

# Chapter 9

## Biological Control of Insect Pests for Sustainable Agriculture

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**Abstract** Maintenance of agricultural productivity is currently based mainly on extraneous application of fertilizers and pesticides. However, indiscriminate use of agrochemicals for controlling the pests and diseases led to pollution of soil, water, and food sources, poisoning of nontarget beneficial insects, and development of insect population resistant to insecticides. To obviate the pollution problem and obtain higher yields in a sustainable manner, biological control of insect pests using specific antagonistic microorganisms is an effective alternate approach with minimum deleterious effects. Microorganisms have been obtained from the rhizosphere of different crop plants that inhibited insect pests by producing toxins, bacteriocins, siderophores, hydrolytic enzymes, and other secondary metabolites. Moreover, plant hormones salicylic acid, jasmonic acid, and ethylene orchestrate a complex transcriptional programming that eventually leads to pest-induced SAR (systemic acquired resistance) and ISR (induced systemic resistance) in many plant species. Microbial genes involved in the biosynthesis of secondary metabolites and enzymes have been cloned and transferred to other microorganisms and plants to enhance the suppression and killing of insects. The efficiency of these biocontrol products can be further increased through genetic improvement, manipulation of the soil and plant environment, using mixtures of biocontrol agents, and optimization of formulations and by integration of biocontrol agents with other alternative methods that provide additive and synergistic effects. Thus, the application of effective biocontrol agents may reduce the use of chemical insecticides and support sustainable agriculture in an eco-friendly manner in tandem with improved crop productivity.

**Keywords** Biological control · Insect pests · Rhizosphere microorganisms · Sustainable agriculture

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## 9.1 Introduction

Global crop yields are reduced by 20–40% annually due to pests and diseases (FAO 2012). For the control of insect pests in agriculture, farmers have mostly relied on the application of synthetic pesticides, and the global pesticide market is presently growing at a rate of 3.6% per year (Lehr 2010). However, indiscriminate use of chemical pesticides to control the pathogens/insects has generated several problems including resistance to insecticides/fungicides, outbreak of secondary pests, as well as safety risks for humans and domestic animals. Moreover, the long persistence of applied pesticides in soil leads to contamination of groundwater and soil, and the residual toxic chemicals enter into the food chain. Excessive pesticide application also decreases the biodiversity due to destruction of nontarget entomofauna. These problems have increased the interest of scientists for development of eco-friendly microbe-based insecticides or biocontrol agents, which act differently from known chemicals (Ruiu et al. 2013). Sustainable agriculture in the twenty-first century will rely increasingly on alternative interventions for pest management that are environment-friendly and will reduce the human contact with chemical pesticides. Therefore, microorganisms are currently being explored for their possible use as biocontrol agents in the integrated pest management programs.

Some of the microorganisms obtained from the rhizosphere of crop plants provide the frontline defense to plant roots against the attack by various plant pathogens and insects (Compant et al. 2005). Several microorganisms including bacteria, actinomycetes, fungi, viruses, protozoa, and nematodes have been identified to control various root, foliage, and postharvest diseases of agricultural crops (Glick and Bashan 1997), and many microorganisms have been found to act as potential entomopathogens (Vaga and Kaya 2012; Lacey et al. 2015; Mascarin and Jaronski 2016). Among the various microbial control agents (MCAs), *Bacillus thuringiensis* (Bt), *Pseudomonas fluorescens*, *Serratia marcescens*, *Streptomyces* sp., *Lecanicillium lecanii*, *Trichoderma virens*, *Metarhizium* sp., *Beauveria bassiana*, and nuclear polyhedrosis virus are popularly used in plant protection (Mascarin and Jaronski 2016; Sindhu et al. 2016). So far, about 175 biopesticide active ingredients and 700 products have been registered worldwide.

## 9.2 Characterization of Microorganisms Involved in Biological Control of Insect Pests

Several microorganisms inhabiting either the soil or plant rhizosphere have been identified to act as entomopathogens, and some of the microbes have also been found to suppress the diseases of agricultural crops (Borneman and Becker 2007; Lacey et al. 2015). The microorganisms isolated from the rhizosphere soil could be screened for their biocontrol activities for subsequent use as biocontrol agents (Fig. 9.1). Li et al. (2015) demonstrated distinct variations in the microbial

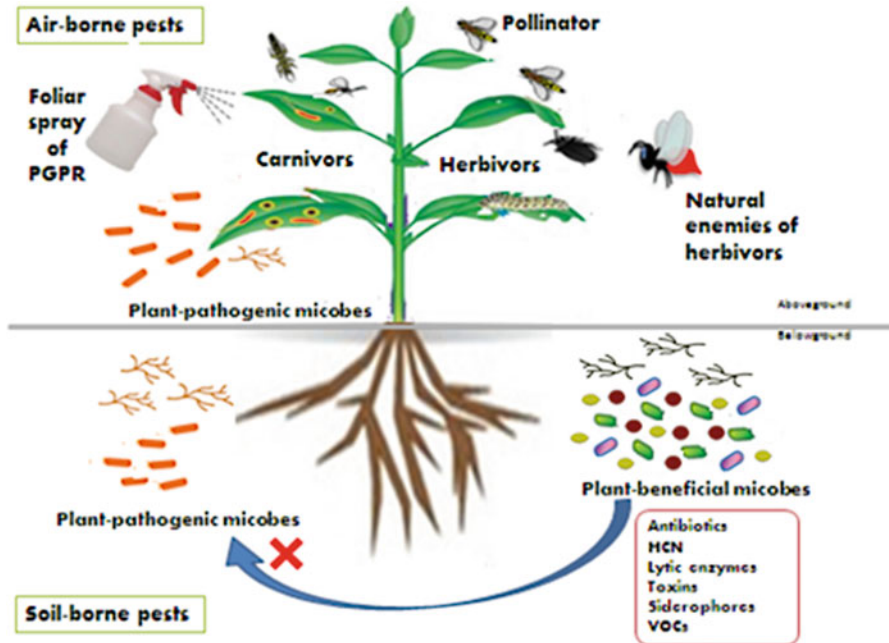


Fig. 9.1 Interactions of pathogens, insects, and biocontrol agents affecting the plant growth

community of cotton rhizosphere between monocropped (4 and 15 years) rhizosphere soils and fallow (control) agricultural soil. The monocropped soils significantly influenced the composition of root exudates and also reduced soil suppressiveness to *Fusarium* wilt. A significant correlation existed between the presence of certain amino acids (e.g., glutamic acid and alanine) and predominant bacterial taxa in the rhizosphere, indicating that some constituents in root exudates influenced the microbial compositions of the cotton rhizosphere to manage the disease status of plants in monocropped soils.

