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Review

Microbes as interesting source of novel insecticides: A review

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Microbes are ubiquitous, survive in all sorts of environments and have a profound influence on the earth. In the present day plant protection scenario, development of resistance to chemical pesticides is the major hurdle in insect pest management. In recent years, several microbes with potential insecticidal properties have come to light. Viruses, bacteria, fungi and protozoa that are known to produce an array of metabolites or toxins, form the basis for microbial insecticides. Since these versatile organisms are amenable for genetic engineering, strains with good insecticidal properties can be identified, evaluated and utilized for pest control. This paper reviews the insecticidal properties of microbes and their potential utility in pest management.

Key words: Microbes, insecticides, metabolites, pest management.

INTRODUCTION

Plant pests and diseases have a serious effect on food production-global crop yields are reduced by 20 to 40% annually due to plant pests and diseases (FAO, 2012). Indiscriminate use of pesticides to combat pest challenges have increased the selection pressure leading to resistance in insects. In such a situation, alternate options of pest control are much awaited. Though biopesticides cover about 1% of the total plant protection products globally, their number and the growth rate have been showing an increasing trend in the past two decades (Ranga Rao et al., 2007). About 175 biopesticide active ingredients and 700 products have been registered worldwide. Among various bio-products, *Bacillus thuringiensis* (Bt), *Trichoderma viride*, *Metarhizium* spp., *Beauveria bassiana* and nuclear polyhedrosis virus are popularly used in plant protection (Anonymous, 2007). Microbes often produce epizootics which is a natural control phenomenon of some insect pests. The need of the hour is development of environment-friendly, microbe-based insecticides, which act differently from known chemicals, thereby providing insect the least chance to develop resistance. So far, prevailing microbial pesticides are being used mainly as foliar applicants. However, new products suitable for varied methods of application such as seed treatment, whorl application, bait treatment etc. are essential. Microbial-based pesticides, their mode of action, application in pest control are described below.

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VIRUSES

Baculoviruses, which are considered to be the largest and most broadly studied insect viruses, are environment-friendly insect control agents. Individual isolates normally show a limited host range and infect only closely related species (Chang et al., 2003). They induce lethal epizootics that can devastate host insect populations and were used successfully to control different insect pests. Nucleopolyhedrovirus and Granulovirus were isolated from lepidopteran insects. The viral insecticide Elcar™ (*Heliothis zea* NPV) introduced during 1980s, provided control of cotton bollworm; its production and usage was later limited. Another baculovirus, HaSNPV (*Helicoverpa armigera* single nuclear polyhedrosis virus), almost identical to HzSNPV (*H. zea* single nuclear polyhedrosis virus), was registered in China as a pesticide in 1993 (Zhang et al., 1995) and has been extensively used on cotton and many crops in India (Grzywacz et al., 2005; Rabindra et al., 2005; Srinivasa et al., 2008). Commercial preparations based on *Spodoptera* NPV were used to protect cotton, corn and vegetables globally (Moscardi, 1999; Kumari and Singh, 2009). *Autographa californica* and *Anagrapha falcifera* NPVs with relatively broad host spectrum activity were used on a variety of crops infested with *Spodoptera* and *Helicoverpa*.

Granulovirus CpGV was effective against the coddling moth (*Cydia pomonella*) on fruit crops. Another granulovirus (GV), *Erinnyis ello* (cassava hornworm) granulovirus, was found to be very efficient for protection of cassava plantations (Bellotti, 1999) in South American countries. The GVs of tea tortricids, *Homona magnanima* and *Adoxophyes honmai* were used in Japan. Entomopoxvirus, a large DNA genome virus is found pathogenic to insects. *Amsacta moorei* entomopoxvirus, has been reported to infect agriculturally important lepidopteran pests such as *Estigmena acrea* and *Lymantria dispar* (Muratoglu et al., 2010). Among all the viruses studied, NPV was effective on lepidopteran pests in different agroecosystems. However, favourable weather, slow action and microbial contamination are the major constraints in baculovirus production and use. To address such constraints, engineering viruses to express insecticidal genes is one of the best approaches.

