



## Potential of entomopathogenic fungi in insecticide resistance management (IRM): A review

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### ABSTRACT

Insect pests have remarkable capacity to develop resistance to insecticides. More than 600 species of plant feeding insect pests have developed resistance to insecticides. Management of insecticides resistance offers great promise as a complementary extension of integrated pest management (IPM). Insecticide resistance management (IRM) attempts to prevent or delay the development of resistance. A revival of interest in the use of microbial agents, especially entomopathogenic fungi in combination with sublethal doses of insecticide is increasing in insecticide resistance management. Integration of selected strain entomopathogenic fungi with selective insecticides can improve the control efficiency, besides decrease the amount of insecticides required, minimize the risks of environmental contamination and delay the expression of insecticide resistance in insect pests. Co-application of fungi like *Beauveria bassiana*, *Metarhizium anisopliae*, *Nomuraea rileyi*, *Paecilomyces* spp., *Lecanicillium* (= *Verticillium*) *lecanii* and *Hirsutella thompsonii* with suitable sublethal concentration of selective insecticide as two-in-one tank mix have been successfully employed against various insect pests to reduce the selection pressure insecticides and to avoid concurrent resistance risks in target pests. Majority of mechanisms of resistance occur through induction of enzymes especially mono-oxygenases and to some extent the esterases. Entomopathogenic fungi have ability to induce high degree of susceptibility to insecticides in target pests by suppressing enzyme activities and predispose them for fungal infection. This review outlines the current state of knowledge on the development of insecticide resistance in insect pests and discusses strategies for manipulation of certain important fungal entomopathogens as potential microbial tool in the insecticide resistance management programme for sustainable pest management systems.

**Key words:** Entomopathogenic fungi, insecticide resistance management

### INTRODUCTION

One of the practical means of increasing crop production is to minimize the pest-associated losses, which are estimated at 14% of the total agricultural production. The actual losses have been estimated at 51% in rice, 37% in wheat, 38% maize, 41% in potato, 38% in cotton, 32% in soybean, 32% in barley, and 29% in coffee (Sharma *et al.*, 2001).

Without pesticides, an estimated two-thirds of all crops would be lost, depriving food for millions of people (Deedat, 1994). About 50% of all pesticides produced are used for the protection of agricultural commodities. In India, an array of insecticides based on chlorinated hydrocarbons, organophosphates and carbamates have been successfully used against agricultural pests. Nearly 54% of the total pesticides produced in the whole country are applied to merely 5% of the production area (Sundaramurthy and Gahukar, 1998). Crop-wise market share of pesticide usage in India indicates highest use

pattern of 45% in cotton followed by 22% in rice, 9% in vegetables, 7% in plantations, 4% each in wheat and pulses, and 9% constituting other crops (David, 2008). As a consequence of heavy reliance on insecticides, a number of ecological complications including development of insecticide resistance in insect pests have surfaced in diverse agro-ecosystems. Resistance develops in all agricultural pest groups—insects, mites, fungi, bacteria, nematodes and weeds.

Insecticide resistance is a measurement of an insect's ability to tolerate the toxic effects of particular insecticide, resulting in repeated failure of the product to achieve an effective level of control when used according to the label recommendations (Luckmann and Metcalf, 1982). Perusal of literature indicates that more than 600 species of injurious insects have developed resistance to different group of insecticides (Sharma *et al.*, 2001). An individual insect does not become resistant but, through multiple applications of an insecticide over multiple generations

of the pest, susceptible individuals are removed from the population and resistant individuals remain to reproduce, resulting in flare up of insect populations that can no longer be controlled with that insecticide (Riley and Sparks, 2006). As modern agriculture moves to adopt more environmentally benign practices, there is a resurgence of interest to search for biologically compatible forms of pest control options to abate resistance to insecticides in insect pests of crops. In all pest control programme where insecticides are to be used, insecticides management must be paramount importance to: (i) preserve ecological balance, (ii) minimize adverse effects on non-target organisms, and (iii) ensure human safety and health (Deedat, 1994). In this context, the use of microbial agents in combination with synthetic insecticides would be an ideal option for reducing insecticide induced resistance in insects. This review outlines the current state of knowledge on the development of insecticide resistance in insect pests and discusses strategies for manipulation of certain important fungal entomopathogens as potential microbial tool in the insecticide resistance management programme for sustainable pest management systems.

#### RESISTANCE DEVELOPMENT IN INSECT PESTS

Resistance development in arthropods is effected by the frequency of application, rate or dose of pesticide and certain pest characteristics (Regupathy, 1995). Arthropods like mites, aphids, whiteflies, and thrips have similar traits that contribute to resistance development, such as having many generations per year, exposure of multiple generations to a pesticide, having a lot of offspring, limited dispersal, and exposure to sublethal pesticide doses (Metcalf, 1982). Before exposure to a new insecticide, resistant individuals are rare. Repeated applications of the same insecticide or of insecticides with common mode of action provide an insect pest population a chance to develop resistance (Georghiou and Taylor, 1986). Pesticides are designed to kill pest populations, but they are seldom 100% effective; a few individuals usually survive which may have a behavioral trait that helps them avoid the pesticide (or) a biochemical trait that allows them to detoxify the pesticide (or) some other genetic characteristic that reduces their susceptibility to the pesticide (Kranthi *et al.*, 1997). If these survivors mate and pass on this resistance to their offspring, then subsequent generations will contain fewer susceptible individuals; eventually, the entire population may become resistant.

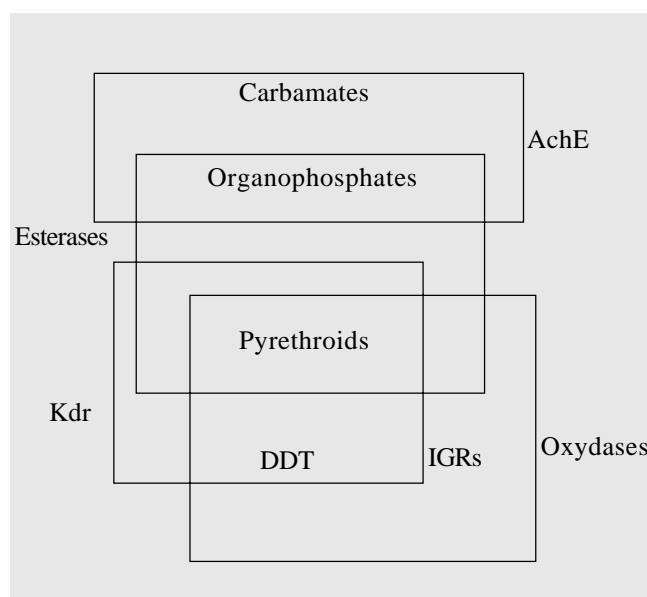
#### Resistance Mechanisms

Insects and mites acquire resistance via metabolic, physical, physiological, behavioral and biochemical

mechanisms (Soderhall and Smith, 1986). Insecticide resistance is the inherited ability of an individual insect to survive exposure to a concentration of insecticide that is lethal to other individuals that lack this gene (Brogdon and McAllister, 1998). An individual insect inherits this resistance gene from its parents. Most of the insect and mite pests have very short generation times, reproducing rapidly with high birth rates (Ahmad *et al.*, 1999), and many overlapping generations can occur during a growing season when abundant food sources are available in any cropped ecosystems.

