved efficiently. Thus, formation of the appressorium plays a pivotal role in establishing a pathogenic interaction with the host. Appressorium formation may be influenced by host surface topography, and biochemical investigations indicate the involvement of the intracellular second messengers Ca^{2+} and cyclic AMP (cAMP) (Leger *et al.*, 1991) or in general when the cuticle is hard (Sandhu, 1995). After the successful penetration, the fungus is then distributed into the haemolymph by formation of blastospores (Bhattacharyya *et al.*, 2004).

The mechanism of antagonism of *B. bassiana* includes antibiosis (Vesely and Koubova, 1994; Bark *et al.*, 1996), competition (Ownley *et al.*, 2004) and induced systemic resistance (Ownley *et al.*, 2008). In 1969, Hamill *et al.* first discovered the insecticidal activity of BEA against a model organism *Artina salina*. BEA is a cyclic hexadepsipeptide that contains three D-hydroxy-isovaleryl and three N-methyl-phenylalanyl residues in an alternating sequence, BEA belongs to the enniatins (ENNs) antibiotic family (Wang and Xu, 2012; Tao *et al.*, 2015; Patocka, 2016). Further evidence shows that this mycotoxin beauvericin (BEA) has a variety of biological activities and is being considered a potential candidate for medicinal and pesticide research (Hamill *et al.*, 1969; Peczynska-Czoch *et al.*, 1991). *B. bassiana* grows saprotrophically within the host and produced other metabolites such as bassiacridin, bassianin, bassianolide, isarolides, beauverolides, tenellin and oosporein (Elsworth and Grove 1977; Strasse *et al.*, 2000; Quesada and Vey, 2004).

Conclusion

For biocontrol of insect pests to become an integral part of the organic agriculture, a few goals must be met such as the selection and development of improved or superior biocontrol agents, the development of mass production technology, and the development of formulation and delivery systems, which are compatible with microorganism requirements as well as with common agriculture practices. Two types of improvements could be considered: (i) efficacy of the biopesticides, by reducing the time to kill the pest (ii) expanding the host range. Genetic transformation systems, which are an essential part of modern fungal research, are necessary for the experimental manipulation of virulent genes *in vitro* and *in vivo*.

The success of utilizing these procedures depends on the availability of selectable transformation markers (Sandhu *et al.*, 2000). Genetic engineering technology which identifies the regulatory gene in the biosynthesis pathway of the active compound can lead to increase production of the metabolite (Yu *et al.* 2010; Radic and Strukelj 2012). There is a strong urge to reveal the essence of these factors to improve the overall efficacy of these biocontrol agents along with developing accurate methods to deliver sufficient inoculum at the target sites. *B. bassiana* are the biological control agent that may be a potentially good source of a microbial nematicide that can be harnessed for successful plant-parasitic nematode control.

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