Recombinant viruses

The insertion or deletion of a single gene from the viral genome can alter the biological properties of the virus. Insertion of insect-specific toxin genes such as juvenile hormone esterase, diuretic hormone and prothoracicotropic hormone, genes encoding enzyme inhibitors, neuropeptides or toxins improve the efficiency of viruses. *Autographa californica* multicapsid nuclear polyhedrosis virus (AcMNPV) recombinants with wild type and mutated mutated versions of Juvenile Hormone Esterase (JHE) reduced the consumption of food by *H. virescens* and *Trichoplusia ni* (Bonning et al., 1999). The gene coding for a toxin from scorpion *Androctonus australis* reduced the feeding damage by about 40-60% in lepidopteran larvae (Choi et al., 2008). Toxin genes isolated from other scorpions, for example *Leiurus quinquestriatus hebraeus* (Imai et al., 2000), straw itch mite *Pyemotes tritici* (Burden et al., 2000), ants (Szolajska et al., 2004) or spiders (Prikhod'ko Prikhod'ko et al., 1998), sea anemones and *B. thuringiensis* (Chang et al., 2003) have been intensively studied as potential enhancers of baculovirus activity. Genetically modified *Anticarsia gemmatalis* multicapsid nuclear polyhedrosis virus (AgMNPV) by the inactivation of ecdysteroid glucosyltransferase (egt) gene, were shown to kill infected larvae more rapidly when compared to wild-type virus infections (Pinedo et al., 2003). Ignoffo et al. (1995) found that AcMNPV *pp34* deletion mutants have an increased infectivity in *Trichoplusia ni* larvae due to the increased loads of occlusion-derived virions (ODV) from the polyhedra in the midgut to start the infection process. Application of recombinant baculoviruses, vAPcmIT2 and vAP10IT2 against two major pesticide-resistant vegetable pests, *Plutella xylostella* (Linnaeus) and *S. exigua* resulted in shortening of the lethal time (Tuan et al., 2007).

Two recombinant baculoviruses containing the ScathL gene from *Sarcophaga peregrina* (vSynScathL) and the keratinase gene from the fungus *Aspergillus fumigatus* (vSynKerat), against third-instar and neonate *S. frugiperda* larvae showed protease activity in the haemolymph and reduced the time of kill (Gramkow et al., 2010). Report of Seo et al. (2005) documented higher pathogenicity for recombinant baculovirus containing a fusion protein with polyhedrin and Bt toxin than wild type strains.

BACTERIA

Bacillus thuringiensis

Among the bacteria, *B. thuringiensis* (Bt) is the most important and also the most extensively studied and used in the integrated pest management programs. It has long been regarded as a bonafide entomopathogen that can produce an array of virulence factors including insecticidal parasporal crystal (Cry) toxins, vegetative insecticidal proteins, phospholipases, immune inhibitors and antibiotics. Bt produces delta-endotoxin that reacts with the cells of the gut lining of susceptible insects. There are about 200 registered Bt products in the USA and, at the end of the last century, worldwide sales amounted to about 100 million dollars (about 2% of the total global insecticide market) (Anonymous, 1998). Though this versatile organism has multiple insecticidal properties, Ultra violet (UV) radiation inhibits its activity. UV resistant mutant strains with high melanin, which absorb light of any wavelength, can be used for large scale production of light stable insecticides (Liu et al., 2013). Other than Cry toxins, orally active insecticidal proteins that meet the efficacy hurdles required for pest control have also been reported from Bt. Vegetative insecticidal proteins (Vips) are produced by *B. cereus* and *B. thuringiensis* which show similar activity to endotoxins. Vip1and Vip2 are toxic to coleopteran insects and Vip3 is toxic to lepidopteran insects (Zhu et al., 2006). VIPs have excellent activity against black cutworms and armyworms (Yu et al., 1997) *S. frugiperda* (Barreto et al., 1999). *S. litura* and *Plutella xylostella* (Bhalla et al., 2005), *Helithios zea, Trichoplusia ni*, *Ostrinia nubilalis* (Fang et al., 2007; Sellami et al., 2011).