#### Types of Insecticide Resistance

Insect population resistance to one pesticide may also prove to be resistant to similar compounds that have the same mode of action; this is termed as ‘class-resistance’ which occurs in pest populations that develop resistance either to organophosphates (or) carbamates (or) pyrethroids. Some insect population develops resistance to more than one pesticide within a chemical family (e.g., organophosphates, carbamates, synthetic pyrethroids etc.), which is known as ‘cross resistance’ (Fig.1). Cross resistance occurs when resistance to one insecticide confers resistance to another insecticide, even where the insect has not been exposed to the latter product (Zhang *et al.*, 2000). Cross resistance may produce an insect population that can no longer be controlled with any of these chemical insecticides.



**Fig. 1.** Cross-resistance relationships of commonly used classes of insecticides

Some insects acquire 'multiple resistance' which involves multiple, independent resistance mechanisms, leading to resistance to chemicals from different families (e.g. organophosphates, carbamates and synthetic pyrethroids). Tolerance is the state of physiological and/or behavioral adaptations leading to increased survivorship relative to some toxicity baseline.

#### Insect pests acquired resistance to insecticides in India

Many species of insect pests have shown resistance to currently available insecticides belonging to different chemical groups (Georghiou and Taylor, 1986). About 645 cases of resistance have been documented until 1996 (Sharma *et al.*, 2001). Populations of lepidopterous worms, leafhoppers, planthoppers, aphids, whiteflies, mealy bugs, thrips and mites have developed resistance to organophosphates, carbamates and synthetic pyrethroids (Regupathy and Paramjothy, 1977). Maximum reports of resistance development pertain to organophosphates (250), followed by synthetic pyrethroids (156), carbamates (154) and others (85) including chlorinated hydrocarbons (Sharma *et al.*, 2001). Resistance may develop rapidly in one species, more slowly in another and not at all in a third organism. Many species of plant feeding insects

have developed resistance to more than two groups of insecticides (Sharma *et al.*, 2001). In India, there are several documented cases of development of resistance in insects to insecticides; the following are major example (Table 1). Maximum number of insects showing resistance to insecticides has been reported on cotton, vegetables, tobacco followed by fruit crops, cereals and ornamentals (Regupathy, 1995). The cotton bollworm (*Helicoverpa armigera*), which is the most severe pest on cotton has shown resistance to several groups of insecticides in cotton, tobacco, vegetables, chillies, sunflower, groundnut, pigeonpea and chickpea (Ahmad *et al.*, 1995; Kranthi, *et al.*, 1997). This has resulted in widespread failure of insect control causing extreme debts, at times even forcing the framers to commit suicide (Sharma *et al.*, 2001). The tobacco caterpillar (*Spodoptera litura*) has been found to be resistant to insecticides on cotton, cauliflower, groundnut and tobacco (Regupathy, 1995). In several cruciferous crops, the diamond back moth (*Plutella xylostella*) has shown significant resistance to almost every insecticide applied in the field (Sarfraz and Keddie, 2005). The cotton whitefly (*Bemisia tabacci*) has shown resistance to insecticides in cotton, brinjal and okra. Green peach and potato aphid (*Myzus persicae*), cotton aphid

**Table 1.** Examples of insect pests developed resistance to insecticides in India

Order	Insect Pests		Possessing resistance to			
	Common Name	Scientific Name	OP	Car	Pyr	Chc
Lepidoptera	Rice leaf folder	<i>Cnaphalocrocis medinalis</i>	x	-	x	-
	Rice leaf folder	<i>Marasmia patnalis</i>	x	-	x	-
	Cotton boll worm	<i>Helicoverpa armigera</i>	x	x	x	x
	Tobacco caterpillar	<i>Spodoptera litura</i>	x	x	-	-
	Diamond back moth	<i>Plutella xylostella</i>	x	x	-	x
	Brinjal fruit borer	<i>Leucinods arbonalis</i>	x	-	-	-
Homoptera	Cotton jassid	<i>Amrasca bigutula</i>	x	-	-	-
	Cotton jassid	<i>Amrasca devastans</i>	x	-	-	-
	Cotton aphid	<i>Aphis gossypii</i>	x	-	-	-
	Cotton white fly	<i>Bemisia tabaci</i>	x	-	-	-
	Mustard aphid	<i>Lipaphis erysimi</i>	x	x	-	-
	Potato aphid	<i>Myzus persicae</i>	x	-	-	-
	Tobacco aphid	<i>Myzus nicotianae</i>	x	x	-	-
Thysanoptera	Tobacco thrips	<i>Thrips tabaci</i>	x	-	x	-
Coleoptera	Singhara beetle	<i>Galerucella birmanica</i>	x	-	-	-

OP = Organophosphorus; Car = Carbamates; Pyr = Pyrethroids; Chc = Chlorinated hydrocarbon

(*Aphis gossypii*) and mustard aphid (*Lipaphis erysimi*) have also been found to exhibit resistance to insecticides in several crops.

#### **Need for developing alternate control strategies**

Development of resistance to insecticides has necessitated the application of higher dosages of the same pesticide or increased number of pesticide applications. The farmers often resort to insecticide mixtures to minimize the insect damage to crops, which leaves harmful residues in the food and causes adverse effects on associated natural enemies. This has resulted in widespread failure of insect control causing extreme debts, at times even forcing the farmers to commit suicide (Sharma *et al.*, 2001). Massive application of pesticides not only increases the cost of pest control, but also results in insecticidal hazards and pollution of the environment. It is in this context developing alternate strategies is important to obviate insecticide resistance problems.

