Chapter 15
Biopesticides: Microbes for Agricultural Sustainability

Fatemeh Salimi and Javad Hamedi

Abstract The human population is growing over time. In this regard, the agricultural yield should be improved and effective strategies must be intended to minimize crop loss to meet the food demand of this population. One of the detrimental groups that adversely affect agricultural yield is pest. Therefore, pesticide application can be considered as a promising approach in diminishing pests corresponding to damages to agricultural yield. Although improper and extensive usage of non-biodegradable chemical pesticides can adversely affect ecosystem and health of human, animal and non-target organisms. Therefore, alternative strategies should be considered to augment plant growth, preserve agricultural yield and compensate for reduced consumption of chemical fertilizers. The most suitable substituent for chemical pesticides is biopesticides. They are formulated pesticides containing various microorganisms (nematodes, bacteria, fungi and viruses) or plant, animal, bacteria and fungi-derived compounds that ecofriendly control insect, weed, nematode and plant disease by various mechanisms and, therefore, gaining importance all over the world. Some of the biopesticides have equal efficiency comparing with chemical pesticides while having no pathogenicity or toxicity on non-target micro- and macroorganisms, so they can be applied near harvesting time. In addition, due to their decomposability feature, they do not remain in agricultural products and do not compromise air, groundwater and soil quality. Microorganisms in biopesticides impose their effects via producing antimicrobial compounds, lytic enzymes or compete with phytopathogens for uptake nutrients, attachment, establishment, and colonization on plants. Interfering in communication of pathogens via degrading of chemical signal messenger or inducing resistance in plants are other strategies which are applied by biofertilizers. In this chapter, we reviewed the types of biofertilizers,

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their mode action and their limitation as well as molecular and culture-based monitoring strategies, fermentation procedures by which microbial cells are multiplied, types of formulation, their advantages and limitation are also discussed.

Keywords Agricultural applications · Bacteria · Biopesticides · Fermentation · Formulation · Fungi

15.1 Introduction

Agricultural productivity can be enhanced through applying high-yielding varieties, optimum irrigation, managing fertilization and minimizing pest-associated losses. In best condition, a meaningful portion of agricultural productivity is lost because of the influential effect of the pests. It has been estimated that pests cause a dramatic reduction in rice (51%), wheat (37%), maize (38%), potato (41%), cotton (38%), soybean (32%), barley (32%) and coffee (29%) (Sharma et al. 2001). According to Food and Agriculture Organization (FAO), pests, weeds and phytopathogens are responsible for a significant loss (20–40%) of the world's potential crop production annually (Fao 2012). Therefore, effective crop protection strategies should be intended to minimize crop loss in the field (pre-harvest losses) and during storage (post-harvest losses) (Oerke 2006). It seems that two-thirds of all crops will be lost if not using pesticides (Deedat 1994). Before the development of chemical pesticides, natural enemies of those specific pests were considered as a crucial strategy to biologically control pests and their corresponding damages to agricultural yield. Pesticide usage has profoundly improved the yield and quality of agricultural production.

According to FAO definition, the pesticide is any pure compound or their mixture, which is applied to prevent, destroy, repel or mitigate insect pests (insecticides), plant diseases, weeds (herbicides), rats, fungal infections (fungicides) or other unwanted organisms and interfering agents in the critical process of production, processing, storage, transportation or marketing of food and agricultural commodities to increase crop yield. They can act as a regulator of plant growth, defoliant, desiccant or preservation compounds, which preserve the agricultural products from spoilage during storage and transport. Pesticides are divided into two groups: chemical and biological pesticides according to their origins (Thakur et al. 2020). However, they can be also categorized into distinct classes according to their target organism (insecticides, herbicides, fungicides, rodenticides and fumigants), chemical structure, physical state, mode of action and application route. Chemical fertilizers that act very effective, affordable and rapid play an undeniable role in the yield of agriculture to meet the enhancing requirement of increasing world population to the food. Meanwhile, the use of biofertilizers is promising and increasing due to the limitations of chemical fertilizers.

Improper and extensive usage of non-biodegradable chemical pesticides including chlorinated hydrocarbons, organophosphates and carbamates can impose deleterious effect on human and animal health (neurological, psychological, behavioral and immune system dysfunctions and hormonal imbalances, reproductive system defects, genotoxicity and blood disorders) as well as ecosystems via enhancing hazardous residue through food chain, contaminating soil and groundwater (Barnawal et al. 2016; Sharma et al. 2021), destroying soil quality and fertility, creating hard water, emerging pesticide-resistant insects, mites, pathogenic fungi, pathogenic bacteria, pathogenic nematodes and weeds (which is due to modification of their target receptors involved in pesticide activity and results in consecutive failures of the commercial controlling agents to gain an effective rate of control when applied based on the label recommendations and necessitate new pesticide) (Kogan et al. 1982), reducing biodiversity as well as beneficial microbial activities like nitrogen fixation and disturbing biological balances by their non-specific effect on non-target organisms and acute poisoning (Carvalho 2017). In addition, through their non-specific action, it is possible that they induce a harmful effect on non-target organisms like insects/pests predators or parasites. Therefore, alternative strategies should be considered to augment plant growth, preserve agricultural yield and compensate for reduced consumption of chemical fertilizers like organochlorine, organophosphate, carbamate, pyrethroid, halogenated insecticides (Smith and Gangolli 2002) through inhibiting the growth of detriment pests. Biological pesticides are environmentally friendly alternatives to chemical pesticides (Gupta and Dikshit 2010; Kumar et al. 2021; Yadav 2021).

By revealing various adverse effects of chemical pesticides, a lot of studies are conducting to find and introduce efficient and safe biocontrol agents as biopesticides. Biopesticides are formulated pesticides containing various microorganisms (nematodes, bacteria like *Bacillus thuringiensis* and *Bacillus sphaericus*, fungi like *Trichoderma* and virus-like nucleopolyhedrosis) or plant, animal, bacteria and fungi-derived compounds that ecofriendly control insect, weed, nematode and plant disease by non-toxic mechanisms and, therefore, gaining importance all over the world for turf, field crop, orchard and garden (Grewal et al. 2005). A lot of bacterial (>100), entomopathogenic fungal (>800), viral (>1000) and protozoan species (>1000) have been known as insect pathogens. Biopesticides are frequently used along with other controlling substances like chemicals (Senthil-Nathan 2015).

Biopesticides have equal efficiency comparing with chemical pesticides while having no pathogenicity or toxicity on non-target macroorganisms (including predators, parasitoids, pollinators, animals and humans), beneficial microorganisms, communities and ecosystems as they have a narrow activity spectrum (target-specific) and their toxic action is mostly specific on pest of interest; also they can be applied near harvesting time. In addition, they have no residue problem that is an issue of substantial concern for consumers. They are usually effective in very small quantity and, therefore, biofertilizer application leads to lower exposures of non-target organisms and minimized pollution problems. In some cases, the establishment of biopesticides in a pest population or their habitat assures efficient control of pest in subsequent generations or seasons. Biopesticides can promote plant growth and agricultural yield by acting at the same time as biofertilizers and improving the growth of plant roots and beneficial microorganisms (Hesham et al. 2021; Yadav et al. 2021). They do not decline air, groundwater and soil quality because of their naturally and quickly decomposability feature. Finally, they can be considered as a constituent of integrated pest management (IPM) (Usta 2013).

The introduction of live organisms or their derived compounds as a commercial pesticide requires comprehensive investigations including systematic studies on biological agent properties, its pesticide mechanism and its probable pathogenicity on non-target macro- and microorganisms. Ecological investigations on the dynamics of diseases in pest population of interest should be conducted due to the significant effect of environmental factors on disease outbreaks; also a wide range of studies should be evaluated biopesticides persistence and dispersal potential. High-qualified technologies should be considered for large-scale production of viable agents or their derived products to make biopesticides without contamination.

