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Antagonistic potential of *Beauveria* sp. against phytopathogens

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ABSTRACT

Beauveria bassiana is the most widely studied entomopathogenic fungi which have drawn attention worldwide not only as an insect-pest control but also as management of plant pathogenic fungi and bacteria along with their plant growth promotion activities. Mechanisms of plant disease suppression by *Beauveria* sp. involve antibiosis, mycoparasitism, competition, endophytism and induced systemic resistance. Moreover, production of wide array of volatile organic compounds, hydrolytic enzymes (chitinases, amylases, lipases, cellulases, caseinases and proteases) and various secondary metabolites (beauvericin, bassinolide, beauvolide, beauviroloide, oosporein, bassianin and tenellin) triggers its antifungal and antimicrobial properties against various plant pathogens. *Beauveria* sp. have been reported to suppress diseases caused by various foliar and soil borne phytopathogens belonging to Oomycetes (*Phytopthora infestans*, *Pythium myriotylum, P. debaryanum, P. irregular* and *P. ultimum*), fungi (*Fusarium oxysporum, Rhizoctonia solani, Gaeumannomyces graminis* var. *tritici, Armillaria mellea, Rosellinia necatrix, Thielaviopsis bassicola, Botrytis cinerea, Septoria nodorum, Phoma betae, P. exigua* and *Colletotrichum falcatum*, bacteria (*Xanthomonas campestris* pv. *malvacearum)* and nematodes (*Melodogyne incognita).* Considerable efforts are made in the utilization of the potential hypomycete *B. bassiana* as one of the most promising dual purpose biocontrol agents in terms of large scale applications in crop protection.
Keywords: *Beauveria bassiana,* Hypomycete, Biocontrol, Antimicrobial, Mechanism, Mycoparasitism, Hydrolytic enzymes, Secondary metabolites.

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INTRODUCTION

Beauveria bassiana was initially discovered by Agostino Bassi di Lodi in 1834 as white muscardine disease causing pathogen of silkworm (*Bombyx mori*) (Porter, 1973). The initial identification was done by Balsamo-Crivelis as *Botrytis bassiana,* while Spegazzini named it as *Sporotrichum globoliferum* and Petit named it as *Isaria vexans* but later named as *B. bassiana* by Vuillemin in 1912. The taxonomy of *Beauveria* was determined as phylum Ascomycota, class Pyrenomycetes (perithecia), order Hypocreales, family Clavicipitaceae, *Cordyceps bassiana* (teleomorph) and *B. bassiana* (anamorph) (Yokoyama *et al*., 2004). De Hoog (1972) also recognized two entomogenous species of *Beauveria* as *B. bassiana* and *B. brongniartii* and one non-entomopathogenic species as *B. alba.*

Beauveria in plant disease control

B. bassiana has been titled as an organism functioning as dual controlling agent to biological elements for insects and pathogens in crops (Ownley, 2004). Among all, the two important species *i.e., B. bassiana* and *B. brongniartii* are easily cultured and highly virulent making it as a very important candidate for microbial pest control (Li *et al.,* 2001). The ability of mycosis initiation and production of biologically active volatile and non-volatile metabolites are related to its mechanism of pathogenicity (Inglis *et al.,* 2001). The aerial conidia of *Beauveria* spp. are effective agents in controlling biological elements due to their resistance to varying environmental conditions and prolong shelf life (Affandi *et al.,* 2012). Apart from having entomopathogenic properties, *Beauveria* also play additional roles as plant endophytes, antagonists for plant diseases, beneficial rhizosphere colonizers and plant growth promoters (Vega *et al.,* 2009). *B. bassiana* provide protection against fungal and bacterial plant pathogens (Griffin *et al.,* 2006; Griffin, 2007; Ownley *et al.,* 2008, 2010; Vega *et al.,* 2009).The antagonistic property of *B. bassiana* against soil borne pathogens have already been studied by earlier workers (Ownley *et al.,* 2004; Ownley *et al.,* 2008; Vega *et al.,* 2009).

