11 Microbial Inoculants as Biofertilizers and Biopesticides

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Abstract

Bioinoculants are ecofriendly as they don't have any adverse effect on soil fauna and flora. These bioinoculants can also be used as biopesticides which do not have any residual effect on crop products. But the main problem with the bioinoculants is its quality, as the private agencies which supply various biofertilizers and biopesticides don't care for their quality parameters. The availability of good quality bioinoculants to the farmers is main hurdle in their success. There is lack of co-ordination between the extension workers and scientists. Due attention is needed regarding *Azotobacter*, *Azolla*, *Acetobacter*, *Trichoderma*, *Bacillus thuriengensis*, and *Azospirillum* and their application in various cereal and vegetable crops. These biofertilizers should be integrated with organic manures and chemical fertilizers to enhance the soil organic carbon and maintain sustainability in field and horticultural crops.

Keywords

Biofertilizers • *Azotobacter* • *Azospirillum* • Biopesticides • *Trichoderma* • *Bacillus*

11.1 Introduction

Agricultural productivity in Indian subcontinent has gained encouraging trends during last four decades. High-yielding variety seeds, availability of more water for irrigation, and enhanced use of chemical fertilizers have been the main factors for achieving high productivity. However, the

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pathway adopted by us has been dependent on nonrenewable energy resources, resulting in an exponential increase in the consumption of petroleum products. Urea is the main fertilizer being used across the globe in maximum quantities as compared to any other fertilizer. All the urea-manufacturing units depend upon petroleum products. According to an estimate, the manufacture, transportation, and application of one 1.0 kg urea involve an expenditure of 1.0 l petroleum products. Besides, an excessive use of urea to supplement nitrogen to the soil may render the groundwater polluted. Nitrate pollution in water may cause awful diseases like methemoglobinemia and hypertension among the infants, rendering them handicapped. In other words, excessive use of urea is not only expensive but also unsafe for human health and environment.

In view of sky rocketing population and growing grain demand, the necessity of intensive agriculture is likely to continue. Regular replenishment of plant nutrients to maintain the soil fertility is unavoidable. Consequently, any curtailment in the consumption of urea and other chemical fertilizers would not be feasible. In view of the necessity of intensive agriculture and keeping economy, health, and environment in mind, the need of the hour is to exploit all possible sources of plant nutrients so as to achieve the required productivity through intensive agriculture. Agriculturists suggest that the requirements of plant nutrients can be fulfilled only when the chemical fertilizers are judiciously used along with green manure, organic manure, and biofertilizers.

Biofertilizers are environment friendly, highly efficient, and low-cost agricultural inputs. The use of biofertilizers for various crops is, directly or indirectly, a true service to the soils of nation and the environment. Biofertilizers are mainly concerned with the nitrogen fixation in cereals and legume crops. Hence, to start with the biofertilizers, it is necessary to understand the mechanism of nitrogen fixation, so in the first part of the chapter, various aspects like biochemistry of nitrogen fixation, nodulation, and genetics of nodulation have been dealt with.

11.2 Biological Nitrogen Fixation

In the environment, nitrogen concentration is 78 % by volume; but the plant kingdom is unable to utilize it directly as the plant lacks the enzyme system required to convert N_2 into ammonia. Dinitrogen (N≡N) cannot be utilized as such because of the extremely stable triple-bonded structure of this gas and only certain prokaryotes have the utility to convert N_2 into ammonia with the help of nitrogenase system. Conversion of atmospheric elemental nitrogen into ammonia through a reductive process with the help of microbes is known as biological nitrogen fixation. These microbes include some eubacteria, blue-green algae, and actinomycetes (Table 11.1).

It was first discovered by Beijerinck in 1901 (Wagner 2012). In atmosphere the amount of free nitrogen present accounts to $4x10^{21}$gN out of which around 2.5×10^{11}kg NH_3 is fixed annually by biological means (Schlesinger 1991). In nature, 70 % of total nitrogen is fixed by biological means, the rest by chemical means and traces by physical means. Biological nitrogen fixation (BNF) is divided mainly in three groups: asymbiotic nitrogen fixation or free-living nitrogen fixers, associative nitrogen fixation, and symbiotic nitrogen fixation. The amount of nitrogen fixed by different modes has been shown in Table 11.2.