Since the formulation tremendously affects biopesticide efficiency and shelf life, extensive studies should be performed to design a suitable formation. In this regard, dry formulations are preferred comparing to liquid ones. In addition, the speed of killing pests should be improved to meet farmers' requirements.

Co-application of biopesticides along with chemical pesticides may be inappropriate, in some cases due to incompatibility occurrence, which includes the adverse effect of chemical compounds on the living organism. There are some physicochemical conditions like heat, desiccation or exposure to ultraviolet radiation, which deactivate biopesticides. Formulation and storage procedures can profoundly affect the efficiency of biopesticides. Since applying one biopesticide cannot control several pests due to their pest-specific activity, it is possible that their potential market be limited. Also, complicated production, formulation, and storage processes of biopesticides lead to their high cost in comparison with chemical pesticides (Fig. 15.1).

15.2 Classification of Biopesticides

According to active ingredients in the biopesticides or their origin, they can be divided into three categories including microbial pesticides (bacterial, fungal, viral, nematode, protozoan), biochemical pesticides including compounds derived from animals or plants and plant-incorporated protectants, which are the results of incorporation of pesticide coding genes into the plant's genetic material.

15.2.1 Microbial Pesticides

It has been estimated that the portion of bacterial, fungal, viral, predator and other biopesticides from global biopesticide market is 74%, 10%, 5%, 8% and 3%, respectively (Thakore 2006). Active ingredients in microbial pesticides, whether the microorganism itself or its product, maybe native or genetically engineered. Currently, 73 active microbial ingredients with significant pesticide activities have

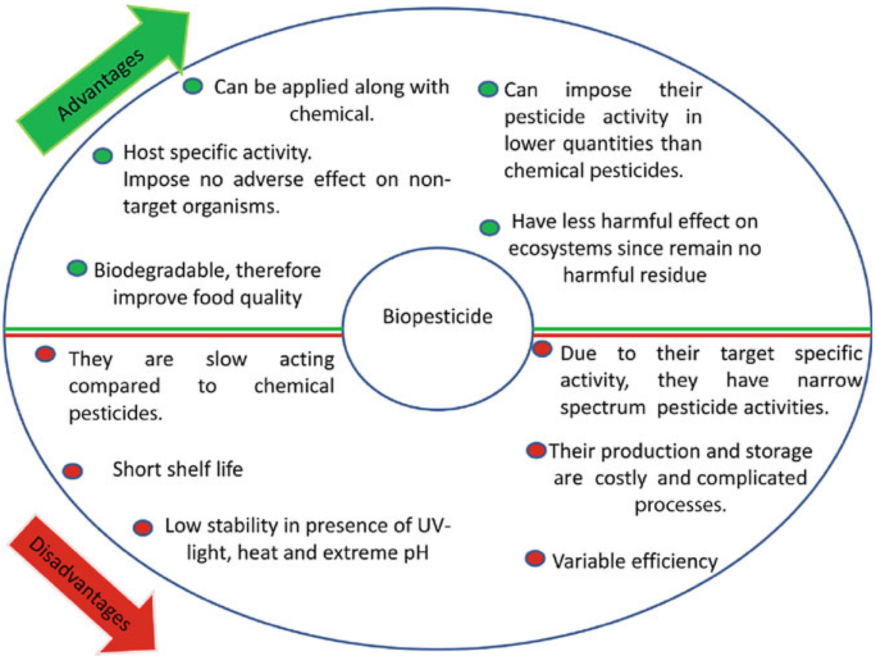

Fig. 15.1 Advantages and disadvantages of biopesticides

been registered by the United States Environmental Protection Agency (US EPA). Microbial pesticides include mainly biofungicides (*Trichoderma*, *Pseudomonas*, *Bacillus*), bioherbicides (*Phytophthora*) and bioinsecticides (*Bacillus thuringiensis*) (Gupta and Dikshit 2010). They inhibit pests through synthesizing specific toxic, antibacterial or antifungal bio compounds, blocking attachment, establishment and colonization of other microbial cells via parasitism or competition. Insecticides usually have specific activity on various species of moths, butterflies, beetles, flies and mosquitoes. A lot of microbial insecticides can preserve their bioactivities in the presence of synthetic chemicals, which make their usage as a mixture possible to achieve better pest management (Kachhawa 2017).

Microorganisms through producing various antimicrobial compounds like cyclolipopeptides, phenolic compounds, bacteriocins or degrading enzymes against pathogenic bacteria and fungi limit their growth. Production of fengycins (produced by *Bacillus subtilis*) (Fan et al. 2017), pyrrolnitrin (produced by *Pseudomonas cepacia*) (Cartwright et al. 1995) herbicolin, pantocins (produced by *Pantoea agglomerans* and *Pantoea vagans*) (Ishimaru et al. 1988; Smits et al. 2010; Wright and Beer 2001) and lytic enzymes (produced by some yeast and fungi like *Trichoderma harzianum*) (Batta 2004) are some examples for this strategy, which are applied by several microorganisms. In another strategy, they compete with plant pathogens for assimilation of nutrients, attachment, establishment and colonization on plants

(Sharma et al. 2009). Some microorganisms like *Pichia* and *Trichoderma* via interfering in communication of pathogens through degrading of chemical signal messengers (which are essential for communication through quorum sensing e.g. acylhomoserine lactones) (Molina et al. 2003) or inducing resistance in plants (through producing either elicitors or messenger molecules e.g. salicylic acid) (Spadaro and Gullino 2004) limit the unfavorable effect of pests on plants (Harman et al. 2007). Viral-based biopesticides that containing fungal, bacterial or insect viruses can limit the growth of phytopathogens through parasitism and lysis of pathogenic bacteria, fungi or insects (Ghabrial and Suzuki 2009).

15.2.1.1 Bacterial Pesticides

Bacterial pesticides are the most common and cost-effective pesticides. These pesticides are usually applied as biological agents to kill insects, insecticides; also they can also be used to control unwanted bacteria, fungi or viruses. Producers mostly belong to *Bacillaceae*, *Pseudomonadaceae*, *Enterobacteriaceae*, *Streptococcaceae* and *Micrococcaceae* genera (Tanada and Kaya 2012). Bacterial pesticides colonize various organs of plants including roots and leaves to obstacle phytopathogen attachment, establishment, colonization and finally pathogenesis (O'Brien et al. 2009). Microbial insecticides specifically kill particular species of moths and butterflies or species of beetles, flies and mosquitoes. For this purpose, they should come into contact with pests of interest or be ingested by them. In this regard, bacteria via producing endotoxins specifically damage the digestive system of insects.

Most commercial microbial pesticides are produced by the subspecies and strains of the *Bacillus* genus, which frequently exist in soil and possess wide genetic biodiversity. They can create spores that are tremendously tolerant dormant forms able to resist extreme temperatures, pH, drought and starvation. Therefore, they could be significant sources of potential microbial biopesticides (Piggot and Hilbert 2004).

Almost 90% of commercial pesticides in the USA are *B. thuringiensis* (Bt) containing pesticides (Kumar and Singh 2015). *B. thuringiensis*is an aerobic, Gram-positive, spore-producing soil bacterium whose biopesticides are extensively applied to control agriculturally and medically important insects (Mazid et al. 2011). Its biopesticide action is based on the production of crystalline inclusions that contain δ endotoxins or cry proteins during sporulation. They have no toxicity to other organisms, including vertebrates and beneficial insects. A different mixture of proteins is produced by each strain of *B. thuringiensis*, which are capable to particularly destroy one or a few related species of insect larvae. The generated toxin can bound to the receptors of larval gut, so lead to its starvation. Whenever the insect feeds on the *B. thuringiensis* contaminated foliage, Cry proteins are hydrolyzed in the midgut of insect and consequently an active endotoxin is produced and its attachment to receptor sites on epithelial cells in the gut is resulted in ionic disbalance of the cell via forming transmembrane pores or ion channels. This event leads to cell lysis due to osmotic shock. Paralysis of the insect's mouthparts and gut is considered as subsequent symptoms (Lambert et al. 1992). High efficiency and environmental safety of

B. thuringiensis and cry proteins make them suitable alternatives to chemicals with pesticide activity to kill insect pests (Roy et al. 2007).

