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COMBINED EFFECT OF *BEAUVERIA BASSIANA* WITH NEEM ON VIRULENCE OF INSECT IN CASE OF TWO APPLICATION APPROACHES

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ABSTRACT

Biological control, particularly by entomopathogenic fungi is important for reducing the population density of pests in Integrated Pest Management (IPM) programs. The compatibility of entomopathogenic fungi with crop production techniques such as the use of insecticides is needed to understand, which may inhibit to a smaller or larger extent the development and reproduction of pathogen. The efficacy of microbial control agent could be enhanced by applying them in conjunction with reduced rates of insecticides. The interaction between these control agents could be additive, synergistic or antagonistic. Synergistic interactions would enhance the effectiveness of the microbial control agent while reducing the adverse effects of pesticides. In this review, we will describe the compatibility of entomopathogenic fungus, *Beauveria bassiana* and botanical insecticide, neem.

Key words: Neem, *Azadirachta indica*, entomopathogenic fungi, *Beauveria bassiana*, compatibility, IPM.

INTRODUCTION

The entomopathogenic fungus *Beauveria bassiana* (Hypocreales: Cordycipitaceae) (Balsamo) Vuillemin is exploited in greenhouse and outdoor crops as a tool for the control of many agricultural pest arthropods, including whiteflies, aphids, thrips, psyllids, weevils, and mealybugs (Shah and Goettel, 1999). It is a common, soil-borne entomopathogenic fungus that occur worldwide (Fuxa and Kunimi, 1997). The primary reasons for interest in this fungus include its portal of entry, which is by contact instead of ingestion (Fuxa, 1987), its wide host range, replication in target insects (Ferron, 1978; Roberts and Humber, 1981), safety to non-target organisms (Hokkanen and Lynch, 1995), in vitro mass-culture (Jackson *et al*., 2000), and the availability of abundant strains (St. Leger *et al*., 1992). Neem, a steroid-like triterpenoid, is derived from the neem tree, *Azadirachta indica* A. Juss (Sapindales: Meliaceae). It can have many effects on susceptible insects, including repellency, moulting disruption, growth reduction, interference with development and oviposition, and high mortality, particularly in immature insects, as documented for a wide group of phytophagous insects (Liu and Stansly, 1995; Mitchell *et al*., 2004). It is widely used around the world today either as a stand-alone treatment (Nadia *et al*., 1996; Kumar *et al*., 2005; Kumar and Poehling, 2006) or in conjunction with synthetic pesticides or entomopathogens (Depieri *et al*., 2005; Filotas *et al*., 2005; Mohan *et al*., 2007; Islam, 2009; Islam *et al*., 2010a, b).

An alternative eco-friendly strategy for the management of noxious insect pests has been searched to reduce harmful effects of chemical insecticides on humanity. The appropriate use of environment-friendly microbial pesticides can play a significant role in sustainable crop production by providing a stable pest management program. Because, biological control is generally perceived as providing both long-lasting insect control and having less potential for damage to the environment or non-target organisms than chemical interventions (Grace, 1997; Hokkanen and Lynch, 1995; Howarth, 1991; Khetan, 2001). There is worldwide interest in the use of entomopathogenic fungi as biological control agents, and a significant advance in development and manufacturing of these agents in the future is expected with recent biotechnological innovations (Khachatourians, 1986). In IPM, biological control with entomopathogens represents a potentially important reduction factor of pest population density. Entomopathogenic fungi are ideal for IPM programs because they are relatively safe to use and have a narrower spectrum of activity than chemical insecticides (Lacey and Goettel, 1995). Microbial organisms such as *B. bassiana* are sustainable in IPM programs through their dynamic relationship with insects. In some cases, compatible products may be associated with entomopathogenic fungi, increasing the control efficiency, decreasing the amount of insecticides required and minimizing the risks of environmental contamination and pest's resistance expression (Moino and Alves, 1998; Quintela and McCoy, 1998). When an IPM strategy is devised, it is important to take into account the compatibility of products sprayed on the crop, avoiding the use of the most toxic, or using them during seasons when the effect over a natural control agent is minimized. Therefore, the toxic effect impact on the control agent will be smaller, contributing indirectly to control the host pest-insect and, consequently, to reduce damage in the cultivated field.

The integration of microbial pesticides with chemical pest management practices requires detailed compatibility studies. Data from such studies would enable farmers to select appropriate compounds and schedule microbial and chemical pesticide treatments such that benefits from compatible sets can be accrued and, with noncompatible pairs, the deleterious effect of the chemical on the microbe in the biopesticide can be minimized. From our detailed study concerning the compatibility of *B. bassiana* with neem would help to choose the optimal combination to improve IPM efficiency and to achieve a higher level of reliability and sustainability in pest management. For better understanding, the compatibility of these two products has been described by two major categories- *in vitro* and *in vivo*.

