Microbial agents against *Helicoverpa armigera*: Where are we and where do we need to go?

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Plants are prone to various biotic stresses in nature by bacteria, viruses, fungi, parasites, harmful insects and weeds. The biggest percentage loss (70%) in plants is attributed to insects. Lepidoptera is one such diversified phytophagous insect group, which include *Helicoverpa armigera*, a key pest of many food crops including chickpea, pigeonpea, pea, lentil, chillies, sunflower, tomato, tobacco and cotton. Controlling this insect has been a big task for farmers leading to the manufacture of a plethora of pesticides. However, over reliance on chemical pesticides has resulted in problems including safety risks, environmental contamination, outbreaks of secondary pests, insecticide resistance and decrease in biodiversity. Hence, there is an urgent need for the development of eco-friendly methods such as entomopathogens, antagonist or competitor populations of a third organism and botanicals to suppress *H. armigera*. Also, many compounds from microorganisms have been found to be effective in crop production, and these have a role in controlling *H. armigera*. The actinomycetes play an astounding role in controlling the key plant pathogens. They are the representative genera of higher microbial mass in the soil. Numerous studies have shown that these productive actino-bacteria can generate an impressive array of secondary metabolites such as antibiotics, antitumor agents, insecticides etc. This review emphasizes the mechanism behind resistance to insecticides along with actinomycetes and its potential as a biocontrol agent against *H. armigera*.

**Key words:** *Helicoverpa armigera*, actinomycetes, biocontrol, metabolites.

**INTRODUCTION**

Since the commencement of agriculture about 10 000 years ago, insect pests have been a major problem for crop production. The situation still exists and is crucial to manage them due to the rising populations. Malnourishment due to scarcity of food and feed is a major problem especially in poor countries. A recent survey has reported that there are 3.7 billion malnourished people in world (WHO, 2005). Hence, it is important to control crop damages to maintain the quality and abundance of food, feed and fibre around the world. Different approaches namely: chemical based control, host-plant resistance, Integrated Pest Management (IPM) strategies and transgenic plants may be used to prevent, mitigate or control plant infections. The agriculture related pest and patho-
Insects known so far include 2000 species of insects (Revathi et al., 2011). FAO has reported a worldwide loss of US$120 billion in crop yield, where 20 to 40% was caused by the attack of insect pests and pathogenic organisms (Zhou, 2001). There is a close relationship between insect and host interaction, which leads to crop damage. Much of the plant source (leaves and flowers) has been exploited as food by insects preventing normal growth and products of the plant. The major damaging insect species belong to Lepidoptera (Pimental, 2009). Insect pests affect the food productivity by either generating diseases or by reducing the quality and quantity of food. Low intensification in agriculture on a global level is the root cause for the enhanced damage. An extensive research resulted in broad spectrum chemical insecticides, which have been a primary control agent. About 40% of the insecticides were targeted towards Lepidopteran insects (Srinivasan et al., 2006). An outstretched usage led to pesticide-resistant insects, a reduction in the beneficial insect populations, and harmful effects to humans and the environment (Brooks and Hines, 1999). These problems have encouraged researchers to develop different insect control strategies using both synthetic and natural molecules that are more eco-friendly.

**HELCOVERPA ARMIGERA (HUBNER), THE CHALLENGING PEST**

*H. armigera* (Kingdom: Animalia, Phylum: Arthropoda, Class: Insecta, Order: Lepidoptera, Family: Noctuidae) is the key pest of agriculture and horticulture in many parts of the world. These pests behave like ‘eating machines’ and have rapid growth and metabolism (Haq, 2004). The pest status is rooted in its mobility, polyphagy, high reproductive rate, diapause and high fecundity. These factors make it particularly well adapted to exploit transient habitats such as man-made agro-ecosystems. Its predilection for harvestable parts of essential food and high-value crops like cotton, tomato, pulses and tobacco confers a high economic cost to its depredations. It infects dianthus, rosa, pelargonium, chrysanthemum and a range of monocot and dicot crops as well. As per the survey of European Plant Protection Organization (Chamberlin, 2004), *H. armigera* has been widespread in Asia, Africa and Oceania. In India, *H. armigera* commonly destroys over half the yield of pulse crops like pigeon pea and chickpea, which leads to $US 300 million loss per annum (EPPO, 2006), while in the late 1980s losses of both pulses and cotton were estimated to exceed US$500 million in addition to the investment of US$127 million in insecticides.

