Chapter 14
Biofertilizers and Biopesticides in Sustainable Agriculture

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Abstract Green revolution has revolutionized the world agriculture by increasing the yields of food crops by the development of high-yielding varieties, chemical fertilizers, synthetic herbicides, and pesticides. The continuous and excess use of chemical fertilizers has changed the soil characteristics to acidic/alkaline leading to the reduction in the naturally occurring microorganisms in soil that resulted in the stagnation/reduction in crop yields. Use of microorganisms (biofertilizers and biopesticides) as an alternate to synthetic fertilizers and pesticides to increase the soil fertility and disease and pest control in agriculture is gaining prominence. Biofertilizers and biopesticides are environmental friendly products and can be used in integrated nutrient management (INM) and integrated pest management (IPM) techniques. This chapter reviews the microorganisms and their role in enhancing soil fertility and disease and pest control for sustainable agriculture.

Keywords Green revolution · Biofertilizers · Biopesticides · Soil fertility · Sustainable agriculture

14.1 Introduction

In the first half of the twentieth century, the world has witnessed many famines (widespread shortage of food that may apply to any faunal species, a phenomenon which is usually accompanied by regional malnutrition, starvation, epidemic, and increased mortality) resulted in the mortality of millions of people (World Ecology Report 2008). Due to the tireless efforts of Norman Borlaug (considered as "Father of Green Revolution") in the 1940s, Mexico became self-sufficient in wheat production and saved millions of lives in India, Pakistan, and elsewhere from starvation through high-yielding wheat varieties. By his efforts Mexico became the exporter of wheat by 1963, and in India and Pakistan, wheat yields were doubled between 1965

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and 1970. The miracle rice developed by Hank Beachell and colleagues at International Rice Research Institute (IRRI) has significantly increased the rice yields and benefitted poor people across Asia (Philips 2013). The green revolution relied on crops with high-yielding varieties, response of crops to chemical fertilizers and water, and synthetic herbicides and pesticides, for weed, disease, and insect control. The tolerance of the wheat to biotic and abiotic stresses has made it possible to double the food grain production worldwide. The green revolution (first "wave") in India was started in the late 1960s, and by the late 1970s, India became self-sufficient in wheat production, and the impact of green revolution was confined to Northern India. The second "wave" of green revolution covered almost all the crops including rice covering the whole country, enabling the rise in rural income and alleviating rural property (Fujita 2010; Meena et al. 2013a; Bahadur et al. 2014; Maurya et al. 2014; Jat et al. 2015; Kumar et al. 2016b).

The green revolution relied on the package of inputs such as high-yielding varieties (HYVs) developed through breeding techniques; chemical fertilizers and irrigation; chemicals to control weeds, diseases, and pests; and mechanization. These HYV were irrigated using canals or tube wells. Tube wells have been made by the farmers wherever the electricity was available. Canals received water from the dams/reservoirs made by the government. The chemical fertilizers supplied nutrients such as nitrogen (N), phosphorus (P), potash (K), etc. which increased the productivity. The monoculture has resulted in the increase in pest incidences in HYV, and pesticides (synthetic chemicals) were used to control the pests. Even though green revolution had fed the world by increasing the crop productivity worldwide, the use of chemical fertilizers had reduced the soil fertility. It increased the soil salinity. Salinity reduces the availability of micronutrients to the crops (Kumar et al. 2015, 2016a; Ahmad et al. 2016; Meena et al. 2016a; Parewa et al. 2014; Prakash and Verma 2016; Jaiswal et al. 2016; Jha and Subramanian 2016).

The continuous use of chemical pesticides had reduced the naturally occurring organisms which control the pests. It also resulted in environmental pollution. The use of excess chemical fertilizers and pesticides have reached streams through run-off water from the fields causing the eutrophication. Intensive commercial irrigation resulted in soil erosion from irrigation on slope land, reduced soil nutrient content, and compaction of soil by the use of heavy machinery (Moore and Parai 1996). The chemical fertilizers are not used fully by the plants. When urea is used as nitrogen fertilizer, some of it will be evaporated, a part will be utilized by the plants, and the remaining will reach the streams through runoff water. Similarly when single superphosphate (SSP) and muriate of potash (MOP) are used as phosphate and potash fertilizers, part of phosphate and potash are utilized by the plants, and the remaining will be fixed in the soil through various chemical reactions making these fertilizers unavailable to plants. Even though the soils are rich in phosphorus and potash, due to their unavailability, the farmers are adding these fertilizers continuously to the soil making the soils saline/alkaline (Meena et al. 2017).

At the same time, the continuous use of chemical pesticides is making the insects resistant to the pesticides. To control these pests, the more powerful pesticides are being developed and used. These pesticides are not biodegradable and are causing environmental pollution. Substituting/supplementing the use of chemical fertilizers and pesticides with biofertilizers and biopesticides along with organic manure can make the agriculture sustainable by maintaining the soil fertility and alleviating the various abiotic and biotic stresses.