#### **STRATEGIES FOR INSECTICIDE RESISTANCE MANAGEMENT**

Management of insecticide resistance offers great promise as a complementary extension of integrated pest management (IPM), and it must be attacked in a variety of ways that could prevent or delay the development of resistance (Regupathy, 1995). Traditionally used resistance management strategies are can be grouped as: (1) management by moderation, (2) rotation and mixtures, and (3) saturation. These strategies, however, are often difficult to follow, especially in high-value commercial crops like fruits, nuts and beverages, where high quality standards and limited numbers of registered pesticides make the task more challenging. Integrated pest management combining cultural, physical, mechanical, chemical and microbial methods are desired long-term philosophy (Pedigo, 2002) to slash-down the number of insecticide applications needed, and reduce the gravity of selection pressure placed on insect populations. The use of microbial pest control agents especially, entomopathogens and their products are considered as an important component of pest management (Jayaraj *et al.*, 1985). One of the most promising aspects of microbial control of insects is its integration with other pest control measures, particularly the chemical method (Laird, 1962; Bajan *et al.*, 1977). Among several entomopathogens, fungi offer promise in microbial control of a wide range of economic crop pests (Agarwal, 1990; Ambethgar, 2001). Selective insecticides can be associated with candidate entomopathogenic fungi to increase the efficiency of pest control, and to reduce the use of required insecticides

(Balikai and Sattigi, 2000). Integration of compatible insecticide-fungal combinations can improve the control efficiency, besides decrease the amount of insecticides required, minimize the risks of environmental contamination and delay the expression of insecticide resistance in insect pests.

#### **POTENTIAL BENEFITS OF ENTOMOPATHOGENIC FUNGI**

Entomopathogenic fungi are important natural regulators of insect populations and have potential as mycoinsecticide agents against diverse insect pests in agriculture. These fungi infect their hosts by penetrating through the cuticle, gaining access to the hemolymph, producing toxins, and grow by utilizing nutrients present in the haemocoel to avoid insect immune responses (Hajek and St. Leger, 1994). Entomopathogenic fungi may be applied in the form of conidia or mycelium which sporulates after application. The use of fungal entomopathogens as alternative to insecticide or combined application of insecticide with fungal entomopathogens could be very useful for insecticide resistant management (Hoy, 1999). Entomopathogenic fungi in combination with low doses of insecticides are useful to abate insecticide resistance and non-selectivity problems in pest control (Ramaraje Urs *et al.*, 1967). Since small arthropod pests are known to easily develop resistance to chemical insecticides, interest in utilization of entomopathogenic fungi against them is increasing worldwide.

Steinhaus (1958) outlined the compatibility of pathogens and insecticides as one of the advantages of microbial pest control. Franz (1961) provided an excellent review on several fungal entomopathogens in combination with sublethal doses of insecticides against important insect pests. The principles underlying the integration of microbial pathogens with insecticides and use of specific adjuvants admitting the maximum effectiveness of the pathogen have been discussed (Laird, 1962; Hall, 1963). Hall (1963) described the possibility of two materials assisting each other, the pathogen making the pest sick enough to become less resistant to the chemical and the chemical in turn weakening the pest sufficiently to make it more susceptible for the infection by the pathogen. Fargues (1975) demonstrated that the effects of fungal-insecticidal combinations are additive and delay the expression of insecticide resistance in insect pests. In addition to the reduction in insecticide resistance in insects, the joint application of insecticides and fungi will also lead to: (i) a major reduction in pesticidal usage; (ii) reduced exposure of non-target organisms to pesticides; (iii) increased activities of natural enemies; (iv) reduced

amounts of pesticide residues in food; and (v) a safer environment to live.

### INSECT IMMUNE REACTIONS TO FUNGAL INFECTION

Insect immune responses are one of the potential factors involved in the inability of a fungus to infect the nonpermissive host insects (Bidochka and Khachatourian, 1988). The insect immune reaction against entomopathogenic fungi is activated in two phases (Bidochka and Hajek, 1998; Wago, 1995). The first is the cellular response in which insect hemocytes react to a foreign body by degranulation, phagocytosis, and/or encapsulation (Gotz and Boman, 1985; Gunnarsson and Lackie, 1985; Wheeler *et al.*, 1993). With reference to fungi, targets for hemocytic encapsulation may be identified by certain structural components of the fungal cell wall, such as chitin and  $\beta$ -1,3, glucan (Beauvais *et al.*, 1989). The second response is the adaptive humoral or noncellular immunity in which certain proteins are induced in response to a “non-self” elicitor or the activation of an immune protein. Majority of mechanisms of resistance to insecticides occur through induction of enzymes especially mono-oxygenases and to some extent the esterases (Soderhall and Smith, 1986). Fungi have ability to induce the susceptibility of target host to the insecticides by suppressing enzyme activities and predispose the pests to pathogenic infection (Kanost *et al.*, 1990). Stress effects depend on sublethal dosages of insecticide may alter the immune reaction including hemocytes and humoral defense.

### THE CONCEPT OF JOINT ACTION

The term ‘joint application’ is used as a total effect of two or more types of insecticides applied together in mixture or simultaneously. When two kinds of agents are used together to obtain joint action, sometimes one agent may act as a stress inducer to the target insects and the other agent as the major control agent (Hurpin and Robert, 1968; Thurston *et al.*, 1993). Joint action has been investigated between the biological control agents as arthropod natural enemies and microbial agents and chemical pesticides. Information on the effect of agricultural chemicals on the microbial insecticides is gradually gathering. Data published on the interactions of microbials-chemical pesticides, prior to 1976 have been summarized by Roberts and Campbell (1977), who concluded that susceptibility varied between pesticides and fungal isolates. Most pesticides-pathogens compatibility studies have been used solid or liquid medium containing various concentrations of pesticides and employed mycelial

growth as criterion for inhibition status (Hall, 1963; Roberts and Campbell, 1977; Gardner *et al.*, 1979; Clark *et al.*, 1982), and consequently extrapolation to the field is very difficult. Fungal entomopathogens, especially mitosporic hyphomycetes can be used as an important component in integrated pest management either alone or in combination with reduced amounts of insecticides. Mycoinsecticides containing viable conidia and sublethal 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 interspecific economic synergism (Benz, 1971). Fargues (1975) demonstrated that the effects of the components in these combinations are additive. Suffice to say that pesticides that are compatible under laboratory conditions will be unlikely to be harmful in the field conditions.