11.2.1 Asymbiotic Nitrogen Fixation

Free-living nitrogen fixers exist in the rhizosphere zone of plants. They take up carbon exudates from plants as nutrients and in return fix nitrogen under free-living state. *Azotobacter* is

Table 11.1 Nitrogen fixing microorganisms

Free living	Symbiotic
Azotobacter	*Rhizobium*
Azospirillum	*Azorhizobium*
Cyanobacteria	*Frankia*
Bacillus	*Acetobacter*
Clostridium	*Herbaspirillum*
Klebsiella	

Table 11.2 Amount of biological N_2 fixed by different inoculants

State	Aerobic/anaerobic	Bacteria	Amount of N_2 fixed Kg/ha/year
Free living	Anaerobic	*Clostridia*	2–5
	Aerobic	Azotobacter	10–20
	Facultative	*Klebsiella*	5–10
Associative	Legumes	*Rhizobia*	50–500
	Nonlegumes	*Azospirillum*	5–20
		Acetobacter	150
Blue green algae		*Anabaena*	20–25
		Azolla	70–100

the best example of this type which has potential to fix atmospheric nitrogen because it posses more than one type of nitrogenase enzyme. *A. chroococcum* possesses other properties like ammonia excretion (Narula et al. 1991), production of vitamins and growth substances (Shende et al. 1977; Martinez-Toledo et al. 1988), antifungal substances (Sharma et al. 1986), and siderospore production. All these properties favor its performance, increasing the biomass and grain yield of various crops (Lakshminarayana 1993; Goel et al. 1999). Other microorganisms involved in nitrogen fixation are *Clostridium*, *Rhodospirillum*, *Anabaena*, *Klebsiella*, and *Nostoc*.

11.2.2 Associative Nitrogen Fixation

Azospirillum, *Herbaspirillum*, and *Acetobacter diazotrophicus* are associated with the roots of Gramineae family. *Azospirillum* inoculation has sown marked effects on the seedlings of corn, wheat, sorghum, and other grasses. These bacteria can supply 20–25 % of total nitrogen requirements in rice and maize (Saikia and Jain 2007; Montanez et al. 2012). *Herbaspirillum* is beneficial to pearl millet. *Acetobacter diazotrophicus* is found to occur in the root and stem of sugarcane (Cavalcante and Dobereiner 1988; Gillis et al. 1989). It has high ability of nitrogen fixation which can fix up to 150 kg N/ha (Pathak et al. 1997).

11.2.3 Symbiotic Nitrogen Fixation

Rhizobium is the main contributor to the symbiotic nitrogen fixation in legume crops. Moore and Moore (1992) have divided it into four groups. They are fast-growing *Rhizobium*, six species; slow-growing *Bradyrhizobium*, a single species; *B. japonicum* and *Azorhizobium* (stem nodule forming), one species; and *Sinorhizobium*, two species (Table 11.3). On a global basis, of the total 17.2×10^7 tones of biologically fixed nitrogen, about 70–80 % is contributed by rhizobia in symbiosis (Ishizuka 1992). The details of nodulation, biochemistry, and genetics of nitrogen fixation also have been described in the chapter.

Azolla, a small, tree-floating aquatic fern, fixes nitrogen in association with nitrogen-fixing Cyanobacterium, *Anabaena azollae. Azolla* provides the suitable environment and nutrients to *Anabaena* in exchange of the fixed N and certain growth hormones. The heterocyst of symbiotic *Anabaena* is the site of nitrogen fixation. *Azolla* mainly contributes to rice crop by providing nitrogen and adding biomass to the soil.

Frankia, an actinomycete, is capable of forming nodules to actinorhizal plants, alders (*Alnus* sp.). The other genera which can be nodulated by *Frankia* include *Allocasuarina*, *Eleagnus*, *Myrica*, *Gymnostoma*, *Casuarina*, and *Coriaria*. All are monocots which have great future in agroforestry and land reclamation.