14.2 Biofertilizers

Biofertilizers (also called as "bioinoculants") are the living organisms of bacterial, fungal, or algal origin. They are not the nutrients by themselves, but they help in plant nutrition by various biochemical processes like nitrogen fixation, phosphate solubilization, potash mobilization, zinc solubilization, phosphate and micronutrient mobilization, etc. (Kumar 2013b; Meena et al. 2015a, 2016b; Priyadharsini and Muthukumar 2016; Kumar et al. 2017; Raghavendra et al. 2016; Dotaniya et al. 2016; Meena et al. 2015f). The partial list of different biofertilizers with their function are given below (Table 14.1).

Apart from the above functions, the above bacteria and fungi are useful in plant growth promotion by secretion of hormones such as auxins, cytokinins, gibberellins, and abscisic acid which directly promote growth of the plants. These bacteria also promote the plant growth by (a) antibiotic production, (b) siderophore secretion, (c) production of low molecular weight metabolites such as hydrocyanic acid (HCN), (d) production of lytic enzymes, (e) successfully competing with plant pathogens for nutrients and colonizing surfaces on the roots, and (f) induced systemic resistance (ISR) in plants (Gopalakrishnan et al. 2015; Zahedi 2016; Meena et al. 2015b; Rawat et al. 2016; Yasin et al. 2016; Bahadur et al. 2016b; Das and Pradhan 2016;

Table 14.1 Partial list of biofertilizer organisms and their functions

Name of bacteria	Function
Bacterial biofertilizers	
Rhizobium, *Azotobacter*, *Azospirillum*, *Acetobacter* (*Gluconacetobacter*), *Frankia*, etc.	Nitrogen fixation
Bacillus megaterium, *Pseudomonas* sp., *Rhodococcus*, *Arthrobacter*, *Serratia*, *Phyllobacterium*, *Paenibacillus*, *Xanthomonas*, *Micrococcus*, etc.	Phosphate solubilization
Frateuria aurantia, *Bacillus mucilaginosus*, etc.	Potash solubilization
Bacillus sp., *Pseudomonas* sp., *Xanthomonas* sp., *Enterobacter* sp., *Mycobacterium* sp., *Stenotrophomonas* sp., etc.	Zinc solubilization
Fungal biofertilizers	
Mycorrhiza (arbuscular mycorrhizal fungus – AMF)	Phosphate solubilization, mobilization, and micronutrient mobilization
Penicillium	Phosphate solubilization
Piriformospora indica	Phosphate solubilization

Dominguez-Nunez et al. 2016). Due to their potential in improving the plant growth by nutrition, as well as alleviating biotic and abiotic stresses, these microorganisms are called as plant growth-promoting microorganisms (PGPM). The bacteria and fungi having potential in improving the plant growth are called as plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF).

14.2.1 Bacterial Biofertilizers

14.2.1.1 Nitrogen Fixation

Nitrogen is the essential element in the growth and development of all living organisms, as it is a constituent of DNA, RNA, ATP, and proteins. In plants it is an essential constituent in chlorophyll, growth hormones, alkaloids, and glucosinolates. It is present ~78% in the atmosphere in gaseous form making it the largest pool of nitrogen. But this nitrogen gas can't be utilized by plants and animals. For nitrogen to be available to make proteins, DNA, and other biologically important compounds, first it must be converted into a different chemical form. The nitrogen is converted to ammonia by reaction with hydrogen by catalytic reaction. Urea is produced by the reaction of ammonia with carbon dioxide in industrial process that can be used in agriculture as nitrogen fertilizer for increasing the crop yields (Meena et al. 2015e, 2016c, d; Saha et al. 2016a; Yadav and Sidhu 2016; Teotia et al. 2016).

The atmospheric nitrogen is also reduced to ammonia in the presence of nitrogenase by a process known as biological nitrogen fixation. Nitrogenase is an oxygen-sensitive enzyme (a biological catalyst) found naturally in certain microorganisms. The oxygen sensitivity is overcome by compartmenting in cyanobacteria (heterocysts in *Anabaena azollae*), active respiration (*Azotobacter*), and synthesis of leghemoglobin (*Rhizobium*).

The NFR are either free living or symbiotic or associative symbiotic. *Azotobacter* is an example of free living NFR. *Rhizobium*, *Bradyrhizobium*, and *Sinorhizobium* are the symbiotic NFR forming the root nodules in leguminous plants. Similarly actinomycete filamentous bacteria, *Frankia*, form root nodules in actinorhizal plants in the genus *Alnus* (Alder, whereas *Azospirillum*, *Klebsiella*, etc. are the associative symbiotic NFR living on the surface of the plant roots or sometimes invade the outer cortical cells of the roots. The PGPR strains *Acetobacter* (*Gluconacetobacter*) are a root-endophytic NFR isolated from sugarcane. Application of *A. brasilense* in wheat in a greenhouse experiment, isolated from the rhizosphere soil, has promoted sheath elongation, root depth, fresh weight of roots, fresh and dry weight of shoots, total nitrogen, and bacterial counts in soil. In the absence of supplemented nitrogen source, the wheat plants inoculated with *Azospirillum* had higher growth, mineral, and chlorophyll (Sayed et al. 2015; Saha et al. 2016b; Verma et al. 2014, 2015b; Meena et al. 2014a, 2016e; Masood and Bano 2016).