The extensively applied microorganisms with effective biopesticide activity are strains of *B. thuringiensis.* They can efficiently kill three genera of mosquitos including, *Culex*, *Culiseta* and *Aedes*. *B.thuringiensis* var. *tenebrionis* strain Xd3 (Btt-Xd3) also exhibited biopesticide activity on *Agelasticaalni* (Eski et al. 2017). It has been proved that the bacteria can survive for a considerable time. Five percent of applied *B. thuringiensis* can survive after a year in the form of spores. Nowadays, using genetic engineering, insect-resistant crops such as cotton, maize, potato and rice have been produced through transferring coding genes of the insecticidal crystal proteins into their genetic material. The first developed *B. thuringiensis* insecticidal agent was a mixture of *B. thuringiensis* spores and its toxin. *B. thuringiensis*-based formulated pesticides are present in solid (powdery or granulated) or liquid forms. These products contain spores and toxin crystals and are used on feeding sites of larvae like leaves (Usta 2013).

Another *Bacillus* with larvicidal characteristics is *B. sphaericus*. This bacterium is frequently found in the soil and has been applied to biologically control *Culex* and *Anopheles* populations in diverse geographical regions. Although there are *B. sphaericus* resistant insects like *Psorophora*, *Aedesaegypti* and *Ae. albopictus.* It is first isolated from *Simulium* in Nigeria with low larvicidal activity. *B. sphaericus* 1593, which was isolated from dead mosquito larvae in Indonesia exhibit a significantly higher mosquitocidal activity on *Culexquin quefasciatus.* This strain has been applied as an insecticide in the field as part of vector control programs (Kellen et al. 1965). This bacterium produces a fetal pro-toxin during its sporulation, which is causative agent of fatal cellular alterations in the cells of insects. It has been revealed that some toxins may be located in several parts of the cell like cell wall but the spore possesses the most concentration of the toxin (Brownbridge and Margalit 1987; Charles et al. 1993). Vectolex is a commercial biopesticide with a larvicidal activity, which contains *B. sphaericus.*

Pseudomonas syringae Van Hall with the commercial name of Bio-Save has been used to control fungal infection in various fruits like apples, pears and citrus (Koul et al. 2001). Antinsectan compounds derived from actinobacteria and some fungal strains (e.g. milbemycins, actinomycin A, nikkomycin, piericidins, aplasmomycin, avermectins, citromycin, spinosyns, various cyclic peptides, etc.) and other bacteria (e.g. aminolevulinic acid, thiolutin, thuringiensin, xenorhabdins) are compounds with antifeedants, toxic, growth inhibitory effect and physiological disrupter activities on various pests (Dowd 2001; Kirst 2010; Koul and Dhaliwal 2003). In this regard, avermectins and spinosyns are some of the commercialized compounds (Tables 15.1, 15.2, 15.3 and 15.4).

15.2.1.2 Fungal Pesticides

Fungal biopesticides are containing fungal strains, which are capable of controlling insects, pathogenic fungi or bacteria, nematodes and weeds (Table 15.5). These

Table 15.1 Biopesticides derived from bacteria belonged to *Bacillus* genus

Microorganisms	Trade name	Host range
Bacillus thuringiensis var. kurstaki(Bt)	Bactur, Bactospeine, Bioworm, Caterpillar Killer, Dipel, Futura, Javelin, SOKBt, Thuricide, Topside, Tribactur, Worthy Attack, Lepidocid, Rokur, Bio-Dart, Biolep, Halt Taciobio-Btk, Imperial, Tuneup, Gumulmang, Biobit, Bychung, Bigule, Samgong BT, Shuricide, Youngil BT	Larvae of moths and butterflies
Bacillus thuringiensis subsp. *Kurstaki* ABTS 351, PB 54, SA 11, SA12, and EG 2348	Batik, Delfin	Lepidoptera pests
Bacillus thuringiensis subsp. *kurstaki* BMP 123	BMP 123 Prolong	Lepidoptera pests
Bacillus thuringiensis subspp. *Aizawai* and *kurstaki*	Agree	Lepidoptera larvae
Bacillus thuringiensis subsp. *Israelensis*	VectoBac, Tacibio, Technar, Aquabee, Bactimos, Gnatrol, LarvX, Mosquito Attack, Skeetal	Mosquito, Lepidopteran pests, Sciarids, larvae of Aedes and Psorophora mosquitoes, black flies, and fungus gnats
Bacillus thuringiensis var. tenebrinos	Foil, M-One, M-Track, Novardo, Trident	Larvae of Colorado potato beetle, elm leaf beetle adults
Bacillus thuringiensis var. aizawai	Certan, Biocan, Salchungtan, Scolpion Solbichae, Tobagi	Wax moth caterpillars and Lepidopteran pests
Bacillus thuringiensis subsp. *Aizawai* GC-91	Turex	Lepidoptera pests
Bacillus thuringiensis subsp. *Tenebrionis* NB 176	Novodor	Coleoptera pests
Bacillus popilliae and *Bacillus lentimorbus*	Doom, Japidemic, Grub Attack	Larvae of Japanese beetle
Bacillus sphaericus	Vectolex CG, Vectolex WDG	Larvae of *Culex*, *Psorophora*, and *Culiseta* mosquitos, larvae of some *Aedes* spp.
Bacillus subtilis	Defender, Bibong, Ecogent, Ecosmart Topsaver, Teras, Holeinone, Ibsalim Greenall, Cillus, Shootingstar, Jaenotan Gamair SP, Alirin-B, Phytosporin	Powdery mildew, gray mold, *Alternaria* blight, large patch, brown patch, *Pythium* blight, *Phytophthora* blight, Root rot, mildew, bacterioses, phytophtorosis, seed molds anthracnose and microsporiosis
Bacillus subtilis 101	Shelter	Root and leaf diseases

(continued)

Table 15.1 (continued)

Microorganisms	Trade name	Host range
Bacillus subtilis 102	Artemis	Root and leaf diseases
Bacillus subtilis 246	Avogreen	Root and leaf diseases
Bacillus subtilis QST 713	Serenade	*Botrytis* spp.
Bacillus subtilis WG6-14	Bactophyt SP	Bactophyt SP
Bacillus subtilis IPM-215	Bactophit	Mildew, root rots
Bacillus pumilus	Ecosense	*Phytophthora* blight
Paenibacillus polymixa	Topseed	*Phytophthora* blight and powdery mildew

Source Usta (2013)

Table 15.2 Biopesticides derived from bacteria belonged to *Pseudomonas* genus

Microorganisms	Trade name	Host range
Pseudomonas fluorescens	ABTEC Pseudo, Biomonas, EsvinPseudo, Sudo, Phalada 104PF, Sun Agro Monus, Bio-cure-B, PlanrizKS	Root rots, mildew, bacterioses, anthracnose phytophtorosis and microsporiosis
Pseudomonas chlororaphis	Cedomon, Cerall	*Pyrenophora teres, Pyrenophora graminea, Tilletia caries, Septoria nodorum* and *Fusarium* spp.
Pseudomonas syringae	Pentafag–M	*Erwinia amylovora, Pseudomonas* spp., *Xanthomonas* spp.
Pseudomonas sp. DSMZ 13134	Proradix	Root rots
Pseudomonas chlororaphis MA 342	Cerall	Cereal diseases
Pseudomonas aureofaciens	AGAT-25, Pseudobacterin 2Z, Agat 25K, Gaupsin	Root rots, mildew, septoriosis, brown rust, ear fusariosis, cercosporosis, pseudoperonosporosis and larvae of harmful insects, scrub, mildew, fruit rots