Table 11.3 Nodulation host range among legume strains

Group	*Rhizobium* spp.	Host
Rhizobium (fast growing)	*R. meliloti*	Alfalfa
	R. trifolii	Clover
	R. leguminosarum	Pea
	R. phaseoli	Bean
Bradyrhizobium (slow growing)	*B. japonicum*	Soyabean
	B. elkanii	Soyabean
Azorhizobium (fast growing)	*A. Caulinodans*	Sesbania (root and stem nodules)

11.2.4 Nodulation Process of *Rhizobium*

First of all, the legumes secrete root exudates in their vicinity to which host-specific rhizobia are attracted. This is followed by root hair curling and invasion of root hair by host-specific rhizobia. Indole acetic acid (IAA) and lectins are possibly concerned in this process. Following the microbial penetration into the root hair, a hyphae-like infection thread is formed. The bacteria are released into the cortical region of root system. Following the release, a period of rapid cell division takes place in the host cells. The cortical cells into the nodule region become tetraploid. Efficient nodules are pink in color due to the presence of leghemoglobin. The nodules are rounded, lobed, or club shaped depending upon the host. Infection thread branch and distribute themselves over the tetraploid cells. The root nodule results from tissue proliferation induced by the rhizobia via growth hormones. Once liberated from the infection thread, rhizobia assume a peculiar morphology, called bacteriods. These bacteroids proliferate rapidly and are irregularly shaped.

The root nodules formed by the bacteria on legumes fix atmospheric nitrogen and fulfill the nitrogen requirements of leguminous plants. Nodules are formed by an efficient strain of *Rhizobium* to meet the whole nitrogen requirement of the plant, and there is no need to supply nitrogen by other means. The legumes excrete excess amount of organic nitrogen into the soil to nourish the succeeding crop. In return to nitrogen fixation, the bacteria get protection and proper conditions for growth and photosynthate as source of energy. It may be mentioned here that neither the bacterium nor the plant can fix atmospheric nitrogen independently. Nitrogen fixation by different legume crops has been listed in Table 11.4.

Table 11.4 Nitrogen fixation by legumes

System	Nitrogen fixed (kg/ha/year)
Alfalfa	113–297
Red clover	75–171
Pea	72–132
Soybean	57–105
Cowpea	57–117
Vetch	79–140
Sesbania	80–100

11.2.5 Root Nodules in Nonleguminous Plants

Many higher plants, which are not members of leguminosae, also form root nodules with the ability to fix nitrogen. In most cases, these endosymbionts are actinomycetes belonging to genus *Frankia*. The host plant of such actinomycetes includes *Casuarina*, *Albus*, *Myrica*, *Dryas*, etc.

11.2.6 Biochemistry of Nitrogen Fixation

Nodules are generally pink in color because of the presence of an iron containing substance known as leghaemoglobin. Neither the plant nor the bacterium is individually capable of leghaemoglobin synthesis. The apoprotein globin is encoded by a plant gene, and the synthesis of heme moiety is under the control of bacterial genes. Throughout the period during which the bacteriods persist, they actively fix atmospheric nitrogen. The reductant and ATP necessary for nitrogen reduction are derived from photosynthates provided by plants. The fixed nitrogen is excreted from the nodules to the plant vascular system as ammonia. About 15–20 mol of ATP are hydrolased per mole of ammonia fixed. It is provided by aerobic respiration within the bacteriods. Ammonia formed reacts with α-ketoglutarate to form glutamate which may be further converted into glutamine. Similarly, aspartate combines with ammonia to form asparagine. Various other products which are synthesized include glutamine, aspartate, and ureides like allantoin and allantoic acid, subsequently transported to plant tissues. Various steps involved in the nitrogen fixation have been mentioned in Fig. 11.1.

The most important plant bacterial interaction is that between legume plants and bacteria of the genera *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium. Azorhizobium* forms stem nodules. In the nodules, precise oxygen levels are controlled by the oxygen-binding protein leghaemoglobin which functions as an oxygen buffer cycling between the oxidized ferric ions and reduced ferrous ions. These forms keep free oxygen levels within the nodule at a low but constant level. The ratio of free leghaemoglobin to bound form to oxygen in the root nodule is in the order of 10000:1.

Bacteriods are totally dependent on plants for supplying them energy sources for nitrogen fixation. The major organic compounds transported across the peri-bacterial membrane are citric acid cycle intermediates, in particular the C_4 acids succinate, malate, and fumarate. They are used as electron donors for ATP production and are converted into pyruvate. Ammonia is transported from bacteroid to plant cell and is assimilated to glutamine by glutamine synthetase enzyme by the plant and subsequently transported to plant tissue.