Insecticidal bacteria from nematodes

Another interesting source of microbial insecticides is bacterial symbionts of nematodes. *Xenorhabdus* and *Photorhabdus* are gram-negative bacteria that live in nematodes are highly toxic to many insect species (Burnell and Stock, 2000). The bacteria and the nematodes produce a variety of metabolites to enable them to colonize and reproduce in the insect host. The metabolites produced include enzymes such as proteases, lipases and phospholipases to maintain a food supply during reproduction (Bowen et al., 2000), and antifungal and antibacterial agents to prevent degradation or colonization of the insect carcass while the bacteria and nematodes reproduce. The bacteria such as *B. thuringiensis* produce Bt and Vip toxins against insects (Chattopadhyay et al., 2004); similarly, nematodes also produce toxins called Toxin Complex (Tc) by their symbiotic bacterial partners *X. nematophilus* and *P. luminescens* (French-Constant and Bowen, 2000).

Bowen (1995) reported that a soluble protein fraction derived from *P. luminescens* culture medium possessed sufficient insecticidal activity to kill *Manduca sexta* upon injection. The bacterium *Xenorhabdus nematophila* produces novel secreted protein toxin which was found effective against *Galleria mellonella* and *H. armigera*, cabbage white caterpillar *Pieris brassicae*, mosquito larva *Aedes aegypti* and mustard beetle *Phaedon cochleariae* (Sergeant et al., 2006). The reports show that these bacteria are effective on most of the economically important lepidopteran, dipteran and coleopteran insect orders. Hence, there is a wide scope to harness these organisms in the insect pest management. The genome of *P. luminescens* was homologous to hemolysin A, chitinase, Rtx (repeats-in-toxin)-like toxin, and delta endotoxin (Duchaud et al., 2003). It is interesting that novel colicin and immunity proteins are also found associated with insecticidal Tc genes in *Photorhabdus* spp. (Sharma et al., 2002). In future, in areas where the pests are resistant to Bt, these bacteria from nematodes could be the successful option to replace Bt.

Bacteria supplementing the activity of other bacteria

In some instances, one microbe assists other microbe to elevate its significance. In the absence of indigenous midgut bacteria, *B. thuringiensis* does not kill larvae. Elimination of the gut microbial community by oral administration of antibiotics abolished *B. thuringiensis* insecticidal activity, and reestablishment of the same gut bacteria that normally resides in the midgut microbial community restored *B. thuringiensis*-mediated killing (Broderick et al., 2006). Thus, the gut microflora plays an important role in the insecticidal properties of Bt. This is an area where the major research is being focused these days (Broderick et al., 2009; Patil et al., 2013).

Insecticidal bacterial flora of insects

Isolation of insecticidal bacterial flora from the insect itself and its use against the same is becoming popular. Several pathogenic bacteria species from insect samples have been developed as pesticides and used successfully in biological control of insects worldwide (Thiery and Frachon, 1997). A common soil organism, *B. cereus*, has been found pathogenic to insects on several occasions, and this species has been isolated from several insect species (Kuzina et al., 2001; Sezen et al., 2005). The isolates *B. cereus* (Ags1), *Bacillus* spp. (Ags2), *B. megaterium* (Ags3), *Enterobacter aerogenes* (Ags4), *Acinetobacter calcoaceticus* (Ags5), *Enterobacter* spp. (Ags6), *Pseudomonas putida* (Ags7), *Enterococcus gallinarum* (Ags8) and *Stenotrophomonas maltophilia* (Ags9) identified from the flora of *Agrotis segetum* when applied on the same, found to cause 60% mortality after eight days of application (Sevim et al., 2010).