### Interactions of insecticides and entomopathogenic fungi

Many researchers have examined the factors which influence the synergism (Anderson *et al.*, 1989; Hassan and Charnley, 1989; Boucias *et al.*, 1996; Kaakeh *et al.*, 1997; Quintela and McCoy, 1997, 1998a). Use of chemicals as ‘stressors’ to enhance the efficacy of insect pathogens has been proposed earlier (Anderson *et al.*, 1989; Quintela and McCoy, 1998b). In 1982, Walter M. Zeck, a member of the Bayer Research Group, Vero Beach, Florida, discovered that sublethal doses of a number of insecticidal nitroguanidine compounds including imidacloprid increased the susceptibility of subterranean termites to various opportunistic fungi (Quintela and McCoy, 1998b). Insecticides may synergize or antagonize diseases in insects, and hence they may be regarded as epizootiologically relevant factors (Jacobson *et al.*, 2001). Chemical insecticides, botanicals, insect growth regulators and mineral oils at sub-normal doses have been combined with entomofungi for obtaining enhanced control of certain insect pests. For instance, increased mortality of *Melolontha melolontha* L. grubs due to mycosis of *Beauveria tenella* Sacc. (McLeod) had been achieved by addition of reduced doses of insecticides (Ferron, 1971). Quintela and McCoy (1997) indicated that reduced larval mobility and associated conidial avoidance was the basis of synergism of imidacloprid with both *Metarhizium anisopliae* Sorok and *B. bassiana* treated larvae of root weevil *Diaprepes abbreviatus* Linnaeus. Conidial survival can be effected by interaction with agrochemicals, environmental factor or by bio-pesticide and/or chemical product 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 works on joint action of microbials-synthetic insecticides indicate that fungal sporulation is synergized at sub-normal 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.

#### Factors influencing joint action

Many factors may be responsible for the differences observed between laboratory and field experiments. 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. Mietkiewski *et al.* (1997) have opined 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 microorganisms in soils which affect the entomopathogenic fungi indirectly. Certain key biotic and abiotic factors influencing the joint action of fungi-insecticides include type of formulations, carrier materials, emulsifying agents, dosage, soil types and condition of host plants. These factors in combination with variable climate and soil conditions are difficult to separate. Long term field experiments, complimented by parallel laboratory studies are needed to evaluate the ecological impact of pesticides on entomopathogenic fungi. 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.

#### CASE STUDIES ON IRM USING ENTOMOPATHOGENIC FUNGI

Entomopathogenic fungi such as *Beauveria bassiana*, *Metarhizium anisopliae*, *Nomuraea rileyi*, *Paecilomyces* spp. and *Lecanicillium lecanii* have been used solely or in combination with various insecticides as a part of insecticide resistant management against important crop pests in diverse agroecosystems. This review illustrates some selected examples of case studies on the effective

utilization of entomopathogenic fungi in insecticide resistance management programme.

#### *Beauveria bassiana* (Balsamo) Vuillemin

The commercial mycoinsecticide 'Boverin' based on *B. bassiana* with reduced doses of trichlorophon have been used to suppress the second-generation outbreaks of *Cydia pomonella* L. (Ferron, 1971). Foschi and Grassi (1985) found that addition of subnormal doses of chlorpyrifos (0.9 kg ha<sup>-1</sup>) to the fungal sprays of *B. bassiana* increased mortality of the European corn borer, *Ostrinia nubilalis* Hub. When carbofuran was added to plants treated with *B. bassiana*, increased mortality of *O. nubilalis* witnessed (Lewis *et al.*, 1996). Anderson *et al.* (1989) detected higher insect mortality when *B. bassiana* and sublethal concentrations of insecticides were applied to control Colorado potato beetle (*Leptinotarsa decemlineata*), attributing higher rates of synergism between two agents. The practice of combining *B. bassiana*- insecticides has been employed routinely for control of Colorado potato beetle in the USSR (Anderson *et al.*, 1989). Enhanced effects of *B. bassiana* were also observed in combination with imidacloprid against *L. decemlineata* (Steinhaus, 1996). Boucias *et al.* (1996) showed that the synergistic effect of *B. bassiana* and imidacloprid on the termite *Reticulitermes flavipes*, and caused an altered behavior that could be disrupted with sublethal dosages of imidacloprid. Studdert and Kaya (1990) and Krueger *et al.* (1991) found that more chinch bugs, *Blissus leucopterus leucopterus* Say and soybean caterpillars, *Spodoptera exegua* Hubner readily infected by *B. bassiana* when exposed to drier soils compared with wetter soils containing residues of insecticides. Mietkiewski *et al.* (1997) observed spectacular mortality of *Galleria mellonella* larvae occurred due to *B. bassiana* where triademefon had been applied to soils. Steinkraus and Tugwell (1997) showed higher mortality of tarnished plant bug, *Lygus lineolaris* with the combination of Mycotrol WP (based on *B. bassiana*) and imidacloprid. Quintela and McCoy (1998b) demonstrated that the synergistic effects among *B. bassiana*, *M. anisopliae* and imidacloprid to *Diaprepes abbreviatus*, resulted in an insecticidal effect on the behavior of *D. abbreviatus*. Synergistic interactions of imidacloprid with fungal agents in insect control have been demonstrated previously (Kaakeh *et al.*, 1997; Gardner and Kinard, 1998; Quintela and McCoy, 1998a; Lacey *et al.*, 1999; Ramakrishnan *et al.*, 1999; Furlong and Groden, 2001; Gupta *et al.*, 2002; Ying *et al.*, 2003). Quintela and McCoy (1998b) demonstrated the synergistic effects of imidacloprid on *B. bassiana*-treated larvae of a soil inhabiting root