The mid-gut epithelium is large in *H. armigera* and effectively digests and absorbs nutrients, which is responsible for its rapid growth (Reed and Pawar, 1982). *Helicoverpa* females lay eggs singly on leaves, flowers and young pods. The larval form of the insect feeds on the foliage (young leaves) in chickpea and a few other legumes, whereas it feeds on flowers and flower buds in the case of cotton and pigeonpea. Young seedlings of chickpea are destroyed completely when this larva feeds on them. Plants in tropical climates such as the southern part of India are more prone to these insects. Larger larvae bore into pods/bolls and consume the developing seeds inside the pod. With such dire scenario in India, farmers try to control this pest by various classes of insecticides.

**MANAGEMENT TACTICS OF HELICOVERPA**

Synthetic pesticides have been in use for the last 50 years, irrespective of pest types in the field and under post-harvest conditions. For high productivity of crops it was found crucial to apply massive amounts of pesticides to control the pest (Chamberlin, 2006), but this leads to disturbance in the ecology as a huge number of pest and predators have been killed by these pesticides, thereby giving way to secondary pests (Armes et al., 1996). The outbreak of plant damage in 1980 was one such example, which was controlled by overuse of pesticides. This led to the resistance of *H. armigera* against many conventional insecticides such as organochlorine, organophosphate, carbamates and pyrethroid insecticides. These insecticides are broad spectrum neurotoxic with a wide host range from insects to mammals. Many researchers have found that this toxicity acts over insects and also non-target groups of organisms (Jiang and Ma, 2000; Rai et al., 2009). The insect pest on cotton crop in India became tolerant to organophosphate insecticides, which triggered the process of re-thinking about the use of chemicals for pest control. After 1980s, a new concept of pest management evolved, called IPM, which combines the methods of cultural, mechanical, biopesticides and chemical pesticides. A case study by Krishi Vignan Kendra, Medak, India, highlighted some non-pesticidal methods followed by farmers such as deep ploughing, burning of farm refuge, intercropping or mixed cropping, trap cropping, bird perches (to attract predators), manual collection and destruction of larvae, natural extracts, mechanical collection, and collection with pheromone traps.

**MECHANISM OF INSECTICIDE RESISTANCE**

The mechanism of insecticide resistance has been reported on various factors. All the mechanisms have elucidated that the foremost factors for resistance are decreased insensitivity, reduced penetration and metabolic detoxification (El-Latif and Subrahmanyam, 2010). The resistance of *H. armigera* against pesticides has been extensively studied. The strains that are resistant were
found to have higher production of esterases (Scott, 1991) and glutathione S-transferases. The most common type of resistance involves the target site insensitivity (kdr) mechanism and increased enzymatic detoxification. The cytochrome P-450-dependent monoxygenase is an extremely important and most frequent type of metabolic system that also influences resistance (Ellison et al., 2011). The possible mechanism of insecticide resistance in *H. armigera* is depicted in Figure 1. The problem allied with synthetic insecticides has led to the search for a sustainable alternative in pest control. This can be substantiated by the growth of organic farming and banned notices of synthetic pesticides in many areas (Christie, 2010). One among the alternatives for pest control is the biopesticides, which had a global market value of approximately US$1 billion in 2010, and which will increase to US$3.3 billion in 2014 (Lehr, 2010).

**BIOLOGICAL PESTICIDES FROM TREASURE BOX**

For decades natural products have been used for humankind either for food, clothing, cosmetics, shelters, traps, tools, weapons and antibiotics or crop protection agents. Initially, crude mixtures of chemicals were isolated from microbes and used to some extent, but recently, advanced scientific methods have led to the development of pure products, which act effectively (Copping and Duke, 2001). Scientists all over the world are averse to synthetic pesticides and have now diverted their attention to the development of safe and more permanent methods of pest control. They have found that biological control is one of the best options as it is eco-friendly and can be integrated with other pest management strategies. Hence, biological pesticides have been used to manage *H. armigera* (King and Coleman, 1989). Use of parasitoids and predators has failed to be successful pest control methods because of constraints in their mass production, storage and availability to the farmers on time. These circumstances have moved the focus to microorganisms.