11.2.7 Genetics of Nodule Formation: *Nod* Genes

Nodulation in legumes by host-specific rhizobia is directed by a number of genes which are called *nod* genes. These are highly conserved and localized on large plasmid called *sym plasmid*. Cross-inoculation group specificity is controlled by *nod* genes. The *nod ABC* genes are common to all spe-

$$\text{Glutamate+ATP+}NH_3 \xrightarrow[\text{Glutamine synthetase(GS)}]{} \text{glutamine+ADP+Pi}$$

$$\text{Glutamine+}\alpha\text{–ketoglutarate+NAD(P)H} \rightleftarrows \text{2 glutamate+NAD(P)}^+ \quad \text{Glutamate Synthase (GOGAT)}$$

$$\alpha\text{–ketoglutarate+}NH_3\text{+NAD(P)H+ATP} \xrightarrow{\text{GS/GOGAT or glutamate dehydrogenase}} \text{Glutamate+NAD(P)}^+\text{+ADP+Pi}$$

$$N{\equiv}N \xrightarrow[2H]{} HN{=}NH \xrightarrow[2H]{} H_2N\text{-}NH_2 \xrightarrow[2H]{} 2NH_3$$

Overall reaction

$$8H^+ + 8e^- + N_2 \longrightarrow 2NH_3 + H_2$$

$$\text{18-24 ATP} \longrightarrow \text{18-24 (ADP+Pi)}$$

Fig. 11.1 Biochemistry of nitrogen fixation cies of *Rhizobium* and are involved in the production of chitin-like molecules, called nod factors, which induce root hair curling and trigger cortical plant cell division. Nod factors consist of a backbone of N-acetyl-glucosamine to which various substituent are linked. *Nif* genes complex regulate the nitrogenase enzyme synthesis (Fig. 11.2).

11.2.8 Nitrogenase

In the fixation process, nitrogen is reduced to ammonia and ammonia is converted to organic form. The reduction process is catalyzed by the enzyme complex called nitrogenase, which consist of two separate proteins called dinitrogenase and dinitrogenase reductase. Dinitrogenase is the Mo-Fe protein, while dinitrogenase reductase is Fe protein. Some nitrogen-fixing bacteria can synthesize nitrogenase that lack molybdenum but contain vanadium.

11.3 What Are Biofertilizers?

All the microorganisms which add or make available different nutrients to the plants are called biofertilizers. These biofertilizers differ from the chemical fertilizers as the chemical fertilizers are manufactured in the factories and are direct source of nutrients, while the biofertilizers are the living or latent form of microorganisms which either mobilize different elements fixed in the soil or add nutrients from the environment to the soil. They also provide plant growth hormones and induce the plant protection mechanism and thus help them from plant pathogens. These biofertilizers improve the soil fertility by fixing atmospheric nitrogen, mineralization of various elements like phosphorus, sulfur, zinc, potash, and iron. These biofertilizers are also known as inoculants which are produced either on small scale under laboratory conditions or on large scale by batch fermentation (Hilda and Fraga 2000). The use of inoculants is ecofriendly and is not harmful to the environment (Rodríguez and Fraga 1999). Biofertilizers may be applied to the soil through seeds, roots, or directly to soil where microbes multiply and mobilize the inert nutrients. Commonly used biofertilizers which are made available to farmers by the government, semi-government, or private agencies have been mentioned in Table 11.5. The media used for commercialized production of bioinoculants is listed in Table 11.6.