Molina et al. (2012) reported the colonization of *Azospirillum brasilense* from inoculated mother plants to new daughter uninoculated plants via stolons by colonizing the inner tissues of roots and stolons in strawberry plants. Inoculation of corn with *Azospirillum* and *Azotobacter* at 1, 2, and 3 kg/ha has increased the grain yield, 1000 grain weight, grains/corn, and grains/row compared to the various doses of nitrogen fertilizer application (Amiri and Rafiee 2013). The seed biopriming by efficient PGPR strains leaf-sprayed inoculation of *Azospirillum brasilense* at V4 stage in maize has shown increased height and shoot and root dry mass. Also it increased the ear size, chlorophyll content, 1000 grain weight, and grain yield (Costa et al. 2015; Sharma et al. 2016; Verma et al. 2015a; Meena et al. 2013b, c; Singh et al. 2015; Bahadur et al. 2016a). Inoculation of *Azospirillum brasilense* in *Cymbopogon winterianus* with 2,4-D increased the N-fixation and contributed to higher chlorophyll content and NR activity leading to higher yield and oil content compared to the control treatment. Nitrogen content of stem, leaves, and roots increased compared to control (Saikia et al. 2014). The application of *Azotobacter* in tomato has increased the germination, shoot height, no. of leaves per plant, and length and width of leaves compared to the control treatment (Mahato et al. 2009). The three *Rhizobium* isolates YSY-25, YSY-26, and YSY-27 isolated from the rhizosphere soil of pigeon pea have shown the production of IAA, YSY-25, and YSY-27 produced NH_3 and YSY-26 produced HCN (Singh et al. 2013). The efficient NFR strains *Gluconacetobacter diazotrophicus* have been isolated from sugarcane roots. It is also isolated from the roots, root hair, stem tuber, fruits, and rhizosphere of coffee, sweet potato, tea, pineapple, mango, and banana. It is also found in the internal environment of VAM spores and mealybugs (Muthukumarasamy et al. 2002; Shrivastava et al. 2016; Velazquez et al. 2016; Meena et al. 2015c, d; Singh et al. 2016). Acetic acid-producing NFR isolated from four different rice varieties are identified as *Gluconacetobacter diazotrophicus* on the basis of phenotypic characteristics and PCR assay using specific primers for that species (Muthukumarasamy et al. 2005).

14.2.1.2 Phosphate Solubilization

Phosphorus is the second important essential macro-element required next to nitrogen in plants. It is required for various metabolic processes such as biosynthesis of macromolecules, energy transfer, signal transduction, photosynthesis, and respiration. Phosphorus is present in the soils both in organic and inorganic forms (Sindhu et al. 2016; Meena et al. 2014b). Of these, organic form, as found in humus and other organic materials including decayed plant, animal, and microbial tissues, is an important reservoir of immobilized P accounting ~20 to 80% of total soil P. A part of the phosphorus fertilizer on application to soil will be utilized by the plants, and the rest will be fixed/forms complex with other chemicals in soil, making it unavailable to plants. The bacteria have the potential of mineralization and solubilization of organic and inorganic phosphorus, respectively. Inorganic P is solubilized by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelate cations (Al, Fe, Ca) and decrease the pH in basic soils (Khan et al. 2009).

Bacillus megaterium is the most widely used bacterium for P solubilization. *Pseudomonas* is another important PSB. The other bacteria having P-solubilizing potential are *Arthrobacter* sp. (Banerjee et al. 2010), *Pantoea* (Castagno et al. 2011), *Serratia* (Farhat et al. 2009), *Paenibacillus* (Zhang et al. 2013), *Xanthomonas* (Sharan et al. 2008), and *Micrococcus* and *Bacillus* sp. (Chatli et al. 2008). The *Bradyrhizobium* isolated from the roots of soybean growing in Latur has shown the phosphate solubilization efficiency. Out of the ten isolates, three isolates of *Bradyrhizobium* (RSB02, RSB04, and RSB08) have shown the phosphate solubilization ability (Jadhav 2013). The principal mechanism in soil for mineral phosphate solubilization is lowering of soil pH by microbial production of organic acids and mineralization of organic P by acid phosphatases. Inorganic P is solubilized by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelate cations (Al and Fe) and decrease the pH in basic soils. The PSB dissolve soil P through production of low molecular weight organic acids mainly gluconic and ketogluconic acids, in addition to lowering the pH of rhizosphere. pH of the rhizosphere is lowered through biotical production of proton/bicarbonate release (anion/cation balance) and gaseous exchanges.

Phosphorus solubilization ability of PSB has direct correlation with the pH of the medium (Khan et al. 2009). Release of root exudates such as organic ligands can also alter the concentration of P in the soil solution. Organic acids produced by PSB solubilize insoluble phosphates by lowering the pH, chelation of cations, and competing with phosphate for adsorption sites in the soil (Nahas 1996). Inorganic acids, e.g., hydrochloric acid, can also solubilize phosphate, but they are less effective compared to organic acids at the same pH. An enzyme glucose dehydrogenase (GDH) was induced fivefold by phosphate starvation by the bacterium *Enterobacter asburiae*, isolated from alkaline Indian vertisol soils.