Microbes are treasure boxes that contain pharmacologically active drugs and antibiotics. A new arena with microbial pesticides isolated from beneficial microbes has been introduced for crop protection. Viruses, bacteria and fungi can act as biocontrol agents against insects and fungi. The mode of infection of virus and bacteria is via their digestive tract while fungi make entry into the host through

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Figure 1. Schematic diagram of all possible mechanism of insecticide resistance in comparison with sensitive insects. 1. Reduced penetration. 2. Acetyl choline esterase (ACHE) modification. 3. Metabolic detoxification or sequestering of insecticide by group of enzymes (esterase, phosphotriester hydrolase, cytochrome P-450 monooxygenase) 4. Modification of sodium channel conferring kdr target site resistance to pyrethroids.
the cuticle (Deshpande, 1999). Investigation of our research group on PGP bacteria *B. subtilis*, *B. megaterium*, *Serratia mercescens* and *Pseudomonas* spp.; fungus *Metarhizium anisoplae* and actinomycetes *S. cavourensis* sup sp. *cavourensis*, *S. alboglutosis*, *S. hydrogenans*, *S. antibioticus*, *S. cyaneofuscatus*, *S. carpaticus*, *S. bacillaris* and *Streptomycete* spp. isolated from herbal vermicomposts and organically cultivated fields documented the broad-spectrum insecticidal of all these microbes against lepidopteran pests such as *H. armigera*, *Spodoptera litura* and *Chilo partellus* (Gopalakrishnan et al., 2011; Vijayabharathi et al., 2014). This proves the efficiency of microbes as treasure box for biological pesticides.

**FUNGAL INSECTICIDES**

The study of microbes as insecticides has brought to light that fungal populations are efficient producers of ubiquitous defensive proteins called lectins. Though plants themselves produce these proteins, they have a limitation on nonspecific activity, hence fungal lectins were concentrated upon (Carlini and Grossi-de-Sa, 2002; Peumans et al., 2000). At a suitable concentration, fungi like *Beauveria bassiana*, *Lecanicillium lecanii* *M. anisoplae*, *Nomuraea rileyi*, *Paecilomyces* spp. were proven to show an effective reduction in the number of insect pests (Ambethgar, 2009). However, the success of these fungi depends on the conidial viability (Olivera and Neves, 2004) because germination decides the pathogenesis (Alizadeh et al., 2007).

The efficacy of fungal pathogen *N. rileyi* (Farlow) Samson has been proven against *H. armigera* by a number of researches in groundnut, soybean and cotton ecosystems (Patel, 2001; Hegde et al., 2004; Ramigowda, 2005). Since *N. rileyi* is a facultative fungus, it can be easily multiplied on rice or sorghum grains under laboratory conditions, and can be utilized in the management of this polyphagous pest. Shekarappa (2009) conducted a field study with *N. rileyi* in sorghum against *H. armigera* and found that mycoinsecticide *N. rileyi* was highly infective at both dosages of $1 \times 10^8$ conidia per litre and $2 \times 10^8$ conidia per litre against *H. armigera* compared to the chemical control malathion in mitigating the pest population as well as in obtaining higher yields. The fungi *N. rileyi* and *Isaria tenueipes*, pathogens for lepidopteran were evaluated for their activity of oil suspended conidia against *Helicoverpa zea*, and this could also be successful against *H. armigera* (Aquino et al., 2010).

*M. anisoplae* and *B. bassiana* were found to be less efficacious insecticides on chickpea (Kale and Men, 2008), but they were more potent on combinations with nucleopolyhedrosis virus and *Bacillus thuringiensis* (*Bt*). Ensuring survival and persistence of the fungi is one of the greatest challenges in using fungal insecticides, where optimization strategies are lacking in this arena. It has been recorded that about 500 (Davis, 1996) host species of Lepidoptera and 200 (Zimmermann, 1993) other insect families have been infected by two types of fungi, white muscardine (*B. bassiana*) and green muscardine (*M. anisoplae*), respectively. The chitin deacetylase of *M. anisoplae* was found to contribute to pathogenesis. This initiates the infection by softening the insect cuticle to aid penetration (Nahar et al., 2004). Enzyme-based biocontrol agent from the fungus *Trichoderma harzianum* was also evaluated against the growth and metamorphosis of *H. armigera* (Pandey et al., 1999).