Table 15.3 Biopesticides derived from bacteria belonged to *Streptomyces* genus

Microorganisms	Trade name	Host range
Streptomyces colombiensis	Mycocide	Powdery mildew, gray mold, brown patch
Streptomyces kasugaensis	Safegrow	Sheath blight, large patch
Streptomyces griseoviridis K61	Mycostop	*Fusarium* wilt, *Botrytis* grey mold, root rot, stem rot, stemend rot, damping off, seed rot, soil-borne damping off, crown rot, *Rhizoctonia*, *Phytophthora*, wilt, seed damping off and early root rot

Table 15.4 Biopesticides derived from bacteria belonged to other genera

Microorganisms	Trade name	Host range
Agrobacterium radiobacter	Crown Gall Inoculant	Crown gall
Aureobasidium pullulans	*Aureobasidium pullulans*	Fire blight and postharvest diseases in apples
Klebsiella oxytoca and *Bacillus mucilaginosus*	Kleps	Enhance resistance to root diseases
Flavobacterium, *Phytobacteriomycin*	Phytoflavin-300	Bacterioses and fungal diseases
Salmonella enteriditis subsp. *Danysz* (LABIOFAM 101-04)	BioratG	Rats
Actinomyces levendula	Phytobacteriomycin	Root rots and bacterioses
Pseudomonas fluorescens, *Streptomyces albus,* and *Micrococcus roseus* bacterial complex	Bactophil	Seed germination diseases
Achromobacter album	Albobacteryn	Sprouting inhibition

fungi kill and control various pests via producing antimicrobial compounds, enzymes or parasitism e.g. *Trichoderma* produces and releases cell wall degrading enzymes (Kawalekar 2013; Kumar 2015; Sharma et al. 2019).

Insect-associated fungi are known as entomopathogenic fungi (also known as mycoinsecticide agents) and classified into four main groups including *Laboulbeniales*, *Pyrenomycetes*, *Hyphomycetes* and *Zygomycetes* (Sharma 2012). These fungi have commensalism or symbiotic relationship with insects (Pucheta and Navarro 2016). They attack, infect and consequently kill the interested insects and regulate their population. Entomopathogenic fungi control sucking pests including aphids, thrips, mealybugs, whiteflies, scale insects, mosquitoes and mites via their infecting and killing. *Beauveria bassiana, Metarhizium anisopilae, Nomuraearileyi, Paecilomyces farinosus* and *Verticillium lecanii* are some of the most widely used entomopathogenic fungi. They penetrate through integument (cuticle), ingestion wounds or trachea and then enter to hemolymph and generate toxins (Meadows 1993). They are regarded as crucial agents in controlling insect populations. There are a lot of obligate and facultative fungal pathogens for insects (90 genera and almost above 750 species). The first commercial mycoinsecticide 'Boverin' contained *Beauveria bassiana*, white muscardine fungus, along with the declined amount of trichlorophon has been successfully applied to inhibit the second-generation outbreaks of *Cydiapomonella* L. (Ferron 1971). Various studies have been conducted on *B. bassiana.* Spores of this fungal strain germinate, grow and proliferate in the body of insects and via producing lethal toxins and draining nutrients lead to their death (Wakefield et al. 2010). Insect-pathogenic fungus *Metarhizium anisopliae* can successfully control the population of adult *Aedesa egypti* and *Aedesa lbopictus* through reducing their

Table 15.5 Commercial fungal biopesticides

Microorganisms	Trade name	Host range
Fungi		
Beauveria bassiana	Botanigard, Mycotrol, Naturalis,Myco-Jaal, Biosoft, ATEC Beauveria, Larvo-Guard, Biorin, Biolarvex, Biogrubex Biowonder, Veera, Phalada 101B Bioguard, Bio-power, Bb Plus, Bb weevil, Sparticus, Ceremoni, Boverin	Aphids, fungus gnats, mealybugs, mites, thrips, whiteflies, coffee berry borer, diamondback moth, thrips, grasshoppers, whiteflies, aphids, codling moth larvae of most pest mosquito species, thrips, greenhouse whitefly, two-spotted spider mite, insect pests, larvae of Colorado potato beetle
Beauveria bassiana strain GHA and *Bacillus thuringensis*	Bitoxibacillin	Colorado potato beetle
Metarhizium anisopliae	Green Muscle, ABTEC, Verticillium Meta-Guard, Biomet, Biomagic, Meta, Biomet, SunAgroMeta, Bio-Magic	Locust, Coleoptera and lepidoptera, termites, mosquitoes, leafhoppers, beetles, grubs
Paecilomyces fumosoroseus	Nemato-Guard Priority Bangsili	Whitefly, two-spotted spider mite, greenhouse whitefly
Paecilomyces fumosoroseus Apopka 97	Preferal WG	Greenhouse whiteflies (*Trialeurodes vaporariorum*)
Paecilomyces fumosoroseus Fe9901	Nofly	Whiteflies
Monacrosporium thaumasium	Ddangumi	Root knot nematode
Lecanicillium muscarium	Mycotal, Vertalec	Whiteflies, thrips, aphids (except the Chrysanthemum aphid: *Macrosiphoniella sanborni*)
Paecilomyces lilacinus	Bio-Nematon, Yorker, ABTEC, Paceilomyces, Paecil, Pacihit, ROM biomite, Bio-Nematon	Nematodes and Whitefly
Paecilomyces lilacinus 251	PL Plus	Nematodes

(continued)

Table 15.5 (continued)

Microorganisms	Trade name	Host range
Trichoderma harzianum	Eco-77, Eco-T, Promot, Romulus, Rootgard, Trichoplus, Trykocide, TrianumP, Trichodex, Rootshield, Gliocladin, Biozim, Monitor, Trichoguard, NIPROT, Bioderma Biovidi, EswinTricho, Biohit Tricontrol, Ecoderm, Phalada 106TV Sun Agro Derma, Defense SF Mycofungicyd, T-Gro	Root diseases *Botritiscinerea, Collectotrichum* spp., *Fulviafulva, Monilialaxa, Plasmoparaviticola, Pseudoperonospora cubensis, Rhizopus stolonifer, Sclerotinia Sclerotiorum*
Trichoderma aspellerum (ICC012) (T25) (TV1) (formerly *T. harzianum*)	Tenet	Fungal infections (*Pythium, Phytophthera, Botrytis* and *Rhizoctonia*)
Trichodermaatro viridae	Binab T Pellets, Esquive	*Botrytis cinerea,* pruning wound infection *Chondrostereum purpureum*. Fungal infections (*Pythium, Phytophthera, Botrytis, Rhizoctonia*)
Trichoderma gamsii	Remedier	Fungal infections (*Pythium, Phytophthera, Botrytis, Rhizoctonia*)
Verticillium lecanii	Verisoft, ABTEC, Verticillium, Vert-Guard, Bioline, Biosappex, Versitile Ecocil, Phalada 107 V, BiovertRich ROMVerlac, ROMGurbkill, SunAgroVerti, Bio-Catch, Mycotal	Whitefly, coffee green bug, homopteran pests Whitefly, thrips, scale insects, Mealybug
Verticillium albo-atrum (WCS850) (formerly *Verticillium dahliae*)	Dutch Trig	Dutch elm disease
Ampelomyces quisqualis	Bio-Dewcon	Powdery mildew due to fungal pathogens
Coniothyrium minitans C ON/M-91-05	ContansWG	*Sclerotinia sclerotiorum, Sclerotinia minor*

(continued)

life span (Shi and Feng 2004). Now, there are many commercial fungal biopesticides mostly from Zygomycota, Deuteromycota (Samson et al. 1988), Oomycota and Chytridiomycota (Barr 2001).