Concomitant with the release of GDH, glucose was converted to gluconic acid, causing the reduction in soil pH and release of phosphate and iron (Gyaneshwar et al. 1999). Phosphate ions readily precipitate with metal cations forming a range of P minerals. In neutral or alkaline soils, P ions will precipitate as Ca phosphate, dicalcium or octacalcium phosphates, hydroxyapatite, and eventually least soluble apatites. Under acidic conditions P ions will precipitate as Fe and Al phosphates such as strengite, vivianite, variscite, and various minerals of the plumbogummite group. The Fe and Al phosphates have an increasing solubility with increasing pH, while Ca phosphates have a decreasing solubility with increasing pH, except for pH values above 8 (Hinsinger 2001).

Mineralization of soil organic P (Po) plays an imperative role in P cycling of a farming system, which may constitute about 4–90% of the total soil P. Alkaline and acid phosphatases secreted by the soil microorganisms use organic phosphate as a substrate to convert it into inorganic form. Principal mechanism for mineralization of soil organic P is the production of acid phosphatases. Release of organic anions and production of siderophores and acid phosphatase by plant roots/microbes or alkaline phosphatase enzymes hydrolyze the soil organic P or split P from organic residues. The largest portion of extracellular soil phosphatases is derived from the microbial population. Mixed cultures of PSMs (*Bacillus*, *Streptomyces*, *Pseudomonas*, etc.) are most effective in mineralizing organic phosphate (Mohammadi 2012).

Inoculation of phosphate-solubilizing and phytohormone-producing mutants of *Azotobacter chroococcum* in wheat increased the grain, straw, biological yield, spike length, spikelets spike^{-1}, 1000 grain weight, and root biomass over control (Kumar et al. 2001). Inoculation of alfalfa seedlings with phosphate-solubilizing and nitrogen-fixing bacteria, *Klebsiella pneumonia* and *Rhizobium meliloti*, has increased the survival rate of seedlings, shoot height, root length, root volume, leaf area, individual number of leaves per plant, and biomass, and P uptake percentage of the two *Medicago sativa* varieties were found remarkably increased than *control group* (Li et al. 2013).

14.2.1.3 Potassium Solubilization

Potassium (K) is the very important mineral required for plant growth next to nitrogen and phosphorus. The K exists in several forms in soil such as mineral K, non-exchangeable K, exchangeable K, and dissolved or solution K (K^+ ions). Soil has abundant reserves of K, among which only 1–2% can be directly absorbed by the plants. However, ~90 to 98% of the soil K exists in silicate minerals such as K feldspar and mica, which only release K slowly (Zhang and Kong 2014). The dissolution of organic matter in soil produces organic acids such as citric acid, formic acid, malic acid, and oxalic acid. These organic acids enhance the dissolution of K compounds by supplying protons and by complexing Ca^{2+} ions (Shanware et al. 2014).

Potassium-solubilizing bacteria (KSB) *Bacillus mucilaginosus* solubilize potassium by secreting organic acids from rock K mineral powders such as mica, illite, and orthoclases. Wild type and mutant strain of *Bacillus edaphicus* solubilized the fixed form of K by producing organic acids and capsular polysaccharides. Oxalic acid seemed to be more effective with the Nanjing feldspar, whereas oxalic and tartaric acids were responsible for mobilizing K in the Suzhou illite (Sheng and He 2006).

Biopriming through KSB strains *Bacillus edaphicus* in cotton and rape has increased the potassium content by ~30% and 26%, respectively, in soils supplemented illite as potassium source. Shoot and root growth and N, P, and K uptake were improved in both cotton and rape (Sheng 2005). Out of the seven KSB isolated from potash-rich soil samples nearby ceramic industries, two isolates, KSB-1 and KSB-7, are able to solubilize potash under in vitro conditions. The KSB-7 released ~33 mg/L from feldspar and KSB-1 released ~31 mg/L under control condition. Inoculation of these isolates in mung bean has increased the plant growth and K uptake compared to the control. KSB-1 is identified as a gram-negative bacterium and KSB-7 is a gram-positive *Bacillus* sp. (Prajapathi 2016).

Inoculation of KSB in tea (*Camellia sinensis)* at 75% K fertilizer has recorded the high chlorophyll, carotenoid, N, P, and K contents in the crop shoots. All the quality parameters of tea such as theaflavin, thearubigin, highly polymerized substances, total liquor color, caffeine, briskness, and color and flavor indexes were greatly improved in KSB-treated plants, which in turn improve the tea quality as well (Bagyalakshmi et al. 2012).

14.2.1.4 Zinc Solubilization

Zinc is an essential micronutrient for prokaryotic and eukaryotic organisms. It is present in the enzyme systems as cofactor and metal activators of many enzymes. Exogenous application of soluble zinc sources, similar to fertilizer application, has been advocated to various crops. This causes transformation ~96 to 99% of applied available zinc to various unavailable forms (Saravanan et al. 2003). High pH and high content of $CaCO_3$, organic matter, phosphate, and copper can fix zinc in the soil giving rise to the reduction of available Zn. These efficient rhizospheric microorganisms play a key role in solubilization of unavailable form of Zn to available forms. This Zn solubilization was due to the production of organic acids and pH drop by the organisms. The release of organic acids that sequester cations and acidify the microenvironment near root is thought to be a major mechanism of Zn solubilization.

A number of organic acids such as acetic, citric, lactic, propionic, glycolic, oxalic, gluconic acid, etc. have been considered due to its effect in pH lowering by microorganisms. Organic acid secreted by microflora increases soil Zn availability in two ways; they are probably exuded both with protons as counterions and, consequently, reduce rhizospheric pH. In addition, the anions can chelate Zn and increase Zn solubility which results in the conversion of available form (Zn^{2+}) to plants.

Bacillus and *Pseudomonas* sp. are widely used bacteria for Zn solubilization. *Aspergillus* sp. is also studied for zinc solubilization potential (Kumari et al. 2014). The endophytic bacteria (*Klebsiella*, *Bacillus*, *Pseudomonas*, *Paenibacillus*, and *Enterococcus* sp.) isolated from soybean and mung bean have shown zinc-solubilizing potential. *Klebsiella* and *Pseudomonas* sp. has solubilized both the inorganic sources of Zn supplemented in Tris mineral medium and P solubilization and IAA production (Sharma et al. 2014). Application of *Azospirillum*, *Pseudomonas*, and *Rhizobium* to wheat along with various concentrations of N and P has considerably increased zinc content in different parts of wheat plant at different growth stages.

Zinc concentration was increased in all the microbial treatments compared to controls in wheat shoot, flag leaves, straw, grain, and roots compared to chemical fertilizer treatments (Naz et al. 2016). *Burkholderia* (one strain) and *Acinetobacter* (two strains) isolated from the Zn-deficient rice-wheat field, when applied to rice either individually or in combination, have significantly increased the mean dry matter yield/pot (~13%), productive tillers/plant (~15%), number of panicles/plant (~13%), number of grains/panicle (~13%), grain yield (~17%), and straw yield (~12%) over the control and Zn fertilizer treatment, respectively.

The bacterial inoculations also significantly enhanced the total Zn uptake/pot (~53%) as well as grain methionine concentration ~39% (Vaid et al. 2014). Different strains of *Pseudomonas* sp. (*P. putida*, *P. fluorescens*, *P. aeruginosa*) have shown the

Zn-solubilizing ability by forming clearing zone in medium with zinc oxide and zinc carbonate in plate assay. The shift in the pH of the medium from 7.0–7.2 to 4.5–6.5 is the clear indication of secretion of acids by the *Pseudomonas* sp. which solubilized the Zn in the medium (Bapiri et al. 2012).

14.2.2 Fungal Biofertilizers

Mycorrhiza (arbuscular mycorrhizal fungus – AMF), *Penicillium*, *Aspergillus*, *Chaetomium*, *Fusarium*, *Mucor ramosissimus*, and *Trichoderma* sp. are the fungi having good P-solubilizing potential and can be used as biofertilizer.

14.2.2.1 Mycorrhiza

The mycorrhiza (commonly called as "fungus root") is the symbiotic association between plant roots and soil fungus. Seven types of mycorrhiza were identified so far. They are (a) ectomycorrhiza, (b) endomycorrhiza (AMF), (c) ectendomycorrhiza, (d) ericoid mycorrhiza, (e) arbutoid mycorrhiza, (f) monotropoid mycorrhiza, and (g) orchidoid mycorrhiza.

Out of the seven types, endomycorrhiza is the most important one as AMF associations were found in the roots ~85% of the land plant families. An AMF fungus lives within the plant roots. The hyphae of the fungi extend outside into the soil beyond the nutrient depletion zone for exploration of mineral nutrients in a greater volume of the soil (Habte 2000). The AMF hyphae enter into the cortical cells of the root forming arbuscules, which are dichotomously branched structures. Arbuscules are the sites of nutrient exchange. In the intercellular spaces of root cortical cells, deeply stained bodies formed by hyphae are called as vesicles. They are the storage organs. They store lipids and phosphorus in the form of polyphosphate granules. This polyphosphate is converted into inorganic phosphate by enzymatic action and will be utilized by the plants under phosphate deficient conditions.

The primary function of AMF is phosphate nutrition (Whitman 2009). The following are the benefits of AMF: increased phosphorus and micronutrients uptake (zinc, copper, iron, sulfur, manganese, cobalt, molybdenum, etc.); increased water uptake; increased resistance to pathogens and pests; enhanced tolerance to soil stress, viz., high salt levels, heavy metal toxicity, drought, high temperatures, etc.; improved seedling survival on transplantation; and enhanced beneficial microbial population in the root zone.

The soil phosphorus levels are critical for obtaining the benefits of mycorrhizal inoculation; these benefits of mycorrhizae are greatest when soil phosphorus levels are at or below ~50 ppm. Mycorrhizal infection of roots declines above this level. Little infection occurs above ~100 ppm P even when soil is inoculated with a mycorrhizal mix (Swift 2004). The AMF hyphae, due to their smaller diameter of 2–5 μm, can penetrate soil pores inaccessible to root hairs (~10 to 20 μm diameter) and absorb water that is not available to non-mycorrhizal plants. The rate of water transport by extraradical hyphae to the root was 0.28 ng/s per entry point, a level sufficient to modify plant water relations (Lozano 2003).

Inoculation of AMF *Glomus* and *Acaulospora* sp. in tomato seedlings in the presence or absence of pathogen (*Fusarium oxysporum f.sp. lycopersici*) increased the stem diameter, leaf area, and shoot and root dry weight. Percent colonization was decreased in the presence of pathogen from ~82% to 64% in *Glomus fasciculatum* and from ~90% to 78% in *Acaulospora laevis* (Manila and Nelson 2013). AMF has densely colonized the roots of *Lotus glaber* Mill (~90%) and *Stenotaphrum secundatum*. The percentage of colonized root length in *L. glaber* was higher (90%) than in *S. secundatum* (73%) at high values of soil pH of 9.2 and at an exchangeable sodium percentage (65%). The arbuscular colonization fraction increased at the beginning of the growing season and was positively associated with increased P concentration in both shoot and root tissue.

The vesicular colonization fraction was high in summer when plants suffer from stress imposed by high temperatures and drought periods and negatively associated with P in plant tissue (Garcia and Mendoza 2007). Silva et al. (2008) had studied the effect of AMF isolates (*Scutellospora heterogama* SCT120E, *Gigaspora decipiens* SCT304A, *Acaulospora koskei* SCT400A, *Entrophospora colombiana* SCT115) individually or mix and by the addition of P on the development and oleoresin production in micropropagated *Zingiber officinale.* In all the mycorrhizal treatments, oleoresin production was high compared to control except in the treatment with *Ec*. Oleoresin production was 3.48 and 1.58% higher in the treatments with *S. herogama* and *G. decipiens* compared to control after 210 days. The higher fresh biomass was recorded in all the treatments except *Sh* compared to control; higher oleoresin and higher content of total extracted oils were recorded in all treatments except *Ec* compared to control after 210 days.

Among 62 fungal isolates, 253 bacterial isolates obtained from heavy metal soils of Orissa were screened for P-solubilizing ability. Among the fungal isolates of *Penicillium* sp. 21 have solubilized tricalcium phosphate (TCP) and released ~82 μg P mL^{-1}; *Aspergillus* sp. MNF1 has produced ~37 μg P mL^{-1} from TCP. *Penicillium* sp. 2 isolate has solubilized rock phosphate (RP) and released ~5 μg P mL^{-1} in liquid culture medium. These rhizobacterial cultures were poor solubilizers of phosphate in both solid and liquid media (Gupta et al. 2007)**.** The 47 fungal isolates were collected from the mangrove and screened for P-solubilizing ability. Among these isolated MPF-8 showed maximum P solubilization, and it was identified as *Aspergillus niger* based upon molecular identification using 16S rDNA sequencing.

Supplementing Pikovskaya's broth with glucose and ammonium sulfate as carbon and nitrogen source recorded maximum P solubilization of ~401 and 427 μg mL^{-1}, respectively. At optimum pH (7.0) and temperature (30 °C), *A. niger* solubilized and liberated ~443 and 468 μg mL^{-1} of soluble phosphate (Bhattacharya et al. 2015). The P-solubilizing fungi *Penicillium expansum*, *Mucor ramosissimus*, and

Candida krissii isolated from phosphate mines of People's Republic of China promoted growth, soil available phosphorus, and phosphorus and nitrogen uptake in wheat seedlings in field soil containing rock phosphate under pot culture conditions (Xiao et al. 2009).

Cane yield and sugar yield (t/ha) were increased by the application of mycorrhiza at 12.5 kg/ha at 75% P + 100% NK at the time of planting in sugarcane. The cane yield and sugar yield were increased from ~79 to 94 t/ha and 10–12 t/ha in control and mycorrhiza-applied plot, respectively. Available P content was increased in mycorrhiza applied plots compared to control (Rani et al. 2011).

Biofertilizers are synergistic to each other and can be applied as consortia to obtain maximum benefits. The application of NPK biofertilizers and AMF together will improve the nutrient uptake, yield, and quality of the produce. The coinoculation of *Bacillus megaterium var. phosphaticum* (PSB) and *Bacillus mucilaginosus* (KSB) in pepper and cucumber in nutrient limited soil has resulted in higher P and K availability than in control (Han et al. 2006). Similarly coinoculation of *Bacillus sphaericus* and *Pseudomonas* sp. in rice at half dose of inorganic fertilizer input has recorded enhanced shoot biomass, leaf chlorophyll content, and N, P, K, Ca, and Mg content (Adzmi et al. 2014).

Inoculation of *Azotobacter* and AMF either singly or in combination in wheat has increased spike as compared to control. *Azotobacter* + mycorrhiza treatment increased grain protein by ~13% than control. The significantly higher kernel weight was found in *Azotobacter* and *Azotobacter* + mycorrhiza and minimum in control and mycorrhiza treatments. Ammonium nitrate and *Azotobacter* + mycorrhiza treatments gave significantly higher grain yield than the other N sources and biofertilizers (Bahrani et al. 2010). The list of commonly used biofertilizers is given below (Table 14.2).

14.2.3 Advantages of Biofertilizers

The following are the advantages of biofertilizers:

1. Improvement in nutrient uptake (up to ~25%).
2. Reduction in fertilizer usage (up to ~25%).
3. Improvement in crop yield (up to ~15%).
4. Improvement in the quality of the produce.
5. Tolerance to biotic and abiotic stresses.
6. Better acclimatization of transplants.
7. Reclamation of degraded soils, sodic soils, habitat restorations, etc.
8. Improves the soil fertility.

Table 14.2 List of commonly used biofertilizers

Name of biofertilizer	Bacteria/ fungi	Useful for crops	Benefits	Remarks
Rhizobium	Bacteria	Leguminous crops like ground nut, soybean	10–35% yield increase, 50–200 kg N ha^{-1}	Leaves residual nitrogen in soil
Azotobacter	Bacteria	Non-leguminous crops, useful for soils containing high organic matter	10–15% yield increase, improve 20–25 kg N ha^{-1}	Also controls certain diseases
Azospirillum	Bacteria	Soil treatment for non-leguminous crops and maize, barley, oats, sorghum, sugarcane, millets	10–20% yield increase	Produces growth-promoting substances
P-solubilizers	Bacteria/ fungi	Soil application for all crops	5–30% yield increase	Can be applied with NFB along with rock phosphate
AMF	Fungi	Many trees, some crops, and some ornamental plants	30–50% yield increase, enhances uptake of P, Zn, S, and water	Can be applied in combination with bacterial biofertilizers

9. Reduces environmental pollution.
10. They are cost-effective, eco-friendly, and easy to handle and apply.
11. No residues are left in the soil.

14.3 Biopesticides

Biopesticides or biological pesticides are a form of pesticides based on microorganisms or natural products. They are categorized into (a) microbial biopesticides containing microorganisms in controlling diseases and insects, (b) botanical biopesticides, and (c) plant-incorporated protectants.

The microbial biopesticides are the formulations containing bacteria, fungi, or viruses for controlling disease-causing fungi/bacteria and insects. The biofungicides control the fungal pathogens by various mechanisms such as competition, mycoparasitism, antibiosis, and lysis. *Bacillus thuringiensis* control the insect larvae belonging to the orders Coleoptera, Diptera, and Lepidoptera by secretion of crystal proteins also known as δ-endotoxins (Mathew et al. 2014). The primary action of crystal proteins (toxins) is to lyse midgut epithelial cells in the target insect by forming pores in the gut cell membrane, followed by destruction of the epitherlial cells.

B. thuringiensis subsp. *israelensis* is highly toxic to *Aedes*, *Culex*, and *Anopheles* mosquito species that are vectors of human diseases (Bravo et al. 2007). The toxin-producing genes from *Bacillus thuringiensis* are introduced into cotton, corn, brinjal, and other economically important crops through genetic engineering techniques. The plants have the inherent capacity to produce crystal proteins in all the plant parts. When insects feed on the leaves and other plant parts, the crystal proteins act on the insects and the insects will be killed.

Beauveria bassiana and *Metarhizium anisopliae* are the entomopathogenic fungi. *B. bassiana* spores upon contact with the body of an insect host; they germinate, penetrate the cuticle, and grow inside the host killing the insect. Afterward, a white mold emerges from the cadaver and produces new spores. *M. anisopliae* spores when come into contact with the body of an insect host, they germinate, and the hyphae that emerge penetrate the cuticle. The fungus then develops inside the body eventually killing the insect after a few days. *Trichoderma* and *Pseudomonas* are the most widely used biopesticides for controlling the soilborne diseases (Handelsman and Stabb 1996).

Trichoderma is a filamentous fungus isolated from soil, dead woods and organic matter. Different species of *Trichoderma* such as *T. viride*, *T. harzianum*, and *T. virens* have the good biocontrol potential (Kumar 2016). The biocontrol abilities have been attributed to various mechanisms such as competition for nutrients; mycoparasitism by secretion of cell wall-degrading enzymes chitinase, glucanases, proteases, etc.; and antibiosis by production of antibiotic compounds such as harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-pentyl-α-pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid, etc. (Benítez et al. 2004).

Trichoderma successfully controls the pathogenic fungi such as *Fusarium*, *Phytophthora*, *Sclerotia*, etc. by the above mechanisms. *Pseudomonas* sp. has controlled the *Fusarium* sp. causing wilt in carnation and *Pythium* sp. and *Rhizoctonia* sp. causing damping-off in cotton. It induced resistance to anthracnose disease caused by *Colletotrichum* sp. in cucumber. *Pseudomonas* suppressed pathogens by secretion of antibiotics such as phenazine-1-carboxylic acid (PCA) and other derivatives, 2,4-diacetylphloroglucinol (DAPG), pyrrolnitrin (Prn), and/or pyoluteorin (Plt), and by induced systemic resistance (ISR) (Weller 2007). The following is the partial list of important categories of biopesticides and their target organisms (Table 14.3).

Other categories of biopesticides include the following (Kumar 2013a).

14.3.1 Predators

Chrysopa carnea and *Chrysopa rufilabris* are found abundantly in the fields. They lay eggs on foliage. After hatching in a day or 2, they feed on aphids, larvae, eggs, small worms, mites, thrips, and immature whitefly.