Likewise, *B. megaterium* is also common in insect populations (Osborn et al., 2002). Several species of *Enterobacter* have been isolated from several insect species and used for biological control purposes (Sandra and Douglas, 2004; Bahar and Demirbag, 2007). *B. cereus*, *B. sphaericus*, *Morganella morganii*, *Serratia marcescens* and *Klebsiella* species isolated from the crop of predatory larvae of the antlion species *Myrmeleon bore* (Neuroptera: Myrmeleontidae) killed 80% or more cutworms *S. litura* (Nishiwaki et al., 2007). The bacterial flora *Leclercia adecarboxylata* of Colorado potato beetle showed highest insecticidal effect (100% mortality) within five days (Muratoglu et al., 2009) and has a potential for the control of several coleopteran pests. *P. pseudoalcaligenes* strain PPA (first isolated from yellow-spined bamboo locust, *Ceracris kiangsu*, in 1991) is an effective biological controlagent for locust (Zhang et al., 2009). Such isolates can rather be multiplied on large scale and used as foliar applications in field to manage important insect pests.

Other bacteria with insecticidal properties

Studies have shown mosquitoes to be the major targets of *Lysinibacillus sphaericus (B. sphaericus)* bacterium that produce insecticidal toxins during the vegetative phase of growth. Sphaericolysin, a toxin from the *L. sphaericus* was found lethal to the common cutworm *S. litura* (Nishiwaki et al., 2007). In addition to endotoxins showing insecticidal properties in Bt, there are bacteria which produce insecticidal exotoxins. The exotoxins of microbial origin from *Pseudomonas* spp. toxic to larvae of mosquitoes as well as lepidopteran insects (Murty et al., 1994) were known to act on the haemolymph proteins. *P. aeruginosa* oxyR mutant revealed its ability to kill the insect *Drosophila melanogaster* (Lau et al., 2003). *P. aeruginosa* strain confers an efficient protection against *Galleria mellonella* and *Batocera oleae* (George et al., 2000; Mostakim et al., 2012) and the potency was due to the presence of quantitatively as well as qualitatively different proportions of bio-surfactants in the crude glycolipids (Desai and Banat, 1997). *B. subtilis*, *B. amylofaciens*, *B. megaterium* and *Pseudomonas* spp. were reported to show more than 50% mortality in *S. litura* and *H. armigera* (Gopalakrishnan et al., 2011). Chitinase is one of the virulent factors in entomopathogens and it is positively correlated with insecticidal activity. Research focused on identifying isolates producing chitinase adds to the benefit of recognition of strains with high insecticidal activity. For example, Bahar et al. (2011) identified chitinase-positive bacteria such as *Serratia marcescens* to be active in killing the coleopteran insects with more chitin in their exoskeleton.

Actinomycetes

Actinomycetes form a large group of Gram-positive bacteria that grow as hyphae like fungi. They play an important role in the biological control of insects through the production of insecticidal compounds. The mortality of insect by actinomycetes may be due to secretion of bioactive materials which stimulate the gamma amino butyric acid (GABA) system or disruption of nicotinic acetylcholine receptors (Herbert, 2010). Actinomycetes was found effective against the house fly *Musca domestica* (Hussain et al., 2002), mosquito larvae (Sundarapandian et al., 2002; Dhanasekaran et al., 2010), and *Drosophila melanogaster* (Gadelhak et al., 2005).

Many actinomycetes strains caused larval mortality of the cotton leaf worm *S. littoralis* (Bream et al., 2001). In addition, considerable lethal effect of some actinomycetes was observed on pupae. Actinomycetes are very well known and successfully exploited as a source of secondary metabolites. The secondary metabolites strain of Streptomyces inhibited the growth of *S. exigua, Dendrolimus punctatus, Plutella xylostella, Aphis glycines* and *Culex pipiens* (Huamei et al., 2008). Besides exhibiting insecticidal activity, Streptomyces metabolites also play the role as antimicrobial compounds for insects and hence protect them from microbial pathogens. For instance, beewalf wasps has been found with antibiotic-producing *Streptomyces philanthi* within specialized glands on its antenna and the antibiotics excreted by *S. philanthi,* into the cocoons, protects the beewalf larvae from harmful pathogens (Kroiss et al., 2010). The potential of using chitinase-producing non-Streptomycete actinomycetes belonging to the genus Actinoplanes, which have chitin as a major component of their cuticle, has been investigated for the biological control of insects in the Arabian Gulf area (Gadelhak et al., 2005).