### 9.2.1 Antagonistic Bacteria

Rhizobacteria have been found to inhibit the growth of various pathogenic bacteria, fungi, and insects resulting in suppression of the diseases caused by different pathogens and insect pests (Sindhu et al. 2014). *Bacillus thuringiensis* (*Bt*) is the most studied entomopathogenic species for biological control of insect pests, and some of the toxin-producing strains have shown high mortality against specific insects compared to conventional insecticides used in the microbial pest management (Vega and Kaya 2012). The insecticidal proteins produced by *B. thuringiensis* are highly specific insect gut toxins and do not affect the nontarget organisms

(Lacey and Goettel 1995). *B. thuringiensis* subspecies *kurstaki* strain HD-1 (De Barjac and Lemille 1970) is most widely used for the management of lepidopteran pests in agriculture and forestry, whereas *B. thuringiensis* subspecies *israelensis* and *Lysinibacillus sphaericus* are the pathogens used for medically important pests including dipteran vectors. Strains of *B. thuringiensis* subsp. *aizawai* (*Bta*) (i.e., ABTS-1857) are used against armyworms and diamondback moth larvae. Similarly, *Bacillus* strains belonging to the subsp. *israelensis* (*Bti*) and *tenebrionis* (*Btt*) have been employed for the management of mosquitoes and simuliids and against coleoptera, respectively (Glare and O'Callaghan 2000).

Other *Bacillus* species have also shown potential for insect pest management. Spherical endospore-producing bacteria *B. sphaericus* (Alexander and Priest 1990) produce parasporal crystals located within the exosporium, and these strains showed toxicity against mosquitoes. The equimolar ratio of the two homologous binary protein toxins (Bin), BinA and BinB, found in parasporal bodies acts in a similar fashion as Cry proteins (Charles et al. 2000). Other entomopathogenic bacteria that possess potential against diverse insect pests include *B. popilliae* with *B. lentimorbus*, the causal agents of milky disease in phytophagous scarab larvae (Zhang et al. 1997). *Serratia entomophila* contains a specific plasmid (pADAP) encoding genes implicated in pathogenicity against the grass-grub, *Costelytra zealandica* (White) (Jackson et al. 1992). *Clostridium bif fermentans* serovar *malaysia* has been found effective against mosquitoes and blackflies (Nicolas et al. 1990). The mosquitocidal activity in this bacterium was associated to the production of a protein with homology to *Bt*  $\delta$ -endotoxins (Cbm71). The encoding gene of this protein was cloned and induced for expression by transformed *Bt*, which exhibited toxicity against mosquitoes (Barloy et al. 1996).

Another group of entomopathogenic bacteria includes the endosymbionts of insecticidal nematodes, especially the members of the genera *Xenorhabdus* and *Photorhabdus* (Burnell and Stock 2000). These entomopathogenic bacteria and the nematodes produce a variety of metabolites that 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). Antifungal and antibacterial agents prevent the degradation or colonization of the insect carcass, while the bacteria and nematodes reproduce. Bowen (1995) reported that a soluble protein fraction purified from *P. luminescens* culture medium possessed sufficient insecticidal activity to kill *Manduca sexta* upon injection. The novel protein toxin secreted by bacterium *Xenorhabdus nematophila* 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). These bacteria were found effective on most of the economically important lepidopteran, dipteran, and coleopteran insect orders, suggesting wide scope of these organisms for application in insect pest management.

Common soil organism *B. cereus* has also been found pathogenic to insects and has been isolated from several insect species (Kuzina et al. 2001; Sezen et al. 2005). Various bacterial isolates, i.e., *B. cereus* (Ags1), *Bacillus* sp. (Ags2), *B. megaterium*

(Ags3), *Enterobacter aerogenes* (Ags4), *Acinetobacter calcoaceticus* (Ags5), *Enterobacter* sp. (Ags6), *Pseudomonas putida* (Ags7), *Enterococcus gallinarum* (Ags8), and *Stenotrophomonas maltophilia* (Ags9), were identified from the flora of *Agrotis segetum* (Sevim et al. 2010), and these isolates caused 60% insect mortality after 8 days of application. *B. cereus*, *B. sphaericus*, *Morganella morganii*, *Serratia marcescens*, and *Klebsiella* species isolated from the predatory larvae of the antlion species *Myrmeleon bore* (Neuroptera, Myrmeleontidae) were found to kill 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 5 days (Muratoglu et al. 2009) and thus showed a potential for the control of several coleopteran pests.

*Pseudomonas entomophila* showed insecticidal properties against insects in different orders and triggered a systemic immune response in *Drosophila melanogaster* Meigen after ingestion (Vodovar et al. 2006). Similarly, biopesticidal potential of *Brevibacillus laterosporus* Laubach has been reported against insects in different orders, such as coleoptera (Boets et al. 2004), lepidoptera (Oliveira et al. 2004), mosquitoes and blackflies (Rivers et al. 1991), and houseflies (Ruiu et al. 2006), nematodes (Singer 1996), and against phytopathogenic fungi (Saikia et al. 2011). *Chromobacterium subsugae* showed its insecticidal potential after ingestion against diverse insect species in different orders (i.e., coleoptera, lepidoptera, hemiptera) (Hoshino 2011). These insects included Colorado potato beetle (*Leptinotarsa decemlineata* Say), Western corn rootworm (*Diabrotica virgifera* Le Conte), Southern corn rootworm (*Diabrotica undecimpunctata* Mannerheim), small hive beetle (*Aethina tumida* Murray), diamondback moth (*Plutella xylostella* L.), sweet potato whitefly (*Bemisia tabaci* Gennadius), and Southern green stink bug (*Nezara viridula* L.) (Martin et al. 2007).