weevil, *Diaprepes abbreviatus*. Larval mobility both in and out of the soil was inhibited due to temporary muscular paralysis caused by imidacloprid. The loss of larval mobility would have interfered with normal conidial avoidance behaviour accomplished by larvae when moving on or within a substrate. According to James and Elzen (2001), imidacloprid had no negative effect on *B. bassiana*. Furlong and Groden (2001) investigated the interaction between the fungal entomopathogen *B. bassiana* and sublethal doses of imidacloprid and cyromazine when applied to larvae of the Colorado potato beetle under *in vitro* conditions. When second instar larvae were fed potato leaf discs treated with sublethal doses of imidacloprid with differential doses of *B. bassiana*, a synergistic action was demonstrated. Sublethal doses of both imidacloprid and the triazine insect growth regulator (IGR) cyromazine prolonged the duration of the second instar, and only imidacloprid interacted with *B. bassiana* produced a synergistic response in larval mortality (Furlong and Groden, 2001). Starvation of larvae for 24h immediately after combined treatment of *B. bassiana* and imidacloprid increased the level of mycosis when compared with control. Jayanthi and Padmavathamma (2001) studied combined effects of fenvalerate and monocrotophos against *Diaprepes abbreviatus* with enhanced mortality. Inclusion of imidacloprid in *B. bassiana* treatments increased the mortality of *Bemisia argentifolii* on cucumbers and cantaloupe melons (James and Elzen, 2001). Dayakar *et al.* (2002) observed synergic effects of fenvalerate, cypermethrin, imidacloprid, quinalphos and endosulfan when combined with *B. bassiana* against *S. litura*, and carbofuran + *B. bassiana* against *Ostrinia nubilalis* Hubner. Thompson and Brandenburg (2006) reported that combination treatments using sublethal doses of imidacloprid and *B. bassiana* resulted for a reduced pesticide approach to mole cricket control. Synergistic interaction was detected when larvae were fed leaf discs treated with imidacloprid and sprayed with *B. bassiana* conidia 24h later. Imidacloprid and other neonicotinoids are also known synergists for *B. bassiana* against the white grub (*Popillia japonica*) larvae in the laboratory and greenhouse, but not in the field (Morales-Rodriguez and Peck, 2009).

Enhanced levels of mycosis with *B. bassiana* were observed in combination with sub-lethal doses of chlorpyrifos against *Cnaphalocrocis medinalis* larvae (Ambethgar *et al.*, 2009). The use of alpha-cypermethrin and thiamethoxam formulations in coffee IPM programs for a *B. bassiana* inoculum conservation strategy are recommended against coffee berry borer (*Hypothenemus*

*hampei*), since these products were compatible with the entomopathogenic fungus *B. bassiana* (CG 425 (Oliveria *et al.*, 2003). Feng *et al.*, (2004) found that *B. bassiana* tended to be more effective against the whitefly, *Trialeurodes vaporariorum* when applied together with the low rates of imidacloprid. Shi *et al.* (2005) illustrated the effects of certain acaricides- *B. bassiana* and their interactions on the hatch rate of spider mite eggs over post-spray days by modeling the time-concentration-hatch relationship to utilize interactions of both agents to mitigate pesticidal resistance in spider mites. Imidacloprid also significantly increased the susceptibility of leaf cutting ant (*Atta sexdens rubropilosa*) to infection by *B. bassiana* (Santos *et al.*, 2007). In all the cases, more desirable and faster control was resulted from fungal sprays containing low rates of imidacloprid, but none of the low imidacloprid rates tested alone suppress the target pests more effectively than any fungal treatments.

#### ***Metarhizium anisopliae* (Metchnikoff) Sorokin**

Instances of enhanced effectiveness of *M. anisopliae* were observed in combination with sublethal doses of certain insecticides (Barbosa and Moreira, 1982). Addition of subnormal doses of chlorpyrifos to the spore suspension of *M. anisopliae* increased the susceptibility of *Ostrinia nubilalis* (Riba *et al.*, 1983). Aguda *et al.* (1988) demonstrated enhanced sporulation and infectivity of three hyphomycetous fungi towards rice insects. Moorhouse *et al.* (1992) observed increased mortality of vine weevil (*Oliorynchus sulcatus*) due to combined application of *M. anisopliae* with recommended insecticide. Soil application of *M. anisopliae* and insecticide mixtures have been reported to increase the mortality of scarab beetle (*Anomala cuprea*) larvae (Hiromori and Nishigaki, 1998). Quintela and McCoy (1998b) demonstrated the synergistic effects of imidacloprid on *M. anisopliae*-treated larvae of a soil inhabiting root weevil, *Diaprepes abbreviatus*. When *M. anisopliae* is applied with insecticides into the soil, the insecticides may impose stress or pressure on the larvae. For example, organophosphates have been known to act as inhibitors of acetylcholine esterase, which would disturb and destroy the grubs defense system and immunity inside the body. Hiromori and Nishigaki (1998) proved that joint use of *M. anisopliae* and insecticides caused a faster spread of *M. anisopliae* infection against grubs of *Adoryphorus couloni*.

Three neonicotinoid insecticides: acetamiprid (Saurus 200 SP), imidacloprid (Confidor 700 WDGr) and thiamethoxam (Actara 250 WG) at average field recommendation rate (AR) and 0.7 time reduced rate to *B. bassiana*, *M. anisopliae* and *Paecilomyces* sp showed little effect

on conidia germination (Pedro *et al.*, 2001). Foliar sprays of *M. anisopliae* in combination with imidacloprid (Kaakeh *et al.*, 1997), chlorpyrifos,  $\beta$ -cyfluthrin, propetamphos (Pachamuthu and Kamble, 2000), boric acid (Zurek *et al.*, 2002) enhanced the mortality of *Blatella germanica* (Linn.). Combination sprays of *M. anisopliae* with fenvalerate, cypermethrin, imidacloprid, quinalphos and endosulfan against *S. litura* (Dayakar *et al.*, 2002) enhanced the virulence of *M. anisopliae*. According to Rachappa *et al.* (2007), imidacloprid and spinosad could be safely used combining with *M. anisopliae* to get enhanced effect, but only after repeated field confirmations. Imidacloprid was shown to be synergist for *M. anisopliae* against the burrower bug, *Cyrtomenus bergi* (Jaramillo *et al.*, 2005) and white grub (*Popillia japonica*) larvae in the laboratory and greenhouse, but not in the field (Morales-Rodriguez and Peck, 2009).