The insecticidal activity of tetranectin, avermectins, faerifungin and macrotetrolides isolated from *Streptomyces aureus*, *S. avermitilis*, *S. albidum* and *S. griseus* respectively, have been reported. Spinosad is a novel insecticide produced from a family of natural products derived from fermentation of the actinomycetes *Saccharopolyspora spinosa* (Snyder et al., 2007) has been accepted in organic farming. It is a neurotoxin with a novel mode of action involving the nicotinic acetylcholine receptor and probably GABA receptors as well (Salgado, 1998).

Exposure causes a cessation of feeding, some 24 hours later, by paralysis and death. It is primarily a stomach poison with some contact activity and is particularly toxic to Lepidoptera and Diptera. The avermectins are a series 16-membered macrocyclic lactone derivatives with potent anthelmintic and insecticidal properties generated as fermentation products by *S. avermitilis* (Pitterna et al., 2009). Cholesterol oxidase derived from Streptomyces broth has shown to have selective, high potency against cotton boll weevil, stunting effect in *H. virescens, H. zea* and *Pectinophora gossypiella* which might be due to disruption of the midgut epithelial membrane (Purcell et al., 1993).

FUNGI

Fungi affect all groups of insects and over 700 species have been recorded as insect pathogens. Fungi do not have to be ingested to infect their host but invade directly through the cuticle, and so can, potentially, be used for the control of all insects including sucking insects. The first step is invasion of host through cuticle by mechanical pressure and enzymatic degradation. Most entomopathogenic fungi are best used when total eradication of a pest is not required, but instead insect populations are controlled below an economic threshold, with some crop damage being acceptable. In addition entomopathogenic fungi have an essential role in Integrated Pest Management (IPM) if they can be used in conjunction with other strategies for sustainable pest control. A substantial number of mycoinsecticides and mycoacaricides have been developed worldwide since the 1960s. Products based on *B. bassiana* (Li et al., 2001), *M. anisopliae*, *Isaria fumosorosea* and *B. brongniartii* are the most common among the 171 products (Faria and Wraight, 2001) available in the market. Strains of the species *B. bassiana*, *M. anisopliae*, *Verticillium lecani, Nomuraea releyi* and *Paecilomyces fumosoroseus* are currently used for insect control (Lacey and Neven, 2006).

Many of the genera of entomopathogenic fungi either belong to the class Entomophthorales in the Zygomycota or the class Hyphomycetes in the Deuteromycota. Most of the entomopathogenic fungi have life cycles which synchronise with insect host stages and environmental conditions. Some of these fungi are obligate; for example, *Aschersonia aleyrodes* infects only scale insects and whiteflies, while other fungal species are facultative with individual isolates being more specific to target pests. Hence, different preservation methods must be standardized to maintain the viability of the most potent isolates. Sub culturing is the best method of conservation of pathogenicity of fungi. For long term storage, glycerol freeze was proved to be good (Oliveira et al., 2011).

Entomopathogenic Hyphomycete fungi, such as *B. bassiana*, are naturally occurring in soil (Klingen et al., 1998) and the fungus is being developed as a biocontrol agent against soil dwelling pests such as scarabs and weevils (Keller, 2000) with no effect on the non-targeted insects (Goettel and Hajek, 2001). Hosts of agricultural and forest significance include the Colorado potato beetle, the codling moth, several genera of termites, American bollworm *H. armigera* (Thakur and Sandhu, 2010), *Hyblaeapara* and *Eutectona machaeralis*, *Ostrinia nubilalis*, pine caterpillars *Dendrolimus* spp. and green leafhoppers *Nephotettix* spp. Spores of entomopathogenic fungi are available as an emulsified suspension or wettable powder and they are applied via spraying. These fungal spores parasitize into a wide range of insects and pests and hence considered as nonselective biological insecticide. *Lecanicillium* (*Verticillium) lecanii* and *Isaria* (*Paecilomyces) fumosoroseus* fungi mainly attacks sucking pests such as aphids and whiteflies (Kim et al., 2002; Nunez et al., 2008) *Isaria* (*Paecilomyces) fumosoroseus* has strong epizootic potential against *Bemisia* and *Trialeurodes* spp. in both greenhouse and open field environments (Faria and Wraight, 2001). Entomopathogenic fungus *P. tenuipes* was documented to control chemical resistant whiteflies on greenhouse vegetables.