Subterranean termites have been found to cause extensive damage to major agricultural crops and forest plantation trees. Khan et al. (1985) reported that a commercial preparation of *B. thuringiensis* (Thuricide-HP concentrate) exhibited 100% mortality within 6 days of exposure against *H. indicola*, *M. championi*, and *Bifiditermes beesonii* (Gardner) (Kalotermitidae). Similarly, the colonies of *M. championi*, *H. indicola*, and *B. beesonii* exposed to suspensions of the spore-forming bacterium *Serratia marcescens* Bizio succumbed completely 7–13 days following infection (Khan et al. 1977). Khan et al. (1992) showed that mortality of *M. championi*, *H. indicola*, and *Coptotermes heimi* (Wasmann) (Rhinotermitidae) termites ranged from 25–52% after 7 days postinoculation to 84–100% 25 days after postinoculation due to the pathogenicity of *Pseudomonas aeruginosa* (Schroeter) in the laboratory. Osbrink et al. (2001) isolated biological control agents from dead termites and revealed the presence of 15 bacteria and 1 fungus in dead termites. Bacteria isolated from termite substrata included *Corynebacterium urealyticum* Pitcher, *Acinetobacter calcoacet/baumannii/Gen2* (Beijerinck), *S. marcescens*, and *Enterobacter gergoviae* Brenner. Devi et al. (2007) observed killing of *Odontotermes obesus* subterranean termites under in vitro conditions by three HCN-producing rhizobacterial species, i.e., *Rhizobium radiobacter*, *Alcaligenes latus*, and *Aeromonas caviae*. Rakshiya et al. (2016) reported that

**Table 9.1.** Entomopathogenic fungi having deleterious effects on different insects

Fungus	Insect	References
<i>Beauveria bassiana</i>	Red flour beetle ( <i>Tribolium castaneum</i> )	Akbar et al. (2005)
<i>B. brongniartii</i> , <i>B. bassiana</i>	<i>Ceratitis capitata</i>	Konstantopoulou and Mazomenos (2005)
<i>Nomuraea rileyi</i> , <i>Mucor hiemalis</i> , and <i>Penicillium chrysogenum</i>	<i>H. armigera</i> , <i>Ceratitis capitata</i> , and <i>Bactrocera oleae</i>	Vimala Devi (2001)
<i>B. bassiana</i> and <i>Clonostachys rosea</i>	Coffee berry borer	Vega et al. (2008)
<i>Verticillium lecanii</i>	<i>Macrosiphum euphorbiae</i>	Askary et al. (1998)
<i>Lecanicillium muscarium</i>	<i>Macrosiphum euphorbiae</i> and <i>Aphidius nigripes</i>	Askary and Yarmand (2007)
<i>L. longisporum</i>	<i>Myzus persicae</i> and <i>Aphis gossypii</i>	Kim et al. (2007, 2008)
<i>L. lecanii</i>	<i>Coccus veridis</i>	Vandermeer et al. (2009)
<i>Aspergillus flavus</i>	<i>Culex quinquefasciatus</i>	Govindarajan et al. (2005)
<i>A. niger</i>	<i>Anopheles aegypti</i> , <i>Culex quinquefasciatus</i>	Seleena and Lee (1994)
<i>Chrysosporium tropicum</i>	<i>Anopheles stephensi</i>	Srivastava and Prakash (2001)

63 bacterial isolates out of 220 bacterial isolates obtained from the soil collected from termite mounds (along with 8 reference strains) killed the termites under Petri plate conditions at 2 days of observation. Killing frequency of different bacterial isolates was found to vary from 5 to 90%. Six bacterial isolates, i.e., PPM119, PPM123, PPM167, PPM194, PPM199, and PPM203, caused even 100% killing at 5 days of observation. Forty-eight bacterial isolates caused 90 to 100% killing of termites at 10 days of incubation.

### 9.2.2 Fungi

Fungi also play a prominent role in insect control, and over 700 species have been recorded as insect pathogens (Table 9.1.). Fungi invade directly through the cuticle and could be used for the control of all insects including sucking insects. Products based on *Beauveria bassiana* (Li et al. 2001), *Metarhizium anisopliae*, *Isaria fumosorosea* and *B. brongniartii*, *Verticillium lecanii*, *Nomuraea rileyi*, and *Paecilomyces fumosoroseus* are the most common fungal species among the 171 products currently used for insect control (de Faria and Wraight 2001; Lacey and Neven 2006). Some of these fungi are obligate for insects; 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. The fungus *B. bassiana* is being developed as a biocontrol agent against soil-dwelling pests such as scarabs and weevils (Klingen et al. 1998; Keller 2000) with no effect on the nontargeted insects (Goettel and Hajek 2001).

Colorado potato beetle, the codling moth, several genera of termites, and American bollworm *H. armigera* are the hosts of entomopathogenic fungi of agricultural and forest significance (Thakur and Sandhu 2010). *Hyblaeparara* and *Eutectona machaeralis*, *Ostrinia nubilalis*, pine caterpillars *Dendrolimus* sp. and green leafhoppers *Nephotettix* sp., *Lecanicillium* (*Verticillium*) *lecanii*, and *Paecilomyces fumosoroseus* fungi mainly attack 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* sp. in both greenhouse and open-field environments (de Faria and Wraight 2001). *Metarhizium* sp. has been found effective in controlling the several economically important insect pests of global importance, viz., *H. armigera* and *S. litura* that attack crops such as groundnut, soybean, sunflower, cotton, and tomato (Revathi et al. 2011). *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).

Some of these entomopathogenic fungi, viz., *B. bassiana*, *M. anisopliae*, and *Lecanicillium lecanii*, have been found to colonize plant tissues as symptomless endophytes (Rodriguez et al. 2009; Vidal and Jaber 2015). Plants harboring these fungi as endophytes have shown detrimental effects against herbivorous insects (Azevedo et al. 2000) or plant parasitic nematodes (Waweru et al. 2014). The endophytic growth of *B. bassiana* Vuillemin (BB) is a common feature in corn cropping systems in the USA, and the natural colonization of corn stalks ranged from 0 to more than 60% of plants sampled in different US federal states (Arnold and Lewis 2005). Plant species harboring BB include coffee (Posada et al. 2007), banana (Akello et al. 2008), sorghum (Tefera and Vidal 2009), cotton, pumpkin and wheat (Gurulingappa et al. 2010), jute (Biswas et al. 2013), and common bean (Parsa et al. 2013). *M. anisopliae* and *B. bassiana* and their related species are also used as biological pesticides to control a number of pests such as termites (Maniania et al. 2002), thrips (Ekesi et al. 2012), locusts (Ouedraogo et al. 2003), and hazelnut weevil (Cheng et al. 2016). *M. anisopliae* and *B. bassiana* do not infect humans or other animals and are, therefore, considered safe for use as pesticide.