Fungal spores germinate on the integument surface and begin an infection, then they deteriorate the insect's cuticle via excreting various degrading enzymes like proteases, chitinases, quitobiases and lipoxygenases and accelerate the penetration process through mechanical forces, which is initiated via a specialized structure formed in the germinative tube, appressorium. The emergence of hyphal bodies in

Table 15.5 (continued)

Microorganisms	Trade name	Host range
Gliocladium catenulatum J1446	Prestop, PrestopMix	Damping off, gummy stem blight, grey mold, root rot, stem rot, wilt, storage diseases, foliar diseases, seed rot
Pseudozyma flocculosa PF-A22 UL	Sporodex	Powdery mildew
Pythium oligandrum	Polyversum	Polyversum
Coniothyrium minitans CON/M/91-08	Contans	*Sclerotinia*
Arthrobotrys spp.	Nematophagin	Nematodes
Chaetomium spp.	Chetomic	Root molds, grey and white molds, fusariosis, common and silver scrub and rhizoctoniosis
Ampelomyces quisqualis	Cufect	Powdery mildew
Yeast		
Candida oleophila O	Nexyl	Post-harvest diseases

Source Usta (2013)

insect body, which disseminate through the hemocoel, is accompanied with their invasion to various muscle tissues, fatty bodies, mitochondria and hemocytes, which lead to the death of the insect within 3–14 days after infection. Fungi invade to insect organs after insect dying and consequently, fungal hyphae pierce the cuticle from the interior of the insect and appear at the surface, where spore formation is initiated in favorable environmental conditions (Diaz et al. 2006). In addition, some fungi kill insects via producing toxins like cycloheximide and novobiocin.

It has been shown that *Talaromyces flavus* SAY-Y-94-01 can act as a biopesticide on *Anthracnose*, which is caused by *Glomerella cingulata* and *Colletotrichum acutatum* (Ishikawa 2013). Entomopathogenic fungi can be used in the conidia or mycelia forms.

15.2.1.3 Viral Pesticides

Viral pesticides contain viruses with the ability to attack insects and other arthropods. A lot of viruses (>1000) have been isolated from insects (Srivastava and Dhaliwal 2010) (Table 15.6). These entomogenous viruses are divided into two categories, including inclusion body (IV)- and non-inclusion body (NIV)-producing viruses. Inclusion body-producing viruses are further subdivided into *polyhedrosis* and granulosis viruses, which produce polyhedral and granular bodies, respectively.

Table 15.6 Viral pesticides (Usta 2013)

Microorganisms	Trade name	Host range
Gypsy moth nuclear plyhedrosis (NPV)	Gypchek virus	Gypsy moth and caterpillars
Adoxophyes orana BV-0001 granulosis virus	Capex	Summer fruit tortrix (*Adoxophyesorana*)
Cydiapomonella granulosis virus	BioTepp, Cyd-X, Cyd-X Extra	Codling moth (*Cydia pomonella*)
Spodoptera exigua nucleopolyhedrosis virus	Spod-X GH	*Spodopteraexigua*
Zucchini Yellow Mosaic Virus, weak strain	Curbit	Yellow mosaic virus
Anticarsia gemmatalis nucleopolyhedrosis virus (AgNPV)	Baculo-Soja, Baculovirus, Nitral, Coopervirus PM, Protégé	*Anticarsiagemmatalis* and Lepidopterans
Tussock moth NPV	TM Biocontrol-1	Tussock moth and caterpillars
Pine sawfly NPV	Neochek-S	Pine sawfly larvae
Pseudomonas resinovorans bacteriophage	Agriphage	Insect pest control
Helicoverpa armigera nucleopolyhedrosis virus	Helicide, Virin-H, Helocide, Biovirus-H, Helicop, Heligard	*Helicover paarmigera*
Spodoptera litura nucleopolyhedrosis virus	Spodocide, Spodoterin, Spodi-cide Biovirus-S	*Spodoptera litura*

Source Usta (2013)

Polyhedrose viruses based on their inhabitance are categorized as nucleopolyhedrosis viruses (NPV) or cytoplasmic polyhedrosis virus (CPV) (GF. 2013). Thirteen NPV-based biopesticides are registered (Thakore 2006). It has been validated that *Spodoptera exempta* (Walker), nucleopolyhedrosis (SpexNPV) possess significant killing ability on armyworms (Mushobozi et al. 2005). Commercial viral pesticides are containing baculoviruses, nucleopolyhedrosis viruses, granuloviruses, acoviruses, iridoviruses, parvoviruses, polydnaviruses, reoviruses, cytoplasmic polyhedrosis viruses, nodaviruses, picrona-like viruses and tetraviruses. Elcar™ was first viral insecticide containing *Helicoverpa zea* NPV (HzSNPV), which is comparatively extent range baculovirus and control many pests which attacking to soybean, sorghum, maize, tomato beans and cotton species. These pests mostly belong to *Helicoverpa* and *Heliothis* genera (Rhodes et al. 1997; Usta 2013).

More than 10% of all viral insecticides contain baculovirus (Moore et al. 1987) which up to 100 insect species are sensitive to it (Usta 2013). These viruses are rod-shaped and have envelope and circular, supercoiled double-stranded DNA genomes (GF 2013). A lot of baculoviruses have been isolated from Lepidoptera (butterflies and moths), Hymenoptera (sawflies) and Diptera (mosquitoes) (Herniou et al. 2011). They are considerably selective and specifically kill insects and some arthropods and exhibit efficient horizontal transmission. Therefore, they are considered as safe for vertebrates and plants. There is no report on their pathogenicity in vertebrates and plants (Krieg et al. 1980). After viral infection, the expression of its protein can occur in three early (0–6 h), late (6–24 h) and very late (up to 72 h) phases. Produced proteins are assembled in late phase to form occlusion bodies. A lot of virions of NPVs are packaged within each occlusion body and form polyhedra while the granulovirus is packaged in one small occlusion body, to develop granules. Once consumption of occlusion bodies (OBs) by insects, their dissolution is triggered under the alkaline condition of insect mid-gut that is resulted in the disruption of covering proteins and release of virion in the midgut lumen (Adams 1991). Then, the released virions enter the midgut cell nucleus where viral proliferation occurs. Various tissues like the hemolymph, fat bodies, nerve cells and hemocytes may be infected by new virions. In this situation, viruses replicate in the nucleus of infected cells. New virions are occluded into polyhedral in the nucleus. Polyhedral containing virions are accumulated into the host and host become to a bag of viruses which with its liqueferation viruses are released and can infect other insects. Dead hosts are contained a high quantity of virions. It is possible that more than 100 occlusion bodies present in a single caterpillar. Negative geotropism is observed in infected larvae before their death which facilitates widespread dissemination of virions.

Environmental conditions affect the speed with that death occurs (3–7 days in optimum conditions and 3–4 weeks in unfavorable environmental conditions) (Kachhawa 2017). Some characteristics of baculovirus-based biopesticides like their killing speed, short stability in field conditions, and high production costs can limit the application of these biopesticides (C 2012; Mills 2010; WJ 2011). Some strategies can be applied to decline these limitations. For example, killing speed can be improved through applying genetic engineered baculoviruses instead of wild types. High cost, pest-specific activity of viral pesticides, which make control of several different pests difficult, low-speed action and instability of occlusion bodies under ultraviolet rays (280–320 nm) of the sun can limit their acceptance by farmers. In this regard, baculoviruses should be encapsulated with UV protectants to make certain a longer field life (Usta 2013). Transgenic baculoviruses have coding genes of hormones, enzymes or insect-specific toxins (El-Sheikh et al. 2011a, b). Engineered baculoviruses containing juvenile hormone esterase have shown promising results since this enzyme leads to a reduced level of juvenile hormone. In this condition, insect feeding and pupation are prevented. However, short half-life of juvenile hormone esterase in the hemolymph has restricted the application of these recombinant baculoviruses (El-Sheikh et al. 2011a, b).

Other influential viral-based pesticides are alphabaculovirus *Anticarsia gemmatalis* multiple nucleopolyhedrovirus (AgMNPV) and *Cydiapomonella* granulovirus (CpGV), which are applied to control *Anticarsia gemmatalis* (velvetbean caterpillar as a very important soybean insect pest in Brazil) and codling moth, *Cydiapomonella* (pest of fruits such as apple, pears and walnuts), which are causing agents of huge economic loss, annually (Arthurs et al.; Moscardi et al. 2011; Yang et al. 2012). It has been shown that viral-pesticide can augment the efficiency of chemical pesticides to control resistant pest e.g. combination of HaMNPV with endosulfan (organochlorine insecticide) has exhibited acceptable results in controlling Cotton Bollworm, *H. armigera*, which is resistant to a wide range of insecticides (Joußen et al. 2012; Mironidis et al. 2013) as well as transgenic Bt cotton (Luttrell 2012). These viral biopesticides have been commercialized in China. Large-scale production of baculovirus is conducted in an open field or laboratory through collecting infected larvae or feeding reared larvae with baculovirus contaminated food (Elvira and Caballero 2010) (Table 15.6).

15.2.1.4 Nematode Biopesticides

Entomopathogenic nematodes are other organisms that can be applied as biopesticides against weevils, gnats, white grubs and different species of the Sesiidae family (Koul 2011). They are soft-bodied, non-segmented roundworms that have an obligate facultative parasitism relationship with insects. These nematodes are frequently found in the terrestrial ecosystem. Species belonging to Heterorhabditidae and Steinernematidae families have been efficaciously applied as bioinsecticides in the management of pest population (Grewal and Shapiro-Ilan 2005). They are considered as a good biopesticide candidate for integrated pest management due to no toxicity effects on humans, their host-specific activity and low possibility of resistant insect emergence (Shapiro-Ilan et al. 2006).

Entomopathogenic nematodes have a free-living lifestyle in their infective juvenile stage. In this stage, they penetrate into the host insect via spiracles, mouth, anus or intersegmental membranes of the cuticle, and subsequently enter into the hemocoel (Bedding 1982). Species belong to Heterorhabditidae and Steinernematidae families are associated with bacteria of *Photorhabdus* and *Xenorhabdus* genera, respectively (Ferreira 2014). In this step, they release their symbiotic microbial cells into the hemocoel of insects. Released microbial cells reproduce in the hemolymph of insect and lead to insect death within 24–48 h. Nematodes continue to feed on the tissue of died host then mature, and consequently multiply. It is possible that one or more generations have emerged within the host cadaver. Released infective juveniles can infect other hosts and consequently resume their life cycle (Bedding 1982). Entomopathogenic nematodes like *Steinernema carpocapsae*, *Steinernema riobrave*, *Steinernema glaseri*, *Steinernema scapterisci*, *Heterorhabditis bacteriophora* and *Heterorhabditis megidis* have been produced in large scale using solid state or liquid fermentation (Lacey and Georgis 2012). Optimization of the application parameters and development of effective strains in order to attain acceptable control of pests via nematodes should be conducted with extensive research (Table 15.7) (Usta 2013).

15.2.1.5 Protozoan Biopesticides

Protozoa are microscopical single-celled organisms and are scarcely applied as biopesticides against an extent spectrum of pests. Entomopathogenic protozoans are a

Table 15.7 Biopesticides containing entomogenous nematodes

Microorganisms	Trade name	Host range
Steinernema feltiae (*Neoaplectana carpocapsae*) *S. riobravis, S. carpocapsae* and other Steinernema species	Biosafe, Ecomask, Scanmask, also sold generically (wholesale and retail), Vector	Larvae of a wide variety of solid welling and boring insects
Heterorhabditis heliothidis	Currently available on a wholesale basis for large-scale operations	Larvae of a wide variety of solid welling and boring insects
Ampelomyces quisqualis AQ10	Bio-Dewcon, AQ10	Powdery mildew and leaf diseases
Paecilomyces lilacinus PL 251	BioActWG	Common plant parasitic nematodes

diverse group of organisms that include more than 1000 species, which attack invertebrates like insects and also are known as microsporidians (WM 1988). Microsporidia are omnipresent, obligatory intracellular parasites that are responsible for diseases in diverse species of insects. *Nosema* and *Vairimorpha* genera are capable of attack lepidopteran and orthopteran insects (Solter et al. 2012). Their act is slow and specific. They produce spores. Germination of spores has occurred in the midgut, and their released sporoplasm invades to the target cells then a chronic infection is triggered by which debilitates their host via reducing their nourishment, vigor, fertility and length of life. A lot of investigations have been conducted on microsporan protozoans as possible constituents of integrated pest management programs. *Nosemapyrausta* is a useful microsporidian that declines fertility and length of life of the adults and also kills the larvae of European corn borer (Siegel and Ruesink 1986). It is sold under the trade names of NOLO Bait, Grasshopper and Attack against European corn borer caterpillars, grasshoppers and Mormon crickets. In sum, germinated spores in the inset midgut develop a polar filament and their sporaplasm is injected into a midgut cell. Then, more spores are generated and infect other tissues. These spores are eliminated along with feces and preserve their viability. They are ingested during larval nourishment; therefore, the infection cycle is repeated in midgut cells of the new host.

15.2.2 Biochemical Biopesticides

Biochemical pesticides are biological compounds that biologically control pests through non-toxic strategies. Sex-pheromones of insect which can interfere with their mating and population build-up, diverse scented extracts which can attract insect pests to traps and also various vegetable oils or extract or their synthesized analogs are some examples of biochemical pesticides (Ritter 2009).

15.2.2.1 Semiochemicals

Semiochemicals are message-bearing substances that can be derived from plants or animals in nature, which can cause a behavioral response like attraction to others or nutrients, locating a mate and sending an alarm in individuals of the same or other species (Chandler et al. 2011; Nerio et al. 2009). Jasmonic acid and sodium alginate application can lead to crop protection through inducing the production of a natural mixture of herbivore-induced plant volatiles and attracting natural enemies. One group of biochemical pesticides is insect pheromones. These chemicals are naturally used by an insect to inter-species communication. Identified sex pheromones can biologically control more than 30 target species pheromones. These chemicals themselves do not kill a target pest. They spread through the air and via attracting insects to traps, which contain a lethal pesticide or disruption mating impose their biopesticide activities. Many pheromones with biopesticide activities have been known and successfully applied in pest management programs (Dhaliwal et al. 2012; Witzgall and Cork 2010). Better pest management can be achieved via conducting a comprehensive study on mechanisms of the communication systems, behavior, mating systems and physicochemical characteristics of target insects as well as their substantial difference with non-target ones.

15.2.2.2 Insect Growth Regulators

As the name denotes, these biochemical pesticides can alter the growth and development of insects. Juvenile hormone-based insecticides are one group of the insect growth regulators, by using them, the developing process is disrupted. For example, precocene through interfering with the action of juvenile hormone-producing glands prevents the emergence of a reproductive adult (Yankanchi and Gadache 2010). The compounds with inhibitory effects on chitin synthesis can restrict the production of a new exoskeleton by insects after their molting. Therefore, they cause insect death through unprotecting the elements and from prey (O'Brien et al. 2009; Yankanchi and Gadache 2010). Cayenne has deterrent activity, others via suffocation or enhancement of the natural immune system of crop control pests (Kawalekar 2013; O'Brien et al. 2009).