Metarhizium spp. popularly known as green muscardine fungus is known to have potential to control several economically important insect pests of global importance viz., *H. armigera*, *S. litura*, that attack crops such as groundnut, soyabean, sunflower, cotton and tomato (Sahayaraj and Borgio, 2010; Revathi et al., 2011). A complete bioactivity of *M. anisopliae* has been tested on teak skeletonizer, *Eutectona machaeralis,* and found to be a potential myco-biocontrol agent of teak pest (Sandhu et al., 2000).

Nomuraea rileyi can cause epizootic death in various insects. It has been shown that many insect species belonging to Lepidoptera including *S. litura* and some belonging to Coleoptera are susceptible to *Nomuraea rileyi* (Ignoffo, 1981). Its mode of infection and development have been reported for several insect hosts such as *Trichoplusia ni*, *H. zea*, *Plathypena scabra*, *Bombyx mori*, *Pseudoplusia includens, Anticarsia gemmatalis, Spilosoma* (Mathew et al., 1998) and hedge plant eater *Junonia orithya* (Rajak et al., 1991). Information on entomopathogenic fungi and their insect hosts is furnished in Table 1.

Fungal metabolites

Fungi also produce secondary metabolites, an inherent property of the organism and these metabolites exhibit insecticidal activities (Vey et al., 2001). For entomopathogens producing these toxins, infection has been shown to result in more rapid host death compared to strains that do not produce these metabolites (Kershaw et al., 1999). The information on toxins or secondary metabolites produced by fungi is furnished in Table 2.

Entomopathogenic fungi (EPF) produce enzymes for converting insect tissue into nutrients for their growth. Catalyzing activity using specific enzymes is considered one of the main mechanisms of fungal infection to insect host. There is large scope for isolates which could produce extracellular enzymes to degrade the host cuticle in pest management. For instance, *M. anisopliae* grown in optimum fermentation conditions could produce host degrading enzymes such as acid phosphatase and phosphatase isoenzymes (Li et al., 2007; Strasser et al., 2000).

Trichoderma produces protease (31 kDa) and chitinase (44 kDa) during the growth phase (Shakeri and Foster, 2007) and it is also known to produce a number of antibiotics, such as trichodermin, trichodermol, harzianum A, harzianolide and peptaibols (Hoell et al., 2005) which were insecticidal on *Tenebrio molitor*. Tanned insect cuticle is poorly utilizable by most fungi; but the EPF which invade the insect host through its cuticle, have evolved powerful cuticle degrading enzymes such as chymoelastase. The crude *Alternaria alternata* chitinase showed 82% mortality against fruitfly (Sharaf, 2005). Quesada-Moraga et al., (2006) used the crude protein extracts of *M. anisopliae* for the control of *S. litura* and Hu et al., (2007) proved contact toxicity to *S. litura. Tolypocladium* and *Isaria fumosorosea* have proved to be toxic to *Plutella xylostella* (Bandani and Butt, 1999; Freed et al., 2012).

Table 1. Entomopathogenic fungi and their insect hosts.