***In vitro* compatibility of *Beauveria bassiana* with neem:** Some highly compatible strains of *B. bassiana* with neem are presented in the Table 1. Conidial germination and hyphal growth are temporally separated, physiologically different stages. Neem can affect these two events in a different way. The emulsible neem oil inhibits mycelia growth and the production and germination of spores of *B. bassiana* (Bajan et al., 1998). There is no fungitoxic effects caused by emulsible oil or by neem seed extract in concentrations above 2.5% (Rodriguez-Lagunes et al., 1997). However, the *in vitro* compatibility of *B. bassiana* with neem is described below.

Effect of neem on germination percentage of *Beauveria bassiana*: The compatibility between the plant protection product and germination of entomopathogenic fungus is necessary, because, insects become infected by means of spore germination, by ingestion or contact (Malo, 1993). The germination percentage of *B. bassiana* is slightly affected (reduction percentage is not more than 12%) by various neem concentrations (Islam *et al*., 2010b). There is no significant effect on conidial germination in the presence of neem in the *B. bassiana* isolates (Mohan *et al*., 2007). The concentrations of emulsible neem oil bellow 5% does not cause significant fungitoxicity effects; and also no significant inhibition in germination of *B. bassiana* due to aqueous neem seed extract at 5% (Rodrígues-Lagunes *et al*., 1997). The significant inhibiting effect on germination of *B. bassiana* spores, caused by the commercial formulation of neem leaves, in concentrations those are equal and greater than 5% a.i. (Castiglioni *et al*., 2003).

Effect of neem on vegetative growth and sporulation of *Beauveria bassiana*: The growth of *B. bassiana* is also slightly affected by various neem concentrations as measured by colony diameter as well as conidiogenesis (Islam *et al*., 2010b). The concentrations of emulsible neem oil bellow 5% does not cause significant fungitoxicity effects; and also no significant inhibition in vegetative growth of *B. bassiana* due to aqueous neem seed extract at 5% (Rodrígues-Lagunes *et al*., 1997). The significant inhibiting effect on vegetative growth and conidiogenesis of *B. bassiana* spores, caused by the commercial formulation of neem leaves; in concentrations those are also equal and greater than 5% a.i. (Castiglioni *et al*., 2003). The concentration of seed aqueous extract at 1% is enough to cause significant inhibition of mycelia growth and conidiogenesis of *B. bassiana*, with greater reductions among the highest concentrations. Incorporation of the seed aqueous extract to the culture medium, however, does not affect the viability of spores produce in none of the concentrations (Depieri *et al*., 2005). Variation in concentration of components with possible fungitoxic activity in neem seeds (Sidhu *et al*., 2004) might explain the smaller negative effect of the seed aqueous extract on the fungus, considering the difference in concentration of azadirachtin in seeds of different origins (Devaranavadagi *et al*., 2003; Ermel *et al*., 2002). Inhibition of vegetative growth might be a less representative indication of fungitoxicity than the viability of spores or the effect on germination (Loria *et al*., 1983). The mycelial growth is significantly reduced in plates inoculated with Agroneem® at 0.5 and 1.0 times the field application rate compared to the control and 0.1 application rate (Al-mazra'awi *et al*., 2009). The enhancing effect of some pesticide formulations on growth is due to the adjuvants in the formulation (Anderson *et al*., 1989). Adjuvants act as mild abrasives and break up conidial agglomerations, which increase number of propagules, thereby promoting better growth. The colonies in culture media containing seed aqueous extract have their vegetative growth and conidiogenesis significantly reduces as compared with the control trial.

Effect of neem on biomass production and enzyme activity of *Beauveria bassiana*: Only morphological parameter like germination, vegetative growth and sporulation is not enough to test the compatibility between the entomopathogenic fungus and chemical pesticide. The most important physiological parameter like enzymatic activity of entomopathogenic fungus is needed to evaluate for confirmation the compatibility between the factors. But the information concerning the compatibility of *B. bassiana* with neem in relation to enzymatic activity and biomass production of *B. bassiana* is very limited. However, the biomass production and enzymatic activity of *B. bassiana* is slightly affected by various neem concentrations. The neem treatment reduces biomass production and proteolytic activity of *B. bassiana* (Islam *et al*., 2010b). The mycelial dry weight of *B. bassiana* is also reduced by the neemgold treatment (Sahayaraj *et al*., 2011).

***In vivo* compatibility of *Beauveria bassiana* with neem:** The compatibility between entomopathogenic fungus and chemical products for IPM system needs to be tested individually using the crop plants on which they will be applied. The optimum combined use of fungi and chemicals for pest control may require sequential rather than simultaneous applications. The *in vivo* compatibility of *B. bassiana* with neem against different insects is described below.

Combined effect of *Beauveria bassiana* with neem on deterrence index (DI) of insect: The deterrence effect of *B. bassiana* and neem has been evaluated against *B. tabaci* by two application approaches on eggplants. In the topical of *B. bassiana* and drenching application of neem tree extract, the treated insect exhibit deterrency higher than using each control agent alone and the two control agents interact synergistically at sub-lethal doses of neem. The deterrence index (DI) is significantly affected by the combined treatment of neem and *B. bassiana*; and it indicates that combined treatment by *B. bassiana* and neem significantly reduces the number of adults and oviposition rates of whitefly on eggplants. Reduced oviposition is a normal consequence if adults avoid setting on a host plant. Overall, the deterrence test clearly demonstrates that adults will preferentially feed on non-treated plants when surrounding plants have been treated with neem and or *B. bassiana*. The overall reduction in egg deposition of *B. tabaci* seems mainly related to the deterrence effect of neem as well *B. bassiana* (Islam *et al*., 2010b).

Table 1. Some highly compatible strains of *Beauveria bassiana* with the different sources of neem, *Azadirachta indica*.

Strain of *B. bassiana*	Origin	Type of neem	Origin	Reference
Unknown	Tamil Nadu, India	Neemgold (0.5%)	Shri Disha Biotect. Ltd., Hydrabad, India	Sahayaraj et al., 2011
GHA	Laverlam International Corp., Butte, MT	Agroneem® (0.15%)	Ajay Bio-tech LTD, Pune, India	Al- mazra'awi et al., 2009
Bb 62	Institute of Guangdong Forestry, Guangzhou, PR China	Azadirachtin EC (0.3% a. i.)	Yunnan Zhongke Bio-industry Kunming, PR China.	Islam et al., 2010 a
ITCC 4688	Hyderbad, India	Margoside® CK 20 EC (0.15% a. i.)	M/s Monofix Agroproducts Ltd, Hubli, India	Mohan et. al. 2007
Unknown	Londrina, PR	Emulsible Dalneem® (0.5%, 1% and 1.5%)	IAPAR experimental fields in Paranavaí, PR	Depieri et al., 2005
CG 252	Embrapa - Cenargen	Neem oil (2%)	-	Hirose et al., 2001

Combined effect of *Beauveria bassiana* with neem on virulence of insect in case of individual application approach: A combined formulation or application of fungal candidate with chemical pesticides may enhance efficacies of both products and effective for the control of sucking pest (Feng *et al*., 2004). The combined effect of *B. bassiana* and neem produces a more rapid mortality response in *B. tabaci* nymphs as indicates by a LT50 value. The combination of *B. bassiana* with neem shows in more nymphal mortality of *B. tabaci* than individual treatments of *B. bassiana* and neem (Islam *et al*., 2010a). In the bioassays on *Spodoptera litura*, combined treatment with neem compatible *B. bassiana* isolate and neem have synergistic effect on mortality. Combination treatment with neem and *B. bassiana* shows in higher mortality and lower LT_{50} and LC_{50} values than single treatments with either of them alone (Mohan *et al*., 2007). The overall interaction of *B. bassiana* and neem in combination treatment (as assessed from insect mortality) is synergistic with the neem tolerant isolate but antagonistic with neem sensitive isolates. The combination of *B. bassiana* with endosulfan is more toxic and performs in higher mortality against *Spilarctia obliqua* than endosulfan alone (Purwar and Sachan, 2006). The combination of insecticides with *B. bassiana* increases in virulence against *Spodoptera litura* (Fab.) over the sole treatment (Dayakar *et al*., 2000). Neemix 4.5 (4.5% azadirachtin) delays pupation and does not reduce the germination rate of *B. bassiana* conidia, but it significantly reduces the mortality of red flour beetle, *Tribolium castaneum* Herbst (Akbar *et al*., 2005).

Combined effect of *Beauveria bassiana* with neem on virulence of insect in case of two application approaches: The combined effect of *B. bassiana* and the neem tree extract has been evaluated against *Thrips tabaci* by two application approaches on potted tomato plants (Al-mazra'awi *et al*., 2009). In the topical of *B.*

bassiana and drenching application of neem tree extract, the treated insect exhibit mortalities higher than using each control agent alone and the two control agents interact synergistically at sub-lethal doses of the neem tree extract. The application method affects the interaction between *B. bassiana* and the neem tree extract, because, drenching neem tree extract while applying *B. bassiana* topically enhance the efficacy of the entomopathogen. The nymph mortality of *B. tabaci* is the highest for combined treatment of *B. bassiana* and neem, in case of the topical application of *B. bassiana* with drenching application of neem (Islam *et al*., 2011). Furthermore, using sub-lethal doses of neem tree extract with *B. bassiana* improve the effectiveness of the control process while reducing the amount of insecticide used.

Conclusions: From this review, we came to know that *B. bassiana* is slightly affected by neem, but the overall control programme of different insect pest with these two factors might be successful. Studies on the compatibility of *B. bassiana* with neem show also conflicting results. For some authors, emulsible neem oil inhibits mycelia growth and the production and germination of spores of *B. bassiana* (Bajan *et al*., 1998). Other authors do not report fungitoxic effects caused by emulsible oil or by neem seed extract in concentrations above 2.5% (Rodriguez-Lagunes *et al*., 1997). Therefore, to solve this contradiction, further research is needed to investigate the effect of neem on enzymatic activity of *B. bassiana* that would help to make sure the compatibility between these two factors.

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