Twenty-two fungal species have been reported as obligate ectoparasites of termites (Blackwell and Rossi 1986). Leong (1966) demonstrated high pathogenicity of *M. anisopliae* to *Coptotermes formosanus*. Mortality exceeded 86% with short exposures (e.g., 5–35 min) and termites succumbed within 3–6 days posttreatment. Exposure over 40 min caused 100% mortality and longer exposure times caused death within 24 h. No significant difference between the pathogenicities of *B. bassiana* and *M. anisopliae* was found. Grace (1991) found that *M. anisopliae* caused greater and rapid mortality than *B. bassiana* in *C. formosanus* to low conidial concentrations. Wells et al. (1995) concluded that



one isolate each of *B. bassiana* and *M. anisopliae* showed the greatest potential for control of *C. formosanus* populations, based on LD<sub>50</sub> (median lethal dose, as conidia per insect), time to death, and conidial production. Similarly, different isolates of *Conidiobolus coronatus* were found pathogenic to *C. formosanus*, *R. flavipes*, and *Nasutitermes exitiosus* (Wells et al. 1995). Milner et al. (1998) tested 93 isolates of *M. anisopliae* (Metschnikoff) obtained from two species of termites. The direct inoculation of the most effective isolate FI-610 by applying  $3 \times 10^{11}$  conidia into termite mounds resulted in successful control of *Coptotermes acinaciformis* (Froggatt) in Australia. Wright et al. (2003) patented *Paecilomyces* sp. for controlling subterranean termites, and these *Paecilomyces* strains were transferred among termites and caused rapid mortality.

Grace and Zoberi (1992) found that living *R. flavipes* workers exposed to sporulating *B. bassiana* cultures effectively spread infection to unexposed nestmates, whereas introduction of fungus-killed workers did not result in sufficient spore transfer or mycelial growth to cause significant mortality. However, the level of mortality achieved in the laboratory was not considered sufficient to control a termite infestation in the field. Rath and Tidbury (1996) found that *Coptotermes acinaciformis* (Froggatt) (Rhinotermitidae) and *N. exitiosus* were equally susceptible to direct conidial applications of both Australian and American strains of *M. anisopliae*. The injection of large quantities of conidia directly into the termite nest had the greatest success in the field studies (Milner et al. 1996). Sun et al. (2002) quantified the sporulation of 22 isolates of *M. anisopliae* and *B. bassiana* on cadavers of the Formosan subterranean termite, *C. formosanus*. Conidial production increased significantly over 11 days post-death. Effects of *M. anisopliae* and *B. bassiana* isolates on in vivo sporulation were significant, and it differed by as much as 89x and 232x among the selected isolates of *M. anisopliae* and *B. bassiana*, respectively.

Wright et al. (2005) reported that *M. anisopliae* (Metschnikoff) isolate, C4-B, caused rapid mortality on Formosan subterranean termite alates. In initial experiments, C4-B was more lethal to both alates and workers as compared with *M. anisopliae* strains ESC 1, previously marketed as the termite biocontrol agent, BioBlast. Dose-response assays to a known concentration of C4-B spores revealed that  $10^6$  spores/ $\mu$ l killed 100% of the Formosan subterranean termite alates in 3 days, both  $10^5$  and  $10^4$  spores/ $\mu$ l in 6 days,  $10^3$  spores/ $\mu$ l in 9 days, and  $10^2$  spores/ $\mu$ l in 12 days. When the transfer of inoculum from infected workers to uninfected nestmates was tested, 62.8% of the workers died in 21 days when only 20% of the workers had been inoculated. Mortality of alates caused by *M. anisopliae* (Metschnikoff) isolate C4-B was studied at two field sites by dispersing fungal spores on grassy lawns and collecting the alates from the treated areas. Infected alates showed 100% mortality by day 5, whereas only 64.8% of untreated control alates from the same collection area were dead on that day. Thus, fungi have the potential for termite control of all the pathogens tested (Milner and Staples 1996).



### 9.2.3 Actinobacteria

Actinobacteria are important part of the microbial community in the rhizosphere soil and produce many secondary metabolites with different disease suppression effects. More than a 1000 secondary metabolites are produced by actinomycetes, which makes 45% of total microbial metabolites. Actinomycetes have been reported as biocontrol agents effective against numerous plant pathogens and insects (Snyder et al. 2007; Prapagdee et al. 2008; de Oliveira et al. 2010; Laid et al. 2016). The potential use of actinomycetes as a biocontrol agent has been reviewed recently (Sabaratnam and Traquair 2015), where inoculation with some of these microorganisms also promoted the growth of plants.

Actinomycetes were found effective against the housefly *Musca domestica* (Hussain et al. 2002), mosquito larvae (Dhanasekaran et al. 2010), and *Drosophila melanogaster* (Gadelhak et al. 2005). The mortality of insects by actinomycetes may be due to secretion of bioactive materials, which stimulate the gamma-aminobutyric acid (GABA) system or disruption of nicotinic acetylcholine receptors (Herbert 2010). Strains of *Streptomyces* inhibited the growth of *S. exigua*, *Dendrolimus punctatus*, *Plutella xylostella*, *Aphis glycines*, and *Culex pipiens* (Huamei et al. 2008). Spinosad is a novel insecticide produced from fermentation of the actinomycetes *Saccharopolyspora spinosa* (Snyder et al. 2007), and it has been accepted for application in organic farming. It is particularly toxic to lepidoptera and diptera insects. Avermectins (a series of 16-membered macrocyclic lactone derivatives) were obtained from the fermentation products by *S. avermitilis* that showed potent anthelmintic and insecticidal properties (Pitterna et al. 2009). Cholesterol oxidase derived from *Streptomyces* broth showed selective and high potency against cotton boll weevil and stunting effect on *H. virescens*, *H. zea*, and *Pectinophora gossypiella*, which might be due to disruption of the midgut epithelial membrane (Purcell et al. 1993).

### 9.2.4 Protozoa

Protozoan diseases of insects are ubiquitous and play an important regulatory role in insect populations (Brooks 1988). They are generally host specific and slow acting, most often producing chronic infections. Entomopathogenic protozoa develop only in living hosts, and many species require an intermediate host. Their main advantages are persistence and recycling in host populations and their debilitating effect on reproduction and overall fitness of target insects. Of the four groups of protozoa containing species parasitic to insects, the phylum Microspora includes species that are potentially most useful in applied insect control (Henry 1990). Desportes (1963) described a gregarine (phylum Apicomplexa) from the hemocoel of the damp-wood termite *Zootermopsis nevadensis* (Hagen) (Termopsidae). Microsporidians were found in the body cavity and proventriculus

of *Microcerotermes championi* collected from the roots of *Saccharum munja* Roxburgh (*Poaceae*) (Jafri et al. 1976). The organisms attacked fat body tissues in the midgut after ingestion with food and caused death. Although protozoa are important biocontrol agents for many insects, they have not been used as soil-applied microbial insecticides because they tend to be slow acting and cause low levels of immediate mortality. Moreover, the protozoa populations are vulnerable to changes in environmental conditions (Klein 1988; Henry 1990).