Table 14.3 Important biopesticides and target pathogens/pests

Name of organism	Target pathogens/pests
Bacterial biopesticides	
Bacillus sp.	*Fusarium*, *Verticillium*, *Pythium*, *Cercospora*, *Colletotrichum*, *Alternaria*, *Ascochyta*, *Macrophomina*, *Myrothecium*, *Ramularia*, *Xanthomonas*, *Erysiphe polygoni*, *Rhizoctonia*, *Phytophthora*, *Botrytis*, *Sclerotiana*, *Erwinia*, etc.
Bacillus thuringiensis	Caterpillars, weevils, leafhoppers, bugs, leaf-feeding insects, etc.
Gliocladium sp.	*Alternaria*, *Chaetomium*, *Penicillium*, *Aspergillus*, *Rhizopus*, *Fusarium*, etc.
Pseudomonas sp.	*Penicillium*, *Botrytis cinerea*, *Mucor*, *Helminthosporium*, *Colletotrichum*, *Pythium*, *Sclerotiana*, etc.
Agrobacterium radiobacter strain 84	*Agrobacterium tumefaciens*
Alcaligenes sp.	*Aspergillus*, *Fusarium*, *Alternaria*, etc.
Serratia sp.	*Sclerotium*, etc.
Trichoderma sp.	*Sclerotinia*, *Rhizoctonia*, *Phytophthora*, *Fusarium*, *Pythium*, *Cercospora*, *Colletotrichum*, *Alternaria*, *Ascochyta*, *Macrophomina*, *Myrothecium*, *Ralstonia*, etc.
Beauveria bassiana	Termites, thrips, beetles, whiteflies, mealybugs, grasshoppers, stem borers, etc.
Metarhizium anisopliae	Root weevils, plant hoppers, Japanese beetle, black vine weevil, white grubs, termites, etc.
Verticillium lecanii	Thrips, whiteflies, aphids, mealybugs, etc.
Viral biopesticides	
Granulosis virus and nuclear polyhedrosis virus (NPV)	Alfalfa looper, corn earworm, imported cabbageworm, cabbage looper, cotton bollworm, cotton leafworm, tobacco budworm, armyworms, European corn borer, almond moth, spruce budworm, Douglas-fir tussock moth, pine sawfly, and gypsy moth
Botanical biopesticides	
Azadirachtin	Thrips, jassids, aphids and whiteflies, flea beetles, *Helicoverpa armigera*, *Helicoverpa zea*, *Spodoptera litura*, *Spodoptera exigua*, *Earias* spp., *Achaea janata*, bunch caterpillars, leaf folders, armyworm, cutworm
Squamocin	*Helicoverpa armigera*, *Helicoverpa zea*, *Spodoptera litura*, *Spodoptera exigua*, bunch caterpillar, green leafhopper, leaf folder, armyworm, cutworm, aphid

14.3.2 Parasitoids

Trichogramma is an exclusive egg parasitoid. It lays eggs in the eggs of various Lepidopteron pests (moths, butterflies). After hatching the larvae feed on the host egg and destroy it. Being an egg parasitoid, it destroys the pest population before it causes any damage to the crops. It is used against sugarcane, paddy, fruits, and vegetable pests.

14.3.3 Entomopathogenic Nematodes

Heterorhabditis is an entomopathogenic nematode used for control of different beetle larvae in soil. It searches the host in the soil, and after active penetration into the larval body through the cuticle, the nematode releases a symbiont pathogenic bacterium (*Photorhabdus*) that multiplies rapidly and kills the host, within 24–72 h. *Heterorhabditis* and *Photorhabdus* then feed upon the insect. Spawned juvenile nematodes then search for new hosts.

14.3.4 Pheromones

These are the biochemical biopesticides. Pheromones are chemical signals that trigger a natural response in another member of the same species. Insects release pheromones to serve many functions. Pheromones are secreted to indicate the location of food sources, to warn others around about potential dangers, or to locate a potential mate for reproduction. Synthetic pheromones can be used to disrupt pest ecology and reduce crop damage. Small amounts of synthetic female pheromone are used to attract males into traps; by measuring trap counts, the data can be used to predict the insect population; and a decision on appropriate pest control measures can be initiated.

14.3.5 Advantages of Biopesticides

1. They are less harmful than chemical fertilizers.
2. They are often effective in small quantities.
3. They give protection throughout the crop period.
4. They multiply easily in soil and leave no residual problem and eliminate the pathogens/pests from the site of infection. The target organisms are only killed/suppressed.
5. They are highly effective against specific diseases/pests and can be used in combination with biofertilizers.
6. They do not cause toxicity to plants and are eco-friendly and easy to handle. They are safe to the environment and the person who applies them.
7. Along with controlling the plant diseases and pests, they can be used as a component in IPM (integrated pest management) and greatly reduce the use of conventional pesticides, while the crop yields remain high.

14.4 Concluding Remarks and Future Prospects

The use of chemical fertilizer and pesticides in agriculture is increasing alarmingly that are causing adverse effect on human health, groundwater quality, and soil fertility. To overcome these adverse effects, there is an urgent need to adopt eco-friendly fertilizers and pesticides. Biofertilizers contain microbial inoculant, which supplies macro- and micronutrients, secretes plant hormones, and increases the soil organic matter, thus restoring the soil fertility. Biopesticides will control the pests without causing any adverse effect to the nontarget pests. The use of biofertilizers in agriculture maintains healthy soils which is a key factor in sustainable agriculture that produces healthy crop plants with optimum vigor and less susceptibility to pests. Availability of quality bioproducts and creating awareness among farmers for using biofertilizers and biopesticides are the key to achieve success in making the agriculture sustainable.

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