Positive interaction between the fungus and insect growth regulators may also occur, that would aid in reducing the resistance build up in certain insects. For example, synergism between *Metarhizium* spp and teflubenzuron, a benzoylphenyl urea insecticide that interferes with chitin synthesis, has been shown against the locust, *Schistocerca gregaria* (Joshi *et al.*, 1992). Dimilin, a chitin synthesis inhibitor containing the active ingredient diflubenzuron, makes infection by hyphomycete entomopathogens faster and easier. For example, Hassan and Charnley (1989) reported that cuticle of *Manduca sexta* treated with Dimilin exhibited diminished resistance to invasion by hyphae of *M. anisopliae*. In the presence of IGRS (Teflutenzuron, flufenoxuron) joint action was observed (Hiromori and Nishigaki, 1998). It is supposed that the grubs of *A. couloni* are easily infected by *M. anisopliae*, because such benzoil urea IGRs serve as inhibitors to biosynthesis of insect chitin. Hence, the scarab larvae surface defense system is probably weathered. In some cases, joint application of *M. anisopliae* (strain AC MA-5) and insecticides showed a faster infection of *M. anisopliae* to larvae than was seen for a single application of AC MA-5 (Hiromori and Nishigaki, 1998). This observation suggests that insecticides are effective for increasing the susceptibility of *A. cuprea* larvae to *M. anisopliae* by preventing from acquiring resistance to applied insecticides.

In subsequent studies, Hiromori and Nishigaki, (2001) observed reduced blood cells in the larval bodies of *Anomala cuprea* inoculated with *Metarhizium anisopliae* after topical application of synthetic insecticides. The phenoloxidase activity of larval hemolymph declined with time after the treatment of *M. anisopliae* and insecticides. One of the mechanisms of this synergistic effect is

attributed to be weakening of the immune system of *A. cuprea* larvae by insecticidal stress, which facilitates infection of *M. anisopliae* to larvae. Enhanced mortality due to the dual application of the fungus *M. anisopliae* and insecticides like teflubenzuron and diflubenzuron was also observed in desert locust, *Schistocerca gregaria* (Forsk.) (Seyoum, 2001). Alternatively, if the mode of action of the biorational can induce morphogenetic damage, preventing moulting and maturation, then successful invasion is more likely to occur (Butt and Brownbridge, 1997). Similarly, insecticidal soaps affect the waxy layers of the insect cuticle. These layers prevent desiccation and are important in the defense system; their disruption could predispose insects to fungal infection. Mineral oils can also kill insects by asphyxiation; but insecticidal oils have been used in the formulation of several fungal species, enhancing efficacy against the target host insect (Johnson *et al.*, 1992).

#### ***Nomuraea rileyi* (Farlow) Samson**

The fungus *Nomuraea rileyi* exhibits host preferential infections primarily in lepidopterous larvae. Ignoffo *et al.* (1975) initiated the work on sensitivity of *N. rileyi* conidia to chemical pesticides by *in vitro* paper disc technique. Field tests against, *Trichoplusia nii* in cabbage and *Heliothis zea* in sweet corn gave significant control of the pests but not the economic damage to the crop. *In vitro* evaluation of the effect of insecticides on the sporulation of *N. rileyi* showed that permethrin and diflubenzuron were compatible, trichlorfon reduced sporulation while profenophos and endosulfan totally inhibited sporulation (Silva *et al.*, 1993). Compatible antifeedants used in combination with the fungus in foliar sprays reduced foliar damage and increased susceptibility under stress (Vimala Devi and Prasad, 1996). Kernel extracts of melia and pongamia as well as oils of plant origin were found to be compatible with *N. rileyi* when used together in terms of larval mortality in bioassays. Despite the known fungicidal activity of neem, its incorporation after sterile filtration in to Sabouraud's maltose agar medium did not cause inhibition of mycelial growth and sporulation of *N. rileyi* (Vimala Devi and Prasad, 1996). Further, foliar application of *N. rileyi* in combination with 1% kernel extract of neem and melia against an artificial infestation of *S. litura* on groundnut crop significantly reduced foliar damage compared to the damage in the untreated crop. Combination of botanical insecticides and *N. rileyi* has been used to stress the *S. litura* populations in fields thus making them more susceptible to mycosis and prevent resistance to synthetic insecticides. Gopalakrishnan and Mohan (2002)



recommended that *N. rileyi* can be used along with endosulfan for effective management of *H. armigera*, *S. litura* and *P. xylostella* on cabbage when they occur in a complex. Manjula and Krishna Murthy (2005) reported that synergistic action of *N. rileyi* and synthetic insecticides such as monocrotophos/acephate on *S. litura* is practically useful to narrow-down the complete dependence on synthetic compounds, and suggested application of insecticides coinciding with onset of natural infection by *N. rileyi* for enhanced larval mortality in the fields.

#### ***Paecilomyces* species**

The sensitivity of *Paecilomyces farinosus* to agricultural disturbances has been described in the presence of larval *Galleria mellonella* (Mietkiewski *et al.*, 1997). Effects of acetamiprid (Saurus 200SP) - a neonicotinoid insecticides, imidacloprid (Confidor 700 WDGr) and thiamethoxam (Actara 250 WG) in three concentrations (AR = average field recommendation: 0.7 AR and 1.3 AR) to *Paecilomyces* sp revealed that these insecticides had no effect on conidia germination, except under the highest concentration (1.3 AR) of acetamiprid, indicating compatibility of both the agents (Pedro *et al.*, 2001). Significant increase in the conidia production of *Paecilomyces* sp fungus was observed in two concentrations, 0.7 AR and AR of acetamiprid; and in the two highest concentrations, AR and 1.3 AR, of thiamethoxam treatments. The use of insecticides in the recommended formulations and other concentrations tested in most cases had no negative effect on conidia germination, vegetative growth and conidia production of *Paecilomyces* sp. Consequently, these products in the formulations and concentrations tested have been used in IPM programs in which the entomopathogens are important pest regulators (Pedro *et al.*, 2001). Oil-based emulsion of *Paecilomyces fumosoroseus* conidia with or without low application rates of imidacloprid were effective in controlling *T. vaporariorum* infesting lettuce crop under greenhouse conditions (Feng *et al.*, 2004). A more desirable control of *T. vaporariorum* was achieved by fungal preparation containing imidachloprid 10% WP at the rates as low as 14.0-23.3% of its labeled rate. For faster control, the rate of imidacloprid to be applied together with the fungal preparations was around 15% of its common application rate but no more than 20 per cent (Feng *et al.*, 2004).

Combination of botanicals and the fungus has also delivers beneficial interactions. For example, in spray trials, sole application of *P. fumosoroseus* was not effective against glasshouse infestations of the aphids, *Aphis*

*gossypii* and *Macrosiphoniella sanborni*, but when applied with azadirachtin (Margosan-O®), fungal efficacy was enhanced and desirable level of control achieved (Lindquist, 1993). Such interactions could potentially expand the pest host range of fungal agents, thereby increasing the cost effectiveness of the control strategy. Moreover, if greater efficacy could be obtained, fewer applications would be required to achieve the desired degree of pest control. Thus, the minimum exposure to chemical toxicants would helps to prevent the chance for development of resistance to synthetic insecticides.