**VIRAL INSECTICIDES**

The narrow host range of most viruses can be both a limitation and an advantage depending on the pests. Viral insecticides are effective biological controls used with insect-specific nucleopolyhedrosis viruses (NPVs). These NPVs are highly virulent and lethal, but are slow acting. Baculoviruses consist of two genera, nuclear polyhedrosis virus (NPVs) and granulo virus (GVs), and are host-specific (Black et al., 1997; Van Reomurnd et al., 1997). The virus specific to *H. armigera*, a single nucleocapsid nucleopolyhedrosis virus (*HaSNPV*), is used commercially, which is a promising alternative since they are target-specific and highly pathogenic (Moscardi, 1999).

Recent trials in Queensland carried out by Commonwealth Scientific and Industrial Research Organization (CSIRO) Entomology, University of Queensland and the Queensland Department of Primary Industry on sorghum have suggested that NPV produced in *vitro* will also be effective as much as NPV cultured through *in vivo*. Although NPV is a promising insecticide, some constraints have made them difficult to use. The slow activity of the pesticides is improved by genetic modifications to generate fast killing (Inceoglu et al., 2001). Recombinant *HaSNPV* has been recently introduced with gene deletion or insertion, which could act at a high speed. Bioassays of these recombinants have shown that they could kill the second instar *H. armigera* at a faster rate than the wildtype (Chen et al., 2000; Sun et al., 2004a). However, these genetically modified viruses are ecologically not successful due to its limited production of polyhedral and alteration in the behaviour of larvae (Sun et al., 2004b; Zhou et al., 2005).

Entomopox virus (EPV) is also found to be a potent insecticide. They have a very small range of hosts and hence they do not affect non-target organisms. Current research is ongoing on EPV’s special protein component, which severely restricts the growth of pest caterpillar, reduces their appetite and thus may result in less damage to the crop. Time taken for effective killing is the limitation of this EPV, and hence an improved technology for speeding the process is needed. Stunt viruses (SVs) are also emerging as insecticides that have a small compact RNA and can therefore self-assemble from their components. This helps in producing them *in vitro*. Expe-
Bacterial Insecticides

Bacillus genera in the bacterial family are found to be effective insecticides from a decade ago. The proteins present in *B. thuringiensis* are found to possess insecticidal properties and show efficacy as spray formulations in agriculture. They have been expressed in crop plants. *B. thuringiensis* (Bt) has been developed to be a potent biopesticide. Bt is Gram-positive and sporulating soil bacterium. The proteins such as d-endotoxins and cytolytic proteins produced by Bt are efficient sources of insecticides for food crops and stored grains (Meadows, 1993). The Cry toxin of Bt is one such endotoxin that acts against a wide range of insects (Federici, 2005). The gut regions of these pests are targeted by the toxins causing death by starvation (Starnes et al., 1993). Many transgenic plants have been developed that can produce insecticidal proteins that are derived from this genera. They have been successfully used in control of pest and protecting important high acre crops (Perlak et al., 2001; Pilcher et al., 2002). Cry toxins are of various types (a, b, c etc.) and differ in their host specificities. Cry 1 Ab and Cry 1 Ac genes were used in the first commercial genetically modified (GM) cotton and corn crops that were produced against Lepidopteran pests (Llewellyn et al., 2007).

There is a long lasting argument that transgenic plants can affect the natural environment. It is obvious that a negative impact can also be created by interference from natural protection. However, severe problems have also occurred in Bt-transgenic crops where pest insects had gained resistance to Bt-toxin just as in the development of the resistance to many chemical insecticides. The most common mechanism of resistance is by the disruption of binding of Bt toxin to receptors in the mid-gut membrane. This disruption might be either due to mutations in the receptors or changes in the expression of the receptors (Fuentes et al., 2011; Tiewsiri and Wang, 2011). The resistance mechanism associated with ABC transporter loci has also been reported (Baxter et al., 2011).