15.2.3 Botanical Biopesticides

These compounds are derived from whole plants neem, custard apple, tobacco, pyrethrum or some parts of them like leaves, barks, seeds, flowers, roots, oil or extract with the ability to control pests (Byrappa and Divya 2012; Kovach et al. 1992). Botanical-based pesticides have diverse composition, target pest and mode of action and were used to control pest in the field or protect the crop and stored products from pests, especially insects for a long time. A large number of plants (>6000) with insecticidal characteristics are known, and some of them are commercialized (O 2012). Nicotine (*Nicotianata bacum* Linnaeus); Rotenone (*Lonchocarpus derris* Benth and *Tephrosia vogelii* Hook f.) and Pyrethrum (*Tanacetum cinerariifolium* Trevir) are the first known botanical pesticides (Khater 2012). Botanical pesticides can be regarded as safer pesticides compared to synthetic pesticides because of their volatile property, low environmental risk, and a minimum residue that minimizes their adverse effects on non-target organisms like predation and pollination insects.

One group of commercialized botanical pesticides is Azadirachtin compounds, which are derived from the neem tree and can be applied on several food and crops in order to control whitefly, thrips, scale and other pests (Sarwar and Tofique 2012). The extraction method and extracted compounds profoundly influence the pesticide activity of neem-based biopesticides. Extracted compounds can be a repellent, regulator of growth, inhibitor of ovipostion or toxin for pests of interest (Isman 2006). Neem leaves (against wide range of pests) (Immaraju 1998), leaf extracts of *Clerodendrum serratum* L. and powdered leaves and leaf extracts of *Olax zeylanica* Wall (against *Sitophilus oryzae* (L.)) (Fernando and Karunaratne 2012), *Cichorium intybus* L., *Melilotus parviflora* L., *Chenopodium album* L. (on *Trogoderma granarium* Everts) (Sarwar and Sattar 2012), methanolic extracts of medicinal plants (against wheat pest), *Tribolium castaneum* Herbst (Padin et al. 2013), *Phthorimaea operculella* Zeller against the potato tuber moth (Thakur and Chandla 2013), extract of the species *Clitoria ternatea* (butterfly pea) (against *Helicoverpa* spp.) (Mensah et al. 2014), stilbenes derived from grapevine extracts (against *Spodoptera littoralis*) (Pavela et al. 2017) and olive mill waste (against various pests) are some examples of botanical pesticides (El-Abbassi et al. 2017).

However, quality control, product standardization and phytotoxicity are the problems in the commercialization of botanical pesticides. For example, tomato, brinjal and ornamental plants are sensitive to a high concentration of neem oil. Also, all plant extracts with pesticide activities are not safe for humans and animals, e.g. *Aconitum spp.* and *Ricinus communis*, possess considerable toxicity for humans and *Tephrosia vogelii*, and impose adverse effects on fish (Stevenson et al. 2012).

15.2.3.1 Genetically Engineered Plants

One eco-friendly strategy to decline the yield loss of crops due to phytophagous arthropod pests is genetically engineering plants to possess genes encoding insecticidal toxins and successfully produce their corresponding products. Plant-incorporated protectants are transgenic plants in whose genome a coding gene of a pesticide is incorporated e.g., insertion of *Bt* gene, protease inhibitor, lectins, chitinase into the plant genome has been conducted. Therefore, these transgenic plants themselves can synthesize pesticide substances. These transgenic plants generate biodegradable pesticides with no detrimental effect on animal and human health and, therefore, can decline the application of chemical pesticides. For example, the lethality of *Bt* endotoxins is significantly related to the alkaline condition of the insect gut. This characteristic assures inactivity of these toxins in vertebrates, mostly in humans (Zhang et al. 2006). Plants incorporated protectants can profoundly enhance food, feed and forage production.

15.3 Improvement of Biocontrol Agents

Once the introduction of biocontrol agents, they should be survived, establish and colonize in the environment (rhizospheric region or phyllosphere) where they are applied. But their survival, establishment and colonization are affected by fluctuations of biotic (host species, nutritional status and competition with indigenous microbiota and pathogens) and abiotic (temperature, wetness and relative humidity) factors. These factors can lead to variability in efficacy or even lack of performance of biological agents and consequently their limited acceptance by farmers (Lugtenberg and Leveau 2007; Sundin et al. 2009). Colonization of biocontrol agents can be augmented by enhancing nutrients or inhibiting the growth of the competing microorganisms. This purpose can be achieved through applying nutrients or inhibitors along with biocontrol agents in their formulation to increase multiplication, survival rate and adaptation of biocontrol agents or suppress competing or antagonistic indigenous microbiota (Druvefors et al. 2005; Guetsky et al. 2002). The inhibitory effect of *P. fluorescens* 62e on *Erwinia amylovora* (causing agents of fire blight infections) was augmented by applying glycine and Tween 80 (Cabrefiga et al. 2011). Applying chemical compounds with stimulatory effects on beneficial characteristics of rhizobacteria like proline synthesis by *P. fluorescens* is another example of this strategy (van Veen et al. 1997).

Improving the efficiency of biocontrol agents via increasing their adaptation to environmental conditions is another approach. These conditions include unfavorable conditions like drought, salinity, freezing and high temperature. Adaptation of biological agents can be enhanced through their cultivation under suboptimal conditions to induce their tolerance mechanisms such as osmoadaptation via accumulating compatible solutes (sugars, polyols, heterosides, amino acids and amino acid derivatives) in their cells (Csonka and Hanson 1991; Miller and Wood 1996). This strategy has been applied to adapt *Pantoea agglomerans* EPS125, *Pseudomonas fluorescens* EPS62e (Bonaterra et al. 2005) and *Candida sake* CPA-1 (Teixidó et al. 1998) to saline, water and osmotic stresses. It is possible that the combination of the above strategies is applied through culturing biocontrol agents in a fermenter by supplementing the salts and osmolytes or adding specific nutrient to the harvested cells to prepare a liquid or dried formulation (Montesinos and Bonaterra 1996). Also, applying a mixture of compatible biocontrol agents can give better efficiency through controlling pathogens via various activities under an extended spectrum of environmental conditions (Stockwell et al. 2011). Finally, the efficiency of biocontrol agents can be improved through genetic engineering to enhance the expression of antibiotic compounds, which has been represented for *T. harzianum* or *P. fluorescens* (Flores et al. 1997; Girlanda et al. 2001) or produce new compounds (Walsh et al. 2001).

15.4 Safety, Detectability and Fate in the Applied Ecosystem

It is critical to determine survival rate, dispersal, genetic stability and horizontal gene transfer as well as effects of biofertilizers and biopesticides on the resident microbiota and fauna, and environmental impact of biocontrol agents including natural or genetically modified organisms before their commercialization and extensive use in agricultural environments. These characteristics should be monitored and validated after the release of biocontrol agents in field conditions via well-designed ecological monitoring programs (Van Elsas et al. 1998). This is while lack of a suitable method to analyze all populations of autochthonous microbiota to estimate which one is essential for qualitatively and quantitatively evaluation of the microbial community structure after the released biocontrol agents makes the monitoring difficult. Toxicity tests are crucial to assure the safety of biocontrol agents toward humans and animals. In this regard, microbial agents should not be phenotypically and genotypically similar to opportunistic microorganisms e.g., strains of *Burkholderia cepacia* (Parke and Gurian-Sherman 2001), *Pseudomonas putida* (Aumeran et al. 2007), *Pantoea agglomerans* (Rezzonico et al. 2009) and *Aureobasidium pullulans* (Gostinčar et al. 2011) that there is not considerable differences between their environmental and clinical isolates, may cause opportunistic infections. Interestingly, some of these microbial cells are frequent in nature and are inhabitants on the surface of many plants (C 1965).

Fate and behavior of released microorganisms as biocontrol agents in the environment should necessarily be monitored to evaluate risk assessment, investigation on traceability, residue analysis and environmental impact, which are perquisites for registration and subsequent commercialization of microbial pesticides (De Clercq et al. 2003). In this regard, various monitoring methods should be applied for accurate identification of biocontrol agents and their population dynamics over time. These methods should be capable of distinguishing biocontrol agents from the native inhabitants into the microbial community.