#### ***Lecanicillium* (= *Cephalosporium* = *Verticillium*) *lecanii* (Zimmermann) Veigas**

The hyphomycetous *Lecanicillium lecanii* is a widespread opportunistic fungus which has a wide host range, including insects, mites, plant pathogens and plant parasitic nematodes (Hall and Dunn, 1959; Hall, 1981). The potentials of *L. lecanii* as a microbial agent for biocontrol was demonstrated for coffee green bug, *Cocus viridis* (Santharam *et al.*, 1977) and banana aphid, *Pentalonia nigronervosa* forma *typica* Coq. (Regupathy and Paramjothi, 1977). *Cephalosporium lecanii* was inhibited by several test insecticides *viz.*, dichlorvos, carbaryl, monocrotophos, malathion and endrin (Easwaramoorthy and Jayaraj, 1977), fenthion and phosphamidon (Easwaramoorthy *et al.*, 1978) at higher concentrations under *in-vitro* conditions. But the efficacy of the fungus was enhanced when applied in combination with sublethal concentrations of the same insecticides to control coffee green scale. Significant increase in the mortality of coffee green bug *C. viridis* was achieved by the combination of phosphamidon and fenthion with *V. lecanii* (Easwaramoorthy *et al.*, 1978). Moino and Alves (1998) suggested two possible explanations for these results: (1) In fungi, as a physiologic mechanism of resistance, insecticides can be metabolized and liberate compounds that can be used by the fungus as secondary nutrients; and (2) In a toxic medium, the fungus could be making a reproductive effort, increasing conidia production. Another possible explanation is that substances present in the insecticide formulations can be used directly as nutrients increasing the vegetative growth and conidia production of the pathogen.

A more practical approach to increasing conidia pick-up appears to be the use of sub-lethal doses of the chloronicotinyl insecticide imidacloprid. One per cent of the recommended dose, dramatically increased aphid movement; quantified by image analysis of video-taped aphid behavior (Moino and Alves, 1998). This resulted in greater mortality where aphids were exposed to

insecticide-treated leaf discs that had been sprayed with fungal conidia. They further pointed out that a comparison with results from an experiment where conidia were sprayed directly onto aphids which were feeding on insecticide-infused pepper discs established that synergy was due to an indirect effect of the insecticide, *i.e.* through increased movement, rather than a direct effect *viz.*, predisposition of insecticide-weakened insects to disease. In a recent study, compatibility between *Lecanicillium muscarium* and chemical insecticides used to control sweet potato whitefly, *Bemisia tabaci* was investigated (Andrew *et al.*, 2005). The effect on spore germination of direct exposure for 24h to the insecticides imidacloprid, buprofezin, teflubenzuron and nicotine was determined. Only exposure to buprofezin was followed by acceptable spore germination. However, all chemicals significantly reduced spore germination when compared to a water control. Infectivity of *L. muscarium* in the presence of dry residues of buprofezin, teflubenzuron and nicotine (imidacloprid is a systemic pesticide) on foliage were also investigated. No significant detrimental effects on the level of control of *B. tabaci* were recorded when compared with fungi applied to residue free foliage on either tomato or verbena plants. Fungi in combination with imidacloprid gave higher *B. tabaci* mortality on verbena foliage compared to either teflubenzuron or nicotine and fungi combinations. Andrew *et al.* (2005) discussed on the use of these chemical insecticides with *L. muscarium* in integrated control programme for *B. tabaci*. Senthilkumar and Regupathy (2007) reported that desired levels of supplemental synergism were enhanced by the sublethal concentration of Thiamethoxam and imidacloprid (25g a.i.) with the fungus at  $8 \times 10^6$  conidia/ml towards coffee green scale, *C. viridis*. In these studies, use of fungus in combination with the insecticides might have slowed down the development of resistance in target host insects. Andrew *et al.* (2008) reported that sequential application of chemicals such as savona, spray oil and majestik with *L. muscarium* produced more than 90% mortality of second instar *B. tabaci*.

#### ***Hirsutella thompsonii* Fisher**

The mite specific pathogen, *Hirsutella thompsonii* Fisher has been proved to be pathogenic to various mite pests in diverse ecosystems. McCoy (1981) listed several insecticides including imidachloprid, dichlorvos, dicofol, and sulphur had low to moderate effect on *H. antonii*. However, insecticides caused less inhibition of the fungus than fungicides (Sreerama Kumar and Singh, 2000). The combination treatment involving nimbecidine (a neem oil based EC formulation containing 0.03% Azadirachtin) and

Bio-catch (*Hirsutella thompsonii* based WP formulation with spore count of  $1 \times 10^7$ ) at the dosage of 500ml and 1000g respectively in 200 litre of water was found to be effective in the control of the Eriophyid mite, *Aceria (Eriophyes) guerreronis* in coconut plantation. Imidachloprid synergized the germination of *H. thompsonii* (Ramarethinam *et al.*, 2000). Significantly higher proportions of conidia germinated at 100, 200, and 500 ppm of imidacloprid compared with the control (Dara and Hountondji, 2001). In bioassay study, *H. thompsonii* alone and in combination with sub-normal concentration of imidacloprid both killed around 95% of cassava green mite, *Mononychellus tanajoa* (Bondar).

#### **ECOLOGICAL IMPLICATIONS**

The success of entomopathogenic fungi depends on conidial viability (Olivera and Neves, 2004), because the beginning of epizootics is conditioned to the capacity of these structures to germinate on the host (Alizadeh *et al.*, 2007). A fungus attempting to infect non-host insect may be interrupted at any one of these factors and this could result in an aborted infection (Bidochka and Hajek, 1998). Conidia from dead cadavers can survive to infect later pest generations. However, fungi intended for use in the management of pest arthropods have to encounter an array of chemicals applied to control pests, pathogens and weeds in the same habit (Majchrowicz and Poprawski, 1993). Documented studies on the interactions between chemical insecticides and entomopathogenic fungi are enormous. Three decades ago, Olmert and Kenneth (1974) have emphasized the necessity of clarifying the effects of pesticides on a wide range of entomopathogenic fungi in with view to integrate the co-application strategies in the pesticide resistance management programme. Several studies have demonstrated that in intensively cultivated arable soils *M. anisopliae* is the dominant species (Mietkiewski *et al.*, 1991; Vanninen, 1995). This is thought to be due to ability of *M. anisopliae* to tolerate agricultural chemicals, mechanical disturbances and to persist more readily outside the host insects (Latch and Fallon, 1976). Germination, mycelial, growth and sporulation are important in insect infection and disease transmission. The impacts of chemical pesticides on the biological processes vary depending on the fungal species and strains (Vanninen and Hokkanen, 1988). Some insecticides, for example, carbamates are fungicidal and analogues are available to control fungal plant pathogens and so some insecticides as well as fungicides may be harmful to fungal biocontrol agents (Butt and Brownbridge, 1997). Fungal sporulation is less affected at low pesticides concentrations (Anderson *et al.*, 1989). Pesticides which

are inhibitory in the laboratory do not always exhibit the same action in the field (Butt and Brownbridge, 1997).