Approaches like ‘high dose/refuge strategy’ (Chilcutt and Tabashnik, 2004) or pyramid by expression of two genes have been tried to prolong the effectiveness of Bt crops (Suresh et al., 2008). Another severe issue is on the transfer of inserted transgenes from crops to wild or weedy plants. Many control measures on transgene flow have been performed with special reference to plastid transformation, but still there is a problem of relocation of plastid into the nuclear genome itself (Huang et al., 2003).

Bt plants also have little effect on soil biota and hence further research is needed to know the effects of GM plants on soil decomposition. Assessment of non-target impacts is an essential part of risk assessment in insect-resistance GM plants (Ócallaghan et al., 2005). There are still miles to travel for the success of transgenic plants, with solutions that dispel all negative impacts.

Actinomycetes and Its Metabolites

Actinomycetes come under the order actinomycetales, which are Gram positive bacteria with high G+C content. They are wonderful resources of biologically active secondary metabolites that are important for chemical, pharmaceutical and agricultural industries. Actinomycetes are omnipresent in nature, but prefer soil components (Lechevalier et al., 1981). They therefore play an important role in soil biodegradation and humus formation (McCarthy and Williams, 1992; Stach and Bull, 2005). These are the bacteria responsible for the “wet earth odour” that emanates from wet soil due to volatile substances like geosmin (Wilkins, 1996) and are potent producers of extracellular enzymes (Sehrempf, 2001). To date, about 20 different genera of actinomycetes have been isolated and many are to be explored (Williams and Wellton, 1992). Actinomycetes produce a variety of antibiotics with diverse chemical structures such as polyketides, β-lactams and peptides in addition to a variety of other secondary metabolites that have antifungal, antitumor and immune suppressive activities (Behal, 2000).

Microbial cells produce synthetases that catalyse transformation of unused substances into molecules called secondary metabolites. Secondary metabolites consist of natural products that (a) are restricted in taxonomic distribution, (b) are synthesized for a finite period by cells that have stopped dividing, and (c) most probably function as convenient disposal packages of excess primary substances. Some of these metabolites act as toxins for non-producer cells. Included in this category are hormones, pheromones, toxins and antibiotics. Substrates of secondary metabolism are primary metabolites as acetate, pyruvate, malonate, mevalonate, shikimate, prephenate, amino acids and purines (Demain, 1992; 1995).

The genus *Streptomyces* is the prime group that comes under actinomycetes. About 70% of the explored actinomycetes were found to be species of *Streptomyces*. They have the capacity to produce significant compounds, especially antibiotics, insecticides and pigments, due to their extra-large DNA complement (Goodfellow and Williams, 1986). They follow a special metabolic pathway, which includes the formation of glycosides and uses the shikimate pathway to aromatic compounds. The distribution of natural products in *Streptomyces* is attributed by the antibiotic biosynthetic gene transfer (Baltz, 2005). Overall 23,000 bioactive components have been reported, of which 10,000 compounds are produced by actino-
mycetes. Of this, 7600 compounds are reported to be from *Streptomyces* species (Berdy, 2005).

The next important genus of actinomycetes is *Micromonospora*. They are best known for synthesizing antibiotics, especially aminoglycoside, enediyne and oligosaccharide antibiotics, and hence they are employed in biocontrol. These genera are yet to be fully explored, after which new light on actinomycetes population might emerge. The secondary metabolites of actinomycetes, namely tetranectin, avermectins, faerifungin and macrotetrolides and flavonoids produced were found to be toxic to many insects. Avermectins are compounds produced by a novel species *S. avermitilis* isolated from soil. They were found to be an effective antihelminthic compound earlier (Burg et al., 1979), but later it was also found to be a potent insecticide, acaricide and nematicide (Putter et al., 1981).