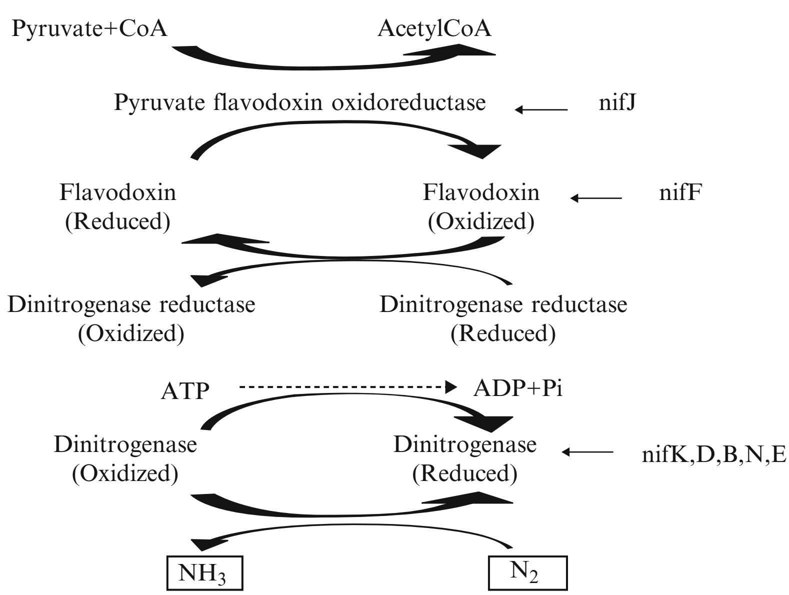

Fig. 11.2 Genetics of nitrogen fixation

Table 11.5 Types of biofertilizers commonly used

Sr No	Biofertilizers	Character specification requirement
a.	*Rhizobium*	Should show effective nodulation on all the species listed on the packet
b.	*Azotobacter*	The strain should be capable of fixing at least 10 mg of nitrogen per g of sucrose consumed
c.	*Azospirillum*	Formation of white pellicle in semisolid N-free bromothymol blue media
d.	PSB	The strain should have phosphate-solubilizing capacity in the range of minimum 30 %, when tested spectrophotometrically. In terms of zone formation, minimum 5 mm solubilization zone in prescribed media having at least 3 mm thickness

Table 11.6 Media for large-scale production

Bacteria	*Media*	*C- source*
Rhizobium	*YEMA*	*Mannitol or molasses, sugar, and glycerol*
Azotobacter	*Jenson*	*Sucrose or mannitol*
Azospirillum	*Malate or Okon's*	*Malate as C-source + yeast extract as vitamin source*
PSB	*Pikovaskaya's*	*Glucose as C-source + tricalcium phosphate*

11.3.1 Types of Biofertilizers/ Biopesticides

Biofertilizers/biopesticides can be generally categorized into four types

(a) Nitrogen supplementing
(b) Phosphate solubilizing
(c) Composting microorganisms
(d) Biopesticides/PGPRs

(a) *Nitrogen-supplementing microorganisms*

These microorganisms have the capability of fixing atmospheric nitrogen which is 78 % of the atmosphere. Most of the plants can utilize nitrogen only in the form of nitrate; hence, unless the nitrogen gas is converted to nitrate, it remains unavailable for plants. Certain microorganisms absorb nitrogen gas as their feed and convert it into ammonia through the activity of an enzyme called nitrogenase. Ammonia is converted into nitrate by nitrification or directly assimilated into the plant system.

11.3.1.1 *Rhizobium*

This bacterium fixes atmospheric nitrogen in the symbiotic association with the leguminous crops. *Rhizobium* enters the root system after germination of seeds and nodules are developed on the roots. These nodules inhabit rhizobia, which fix atmospheric nitrogen and keep supplying ammonia to the plant. Rhizobia are host specific as they form nodules and fix nitrogen on specific hosts. Hence, while procuring *Rhizobium* culture, it should be taken care of that name of the pulse crop should be mentioned on the culture for which it is used.

Benefited crops: Soybean, groundnut, berseem, sesbania, and all other pulse crops

Selection Criteria for ***Rhizobium*** **and** ***Bradyrhizobium***

- Host specificity
- Nitrogen fixation potential
- Adaptation in different environments and soil conditions
- Competance with native *Rhizobium*
- Production of siderophores, auxins, vitamins, and other PGPS
- Production of bacteriocins and other secondary metabolites

11.3.1.2 *Azotobacter*

These bacteria fix atmospheric nitrogen in free-living conditions. They multiply in the vicinity of the root system and convert atmospheric nitrogen to ammonia. Plants assimilate the fixed nitrogen. The capability of fixing nitrogen in free-living conditions accredited to *Azotobacter* as a versatile biofertilizer which can be successfully utilized against a broad range of crops belonging to different groups for supplementing chemical nitrogen. In addition to fixing nitrogen, they also produce plant growth-regulating substances in the vicinity of the plant.

Benefited crops: Wheat, maize, sorghum, pearl millet, mustard, sunflower, cotton, fruits, and flowers yielding crops, tea, coffee, vegetables, etc.

Selection Criteria for *Azotobacter* and *Azospirillum*

- Fix higher amount of N/g of C substrate in growth medium
- Excretion of ammonia
- Faster growth rates, survival, and competence in soil environment
- Tolerance of wider pH and temperature range
- Antibiosis and phosphate dissolving ability

11.3.1.3 *Acetobacter*

Similar to *Azotobacter*, this bacteria also multiply in the soil and fix nitrogen in aerial as well as underground parts of the plant. Most common sp. is *A. diazotrophicus* fixing nitrogen in sugarcane. Various field studies revealed that *Acetobacter* works more efficiently for sugar-yielding crops like sugarcane and sugar beet. It has been estimated that approximately one-fourth of total nitrogen requirement of sugar-yielding crops can be fulfilled by these bacteria. These bacteria are endosymbiont as they remain within the plant.