### 9.2.5 Nematodes

A plethora of nematode species in more than 30 families is associated with insects and other invertebrates (Kaya and Gaugler 1993). They have been found parasitizing species in the orders Hemiptera, Diptera, Hymenoptera, Lepidoptera, Orthoptera, Coleoptera, Thysanoptera, Siphonaptera, as well as Isoptera (Nickle and Welch 1984). Four families of nematodes, i.e., the Mermithidae, Allantonematidae, Steinernematidae, and Heterorhabditidae, have found application in insect control programs (Popiel and Hominick 1992). After infection of the host by nematodes, symbiotic bacteria are released into the insect hemocoel, causing septicemia and death (Kaya and Gaugler 1993). These entomopathogenic nematodes have a number of characteristics that make them especially suitable for biological control and for commercial production as microbial insecticides. These characteristics include a broad host range, especially among soil-dwelling pests; an ease of production, easy to store, and easy in application; and a high degree of safety to vertebrates, plants, and other nontarget organisms and amenability to genetic selection (Kaya et al. 1993).

Fujii (1975) obtained 96% mortality in *C. formosanus* termites within 7 days of exposure to infective-stage *Steinernema carpocapsae* (Weiser) (Steinernematidae) in laboratory experiments. Mortality exceeding 95% was recorded by Georgis et al. (1982) for both *Zootermopsis* sp. and *Reticulitermes* sp. within 3 days after laboratory exposure to *S. carpocapsae*; termites were also found to carry infection back to their colonies. Under laboratory conditions, high rates of infection of *Nasutitermes costalis* and *R. flavipes* were reported with *S. carpocapsae* (Trudeau 1989). Yu et al. (2006) showed that different species of entomopathogenic nematodes, i.e., *Steinernema riobrave* Cabanillas, Poinar, and Raulston (355 strain), *Steinernema carpocapsae* (Weiser) (Mexican 33 strain), *Steinernema feltiae* (Filipjev) (UK76 strain), and *Heterorhabditis bacteriophora* Poinar (HP88 strain), were all capable of infecting and killing three termite species, *Heterotermes aureus* (Snyder), *Gnathamitermes perplexus* (Banks), and *Reticulitermes flavipes* (Kollar), in laboratory sand assays. *S. riobrave* and *S. feltiae* caused low levels of *Reticulitermes virginicus* (Banks) mortality under the same conditions. Nematode concentration and incubation time had significant effects on the mortality of worker *H. aureus*. *S. riobrave* consistently produced highest infection levels and mortality of *H. aureus* in sand assays.

Biocontrol efficacy of nematodes is usually inconsistent due to various abiotic and biotic factors and the complex interactions between these two after application. Cabanillas and Barker (1989) reported that *Paecilomyces lilacinus* was more effective in protecting tomato against *M. incognita* when it was delivered before transplanting or at transplanting stage than after plants were infected by nematodes. Similar results were obtained when tomato or banana plants were treated with the mutualistic endophyte *Fusarium oxysporum* Fo162 at transplanting (Vu 2005; Dababat and Sikora 2007). In comparison, post-planting application of biocontrol agents, especially in the case of endophytes, does not always lead to high levels of biocontrol since the establishment of a biocontrol agent in the rhizosphere is a prerequisite for the control of endoparasitic nematodes (Dababat and Sikora 2007).

### 9.2.6 Viruses

A large number of viruses offer potential as microbial control agents of plant pathogens and insects (Payne 1982). Baculoviruses are widely used as insect pest control agents (Payne 1982; Popham et al. 2016). More than 400 insect species, mostly in the Lepidoptera and Hymenoptera, have been reported to act as hosts for baculoviruses. These viruses are used extensively for control of insect pests in a diverse range of agricultural and forest habitats. Biotechnological techniques are being used for genetic enhancement of baculoviruses for improved insecticidal efficacy. Insects that feed openly on the foliage of host plants are most easily treated, and the most promising results have been obtained against pest of this type (e.g., caterpillars sawfly larvae) (Smith 1967). The efficacy, specificity, and production of secondary inoculum make baculoviruses attractive alternative to broad-spectrum insecticides and ideal components of integrated pest management (IPM) systems due to their lack of untoward effects on beneficial insects including other biological control organisms (Cunningham 1995).

Gibbs et al. (1970) isolated a virus infecting *Coptotermes lacteus* (Froggatt) (Rhinotermitidae), which was similar to acute paralysis virus of the honey bee *Apis mellifera* Linnaeus (Hymenoptera: Apidae). A nuclear polyhedrosis virus, obtained from caterpillars of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), was infective to a laboratory colony of *Kaloterme flavicollis* (Fabricius) (Kalotermitidae) (Al Fazairy and Hassan 1988). Termites died 2–10 days postinfection of viruses under laboratory conditions. The viral insecticide Elcar™ (*Heliothis zea* nuclear polyhedrosis virus, NPV) introduced during the 1980s provided control of cotton bollworm. 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*. *Amsacta moorei* entomopoxvirus has been reported to infect agriculturally important lepidopteran pests such as *Estigmene acrea* and *Lymantria dispar* (Muratoglu et al. 2010).

Insertion of insect-specific toxin genes such as juvenile hormone esterase, diuretic hormone, and prothoracicotropic hormone and genes encoding enzyme inhibitors, neuropeptides, or toxins improved the efficiency of viruses in killing of the insects. Application of recombinant baculoviruses, vAPcmIT2 and vAP10IT2, against two major pesticide-resistant pests *Plutella xylostella* and *S. exigua* resulted in shortening of the lethal time (Tuan et al. 2007). Seo et al. (2005) documented higher pathogenicity for recombinant baculovirus containing a fusion protein with polyhedrin and *Bt* toxin than wild-type strains. 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 hemolymph and reduced the time of insect killing (Gramkow et al. 2010).

### 9.3 Mechanisms Involved in Biocontrol

The mechanisms by which antagonistic microorganisms kill the insects or inhibit the growth of phytopathogenic microorganisms include (1) toxin production, (2) antibiotic production, (3) production of siderophores, (4) production of hydrolytic enzymes, (5) production of secondary metabolites and volatile organic compounds, and (6) phytoalexin production and induction of systemic resistance. Some of the microbial strains produced a wide range of secondary compounds that include siderophores, antibiotics, volatile metabolites, enzymes, etc. (Saraf et al. 2014). Mode of action of different allelochemicals and molecular mechanisms involved in plant-microbe-pathogen/insect interactions will contribute to better disease and insect management.

#### 9.3.1 Toxin Production

*Bacillus thuringiensis* has been found to produce an array of virulence factors including insecticidal parasporal crystal (Cry) toxins,  $\delta$ -endotoxin, vegetative insecticidal proteins, phospholipases, immune inhibitors, and antibiotics (de Maagd et al. 2003). There are about 200 registered *Bt* products in the USA, and worldwide sales of the *Bt* products amounted to about 100 million dollars (about 2% of the total global insecticide market) (Anonymous 1998). Most commercially available formulations are prepared using spore-crystal mixtures with effectiveness against different pest species. Ultraviolet (UV)-resistant mutant strains with high melanin, which absorb light of any wavelength, were used for large-scale production of light-stable insecticides (Liu et al. 2013). Most of the insecticidal activity of *Bacillus thuringiensis* is associated with the proteinaceous toxins located in the parasporal inclusion bodies, also known as parasporal crystals. The toxins found in the parasporal crystals are collectively referred to as

$\delta$ -endotoxins. The Cry1 proteins (protoxins) which are found in the crystals are biologically inactive. Following ingestion and solubilization in the alkaline midgut, cleavage of protoxins by gut proteases produces a 60- to 65-kDa activated protein that recognizes specific binding sites at the brush border membrane surface of epithelial columnar cells lining the gut lumen (van Rie et al. 1989). The activated protein subsequently causes pore formation, membrane transport disruption, and cell lysis leading to the death of the insect (Bravo et al. 2007).

Vegetative insecticidal proteins (Vips) produced by *B. cereus* and *B. thuringiensis* also showed similar activity to endotoxins. Vip1 and 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), and *Heliothis zea*, *Trichoplusia* sp., and *Ostrinia nubilalis* (Fang et al. 2011; Sellami et al. 2011). The pathogenic action of the *B. cereus* bacterium normally occurs after ingestion of spores and crystalline inclusions containing insecticidal  $\delta$ -endotoxins specifically interact with receptors in the insect midgut epithelial cells (Pigott and Ellar 2007). *Lysinibacillus sphaericus* (*B. sphaericus*) produced insecticidal toxins during the vegetative phase of growth, and mosquitoes have been found to be the major targets of bacterium. 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*, the exotoxins of microbial origin from *Pseudomonas* sp. were found toxic to larvae of mosquitoes as well as lepidopteran insects (Murty et al. 1994). *P. aeruginosa* oxyR mutant revealed its ability to kill the insect *Drosophila melanogaster* (Lau et al. 2003). *P. aeruginosa* strain also conferred an efficient protection against *Galleria mellonella* and *Bactrocera oleae* (Mostakim et al. 2012), and the potency was due to the presence of quantitatively as well as qualitatively different proportions of biosurfactants in the crude glycolipids (Desai and Banat 1997). *B. subtilis*, *B. amyloliquefaciens*, *B. megaterium*, and *Pseudomonas* sp. showed more than 50% mortality in *S. litura* and *H. armigera* (Gopalakrishnan et al. 2011).

Hurst et al. (2011) isolated *Yersinia entomophaga* from diseased larvae of the New Zealand grass-grub, *Costelytra zealandica* White (Coleoptera, Scarabaeidae). This bacterium secreted a multi-subunit toxin complex (Yen-Tc) that showed homology with toxin complexes produced by *Photorhabdus* sp. Tc-like proteins were also identified in other entomopathogenic bacteria such as *Serratia entomophila* and the nematode symbiont *Xenorhabdus nematophila* (Morgan et al. 2001). Histopathological studies on the effects caused by the ingestion of Yen-Tc revealed the progressive disorganization and deterioration of the midgut epithelium of *C. zealandica*. Recently, the insecticidal activity of formulations containing *Y. entomophaga* against the pasture pest porina (*Wiseana* sp. larvae) has been reported under the field conditions (Ferguson et al. 2012).

A mixture of soluble endotoxin, spores, and inclusion bodies of *Bacillus thuringiensis* (Berliner) caused greater than 95% mortality of the subterranean termite species, *Reticulitermes flavipes* (Kollar) and *R. hesperus* Banks

(Rhinotermitidae), after 6 days of exposure of the laboratory colonies (Smythe and Coppel 1965). Khan et al. (1978, 1985) employed a commercial preparation of Bt (Thuricide-HP concentrate) which exhibited 100% mortality of *H. indicola*, *M. championi*, and *Bifiditermes beelsoni* (Gardner) (Kalotermitidae) within 6 days of exposure. Grace and Ewart (1996) constructed recombinant cells of the bacterium *Pseudomonas fluorescens* that expressed the  $\delta$ -endotoxin genes of *Bacillus thuringiensis* (Bt). Two commercial agricultural formulations prepared by the CellCap process were evaluated for palatability to the termite *C. formosanus*. The MVP formulation, active against Lepidoptera, contained the *P. fluorescens* encapsulated  $\delta$ -endotoxin of Bt var. *kurstaki*. Similarly, the M-Trak™ formulation, active against Coleoptera, contained the  $\delta$ -endotoxin of Bt var. *san diego*. The palatability of the CellCap formulations indicated that the host bacterium, *P. fluorescens*, is a suitable delivery system for genetically engineered termiticides. Gunner et al. (1994) reported that the spores of entomopathogenic fungi may contain toxins which may kill the termite host when ingested. Insecticidal cyclic depsipeptides were found to be produced by entomopathogenic fungi including the destruxins from *M. anisopliae* var. *major* (Kaijiang and Roberts 1986) and *Aschersonia* sp. (Krasnoff and Gibson 1996) and the beauvericins from *B. bassiana* (Jegorov et al. 1989). It has been suggested that depsipeptides are localized on the surface of *Beauveria* sp. spores (Jegorov et al. 1989), whereas *Metarhizium* destruxins are generally associated with in vivo or in vitro mycelial growth (Chen et al. 1999).

### 9.3.2 Production of Antibiotics

Antibiotic production by microorganisms is one of the major mechanisms postulated for disease control. These antimicrobial compounds may act on plant pathogenic fungi by inducing fungistasis, inhibition of spore germination, and lysis of fungal mycelia or by exerting fungicidal effects. A large number of antibiotics including diacetylphloroglucinol, oomycin A, phenazines, pyocyanin, pyrroles, pyoluteorin, pyrrolnitrin, etc. are produced by rhizobacteria (Bender et al. 1999), which help in suppression of pathogen growth. Kido and Spyhalski (1950) isolated antimycin A from cultures of an unidentified species of *Streptomyces*. The initial tests showed that the antibiotic caused mortality to some insects which ingested the material. The toxicity of antimycin A was not confined to members of the Insecta, as it showed efficacy for the control of the red spider mite, *Tetranychys* sp. Beck (1950) reported that antimycin A had insecticidal possibilities for some species of insects and mites. The antibiotic inhibited either the succinoxidase system or some other essential step in the oxidative metabolic cycle of cockroaches. Inhibition of the oxidative cycle readily explained the depression of oxygen consumption by the poisoned insects. *Photorhabdus* is a virulent pathogen that kills its insect host by overcoming immune responses (Eleftherianos et al. 2007). *Photorhabdus* produces a small-molecule antibiotic (*E*)-1,3-dihydroxy-2-(isopropyl)-5-(2 phenylethenyl)benzene (ST) that also acts as an inhibitor of phenoloxidase (PO) in the insect host *Manduca*

*sexta*. The bacterium inhibits two key immune defenses of the insect: activity of the antimicrobial enzyme PO and formation of melanotic nodules. Due to production of antibiotics, different *Brevibacillus laterosporus* strains showed insecticidal action in different orders, including Coleoptera, Lepidoptera, and Diptera (Ruiu 2013).

### 9.3.3 Siderophore Production

Many microorganisms synthesize extracellular siderophores, in response to iron stress (Neilands 1981), which are involved in disease suppression and plant growth promotion. Extracellular siderophores of the brown-rot wood decay fungus *Gloeophyllum arabeum* (Persoon: Fries) Murnill (Polyporaceae) were found to inhibit feeding by *C. formosanus* termites (Grace et al. 1992). Siderophore-treated filter paper disks showed negligible feeding, whereas untreated disks were almost completely consumed over a 3-day test period.

### 9.3.4 Production of Extracellular Enzymes

Different extracellular enzymes are produced by rhizosphere microorganisms which contribute to killing of the pathogens and insects. Fungal isolates that produce extracellular enzymes to degrade the host cuticle have large scope in pest management. For example, *M. anisopliae* grown in optimum fermentation conditions produced host-degrading enzymes such as acid phosphatase and phosphatase isoenzymes (Strasser et al. 2000; Li et al. 2007). *Trichoderma* produced protease (31 kDa) and chitinase (44 kDa) during the growth phase (Shakeri and Foster 2007), and it also produced a number of antibiotics, such as trichodermin, trichodermol, harzianum A, harzianolide, and peptaibols (Hoell et al. 2005). 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. littoralis*. *Tolypocladium* and *Isaria fumosorosea* were found toxic to *Plutella xylostella* (Freed et al. 2012).

Different microorganisms including bacteria, fungi, and actinomycetes were found to produce proteases from various types of natural resources. Lysenko and Kucera (1971) showed that *Serratia marcescens* produced extracellular proteases that could be a mode of pathogenicity of these bacteria in termites. Osbrink et al. (2001) examined 15 bacteria and one fungus associated with dead termites as possible biological control agents against Formosan subterranean termites, *Coptotermes formosanus* Shiraki. Bacterial isolates obtained from dead termites were primarily *Serratia marcescens* Bizio that caused septicemia in *C. formosanus* and found to contain proteolytic enzymes. Singh (2007) reported chitinolytic activity in some of the bacterial isolates that killed the termites. Bahar et al. (2011) identified chitinase producing *Serratia marcescens* which were found



effective in killing the coleopteran insects with more chitin in their exoskeleton. Jafri et al. (1976) found microsporidians in the body cavity and proventriculus of *Microcerotermes championi* collected from the roots of *Saccharum munja*. These organisms passed into the midgut after ingestion with the food, attacked fat body tissues, and caused death of termites, indicating the role of lipolytic enzymes in termite killing. Rakshiya et al. (2016) reported that some of the bacterial isolates were found effective in termite killing and possessed all the three enzyme activities, i.e., lipase, protease, and chitinolytic activity. Lack of correlation between enzyme activities and termite killing indicated that besides the production of three enzymes, some other metabolites (toxin or siderophore) could also be contributing to the killing of termites.

### 9.3.5 Production of Secondary Metabolites and Volatile Organic Compounds

A large number of secondary metabolites are produced by rhizosphere bacteria, which play important roles in disease control and plant growth promotion. Hydrogen cyanide (HCN) is known to be produced by many rhizosphere bacteria and has been demonstrated to play a role in the biological control of the pathogens and pests.

HCN-producing *P. aeruginosa* was found to have lethal effects on nematodes (Darby et al. 1999; Gallagher and Manoil 2001). Devi et al. (2007) tested three different species of HCN-producing rhizobacteria for their potential to kill subterranean termite *O. obesus*. The three bacterial species, *Rhizobium radiobacter*, *Alcaligenes latus*, and *Aeromonas caviae*, were found effective in killing the termites under in vitro conditions. *R. radiobacter* and *A. latus* caused 100% mortality of the termites following 1-h incubation. *A. caviae*, which produced significantly lower amounts of HCN, caused only 70% mortality. Termites exposed to exogenous HCN showed 80% mortality at cyanide concentrations of up to 2 µg/ml. The observed HCN toxicity in termites could be correlated with the inhibition of the respiratory enzymes.

Daisy et al. (2002) showed that naphthalene, an insect repellent, is produced by a fungus, *Muscodor vitigenus*. Three species of *Muscodor* and one *Gliocladium* sp. that produce volatile organic compounds with biocidal activity were isolated from several host plants in geographically diverse areas. A large number of metabolites are produced by different fungi (Table 9.2), which showed adverse effects on the insects (Boonphong et al. 2001; Shakeri and Foster 2007). For example, bassianin, beauvericin, bassianolide, and bassiacridin were produced by *Beauveria* sp. that controlled the *Culex pipiens*, *Aedes aegypti*, *Calliphora erythrocephala*, and *H. zea* (Quesada-Moraga and Alain 2004). Similarly, *Trichoderma* sp. produced trichodermin, trichodermol, harzianum A, harzianolide, and peptaibol metabolites having deleterious effects on *Tenebrio molitor* (Shakeri and Foster 2007).

**Table 9.2** Secondary metabolites of fungi effective against insects

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

### 9.3.6 Induction of Systemic Resistance

Salicylic acid (SA) and jasmonic acid (JA) hormones control defense responses to different types of microbes, and they orchestrate a different and complex transcriptional reprogramming that eventually leads to plant resistance. Evidences indicate that cyclic precursors of jasmonic acid (JA), the cyclopentenones, can also function as potent signals of plant defense responses (Farmer and Ryan 1992). Similarly, volatile derivatives of JA, such as methyl jasmonate (meJA) and cis-jasmone, can act as airborne signals stimulating plant defenses and repelling insects (Birkett et al. 2000).

The attack of insect herbivores on the plant roots and leaves imposes different selection pressures on plants, which in turn produces contrasting responses in terms of changes in biomass, gene expression, production of secondary metabolites, and wound hormones (Johnson et al. 2016). Different kinds of plant defenses are reported against root herbivores as compared with foliar herbivores (Johnson and Rasmann 2015). Following herbivore recognition, plants configure their metabolism through changes in the phytohormonal networks (Johnson et al. 2016). Jasmonates, which are widely viewed as the master regulators of plant responses to herbivores, are less inducible in the roots than the leaves (Erb et al. 2012; Lu et al. 2015). Salicylic acid signaling can buffer the jasmonic acid response aboveground (Gilardoni et al. 2011). Root herbivores attack induces different signal signature compared with leaf attack. For instance, attacked rice roots do not increase the biosynthesis of abscisic acid and ethylene (Lu et al. 2015), two important synergistic signals in the wound response of leaves. The difference may be explained by the fact that both hormones strongly influence root growth and architecture; plants may therefore be able to maintain root development under herbivore attack by maintaining abscisic acid and ethylene homeostasis. Thus, it is apparent that roots respond to pathogen or insect attack differently than shoots and regulate the defenses through modulating their phytohormonal networks in a tissue-specific manner.

Biological control of termites may also be facilitated if their highly evolved immune systems can be suppressed (Connick et al. 2001). Eicosanoids (C20 polyunsaturated acids) have been found to play an important role in protecting insects from bacterial infections (Miller et al. 1994). In laboratory experiments, the eicosanoid biosynthesis inhibitors dexamethasone, ibuprofen, and ibuprofen sodium salt were each provided along with a red-pigmented isolate of *Serratia marcescens* Bizio to the Formosan subterranean termite, *C. formosanus* Shiraki, by means of treated filter paper (Stanley-Samuels et al. 1991). The increased mortality was observed with dexamethasone and ibuprofen suggesting that the termites' immune systems were suppressed by these compounds, making the insects more vulnerable to infection by *S. marcescens* (Connick et al. 2001). This effect on mortality was noted only at  $3.4 \times 10^{10}$  colony-forming units  $\text{ml}^{-1}$  treatment level. A significant amount of infection and subsequent mortality may have resulted from direct contact with the bacterium and the remainder from its ingestion.

#### 9.4 Approaches to Increase the Efficiency of Biocontrol Agents

Various entomopathogenic microbial species are normally able to persist in the environment, multiply in the host, and spread to other susceptible hosts. These entomopathogens have developed different strategies to attack, enter, and kill the attacking insect. Mycopathogens enter through the cuticle, whereas virus, bacteria, and protozoa enter through the midgut. Connick et al. (2001) reported that *Serratia marcescens* isolate T8 was highly virulent to the *Coptotermes formosanus* and termite mortality was 24% by 2 days and 99% after 19 days of the exposure. Nematodes belonging to the two families, *Steinernematidae* and *Heterorhabditidae*, have shown promise for use in termite control programs (Kaya and Gaugler 1993; Yu et al. 2006). The limited numbers of field trials attempted on insect or pathogen control have failed largely in reducing pathogen densities below economically damaging levels. Another factor that can contribute to inconsistent performance of biocontrol agents is variable production or inactivation in situ of microbial metabolites responsible for killing of insect/pathogen. The inconsistency in performance is a major constraint to the widespread use of biocontrol agents in commercial agriculture. Future strategies are required to clone genes involved in the production of toxins, antibiotics, and other metabolites so that these cloned genes could be transferred into the microbial strains having good colonization potential (Grace and Ewart 1996). Biotechnological approaches used in manipulation of microbial traits could lead to improved biocontrol activity of pathogenic microorganisms in the control of insects. The development of more stable formulations, such as microencapsulation, would be necessary to ensure their long-term, residual action (Grace and Ewart 1996).

## 9.5 Constraints in Development and Application of Biocontrol Agents

Recently, various biocontrol agents have been tested under field conditions on different crops for controlling pests and diseases of crop plants, and some of the antagonistic bacterial strains have led to the development of commercial biocontrol products. The major disadvantages in using microbes as a biocontrol agent include variability of field performance and the necessity to ensure survival and delivery of the product. Moreover, the effectiveness of a given biocontrol agent may be restricted to a specific location due to the effects of soil and climate. Many soil edaphic factors including temperature, soil moisture, pH, clay content, and interactions of biological disease control microorganisms with other rhizosphere bacteria and with pathogens will also affect their viability and tolerance to adverse conditions once applied. During root colonization, introduced biocontrol agents have to compete with indigenous microflora for carbon source, mineral nutrients, and infection sites on the roots. Sometimes, this competition is so severe that introduced biocontrol agents fail to survive in the soil. Another factor that can contribute to inconsistent performance is variable production or inactivation in situ of bacterial metabolites responsible for killing of the pathogen/insect.

Biological control strategies are also emerging as promising alternatives to the use of synthetic pesticides in the preservation of fruits. Antagonists must survive after their exposure to both postharvest treatments and storage conditions. The limitations of these biocontrol products can be addressed by enhancing the biocontrol through manipulation of the environment, using mixtures of beneficial organisms, physiological and genetic enhancement of the biocontrol mechanisms, manipulation of formulations, and integration of biocontrol with other alternative methods that in combination with biocontrol agents may provide additive or synergistic effects for adequate protection.

## 9.6 Conclusion

The role of microbial pesticides in the integrated management of insect pests has been reviewed for agriculture, forestry, and public health. In most cases, no single microbial control agent provides sustainable control of an insect pest or complex pests. As components of an integrated approach in all agricultural practices, entomopathogens could provide significant and selective insect control without interfering with the effectiveness of other practices. In the near future, synergistic combination of microbial control agents with other technologies (in combination with semiochemicals, soft chemical pesticides, other natural enemies, resistant plants, remote sensing, etc.) may enhance the effectiveness and sustainability of integrated control strategies. Till now, the market for microbial insecticides hardly represents only 1% of the total crop protection market. In the near future, microbials

will face even stiffer competition from new pesticide chemistries and transgenic plants. However, several microbial control agents have good potential for use in integrated pest management (IPM) programs.

Complete elimination of chemical pesticides for controlling plant pests and diseases in modern agriculture may be impossible, but a logical reduction in their application is absolutely feasible. Biopesticide use in combination or rotation with synthetic pesticides is likely to be enhanced in the near future, but more research is needed to come up with innovative solutions that can really meet farmer and regulator needs in terms of effectiveness and environmental sustainability (Glare et al. 2012). To have a sustainable agricultural system with minimum contamination and risks to the environment, a combination of all available methods should be applied to manage pest problems, and this can be achieved by integrated pest management. Implementation of IPM strategies may be the safest solution for management of pest problems including insect infestation in every cropping system. Biological control could be one of the most important components of integrated pest management, which can lead us toward a safe, sustainable, and environmentally sound agricultural system in the future.

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