Spinosyn is a large family of unprecedented compounds isolated from two species of *Saccharopolyspora spinosa*. The fermentation of *S. spinosa* produces several metabolites that are called spinosyn A and spinosyn D. They have a novel molecular structure and their mode of action is by affecting nicotinic acetylcholine receptors at the post-synaptic cells. They are very selective towards target insects such as Lepidoptera and Diptera, but generally show very low activity against many beneficial insect predators and non-target species (Thompson, 2000; Salgado and Sparks, 2005). The efficiency of spinosyn depends on the type of species and their stage of development, exposure time and method of administration. The significant advantage of spinosad also includes less toxicity towards mammals, avians and aquatic organisms compared to other insecticides, thus making it safer to use (Thompson et al., 2002). Several studies have shown that spinosyn has no long time persistence ability in plants, soil and other environments due to facile degradation by microbes present, whereby they partition them into organic matter and sediment with subsequent biotic degradation in the absence of light. Overall, they are easily subjected to diverse degradative pathways and metabolic mechanisms, and hence reduce their persistence in plants, and other environments (Kirst, 2010).

### INSECTICIDAL AND LARVICIDAL PROPERTIES OF ACTINOMYCETES

Many studies have used insecticidal and larvicidal compounds extracted from actinomycetes. Larvicidal actinomycetes were explored from marine samples of Muthupet Mangrove, Tamilnadu (Vijayakumar et al., 2010). The actinobacterial extracts isolated from the marine source showed larvicidal effects on 24 h exposure at 1000 ppm. The highest larval mortality was detected in LK-3 extract against the larvae of *Culex gelidus* with an LC50 of 108.08 ppm and against *Culex tritaeniorynchus* at LC50 of 146.24 ppm (Karthik et al., 2011). Valinomycin has been reported to be an insecticidal antibiotic by *Streptomyces griseus var. flexipertum* var. nov (Heisey et al., 1988). *Streptomyces nanchangensis*, a producer of nanchangmycin, was found to be an insecticidal polyether antibiotic. *Streptomyces lavendulae* is another promising species known to release many secondary metabolic compounds that are considered as important sources of antibiotics and pesticides. The antitumor antibiotic mitomycin C (MC) produced by *S. lavendulae* is a bioreductively activated alkylation agent that crosslinks DNA at 5'CpG sequences. It has been widely used clinically for antitumor therapy (Johnson et al., 1997). A high molecular weight transglutaminase inhibitor has also been purified from the culture filtrate of *S. lavendulae* Y-200 (Ikura et al., 2000). *S. lavendulae* SNAK 64297 releases a novel compound, 1100-50 (1), which has been isolated and purified by Takatsu (Takatsu et al., 2003). All these antibiotics are found to have insecticidal properties. A list of compounds recently found to be with potent pesticidal or larvicidal properties has been shown in Table 1 along with the source of the compound.

### CONCLUSION

There is an increasing demand to discover environment friendly insecticides and pesticides in general. *H. armigera* had made this situation still shoddier because of its voracious feeding habit and the extent of damage it can cause to the agricultural community. Therefore, research has been forced in this area where an effective alternative is needed. This review provides handy knowledge of our treasure box microbes with special reference to actinomycetes. With a push for cleaner and greener alternatives to traditional chemicals, insecticides from microbial sources, particularly actinomycetes, will be strong competitors in the future insecticide market. There is a wide diversity of actinomycetes that are still unexplored and hence a detailed study on these diverse valuable actinomycetes by culturable and unculturatable methods will lead to the discovery of novel insecticidal and larvicidal compounds. Few studies have started flourishing in isolation and characterization of the compounds from actinomycetes. Advanced techniques like matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS) are employed to characterize these compounds from actinomycetes (Stafsnes et al., 2012). To exploit these findings for agricultural use, a typical field study and strategies of optimization are necessary.

Many strategies have been applied for improvement of secondary metabolites, which include pathway-specific, or pleiotropic regulators, or enhancing the availability of precursors, ribosomal engineering etc. Recent interest has been focussed on altering the secondary metabolism through addition of small molecules, which has significant advantages. Bacteriophages are also found to be used...
as a powerful tool in the detection of bioactive actinomycetes and help in discovering the novel bioactive compounds. This offers a more significant benefit if improved understanding host-phage ecology is known; hence a sound knowledge on microbial taxonomy is necessary for the effective use of bacteriophage as a tool in the selective isolation procedure. Therefore, we could go far long to meet the major challenges in pest management with biotechnological tools. Microbes will continue to offer valuable versatile products, thereby serving mankind. The authors hope that this review will fill the gap with knowledge still lacking in this area.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES


