Biofertilizers and Biopesticides: Eco-friendly Biological Agents

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Abstract

Chemicals in the form of fertilizers and pesticides have been used in boosting agricultural productivity and crop protection since years. The adverse effects such as environmental toxicity and long residual action resulting from excessive use of these chemicals have prompted the search for nontoxic eco-friendly biological agents. Microbes have emerged as eco-friendly alternate to achieve enhanced plant productivity and protection. Microorganisms colonize rhizosphere/interior of the plant, thereby promoting growth of plants by increasing the availability of essential nutrients such as nitrogen and phosphorus and providing growth regulators. Microbes and their supplements also provide protection against various pests and pathogens. Biofertilizers and biopesticides serve as an eco-friendly substitute to toxic chemicals and form an important component of integrated nutrient management system. Efficiency of both biopesticides and biofertilizers can be increased by molecular approaches. The present chapter highlights the role of biofertilizers and biopesticides in crop improvement and hence achievement of sustainable agriculture.

Keywords

Microbes • Mycorrhizae • Nitrogen • Phosphorus

10.1 Biofertilizers

Biofertilizers are preparations containing strains of microorganisms which are efficient in providing nutrients to plants through rhizospheric interactions. Microbes as biofertilizers improve soil properties, help in expansion of the root

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Table 10.1 Use of various bacteria as fertilizer to improve crop plants

Bacteria	Dose	Crops
Rhizobium	50 to 300 kg N ha^{-1}	Groundnut, soybean, red gram, green gram, black gram, lentil, cowpea, bengal gram, and fodder legumes
Azotobacter	0.026 to 20 kg N ha^{-1}	Cotton, vegetables, mulberry, plantation crop, rice, wheat, barley, ragi, jowar, mustard, safflower, niger, sunflower, tobacco, fruit, spices, condiment, and ornamental flower
Azospirillum	10–20 kg N ha^{-1}	Sugarcane, vegetables, maize, pearl millet, rice, wheat, fodders, oil seeds, fruit, and flower

system, and increase availability of micro- and macronutrients via nitrogen fixation, phosphate and potassium solubilization or mineralization, release of plant growth-regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Arun 2007; Singh and Prasad 2011; Yosefi et al. 2011; Mohammadi and Sohrabi 2012; Kawalekar 2013; Mishra et al. 2013; Raja 2013; Deepak et al. 2014; Patel et al. 2014; Seiber et al. 2014). The use of biofertilizers has proven effective in promoting growth of crop plants such as rice, pulses, millets, cotton, sugarcane, and vegetables crops (Rajasekaran and Sundaramoorthy 2010; Singh et al. 2014) (Table 10.1).

Microbes are applied to seed and plant surfaces (specifically roots) as inoculants (Chen 2006; Khan et al. 2011a, b, c; Mazid et al. 2011a; Raghuwanshi 2012; Gupta and Sen 2013). The supplementation of microbes increases the organic matter of the soil, thereby improving the exchange capacity of nutrients, increasing soil water retention, and buffers the soil against acidity, alkalinity, salinity, pesticides and toxic heavy metals. Longer shelf life (12–24 months), no contamination, easy storage without loss of properties (up to 45 °C), and effective dosage of biofertilizers provide optimum nutrients to plants for their growth and yield (Thakore 2004).

Commercialization of biofertilizers has been promoted by various organizations in different countries. NifTAL (USA) promoted the popularization of *Rhizobium* inoculants. Some of the commercially available biofertilizers include:

1. Rhizonik (*Rhizobium*) – inoculation groups available for legume crops
2. Azonik (*Azotobacter chroococcum*) – nonsymbiotic bacteria used for all cereals
3. Spironik (*Azospirillum brasilense*) – used for grasses and similar type of crops
4. Phosphonive (phosphate-solubilizing inoculant) – bacteria as well as fungi and used for solubilizing P
5. Sulphonik (sulfur-oxidizing inoculant) – bacteria and fungi that enhance the availability of sulfur
6. Phospho-sulphonik – contains equal mixtures of phosphonive and sulphonive
7. Niku-2000 (decomposing culture) – inoculant degrades all cellulolytic and lignolutic organic matter, thereby releasing nutrients to plants
8. Trichonik (*Trichoderma viride*) – restricts the growth of disease-producing organism
9. Vermiculture – mixed with N-fixing inoculant and P solubilizers so that nutrient value is increased

Table 10.2 Biofertilizer use in various countries for improving crop plants

Country	Biofertilizer	Crop	References
Bangladesh	*Bradyrhizobium*	Soybean	
India	*Azospirillum*	*Stevia rebaudiana*	Das and Dang (2010)
	Vesicular arbuscular mycorrhiza (VAM)		
	Phosphorus-solubilizing bacteria (PSB)		
	Azotobacter and PSB 25	*Brassica campestris*	Mondal et al. (2015)
Vietnam	*Rhizobium*	Peanut 1	Nguyen (2006)
Mexico	*Azospirillum* sp.	Corn seed	Caballero-Mellado et al. (1992)
Iran	*Azotobacter* and *Azospirillum*	Canola 21	Yasari and Patwardhan (2007)
	Azotobacter 25	Black cumin	Valadabadi and Farahani (2011)
Turkey	*Azospirillum brasilense* sp. 246	Wheat	Ozturk et al. (2003)
		Barley	
Colombia	*Azospirillum brasilense, A. amazonense*	Rice	Moreno-Sarmiento et al. (2007)
	Azotobacter	Cotton	
Egypt	*Rhizobium* (Rh)	Sweet fennel	Zaki et al. (2010)
	Bacillus megaterium (BM3)75		Gharib et al. (2008)
	Azospirillum	Snap bean	Shaheen et al. (2007)
	Azotobacter		
	Azospirillum sp.		
	Bacillus sp.	Flax	Naseriad et al. (2011)
	50 *Azotobacter*	Maize	
	Azospirillum		
Kenya	*Rhizobia*	Soybean	Majengo et al. (2011)
Thailand	*Bacillus cereus strain RS87*	Rice	Jetiyanon and Plianbangchang (2011)
Pakistan	*Bacillus mucilaginous*	Maize	Jilani et al. (2007)
	Azotobacter		
	Azospirillum		
Colombia	*Azospirillum brasilense*	Cotton, rice	Moreno-Sarmiento et al. (2007)
	A. amazonense		
	Azotobacter		

Besides several positive aspects of biofertilizers, their application in agriculture is restricted because of the variable response of plant species or genotypes to inoculation depending on the bacterial strain used. The success of a bacterial strain usage depends upon the saprophytic competence and competitive ability (Khan and Naeem 2011; Mazid et al. 2012a) (Table 10.2).

10.2 Types of Biofertilizers

10.2.1 Nitrogen Fixers

Many bacterial species are symbiotic in nature and fix atmospheric nitrogen. These mainly include *Rhizobium* (*Rhizobiaceae*) which fix nitrogen in legumes at 50–100 kg ha^{-1}. They colonize the roots of legumes to form tumor-like growths called root nodules. It is useful for pulse legumes and forage legumes. Inoculation of *Rhizobium* significantly increases the yields in crop plants such as pulses; legumes, viz., chickpea, red gram, bengal gram, lentil and black gram; oilseed plants like pea, lentil, soybean, and groundnut; and vegetables such as pea, alfalfa and sugar beet (Ramchandran et al. 2011; Sharma et al. 2011). The yield enhancement was noted as increased number of pods $plant^{-1}$ and number of seed pod^{-1} and 1000 seed weight (g).

Azotobacter (*Azotobacteraceae*) is a genus of nonsymbiotic, aerobic, free-living heterotrophic N-fixing bacteria. The bacterium colonizes the roots and fixes N at level 25 kg ha^{-1}. The N fixation increases the yield (up to 50%). *Azotobacter vinelandii*, *A. beijerinckii*, *A. insignis*, *A. nigricans*, *A. armeniacus*, *A. paspali*, *A. chroococcum* and *A. macrocytogenes* are the species commonly used as biofertilizers. The strain also produces antifungal antibiotics that inhibit the growth of several pathogens present in the root region and prevent seedling mortality. They improve seed germination and plant growth by producing vitamins such as thiamine and riboflavin and plant hormones such as indole acetic acid (IAA), naphthylacetic acid (NAA), gibberellins (GA) and cytokinins (CK) (Mazid et al. 2011a, b). Improvement in cereal and millet crops such as rice, wheat, sorghum, maize, pearl, millet, cotton, sesame, vegetables, cotton and sugarcane has been achieved using *Azotobacter* (Mazid et al. 2012a; Khan et al. 2012a; Sahoo et al. 2013a; Wani et al. 2013).

Azolla (*Cyanobacteria* or Blue-green algae) are phototrophic bacteria and are used as green manure. Reports suggest that one kg of *Azolla* fixes about 40–55 kg N ha^{-1}, 15–20 P ha^{-1}, and 20–25 kg K ha^{-1} (Sahu et al. 2012). It also produces phytohormones such as auxin, indole acetic acid and gibberellic acid. Blue-green alga (BGA) assists in biological nitrogen fixation (BNF) (Chianu et al. 2011; Olivares et al. 2013; Santi et al. 2013). Application of *Azolla* improves the physical and chemical properties of the soil such as N, organic matter, and cations such as Mg, Ca and Na (Carrapico et al. 2000; Bhuvaneshwari and Kumar 2013). The paddy crop has shown an increase in yield (21–34%) after application of *Azolla* as a biofertilizer. The number of pods $plant^{-1}$, number of seed pod^{-1}, and 1000 seed weight increased after application of *Azolla* (Yadav et al. 2014).

Azospirillum (*Spirilaceae*) are heterotrophic microbes. They are associative in nature and possess N-fixing ability (20–40 kg ha^{-1}). Inoculations of *A. amazonense, A. halopraeferens* and *A. brasilense* have been proven beneficial to crop plants via improvement in leaf area index and yield attributes. Inoculation with *Azospirillum* changes the root morphology by producing plant growth-regulating substances via siderophore production. It also increases the number of lateral roots and enhances root hair formation to provide more root surface area for absorption of sufficient nutrients (Saikia et al. 2013). This improves the water status of the plant and aids the nutrient profile in the advancement of plant growth and development. High yield of maize, sugarcane, sorghum (*Sorghum bicolor* L.), pearl millet etc. has been reported after use of *Azospirillum*.

Herbaspirillum is a symbiont and N-fixing bacteria. It enhances the availability of nutrients such as N, K and P and production of growth-promoting hormones (kinetin, gibberellic acid, and auxin) (Khan et al. 2011a, b, c). The N fixation capacity of 15 kg ha^{-1} $year^{-1}$ has been reported. The synthesis of phytohormones such as IAA triggered by this biofertilizer enhances germination and root development which assist in the absorption of plant nutrients. Other nitrogen-fixing cyanobacteria used as biofertilizers include *Aulosira*, *Tolypothrix*, *Scytonema*, *Nostoc*, *Anabaena*, and *Plectonema*.

10.2.2 Phosphate Solubilizers

Bacterial species (both aerobic and anaerobic) possess the capacity to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate. The organic acids produced by these strains convert the insoluble phosphorous compounds such as tricalcium phosphate to di- and monobasic phosphates that can be easily taken up by the plant. The phosphate-solubilizing bacteria include *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aereobacter*, *Flavobacterium* and *Erwinia* (Karpagam and Nagalakshmi 2014; Rowchoudhury et al. 2015).

10.2.2.1 Zinc Solubilizers

The microorganisms such as *Bacillus subtilis*, *Thiobacillus thiooxidans*, and *Saccharomyces* sp. assist in the solubilization of Zn from the compounds like zinc oxide (ZnO), zinc carbonate ($ZnCO_3$) and zinc sulfide (ZnS) (Mishra et al. 2013).

10.2.2.2 K-Solubilizing Bacteria

Bacteria such as *Frateuria aurantia* have shown capacity to solubilize K into a usable form. Potassium-solubilizing microorganisms (KSM) include *Aspergillus*, *Bacillus*, *Clostridium*, *Azotobacter*, *Azospirillum*, *Phosphobacteria* and *Rhizobacteria*. Co-inoculation of *Azospirillum brasilense* and *Rhizobium meliloti* produces a positive effect on grain yield and N, P and K content in *Triticum aestivum* (Mohammadi and Sohrabi 2012; Mazid and Khan 2014).

10.2.2.3 Silicate-Solubilizing Bacteria (SSB)

Microbes degrade aluminum silicates via organic acids produced by them. Hydrogen ions supplied by organic acids promote hydrolysis. These mainly include *Bacillus globisporus* Q12 and *Bacillus* sp. (Sheng et al. 2008; Kalaiselvi and Anthoniraj 2009; Ghouse et al. 2015).

10.2.3 Plant Growth-Promoting Rhizobacteria (PGPR)

Rhizospheric bacteria that exert a beneficial effect on plant growth are referred as PGPRs. These include *Actinoplanes*, *Agrobacterium*, *Alcaligenes*, *Amorphosporangium*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Cellulomonas*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium*, *Bradyrhizobium*, *Streptomyces* and *Xanthomonas*. These inoculants promote growth via different mechanisms which include suppression of plant disease (bioprotectants), improved nutrient acquisition (biofertilizers), or phytohormone production (biostimulants). The growth hormones such as indole acetic acid, cytokinins, and gibberellins produced by PGPR strains act as biostimulants and promote growth by increasing the absorptive surface for uptake of water and nutrients (Adesemoye et al. 2009; Gholami et al. 2009).

10.2.4 Vesicular Arbuscular Mycorrhizae (VAM)

The symbiotic association between plant roots and fungal mycelia is termed as mycorrhiza. VAM fungi infect the plant primarily through roots. Hyphae absorb nutrients such as phosphate and transfer it to internal cortical root cells. The fungal partner is benefited by obtaining its carbon requirements from the photosynthates of the host, and the host in turn is benefited by obtaining nutrients especially P, Ca, Cu and Zn. They possess special structures known as vesicles and arbuscules. The arbuscules help in the transfer of nutrients from the fungus to the root system, and the vesicles (saclike structures) store P as phospholipids. The organic acids create acidic conditions that facilitate mineralization of the nutrients (Smith et al. 2011). The phytohormones such as indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK) produced by them improve photosynthesis performance, confer tolerance to stress and increase resistance to pathogens. Seed inoculation with fungal species of *Glomus mosseae* mycorrhiza improved corn yield via increased plant height, leaf width, number of grains per ear, and 100 seed weight.

10.3 Application of Biofertilizers and Crop Improvement

Biofertilizers are cheaper than chemical fertilizers (cost-benefit ratio of more than 1:10) (Tiwari et al. 2004), and their use results in fewer nutrient losses, economic savings and environmental protection. The effect of biofertilizers on crop plants depends upon several factors such as crop genotype, the microbial strain, and environmental conditions (soil and weather) (Şahin et al. 2004; Cakmakci et al. 2006; Dhanasekar and Dhandapani 2012). Treatment with biofertilizers particularly N fixers, PGPR, co-inoculants of PGPR and AMF increases the crop yield and productivity by enhanced nutrient use efficiency (Bhardwaj et al. 2014).

The positive effect of combined treatments of *Azolla* and BGA on rice yield has been reported (Askary et al. 2009). The application of BGA+ *Azospirillum* improved leaf area index (LAI) and all yield attributes in rice. Pusa Basmati 1 showed good yield with the application of four amendments (*Azolla*, BGA, vermicompost, and FYM). Use of BNF enhanced soybean (2000 kg ha^{-1}) and other crop yields (4000–6000 kg ha^{-1}) in Brazil, Argentina and Zimbabwe (Hungria et al. 2010; Yadav et al. 2014). Vegetables like cauliflower, broccoli, cabbage, and carrot also recorded high productivity with the use of biofertilizers (Naderifar and Daneshian 2012). Increase in maize yield has been achieved with the application of half the recommended N rate and biofertilizer, i.e., *Azospirillum*, in Egypt. The application of P fertilizers in combination with biofertilizers increased soybean yields by ≈47% (Woomer et al. 2014). Inoculation of *Rhizobium* with crop plants reduced the need for N fertilizers leading to cost saving of US$ 3 billion per cropping season in Brazil (Nicolas et al. 2006).

10.4 Role of Biofertilizers in Curtailing Stress

Mycorrhizae and other biofertilizers benefit plants exposed to drought and saline conditions. AM fungi along with N_2-fixing bacteria such as *Pseudomonas putida* or *Bacillus megaterium* have shown potential in combating drought stress in legume plants. In Sudan, inoculation of *Rhizobia* improved yield of alfalfa, fenugreek, cluster bean, field pea and common bean grown under drought conditions (Hussain et al. 2002; Abdelgani et al. 2003). Photosynthetic efficiency and the antioxidative capacity noted an increase after inoculation of arbuscular mycorrhiza in rice plants subjected to drought stress. Inoculation of PGPR alone or along with AM-like *Glomus intraradices* and *G. mosseae* resulted in the better nutrient uptake and physiological processes. *A. brasilense* and AM combination improved plant tolerance to various abiotic stresses (Aroca et al. 2013). *Pseudomonas inoculation* improved the seedling growth and seed germination in *A. officinalis* L. under water stress. *Rhizobium trifolii* inoculated with *Trifolium alexandrinum* showed higher biomass and increased nodulation under salinity stress (Yang et al. 2009). *P. fluorescens* MSP-393 assisted in producing osmolytes and proteins that help plants in overcoming the negative effects of salt stress. *P. putida* Rs-198 enhance germination rate and other growth parameters such as plant height, fresh weight, and dry weight of cotton under alkaline and high-salt conditions via increasing the rate of uptake of K^+, Mg^{2+}, and Ca^{2+} and decreasing the absorption of Na^+. *Pseudomonas* strains conferred tolerance in plants via 2,4-diacetylphloroglucinol (DAPG). Calcisol produced by PGPRs, viz., *Pseudomonas alcaligenes* PsA15, *Bacillus polymyxa BcP26* and *Mycobacterium phlei* MbP18, provides tolerance to high temperatures and salinity stress. A root endophytic fungus *Piriformospora indica* was found to defend plants against salt stress (Ilyas et al. 2012).

Application of biofertilizers provides protection to plants against disease and pathogens (Gao et al. 2012; Youssef and Eissa 2014). *Rhizobium* control seed-borne fungal infection of *Colletotrichum*, *Ascochyta*, and *Helminthosporium* in legume seeds. PGPR strains are found to be effective in managing the spotted wilt viruses and cucumber mosaic virus in tomato and pepper and bunchy top virus in banana.

B. amyloliquefaciens 937b and *B. pumilus* SE-34 provide immunity against tomato mottle virus, *B. megaterium* IISRBP 17 acts against *Phytophthora capsici*, *Bacillus subtilis* N11 was found effective against *Fusarium* infestation, *B. subtilis* (UFLA285) was found to provide resistance against *R. solani* in cotton plants, *Paenibacillus polymyxa* SQR- 21 used for the biocontrol of *Fusarium* wilt in watermelon, and *Glomus mosseae* was effective against *Fusarium oxysporum* sp. causing root rot disease in basil plants (Zhang et al. 2011). *Medicago truncatula* also showed induction of various defense-related genes with mycorrhizal colonization. Addition of arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* to the soil reduce development of root rot disease in *Phaseolus vulgaris* L. (Aravind et al. 2009; Joe et al. 2009; Kohler and Caravaca 2010;Yao et al. 2010).

10.5 Molecular Approaches

Genes responsible for synthesis of certain factors/proteins involved in the functioning of biofertilizers such as mycorrhizae have been identified and expressed to develop symbiotic association with plants and achieve improvement in plant growth. Genome sequencing of two EM fungi (ectomycorrhizae), *L. bicolor* 13 and *T. melanosporum* (black truffle), provides means for identification of factors that regulate the development of mycorrhiza and its function in the plant cell. Genes upregulated during symbiosis were identified as putative hexose transporters in *L. bicolor*. The upregulation of transporter genes during symbiosis indicated the transport of useful compounds like amino acids, oligopeptides and polyamines through the symbiotic interface from one organism to other. Cysteine-rich proteins of fungus play an important role as effectors and facilitators in the formation of symbiotic interfaces. Genes related to auxin biosynthesis and root morphogenesis show upregulation during mycorrhizal colonization. PGPR produce IAA which induces the production of nitric oxide (NO), which acts as a second messenger to trigger complex signaling network leading to improved root growth and developmental processes. Expression of ENOD11 and defense-related genes and root remodeling genes are upregulated, and genes including subtilisin protease, phosphate transporter, or two ABC transporters involved in arbuscule formation are also overexpressed. Sugarcane plantlet inoculated with a wild strain of *G. diazotrophicus* shows N fixation, while *G. diazotrophicus* mutant, i.e., without nif D gene, lacked N fixation fixing capacity.

Many disease resistance genes such as jasmonate/ethylene signaling as well as osmotic regulation via proline synthesis genes are differentially expressed in microbes such as *B. subtilis* (UFLA285). Metallothionein-like protein type 1, a NOD26-like membrane integral protein, ZmNIP2–1, a thionin family protein, an oryzain gamma chain precursor, stress-associated protein 1 (OsISAP1), probenazole-inducible protein PBZ1, and auxin and ethylene-responsive genes are expressed and identified. Gene encoding glucose dehydrogenase (gcd) involved in the direct oxidation pathway was cloned and characterized from *Acinetobacter calcoaceticus*, *E. coli*, and *Enterobacter asburiae*.

Constitutive expression of certain proteins such as HetR driven by gene hetR gene improves nitrogenase activity in *Anabaena* sp. strain PCC7120. *G. versiforme* possesses inorganic phosphate (Pi) transporters on the hyphae that help in the direct absorption of phosphate from the soil**.** Bioactive compounds called Myc factors similar to Nod factors of *Rhizobium* are suggested to be secreted by mycorrhiza and *Rhizobium* and perceived by host roots for the activation of signal transduction pathway or common symbiosis (SYM) pathway (Roberts et al. 2013). The common SYM pathway prepares the host plant to bring about changes at molecular and anatomical level with the contact of fungal hyphae. Calcium acts as secondary messengers via Ca^{2+} spiking in the nuclear region of root hairs. PGPR produce IAA which, in turn, induces the production of nitric oxide (NO), which acts as a second messenger to trigger a complex signaling network leading to improved root growth and developmental processes (Molina-Favero et al. 2007).

10.6 Biopesticides

Biopesticides encompass a broad array of microbes and biochemicals derived from microorganisms that confer protection against pest damage (Gupta and Dikshit 2010). Microbes are formulated in solid carriers like talc, peat, lignite, clay etc., while liquid formulations are prepared in solvents such as water, oil, and organic solvents. Solid formulations have shorter shelf life, susceptibility to environmental conditions, high contamination and low field performance, while liquid formulations offer longer shelf life (up to 2 years), with high purity, carrier-free activity, ease in handling and application (Mazid et al. 2011c, d). They include nutrients, cell protectants, and inducers responsible for cell/spore/cyst formation leading to improved efficacy. The microbes are present in dormant cyst form which gives rise to active cells upon application in the field, and this helps increase its shelf life for more than one year. Liquid formulations showed their efficacy against insect and nematode pests (Rao et al. 2015). They possess high selectivity to target pests, safety to humans and nontarget organisms, amenability to individual applications, integrated pest management, and suitability for organic niche products in contrast to chemical pesticides that possess broad spectrum and affect nontarget organisms including predators, parasites, as well as humans. Biopesticides mainly include fungus used in weed control, bacteria controlling fungal and bacterial diseases, and viruses active against insect pests.

Liquid formulation of pesticides shows high efficacy for longer period of time. *Pseudomonas fluorescens* formulation in glycerol (10 mM) shows high efficacy of pest destruction for longer duration (6 months). Addition of glycerol increased the stability of the liquid formulations of *Bacillus thuringiensis* (*Bt*) used against *Helicoverpa armigera*. Liquid formulations of *Pochonia* (*Verticillium*) *lecanii* in glycerol, Tween 80, and arachnid oil effectively reduced mealy bug (*Maconellicoccus hirsutus*) infection in grapes. Seed treatment with liquid formulation reduced the disease incidence of *Fusarium* (wilt) in tomato and increased fruit yield. Bentonite oil-based liquid formulations (bentonite, corn oil, gum, glycerin) control fungus in *Beauveria bassiana*. Spray application of the bacterial suspensions of *Agrobacterium radiobacter* and *Bacillus sphaericus* isolates cause significant reduction (24–41%) in root infection of potato caused by cyst nematodes, *Globodera pallida*. Emulsion (water in oil) of *M. anisopliae* showed efficacy against whiteflies, *Bemisia tabaci*, red spider mites and *Tetranychus cinnabarinus* in eggplants. Liquid formulations in vitro produced endospores of bacterial bioagent, *Pasteuria penetrans*, that suppressed the host nematode, *Belonolaimus longicaudatus*, more effectively (59–63%). Liquid biopesticides of *B. subtilis*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Paecilomyces lilacinus*, *Trichoderma viride* and *Trichoderma harzianum* with shelf life more than 12 months proved their efficacy in controlling nematode pests like root-knot nematodes (*Meloidogyne incognita*), reniform nematode (*Rotylenchulus reniformis*), and lesion nematodes (*Radopholus similis*). Liquid formulation of *B. subtilis* (1%) reduced root-knot nematode population to a significant extent.

The total world production of biopesticides is over 3000 tons/year. About 1400 biopesticide products are marketed worldwide, and the global market is expected to reach US$ 3.2 billion by 2014. The biopesticide market in India represents 2.89% of the overall pesticide market and is expected to reach more than US$ 1 billion (Shukla and Shukla 2012).

10.6.1 Categories of Biopesticides

Biopesticides have been broadly categorized as follows.

10.6.1.1 Microbial Pesticides

They consist of a naturally occurring or genetically modified microorganisms (e.g., a bacterium, fungus, virus, or protozoan). They are effective against kinds of pests, though each microbe is relatively specific for its target pest.

Bacteria Bacterial biopesticides are cheaper and the most widely used biopesticides. Bacteria belonging to the genus *Bacillus* are most widely used pesticides. The most commonly known microbial pesticides are bacterium *Bacillus thuringiensis*, or Bt. This bacterium produces protein crystals that are harmful to specific insect pest. Several strains of Bt have been developed and applied to plant foliage. The ingestion of the protein crystals by insects paralyzes their digestive tracts, killing them within 24–48 h. Different Bt strains produce crystals specific for each insect or small number of related insect species. It has been successfully used in controlling caterpillars/larvae moths, mosquitoes and black flies feeding on cabbage, potatoes and other crops. The commercial preparations of *B. thuringiensis* contain a mixture of spores, *cry* protein and an inert carrier. Bt is the first commercially used biopesticide throughout the world. Control of diamond-back moths, *Helicoverpa* and *Trichoplausia ni*, on cotton, pigeon pea and tomato is controlled by *Bacillus thuringiensis*. Till date, over hundred *B. thuringiensis*-based bioinsecticides have been developed and used against lepidopteran, dipteran, and coleopteran larvae. The *cry* genes coding for the insecticidal crystal proteins have been successfully transferred into different crop plants. *B. thuringiensis* and *cry* proteins are efficient, safe, and sustainable alternatives to chemical pesticides for the control of insect pests. These proteins form the pores or ion channels in the membrane that lead to osmotic cell lysis. In addition, *cry* toxin monomers promote cell death in insect cells through a mechanism involving an adenylyl cyclase/PKA signaling pathway (Zhang et al. 2006).

Bacillus subtilis, a Gram-positive rod-shaped bacterium, produces endospore. It produces antimicrobial metabolites that target bacterial and fungal soil inhabitants including plant pathogens. It promotes growth of plants by production of phytohormones, sequestration of nutrients, stimulation of systemic induced resistance in plants and suppression of plant pathogen activities. Antimicrobial metabolites from *B. subtilis* have been employed in biocontrol and plant protection by exploiting their antibiosis activity on phytopathogens (Romero et al. 2007).

Fungi Fungi also control insect pests. Entomopathogenic fungi are regulators of insect populations and have potential as mycoinsecticide agents against diverse insect pests in agriculture. They invade their hosts by penetrating through the cuticle, gaining access to the hemolymph and producing toxins, and hence are used for the control of pests with piercing mouthparts such as aphids and whiteflies. *Trichoderma*, a fungicide has been found effective against soilborne diseases such as root rot and wilts in crops such as groundnut, black gram, green gram and chickpea (Nargund et al. 2007). Insect-pathogenic fungus *Metarhizium anisopliae* has been used against adult *Aedes aegypti* and *Aedes albopictus* mosquitoes. Fungal isolates of *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* showed lethal effects on the eggs of the carmine spider mite, *Tetranychus cinnabarinus*. The ovicidal activity of the fungal species acts as biocontrol against spider mites such as *T. cinnabarinus*. Fungi *Beauveria bassiana* SG8702 and *Paecilomyces fumosoroseus* Pfr153 showed efficacy for control of *T. cinnabarinus* eggs. The potato psyllid has been controlled by *Bactericera cockerelli* (Sulc). *Aspergillus fumigatus*, *Alternaria tenuissima*, *Penicillium* spp. and *Fusarium* spp. produce harmful mycotoxins which also aid in pest control. Entomopathogenic fungi act as an alternate to insecticide and vital component of integrated pest management (Mazid et al. 2011b).

The combination of *B. bassiana* suspension and neem gave the highest *B. tabaci* egg and nymph mortalities with lowest LT50 value (Mazid et al. 2011b). The use of the insect-pathogenic fungus *Metarhizium anisopliae* was found effective against adult *Aedes aegypti* and *Aedes albopictus* mosquitoes. Fungal biocontrol agents such as isolates of *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces fumosoroseus* showed lethal effects on the eggs of the carmine spider mite, *Tetranychus cinnabarinus*. The fungal species showed the ovicidal activity and suggested the capacity as biocontrol agents against spider mites such as *T. cinnabarinus*. Isolates of entomopathogenic fungi, *Beauveria bassiana* SG8702 and *Paecilomyces fumosoroseus* Pfr153, showed effectivity against *T. cinnabarinus* eggs. Several other entomopathogenic fungi (*Hypocreales*) have been used for the control of potato psyllid, *Bactericera cockerelli* (Sulc).

Viruses A family of viruses called baculoviruses infect their hosts through ingestion. They are characterized by rod-shaped enveloped virions and a circular dsDNA genome of 80–180 kbp. They control lepidopteran and sawfly forest pests. Virus particles invade the cells of the gut before colonizing the rest of the body. Infection reduces mobility and feeding and insects are killed in five to eight days. The virus kills insect pests by taking over the metabolic processes of the host insect for viral multiplication and transmission. This process involves both an active, replicating virus and the production of variety of enzymes and proteins that lead to enhanced infection and insect death (Hubbard et al. 2014).

Baculoviruses encode a number of proteins and enzymes that enhance their ability to specifically infect and replicate in insects. This increases the efficiency of spread and persistence of viruses within the host insect environment. All baculoviruses encode an occlusion body protein (polyhedrin or granulin) which forms the bulk of the proteinaceous crystal or occlusion bodies (OB) that occludes the virions in the later stages of virus replication. OB protein, polyhedrin, makes up to 30% of infected cell protein and is the powerful promoter element of the gene required for the development of baculoviruses expression system. The virion structure, novel polymerases are required for virus-specific DNA replication, gene transcription, and oral infectivity factors essential for infection of insect midgut cells. Two virion phenotypes, namely, occlusion-derived virions (ODV) and budded virions (BV), occur in baculovirus infections. ODV spread the infection to tissues orally throughout the host and infect midgut epithelial cells after ingestion and dissolution in the alkaline condition of the midgut. Upon ingestion of OB by a host insect larva, the polyhedrin protein is degraded in the alkaline environment of the insect midgut by proteases. The process releases the infectious occluded virions into the midgut lumen. The infection in the midgut epithelial cells of virions occurs via peritrophic matrix (PM), a chitin and glycoprotein layer which lines the midgut lumen. Baculoviruses also encode a family of metalloproteases associated with the OB or are incorporated into the envelope of the occluded virion referred as "viral enhancing factor" or "enhancins." Enhancins increase the oral infectivity of baculoviruses (2- to 15-fold).

Baculoviruses infecting Lepidoptera encode an enzyme referred to as ecdysteroid UDP-glucosyltransferase (EGT). EGT catalyzes the transfer of glucose from UDP glucose to ecdysone, thus inactivating the hormone and blocking the molting process from one larval instar to the next. It blocks the molting process and extends the life span of a baculovirus-infected larva. The development of recombinant baculoviruses with the EGT gene under the control of an inducible promoter allows production of more OB in insects. A gene encoding a protein tyrosine phosphatase has been implicated in the hyperactivity and wandering behavior of infected lepidopteran larvae. Chitinase genes are found in almost all baculoviruses infecting lepidopteran insects (Macedo et al. 2015). Chitinase is responsible for the digestion of chitin which is the major component of insect exoskeleton. A virus-encoded protease, cathepsin, is expressed late in baculovirus infection. The enzyme protease aids in the breakdown of host cellular structure and eventually the integrity of the infected cadaver, thus maximizing baculovirus OB dispersion. Since CaV channels are not highly conserved in insects, this makes them attractive alternatives and represents a novel mode of action to conventional pesticides. Fusion protein technology, in which insecticidal peptides are linked to a plant lectin "carrier" protein, developed to allow proteins such as spider venom toxins to act as orally delivered biopesticides. ω-Hexatoxin-Hv1a (Hv1a) from the Australian funnel web spider *Hadronyche versuta* acts on CaV channels in the insect central nervous system (CNS), causing paralysis. Fusion of this insecticidal molecule to the carrier protein snowdrop lectin (*Galanthus nivalis* agglutinin, GNA) allows Hv1a to traverse the insect gut epithelium and access its sites of action, producing an orally active insecticidal protein. The Hv1a/GNA fusion protein has oral insecticidal activity against insects from a range of orders, including Lepidoptera, Coleoptera, Diptera and Hemiptera (Macedo et al. 2015).

The baculoviruses are classified into two genera, nucleopolyhedroviruses (NPVs) and granuloviruses (GVs) Erayya et al. (2013). Nuclear polyhedrosis viruses include *Helicoverpa armigera*, *Amsacta moorei*, *Agrotis ipsilon*, *A. segetum*, *Anadividia peponis*, *Trichoplusia orichalcea*, *Adisura atkinsoni*, *Plutella xylostella*, *Corcyra cephalonica*, *Mythimna separata* and *Phthorimaea operculella*.

The first viral insecticide Elcar™ that consists of a preparation of *Heliothis zea* is relatively broad range NPV baculovirus and infects many species belonging to genera *Helicoverpa* and *Heliothis*. HzSNPV is a product of choice for biocontrol of *Helicoverpa armigera*. Another baculovirus, HaSNPV, identical to HzSNPV was registered in China as a pesticide. HaNPV has relatively broad host spectrum and potentially used on a variety of crops infested with pests including *Spodoptera* and *Helicoverpa*. Another baculovirus, HaSNPV, has been used for large-scale biopesticide production. It has been extensively used on cotton fields. HzSNPV provides control of cotton bollworm and other pests attacking crops such a soybean, sorghum, maize, tomato, and beans. GV is the active component of a number of biopesticides used for protection of apple and pear orchards against the codling moth, *Cydia pomonella*. GV-based products are available in the trade names of Granusal™ in Germany, Carpovirusine™ in France, Madex™ and Granupom™ in Switzerland, and Virin-CyAP in Russia. *Autographa californica* and *Anagrapha falcifera* NPVs have a relatively broad host spectrum and potentially used on a variety of crops infested with pests belonging to a number of genera, including *Spodoptera* and *Helicoverpa* (Ranga Rao et al. 2015). Baculovirus *Anticarsia gemmatalis*, a nucleopolyhedrovirus (AgMNPV), is used to control the velvetbean caterpillar in soybean. Two commercial preparations of *Spodoptera* NPV are available in the USA and Europe. Two NPVs have a relatively broad host spectrum and used on a variety of crops infested with pests belonging to a number of genera, including *Spodoptera* and *Helicoverpa*. These include SPOD-X™ containing *Spodoptera exigua* NPV to control insects on vegetable crops and Spodopterin™

containing *Spodoptera littoralis* NPV which protect cotton, corn, and tomatoes (Mazid et al. 2011b).

10.6.1.2 Insects

Trichogramma are minute wasps which are exclusively egg parasites. They lay eggs in the eggs of various lepidopteran pests. After hatching, the *Trichogramma* larvae feed on and destroy the host egg. It is effective against lepidopteran pests like the sugarcane internode borer, pink bollworm, spotted bollworms in cotton, and stem borers in rice. They are also used against vegetable and fruit pests. It kills the pest in the egg stage, ensuring that the parasite is destroyed before preventing damage to the crop. Control of sugarcane borers and rots has been reported by *Trichogramma*. Ladybugs and praying mantis have been successful in combating scale insects or aphids which feed on plant sap. Control of mango hoppers and mealy bugs and coffee pod borer is reported by *Beauveria*. *Entomophthora ignobilis* (a fungus) is effective against green peach aphid of potato (*Myzus persicae*) (Macedo et al. 2015).

10.6.1.3 Plant-Based Pesticides

Pesticides obtained from plants mainly include (1) azadirachtin (*Azadirachta indica*), (2) rotenones obtained from the roots of *Derris elliptica* and *Lonchocarpus nicou*, (3) nicotine, (4) pyrethrum (*Chrysanthemum cinerariifolium*, *C. coccineum*, and *C. marshallii*), and (5) thurioside. Neem products have been effective in controlling a large number of insects which include 350 species of arthropods, 12 species of nematodes, 15 species of fungi, three viruses, two species of snails, and one crustacean species. Neem biopesticides are systemic in nature and provide long-term protection to plants against pests. Azadirachtin, a tetranortriterpenoid, is a major active ingredient isolated from neem and is known to disrupt the metamorphosis of insects. Two tetracyclic triterpenoids, meliantetyraolenone and odoratone, isolated from neem also exhibit insecticidal activity against *Anopheles stephensi*. Neem seed kernel extract (NSKE) has also been found most effective in reducing the larval population of *Helicoverpa armigera* in chickpea. Neem formulations also have a significant effect against eggs of peach fruit fly *Bactrocera zonata* (Saunders). Root extracts of *Tagetes* or *Asparagus* exhibit nematicidal properties, while *Chenopodium* and *Bougainvillea* act as antivirus agents (Isman 2000).

10.7 Biochemical Compounds

These are naturally occurring substances that control pests by nontoxic mechanisms. These include substances such as insect sex pheromones that interfere with mating, as well as plant extracts that attract insect pests to traps. Pheromones are chemicals emitted by living organisms used to send messages to individuals of the opposite sex of the same species. These include plant growth regulators or substances that repel or attract pests and interfere with growth or mating. These have been found effective against rice cutworm, tobacco caterpillar, rice green leaf hopper and several species of aphids and mites. Mating disruption has been successful in controlling a number of insect pests (Mazid et al. 2011b).

Recombinant fusion proteins containing neuroactive peptides/proteins linked to a "carrier" protein can act as effective pest controls. The non-peptidic analog can be used in the development of novel insecticides overcoming the bioavailability of peptides penetrating the insect cuticle or gut mucosa. Hv1a/GNA (*Galanthus nivalis* agglutinin), containing an insect-specific spider venom Ca channel blocker (ω-hexatoxin-Hv1a) linked to snowdrop lectin (GNA) a "carrier," is an effective oral biopesticide toward various insect pests. Internalized Hv1a/GNA reach the brain within 1 h of exposure. It is unlikely to cause detrimental effects on honeybees, indicating that atracotoxins targeting Ca channels are potential alternatives to conventional pesticides. Pseudophomins A and B produced by *Pseudomonas fluorescens* strain BRG100 are found to be effective as herbicide and pesticide. Pseudophomin A acts as a bioherbicide, while pseudophomin B is an antifungal compound (Pedras et al. 2003).

10.8 Commercially Available Biopesticides

The success stories of application of biopesticides have been reported all over the world. Some of the commercially available biopesticides include BioNEEM, Azatin XL, Nema-Q and Biomite. BioNEEM is a broad-spectrum biocide isolated from kernel of neem seeds. The main component is *Azadirachtin*, a water-soluble emulsifiable concentrate that provides the most effective control of major pests of agricultural and plantation crops. It possesses broad specificity against red spiders and insect pests like helopeltis, aphids, jassids, thrips and caterpillars, etc. The active ingredient inhibits sensory receptors of mouth parts, resulting in disorientation of normal probing, feeding and intake of food by insects and mites. It has very strong repellent and deterrent effects which prevent the insects and mites from colonizing the treated plants.

The successful utilization of biopesticides and biocontrol agents in agriculture includes:

- Use of *Bacillus thuringiensis* for control of diamondback moth and *Helicoverpa* on cotton, pigeon pea, and tomato
- Use of *Beauveria* for control of mango hoppers, mealy bugs, and coffee pod borer
- Use of neem products for control of white fly on cotton
- Use of nuclear polyhedrosis virus (NPV) for control of *Helicoverpa* on gram
- Use of *Trichogramma* for control of sugarcane borers
- Use of *Trichoderma*-based products for control of rots and wilts in various crops (Kalra and Khanuja 2007)

10.9 Molecular Approaches

Several biotechnological and molecular approaches have been followed to enhance the capacity of microbes to achieve maximum pest control properties. These include the following.

10.9.1 Plant-Incorporated Protectants (PIPs)

The genetic modification of plants by expressing genes encoding insecticidal toxins, namely, δ-endotoxins, derived from the soil bacterium *Bacillus thuringiensis* (*Bt* plants). *Bt* endotoxins prove lethal to the alkaline environment of the insect gut. These proteins have provided protection against major pests of cotton, tobacco, tomato, potato, corn, maize and rice. More than 60 Cry proteins have been identified. Most *Bt* maize hybrids express the Cry1Ab, Cry1Ac, Cry9C and Cry3Bb1 protein targeted against the European corn borer (*Ostrinia nubilalis*) and corn rootworm complex (*Diabrotica* spp.), a major pest of maize in North America and Europe. Maize hybrids express the Cry3Bb1 protein, which is targeted against the corn rootworm complex (*Diabrotica* spp.) (Coleoptera), also a major pest of maize, especially in North America. Cotton expressing the Cry1Ac protein is targeted against the cotton bollworm (*Helicoverpa zea* Boddie) (Lepidoptera), which is a major pest of cotton; potato expressing the Cry3A or Cry3C is targeted against the Colorado potato beetle (*Leptinotarsa decemlineata* Say) (Coleoptera), which is a major pest of potato; and Cry4 proteins are targeted against some Diptera, such as certain flies (e.g., *Lycoriella castanescens* Lengersdorf) and mosquitoes (e.g., *Culex pipiens* L.). The expression of these toxins confers protection against insect crop destruction. Potato expressing the Cry3A or Cry3C is targeted against the Colorado potato beetle (*Leptinotarsa decemlineata* Say) (Coleoptera), which is a major pest of potato; and Cry4 proteins are targeted against some Diptera, such as certain flies (*Lycoriella castanescens*) and mosquitoes (e.g., *Culex pipiens* L.) (Saxena et al. 2010).

10.9.2 Other Proteins and Peptides

Macrocidins and *P. macrostoma* enter weed tissues via locations adjacent to root hairs, colonizing intercellularly the vascular trachea, thereby interfering with tissue functionality. *P. macrostoma* also colonizes resistant plants and only in the outer layers of the root. There are two major groups of macrocidins: macrocidin A (Graupner et al. 2003) and macrocidin Z (Bailey et al. 2011). *P. macrostoma* could provide a valuable alternative to control weeds with resistance to carotenoid biosynthesis targeting synthetic herbicides.

The antimicrobial peptides are synthesized as two distinct systems: (1) - non-ribosomal peptide synthesis, e.g., cyclic lipopeptides, iturin, and fengycin families, and (2) ribosomal peptide synthesis, which is further subdivided into

(a) posttranslationally modified, e.g., subtilisin; (b) non-modified, e.g., thurincins; and (c) large heat-labile proteins. Peptide synthetases constitute the non-ribosomal biosynthetic machinery that produces a diverse array of lipopeptides (LPs), such as iturin, fengycin and surfactin families. The LPs are amphiphilic and have cell wall and membrane surface active properties, facilitating the formation of pores in membranes of phytopathogenic bacteria and fungi. Loss of cell membrane integrity causes a disruption in active transport (i.e., nutrient and ion transport) leading to cell death. Surfactins have antibacterial activity, while iturins and fengycins have antifungal properties. Optimum biocontrol activity and colonization of the rhizosphere by *B. subtilis* require coordinated production and synergy of complementary LPs. Surfactin is a signal molecule in the quorum-sensing (QS) system in *B. subtilis*. Surfactin may be involved in initiating biofilm development by subpopulations of the colony and detection of microbial community diversity in the rhizosphere. QS molecules elicit regulation of genes required for microbial survival and act as microbial interspecies communication signals. Subtilisin exhibits broad-spectrum activity toward Gram-positive bacteria by disrupting membrane function.

10.10 Conclusions

Biopesticides and biofertilizers form an important component of integrated pest management (IPM). Biofertilizers increase soil fertility and sustainability by mobilizing macro- and micronutrients or by converting insoluble form to soluble forms available to plants. They stimulate root growth and produce phytohormones that change the root physiology to increase nutrient and water uptake. The advantages such as easy biodegradability and less pesticide residues resulting in less pollution make them a sustainable, environmentally safe pest control agent (Copping and Menn 2000). More research needs to be carried out to overcome the limitations such as narrow target range, specific mode of action, shorter shelf life and limited field persistence and achieve maximum benefit from microbes. Molecular techniques can help in development of modified insecticides which possess enhanced activity against a wide range of pathogens and diseases.

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