### ENVIRONMENTAL MANIPULATIONS

In diverse agricultural systems such as those prevailing in the tropics, it would be important to understand the biology and behaviour of insect pests in an ecosystem so that informed decisions can be made as to which developmental stages of insects the chemical-fungal biocontrol agents to be deployed. It is also important to consider the resistant management strategies, economic value and environmental impact of insecticides in each crop. Several studies have shown that an insecticide, which is very effective against one insect species, may be weakly active or ineffective against the other insects. Co-application of selective insecticide and a virulent entomopathogenic fungus is expected to reduce selection pressure in host insects, reduce the amount and number of insecticide applications. However, greater research is needed to identify insecticide molecules that are more effective in combination with the fungal entomopathogens. Impact of various pesticides on fungal entomopathogens has been extensively studied employing poisoned food technique by incorporation of chemicals in to mycological agar, although some of the results are conflicting. Many fungal entomopathogens like *Beauveria*, *Metarhizium*, *Paecilomyces* and *Verticillium* are very promising biocontrol agents, but they face several limitations as sole method for management of crop pests due to complex of factors. The deficiency of these pathogens could be overcome by resorting novel approaches by conjunctive use with selective insecticides as validated in potato ecosystem in the USSR (Anderson *et al.*, 1989). Most importantly, synergistic action expressed both in field and laboratory studies suggests that host behavioural modification stimulated by chemical alterations can improve the efficacy of some entomopathogenic fungi and should be tested against other invertebrate hosts. It is possible that such approaches could also be extended to other field and horticultural crops to curtail the development of insecticide resistance in insect-pests populations. Judicious combination of insecticides and fungi not only preserve their compatibility but also conserve the fungal conidia in the environment, contributing to spontaneous epizootics under the favourable conditions. As natural control is implemented, less chemical insecticides will have to be used, resulting in benefits for the farmer and the environment.

### FUTURE OUTLOOK

Fungal entomopathogens are rated as '*the best-bet*' among the microbial entities, as they provide clear scope for

co-application as components of resistance management strategies. The future needs suggested for insecticide resistance management include:

- Conservation of mycopathogens is needed if they occur naturally or co-applied or introduced with selective insecticides with the objective to abate insecticide resistance in insect pests.
- Fundamental studies are called for on the interaction between fungi and insecticides in the soil or on the analysis of the defense system inside the body of target insects under joint application with fungi and insecticides are desirable.
- Insecticides that can be used to control plant pests without any adverse effects on beneficial fungi are need to be screened in favour of germination, vegetative growth and conidia production both *in-vitro* as well as *in-vivo* conditions.
- Systematic field studies complemented by parallel laboratory experiments are essential for clear understanding of the ecological impact of pesticides on the introduced fungi.
- Work should also be concentrated on the characterization and genetic improvement of fungi for spotting pesticide resistant strains to be exploited in the insecticide resistance management programme.

In this context, gene transfer systems have been reported for *B. bassiana* (Daboussi *et al.*, 1989) using nitrate reductase gene of *Aspergillus nidulans*. Sandhu *et al.* (2001) described a comparison of two methods of transformation in *B. bassiana* using  $\alpha$ -tubulin gene of *Neurospora crassa* encoding resistance to benomyl; which discussed on the generation of benomyl resistant *B. bassiana* strains and their infectivity against *Helicoverpa armigera*. Similar such studies with selective insecticides-fungi will enable the development of mutant strains with improved virulence to insects and increased compatibility with other components of pest control especially for insecticide resistance management programme.

### CONCLUSIONS

Co-application of entomopathogenic fungi with low doses of insecticides is gaining importance in insecticide resistance management in insect pest of crops as a component of IPM programme. Several workers have conducted *in-vitro* screening of various agrochemicals to find out their compatibility with entomopathogenic fungi. The use of selective insecticides at suitable concentrations in favour of germination, mycelial growth, conidial production and survival of entomopathogenic fungi is desirable. Several insecticides at sub-normal

concentrations were reported to be compatible with major fungal entomopathogens such as

*B. bassiana*, *M. anisopliae*, *P. farinosus*, *P. fumosoroseus* and *V. lecanii* in different situations. Co-application of these fungi at suitable subnormal concentrations of selected insecticides as two-in-one tank mix could be employed against various insect pests to reduce the selection pressure of insecticides and there by concurrent resistance risks could be avoided on target pests of crops. Many new insecticidal compounds with novel mode of action are currently being used in insect pest management. Steps must be taken to reduce the development of resistance and extent the useful life of such compounds. For implementing IPM, separate applications for insecticides and fungal entomopathogens are impractical for the growers. But, combinations of selective insecticides with candidate fungal strain serve to reduce or delay selection pressure for insecticide resistance by lowering the insecticidal dose; perhaps reduce the number of applications of insecticides. Such combinations also induce multiple mortality factors, so that target pests with gene for insecticide resistance may still fall prey to co-applied fungal entomopathogens. Fundamental studies on the interaction between fungal entomopathogens and insecticides in the soil (or) on the analysis of defense systems inside the body of target insects under joint application with entomofungi and insecticides are desirable. Considering these observations, it is worth exploring the effects of various new molecule insecticides at sub-lethal doses on entomofungi as two-in-one tank mix strategy. While doing so, adequate care should also be taken because insecticides at sub-lethal doses may sometimes end up with complications like resurgence of less important insect pests. However, recent development in biotechnology may be helpful for strain improvement of fungi for tolerance to insecticides, which could overcome common hurdles encountered in insecticides resistance management.

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