Culture-based methods, immunological assays, microsatellite markers examining (Doube et al. 1995; Plimmer 1999), the methods based on fluorescent antibodies or fluorescently labeled oligonucleotide probes, or transforming biological control agents via fluorescence (gfp) or bioluminescence (lux) reporter genes, PCR-based methods including 16S or 18S rDNA sequencing, real-time PCR (qPCR), BIO-PCR method, combined qPCR and plate-counting methods, reverse transcription (RT) coupled to qPCR, nucleic acid sequence-based amplification (NASBA), loop-mediated isothermal amplification (LAMP) are several strategies to monitor microorganisms of interests in soil, rhizospheric region, the phyllosphere and post-harvest of fruit. Although, some of them have limitations like being time-consuming (culture-based methods) and expensive (immunological assays, microsatellite markers examining), possibility of genetically modified microorganism persistence in the environment (methods based on transforming biological control agents via fluorescence or bioluminescence (lux) reporter genes), failure to do quantitative analysis (simple

PCR-based methods), lack of distinction between dead and live cells (conventional qPCR) and inability to estimate population (BIO-PCR method) constrain their application (Malusà et al. 2016).

15.5 Commercialization of Microorganisms as Biocontrol Agents

Large-scale production and formulation are influential steps in biopesticides biotechnology, which can preserve its pesticide activity for a long period (Burges 1998; Powell and Jutsum 1993). To industrially produce biocontrol agents, a suitable submerged or solid-state fermentation, and appropriate formulation, e.g. liquid, dried, peat, encapsulated types should be selected. In addition to microbial cells, a formulated microbial pesticide contains other ingredients called inerts. Therefore, these compounds should possess no hazard for human and animal health, ecosystems, and be free from allergens (Nerio et al. 2009).

Solid-state fermentation (SSF) is generally identified as the most effective and environmentally safe biotechnological strategy for mass production of high-quality biocontrol products like *Bacillus thuringiensis* in a cost-effective manner through employing agro-industrial wastes like wheat bran, rice bran, rice husks, soybean powder, fish wastes, molasses and protein hydrolysates (Morris et al. 1997; Vassilev et al. 2015). Seeds can be coated using fermentation products containing spores. Naturally occurring polysaccharide gels can be used to encapsulate spores. In addition, these spores can be introduced into compost. It has been revealed that the spores produced in SSF have higher efficiency to reduce phytopathogens and significantly preserve survival under unfavorable environmental conditions than that of produced spores in submerged cultivation (Pascual et al. 2000). SSF was used for multiplication of *Coniothyrium minitans*, a biofungicide against the soil-borne plant pathogen e.g. *Sclerotinia* spp. and production of it urin, an antifungal compound (Balakrishnan and Pandey 1996).

Microbial pesticides can be presented as liquid formulated products containing microbial suspensions in water oils or emulsions, which retained viability and efficacy for several months. Microbial pesticides in the liquid formulation should be preserved under refrigerated conditions (Abadias et al. 2003). Microbial pesticides can also be presented as wettable powders, dust or granules (Schisler et al. 2004). Storage and transportation conditions of microbial pesticides in the dry formulation are easier than liquid ones. Dehydration is a perquisite to obtain stable microbial pesticides in the dry formulation. The dehydration can be performed through freeze-drying, spray-drying and fluidized bed-drying.

To prevent cell damages during dehydration process, the compounds like sulfoxides, alcohols, monosaccharides and polysaccharides, amino acids, peptides and glycoproteins with protective activity must be added to preserve cell survival during dehydration (DJ 1993). Among these dehydration techniques, freeze-drying is less damaging but expensive method while, spray-drying is most damaging due to great water loss and temperature gradients, which creates a stressful condition for cells, but it is a cost-effective method. The third technique, the fluidized-bed drying, has been successfully used for desiccation-tolerant yeast. This method is cost-effective and less stressful in comparison with spray-drying (Larena and Cal 2003). In encapsulation formulation, microbial strains are surrounded by a protective inert layer such as alginate, carrageenan or cellulose (Bashan et al. 2002). Via encapsulating, the microbial cells can be protected from abiotic stress and released gradually.

Biopesticides should be introduced in the plant ecosystem, where they should be survived and colonized near or within entry sites of the pathogen in the host plant. Formulated biopesticides can be introduced through helper insects, coating the seeds or root microbial colonization of seedlings before transplanting, spraying or drenching plants with formulated biopesticides. To control post-harvest disease, treatment of products with microbial pesticide can be conducted before and after harvesting. Biocontrol agents can be applied with either low initial population (inoculative and augmentative strategies) or high population (inundative strategy). The success rate of applied biopesticides is directly dependent on the frequency of pathogen and introduced biological agents as well as pathogen aggressiveness (Francés et al. 2006).

In general, the determination of several characteristics including biological characteristics, efficiency, particular analytical strategies, residues, traceability and potential unfavorable effects on human health, non-target organisms and ecosystems is an essential perquisite for registration and commercialization of microorganisms as biocontrol agents. These measurements are pivotal to decline the number of registered biocontrol agents to ones with more selectivity, no toxicity for consumer health, animals and any non-target organisms, and no adverse effect on the environment (Gullino and Kuijpers 1994).

It was estimated that many commercial microbial biopesticides (90%) are derived from *Bacillus thuringiensis*, an entomopathogenic bacterium (Kumar and Singh 2015). It possesses a small portion (5%, $3 billion) of the global market of pesticides (Marrone 2014; Olson 2015). There are 200 and 60 commercial pesticides in the USA and European Union market, respectively. The annual increase in global usage of biopesticide is 10% (Kumar and Singh 2015). The universal market of biopesticides will be increased over time, and dependence on chemical pesticides will be decreased by their substitution with biopesticides with equal efficiency. The global acceptance of biopesticides is increased due to their less detrimental effects on human and animal health as well as environment, their specific activities on target pests, their effectiveness in small amounts and their quick decomposition without leaving hazardous residues. It has been predicted that global market size of biopesticides will be equalized with chemical ones between the late 2040s and the early 2050s (Marrone 2014; Olson 2015). Therefore, annual growth rates of biopesticides must outpace chemical pesticides. In this regard, comprehensive and systematic studies should be conducted to find and introduce new biological agents as biopesticides. Therefore, the collaboration of enterprises and research institutes is necessitated.

In addition, new guidelines should be considered to facilitate registration (from the aspect of time and cost) of biopesticide products (Czaja et al. 2015).

15.6 Conclusion and Future Prospects

Biopesticides compared to their chemical counterparts are more suitable to preserve quality and quantity of agricultural yield. Since they have no harmful residue and do not contaminate terrestrial and aquatic ecosystems. By using these ecofriendly pesticides, resistant weed, insects, mites, pathogenic fungi, bacteria and nematodes do not appear. Due to biopesticide-specific activity, non-target organisms remain healthy and biodiversity as well as beneficial microbial activities can be preserved. Despite having many considerable advantages, biopesticides have some limitations which can be alleviated through comprehensive studies on screening novel and efficient biological agents, determining their pesticide mode action, creating high-performance technologies to produce these biological agents or their derived compounds with high efficiency and without contamination, finding most efficient formulation. Also, appropriate strategies should be intended in order to biological agents (or their derived compounds) can be adapted, survived, established and colonized in the presence of biotic and abiotic stress conditions like chemical pesticides, heat, desiccation or exposure to ultraviolet radiation. Adapted biological agents can be applied in various ecosystems or along with chemical counterparts, which can augment their pest controlling efficiency. These adaptations to environmental conditions also can be achieved by applying suitable fermentation process, their cultivation under suboptimal conditions, their formulation or even genetic engineering of biological agents. Comprehensive investigations of these fields can lead to a constant and acceptable performance of biological agents and consequently their acceptance by farmers. Finally, accurate monitoring methods are extremely needed to detect population dynamics of biological agents over time, their dispersal, genetic stability as well as effects of biopesticides on the resident microbiota and fauna.

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