Mechanism of Action in plant disease control

Antibiosis: Antibiosis is defined as antagonism mediated by the production of specific or non-specific metabolites by microorganisms which are deleterious to the growth or metabolic activities of other organisms. It involves the production of antibiotics, bioactive volatile organic compounds (VOCs), lytic agents, enzymes and toxic substances (Fravel, 1988). The entomopathogenic fungus has the ability to degrade host cuticle which is covered by a layer of lipids consisting of long chain of hydrocarbons, fatty alcohols and fatty acids by utilizing them for energy production and incorporation into fungal components (Napolitano and Juarez, 1997). The VOCs released by *Beauveria* when cultured on glucose-based media were identified as diisopropyl naphthalenes, ethanol and sesquiterpenes but in media with *n*-octacosane, the primary VOCs were *n*-decane and sesquiterpenes (Crespo *et al.,* 2008).

Beauveria sp. are also known to produce various secondary metabolites like beauvericin, beauverolides, bassianolides, oosporein, cyclosporine A and oxalic acid with antibacterial, antifungal, cytotoxic and insecticidal activities (Gupta *et al.,* 1999; Boucias and Pendland, 1998; Copping and Menn, 2000). Beauvericin is an ionophore, which is capable of inserting into lipid of the plasma membrane and causing abnormal ion transport and disrupting cells organelles such as mitochondria (Plattner and Nelson, 1994). It is structurally similar to enniatins which is a class of N-methylated cyclohexadepsipeptides with manifold biological activities (Weckwerth *et al.,* 2000). Whereas, Bassianolone is a precursor of cephalosporolides E and F which has an antimicrobial property against fungi and gram-positive cocci (Oller-Lopez *et al.*, 2005).

Oosporein is a red pigmented dibenzoquinone which can oxidize proteins and amino acids which is having an antibiotic property against several bacteria, particularly gram-positive species by competing with their natural microflora located within the gut of the insect (Brewer *et al.,* 1984; Taniguchi *et al.,* 1984; Wainwright *et al.,* 1986). Oosporein also exhibits antifungal property against *P. infestans* by inhibiting radial growth of *P. infestans* with minimum inhibitory concentration of 16 μM but not affecting *Alternaria solani* and *F. oxysporum* (Nagaoka *et al.*, 2004). Bassinin and tellinin are two yellow coloured pigments released from mycelial extracts of *Beauveria* are capable of inhibiting total ATP*ase* activity in erythrocyte membrane and promoting cell lysis which plays an important role in pathogenesis (Basyouni *et al.,* 1968; Isaka *et al.*, 2005).

Enzyme production: Enzymatic and mechanical degradation of host cuticle plays an important role in causing infection to host insect body (Parani *et al.,* 2011). Furthermore, the degradation of the components of host cuticle such as chitin, protein and lipid layer takes place by the release of series of extracellular enzymes like chitinases, proteases, lipases, caseinases and amylases which are produced during adheration of fungal conidia on cuticle and formation of penetration structure *i.e.,* appresoria (Inglis *et al.,* 2001; Dhar and Kaur, 2010a; Kumar *et al.,* 2011). Many hydrolytic enzymes such as chitinases, lipases and proteases are detected on germtubes which aid in growth of *B. bassiana* by solubilizing tissues (Smith and Grula, 1981). The function of lipase and protease produced by *B. bassiana* in plant pathogenesis was also studied by various authors (Feng *et al.,* 1994; Hameed *et al.,* 1994). Overexpression of the *Bbchit1* gene in transgenic *B. bassiana* can significantly enhance its virulence towards insects and pathogens (Fang *et al.,* 2005*;* Murad *et al.*, 2007). *B. bassiana* are also capable in the production of cellulase enzyme as compared to *Trichoderma viridae* by forming a clear zone (5.0 mm) higher than that of *T. viridae* (4.7 mm) indicating that *Beauveria* spp. have the ability of cellulose degradation in oomyceteous fungi (Petlamul *et al.*, 2017).

Mycoparasitism: Mycoparasitism is defined as the parasitism of one fungus by another by utilization of other fungi or fungal pathogens as a food base. The antagonism activity of *Beauveria* against *P. ultimum and P. debaryanum* takes place by parasitism and lysis induction of the mycelium while actively growing on or beneath the mycelia (Veseley and Koubova, 1994). Within 15 days of initiation of the parasitism assay, *B. bassiana* strain Bb 11-98 was observed coiling around larger hyphae of *P. myriotylum* but no coiling was observed around *R. solani* and *T. bassicola* which produced only endoconidia. In other aspect, *Beauveria* spp. was able to hydrolyze β-1-3 and β-1-6 glucans on chitin based medium suggesting its hyperparasitic activity against *R. solani* and *T. basicola* (Griffin, 2007).