Fungus	Insect	Reference
Beauveria bassiana	Red flour beetle (*Triboleum castaneum*)	Akbar et al., 2005
B. brongniartii, B. bassiana	*Ceratitis capitata*	Konstantopoulou and Mazomenos, 2005
Nomuraea rileyi, Mucor hiemalis and *Penicillium chrysogenum*	*H. armigera, Ceratitis capitata* and *Bactrocera oleae*	Vimala Devi, 2001
B. bassiana and *Clonostachys rosea*	Coffee berry borer	Vega et al., 2008
Verticillium lecanii	*Macrosiphum euphorbiae*	Askary et al., 1998
Lecanicillium muscarium	*M. euphorbiae* and *Aphidius nigripes*	Askary and Yarmand, 2007
L. longisporum	*Myzus persicae* and *Aphis gossypii*	Kim et al., 2007, 2008
L. attenuatum	*Macrosiphum euphorbiae*	
Lecanicillium spp. DAOM 198499	*Aulacorthum solani*	
L. lecanii	*Coccus viridis*	Vandermeer et al., 2009
Aspergillus flavus	*Culex quinquefasciatus*	Govindarajan et al., 2005
A. niger	*Anopheles aegypti, Culex quinquefasciatus*	Seleena and Lee, 1994
Chrysosporium tropicum	*Anopheles stephensi*	Priyanka et al., 2001

Table 2. Secondary metabolites of fungi effective against insects.

Organism	Metabolites	Insects controlled	Reference
Beauveria spp.	Bassianin, beauvericin, bassianolide, beauveriolide, bassiacridin, oosporein, and tenellin	*Culex pipiens, Aedes aegypti, Calliphora erythrocephala, H. zea*	Quesada-Moraga and Vey, 2004
Paecilomyces fumosoroseus	Pecilomicine-B	*Trialeurodes vaporariorum*	Yankouskaya, 2009
Hirsutella thompsonii	Hirsutellin A, hirsutellin B, phomalatone	Mites	Mazet et al., 1995
Aschersonia aleyrodis and *A. tubulata*	Destruxins, dustanin and homodestruxins.	Whitefly	Boonphong et al., 2001
Trichoderma	Trichodermin, trichodermol, harzianum A, harzianolide, and peptaibols	*Tenebrio molitor*	Shakeri and Foster, 2007

Biofumigants

Some fungi produce volatile insecticidal compounds. This property prompts their use as fumigants for stored pest control. The use of *Muscodor albus* as a biofumigant agent for the control of storage insects has been proved (Lacey and Neven, 2006). The fungus produces a mixture of antimicrobial volatile organic chemicals and when tested against potato tuber moth with 15 or 30 g of *M. albus*, development to the pupal stage was reduced by 61.8 and 72.8%, respectively, relative to controls. Three species of *Muscodor* and one *Gliocladium* sp. that produce volatile organic compounds with biocidal activity have been isolated from several host plants in geographically diverse areas (Daisy et al., 2002; Stinson et al., 2003). Daisy et al. (2002) also showed that naphthalene, an insect repellent, is produced by a related fungus, *Muscodor vitigenus*.

Endophytes

The occurrence of endophytic microbes with pesticidal abilities is leading to exciting new opportunities because it overcomes delivery issues often associated with biopesticides. Endophytic biocontrol agents can be cheaply introduced into seeds, tissue culture plantlets and other propagating material, providing some protection for the microbe to the adversities of the external abiotic and biotic environment. Endophytes can also have additional beneficial properties, such as accelerating seedling emergence, promoting plant growth and tolerance to adverse conditions (Harman, 2011; Companta et al., 2010). Fungal endophytes are quite common in nature and several roles have been recognised, including providing protection against herbivorous insects, plant parasitic nematodes (Elmi et al., 2000), and plant pathogens (Dingle and McGee, 2003; Wicklow et al., 2005). It has been shown that endophytic *B. bassiana* is compatible with both Bt and carbofuran applications used to suppress *Ostrinia nubilalis* (Lewis et al., 1996). Use of Bt transgenic corn did not have any detectable effect on the establishment of *B. bassiana* as a corn endophyte (Lewis et al., 2001). Endophytic *B. bassiana* caused no mortality to

Table 3. Fungal endophytes active against insects.

Fungal endophyte	Insect	References
Neotyphodium	*Rhopalosiphum padi* and *Metopopophium dirhodum*	Clement et al., 2005
Ryegrass endophytes, *Neotyphodium* spp.	Porina larvae *Wiseana* spp.	Jensen and Popay, 2004
Acremonium strictum	*H. armigera*	Jallow et al., 2004
B. bassiana	*Ostrinia nubilalis*	Lewis and Bing, 1991
B. bassiana, Clonostachys rosea	*Hypothenemus hampei*	Vega et al., 2008

Coleomegilla maculata, a predator of *O. nubilalis* eggs and larvae (Pingel and Lewis, 1996). Research on *B. bassiana* as a maize endophyte suggests that the reduced tunneling of *O. nubilalis* could be due to the presence of fungal metabolites that cause feeding deterrence or antibiosis rather than direct fungal infection. Other endophytes are mentioned in Table 3.

PROTOZOA

Protozoan diseases of insects are ubiquitous and comprise an important regulatory role in insect populations (Brooks, 1988). They are generally host specific and slow acting, most often producing chronic infections. The biology of most of the entomopathogenic protozoa is complex. They develop only in living hosts and many species require an intermediate host. Species in the Microsporidia are among the most commonly observed. Their main advantages are persistence and recycling in host populations and their debilitating effect on reproduction and overall fitness of target insects. As inundatively applied microbial control agents, only a few species have been moderately successful (Solter and Becnel, 2000). The grasshopper pathogen *Nosema locustae* is the only species that has been registered and commercially developed (Henry and Oma, 1981).

CONCLUSION

Many of the microbial based insecticides perform well *in vitro*. However, their action is negated *in vivo*. To overcome such negatives, efficient entomopathogenic strains and effective formulations are required. Other way, the actual target insect or microclimate of insect can be modified to make it susceptible to microbe. For instance, in mealybugs and wooly aphids, waxy coating prevents the microbial entry. In such a case, some materials with organic/non-polar nature which remove the upper coating of the insect to provide the entry point are useful. In Tropical countries, high temperatures influence the activity of microbial pathogens. Temperature tolerant strains from the areas with high temperatures could be adapted in other localities. Relative humidity is required for the growth of entomopathogenic fungi. Hence, good control of the insects could be achieved in rainy season where congenial humid conditions will prevail for the entomopathogen. Correspondingly, the crop canopy also influences the potency of entomopathogen. For instance, *Nomuraea rileyi* was quiet effective in crops such as groundnut and soybean for insect control with bushy canopy.

Expression of Bt in crops could be checked with available Bt strips in the market. Similarly, diagnosable tools are vital for other microbial pesticides as well. Plant metabolites such as peroxidases may hinder the action of microbial agents (Hoover et al., 1998). The inactivation can be reduced by addition of free radical scavengers such as mannitol or enzyme superoxide dismutase to baculovirus preparations (Zhou et al., 2004). Research should be directed on efficient delivery system to hit the target. It has been shown that honey bee-mediated delivery of the insect pathogen, *M. anisopliae*, increased pollen beetle control (*Meligethes* spp.) in oilseed rape (Butt et al., 1998) compared to conventional sprayers in delivering the inoculum to the pest infested flowers.

A new method for delivering viruses to target insects by using *Trichogramma* spp. as vector has been developed in China (Peng et al., 1998): *Trichogramma dendrolimi* combined with HaNPV to control the cotton bollworm (Zhu et al., 2002). The potency of microbes can be enhanced by combining with insect growth regulators. Chlorfluazuron enhanced the biological activity of AcMNPV against *S. exigua* and SlNPV against *S. litura* (Guo et al., 2007). Though there is lot of scope for microbial control, it is not comparable with chemical control strategies. Genetic engineering of microbes could fill the gap to some extent. However, farmers are interested in chemicals that show quick knock-down effect. For this, factors like enzymes and metabolites that determine pathogen virulence should be identified and used in strain selection and quality control. Advanced chemical technology could be adapted to formulate microbials as best insecticides like spinosad, a microbial based insecticide. Much emphasis should be given by concerned organization for research in development of microbials as pesticides. At the end, it should be noted that microbials with no environmental concerns effectively fits in Integrated Pest Management programs.

Conflict of Interests

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

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