Endophytism: Endophytes are the microorganisms which grow inside the plant tissues without causing any apparent symptoms. *B. bassiana* has been reported as naturally occurring endophyte in variety of plants (Vega, 2008) and it also have the ability to establish itself in the host plant following artificial inoculation in plants *viz.,* maize (Wagner and Lewis, 2000), tomato (Leckie, 2002), cocoa (Posada and Vega, 2005), date palm (Gomez-Vidal *et al.,* 2006), opium poppy (Quesada-Moraga *et al.,* 2006), banana (Akello *et al.,* 2007), coffee (Posada *et al.,* 2007), sorghum (Tefera and Vidal, 2009), wheat, cotton, tomato, bean, pumpkin (Gurulingappa *et al.,* 2010), jute (Biswas *et al.,* 2012) and radiate pine (Brownbridge *et al.,* 2012).

The endophytic growth of *B. bassiana* in corn following seed treatment and foliar application provided an advantage over biotic stress as plant was colonized prior to attack (Bing and Lewis, 1991). The colonization of corn, *Zea mays*, by the entomopathogenic fungus *B. bassiana* and confirmed the movement of *Beauveria* through xylem vessels by the help of light microscope and transmission electron microscope (Wagner and Lewis, 2000). *Beauveria* sp. has the ability to move upwards and downwards from the point of inoculation and systemically colonising the entire plant mainly intercellular spaces and vascular bundles (Quesada-Moraga *et al.,* 2006). It was also found that less than 1% of hyphae were able to form an endophytic relationship with the plant by entering directly through the natural openings like epidermal cuticle or the stomata. The potential of *B. bassiana* 11-98 strain was studied as a biological control agent against pests of tomato after an artificial inoculation and detection of the mycotoxic metabolite beauvericin in tomato plants was done by using High Performance Liquid Chromatography (HPLC) (Powell, 2005). The endophytic nature of *B. bassiana* has the ability to protect plants from various herbivores as well as also emerged as potential plant disease antagonist (Ownley *et al.,* 2010). Endophytic colonisation of *B. bassiana* has resulted in reduction of severity of damping-off caused by *R. solani* and *P. myriotylum* in both cotton and tomato seedlings (Griffin, 2007; Ownley *et al.,* 2008) and grapevine downy mildew caused by *Plasmopara viticola* (Jaber *et al.,* 2013).

Induced Systemic Resistance (ISR): ISR is a plant-mediated biocontrol mechanism whereby the plant reacts to the presence of a pathogen by expressing defense-related genes. *B. bassiana* Bb 11-98 was found capable of inducing ISR in cotton seedlings, studied by Griffin (2007) and Ownley *et al.* (2008) against *X. axonopodis* p.v. *malvacearum* when conidia were applied as root drench at the rate of 10^7 CFU/seedling root resulted in significant reduction in bacterial blight in cotton. It was observed that over six days of disease assay, the disease severity indices were consistently lower for Bb 11-98 treatments as compared to control based on disease progress curve.

Biocontrol potential of *Beauveria* sp. against plant pathogens

B. bassiana suppresses the growth of *G. graminis* var. *tritici* by producing chitinases and *β*-glucanases (Renwick *et al*., 1991). The isolates of *B. bassiana* when applied to onion bulbs in both greenhouse and field condition showed an antagonistic effect on *F. oxysporum* and *B.* cinerea. More than 63% control of the pathogen was exhibited by inhibiting the mycelial growth and spore germination of the fungus and increasing plant growth (Flori and Roberti, 1993). Lee *et al.* (1999) also observed the inhibitory effects of 22 different culture filtrates of *B. bassiana* on mycelial growth of *R. solani in-vitro* condition. In another study, antifungal inhibitory activity of five *B. bassiana* isolates was observed against the mycelial growth of plant parasitic fungi of the genera *Fusarium*, *Armillaria* and *Rosellinia* (Reisenzein and Tiefenbrunner, 1997). The culture filtrates of *B. bassiana* are also capable of inhibiting mycelial growth and spore germination of phytopathogenic fungi *i.e., B. cinerea* and *F. oxysporum* (Bark *et al.*, 1996). *B. bassiana* also suppresses the growth of *P. ultimum* and *P. debaryanum* in *in-vitro* condition by inducing the lysis of mycelium while actively growing on or beneath the media (Vesely and Koubova, 1994). The strain of *B. bassiana* isolate 11-98 when applied as seed treatment by treating tomato seeds with conidial suspension containing 2.5% methylcellulose (MC) solution prior to sowing provides protection against *R. solani* in pot culture conditions (Bishop, 1999; Seth, 2001; Ownley *et al.,* 2005). The isolates of *Beauveria* sp. when applied as seed treatment *in planta* in tomato reduced damping off of seedlings caused by *R. solani* and increased plant growth in greenhouse conditions (Ownley *et al.*, 2000; 2004). Batson *et al.* (2000) has found that *B. bassiana* seed treatments on cotton has provided protection against seedling pathogens like *R. solani* when field trials on cotton were conducted in sandy loam soil, thus concluding that variability in environment as notable factor for overall potential and efficiency of biocontrols (Ownley *et al.,* 2003).

Beauveria strain Bb 11-98 exhibits antagonistic property on *P. myriotylum* when applied as seed treatment in cotton by coiling around the pathogen and inhibiting mycelial growth by hydrolysis of cell walls of fungus, thus, reducing damping-off of seedlings in growth chamber conditions (Griffin, 2007). In another study conducting an antibiosis assay of *Beauveria* isolates against *T. bassicola, R. solani* and *P. myriotylum, T. bassicola* exhibited unusal colony growth pattern indicating that growth of fungus was hindered and also resulting complete elimination in some plates. Whereas, very little activity was observed against *R. solani* after 2 weeks, however, *B. bassiana* continued to maintain its initial colony and hyphae continued to extend outwards into the colony of *R. solani.* Against *P. myriotylum, B. bassiana* continued to maintain its initial colony diameter on antibiosis plate assay which further sporulated and produced hyphae on areas occupied by the fungus concluding that the toxins produced by *Beauveria* spp. Bb 11-98 strain in *in-vitro* antibiosis assay inhibits the growth of *R. solani, P. myriotylum* and *T. basicola.*

Treatment of seedling roots of cotton with *B. bassiana* strain Bb 11-98 in soilless system at the rate of $1X10^7$ CFU/seed has resulted in significant lower disease reduction of *X. axonopodis* pv. *malvacearum* than untreated control (Griffin. 2007). Various secondary metabolites of *B. bassiana* such as crude ethyl acetate extract are also found efficient in exhibiting moderate antifungal activity at concentrations between 1200-1600 µg/ml against *A. tenuis, F. avenaceum* and *F. graminearum* without any significant difference whereas, against *Aspergillus paraziticus, F. moniliforme* and *F. oxysporum* with significant difference (Sahab, 2012). *B. bassiana* exhibits mycolytic effect of extracellular enzymes of entomopathogenic fungi to *C. falcatum,* red rot of sugarcane and observed the antagonistic property of *B. bassiana* among tested 23 strains of entomopathogenous fungi against *C. falcatum.* It was found that *B. bassiana* strain caused a higher level of lysis of pathogen when mycelial discs of pathogen were treated with secondary fungal mycelia resulting in pronounced inhibitory effect when the lytic enzymes were produced by using chitin as carbon source (Sanivada and Challa, 2014). Clark (2006) studied the biocontrol properties of methods for damping off of tomato seedlings caused by *P. myriotylum* and reported the activity of *Beauveria* spp. against *P. myriotylum* in tomato. Endophytic colonisation of *B. bassiana* in cucurbits has significantly reduced the incidence and severity of *Zucchini Yellow Mosaic Virus* (ZYMV) by inhibiting or interfering with the systemic movement of virus from cell to cell which eventually resulted in delayed symptom development in inoculated plants (Jaber and Salem, 2014). Azadi *et al.* (2015) also studied the biocontrol potential of *B. bassiana* against damping-off disease caused by *R. solani* in tomato.

Beauveria sp. has been reported in exhibiting antagonistic activities against various phytopathogens due to antibiosis, competition for space and nutrients, lytic agents, secondary metabolites, endophytism and induced systemic resistance. It has been widely established as most promising dual purpose biocontrol agent against plant pathogens in addition to its entomopathogenic properties against insects pests.

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