Benefited crops: Sugarcane, sugar beet, and pearl millet

(b) *Phosphate solubilizing microorganisms (PSM)*

Phosphate is the second most important plant nutrient. In general, chemical phosphatic fertilizers are used to supplement phosphates to the soil. Experiments have proved that 30–35 % of phosphatic fertilizers applied are actually utilized by the plants, while the remaining 65–70 % of chemical phosphatic fertilizer change to insoluble state and become unavailable to the plants. Certain microorganisms have the capability of resolubilizing this insoluble phosphate, making it available to the plants. PSM is a balanced blend of certain efficient phosphate-solubilizing microorganisms which work under diverse geographical conditions (Table 11.7). Since PSM has the capability of working in various types of soils under free-living conditions, this biofertilizer can be utilized against all the crops with equal efficiency. *Aspergillus* sp., soilborne fungi, is serving as an important phosphate solubilizer of the soil (Arcand and Schneider 2006). These fungi are capable of solubilizing both organic and rock phosphates; co-inoculation of these fungi will enhance the availability of phosphates to plants and in turn will reduce the requirement of synthetic fertilizers. *Aspergillus niger* also serves as phosphate-solubilizing fungi as it causes production of various organic acids like citric, gluconic, succinic, and oxalic acids and thus helps in pH drop (Nahas et al. 1990). Other than fungus, some bacteria are also involved in phosphate solubilization which are known as phosphate-solubilizing bacteria (PSB) or phosphotika, e.g., *Bacillus*, *Pseudomonas*.

Selection Criteria for Phosphate Solubilizers

- Ability to solubilize insoluble rock phosphate and tricalcium phosphate in liquid medium
- Production of organic acids, e.g., mono-, di-, tri-carbonic acids and gluconic acid

(c) *Composting microorganisms*

The use of compost and farm yard manure to replenish the nutrients in the soil is prevailing since ancient times. Dead leaves, plant parts, and other agricultural trash have got sufficient plant nutrients, but these are unavailable to crop plants

Table 11.7 Phosphate solubilizers

Bacteria and fungi	Mycorrhizal fungi	
Produces acidic metabolites	Endo	Ecto
Caused chelation of metal cation	*Mucor*	*Aminita*
Change the soil pH	*Glomus*	*Boletus*
Phosphate ion is released in soluble form		
Bacteria – *Bacillus, Pseudomonas*		
Fungi – *Aspergillus, Penicillin*		

unless their complex form is changed to simpler form through microbial decomposition. This process of decomposition is known as composting and involves specific microorganisms[1]. Composting microorganisms are available in the atmosphere and continue decomposing the dead organic matter. In case the population of efficient composing microorganisms is increased over the heap of agriculture waste, the process of composting becomes faster, and a good quality compost or organic manure is prepared in merely one-fourth time as compared to natural composting. The organic manure so obtained carries almost all the required plant nutrients in balanced quantities. The organic manure preparation can be fastened by the use of *Trichoderma*, *Penicillium*, and *Aspergillus*.

11.3.1.4 Urea-Coating Agents (UCA)

Nitrogen deficiency in soil is generally replenished by application of urea, but approximately 30 % is actually utilized by the plants, while the remaining 70 % either leaches down to groundwater or volatilized back to atmosphere. Immediately, after its application, urea tends to break into nitrates. This process is known as nitrification, which is much quicker than the nitrate assimilation by the plants. Consequently about 70 % of urea goes to waste and causes pollution. The mode of application of biofertilizers affects their quantity used as given in Tables 11.8 and 11.9.

(d) *Biopesticide/PGPRs*

We are aware of the losses due to certain fungal diseases in various crops. Generally, chemical fungicides are used to combat the fungal diseases. These poisonous chemicals persist in the environment for a long time and impose a slow but harmful effect on living beings and ultimately on human health. Biopesticides include bacteria, fungi, and plant viruses.

11.3.1.5 Bacteria

The bacteria which promote plant growth either by production of plant growth hormones or due to induction of plant protection mechanism have been designated as plant growth-promoting rhizobacteria (PGPR) by Kloepper et al. (1980). Various bacteria which have been identified as PGPRs in recent years include *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus*, and *Serratia* (Kloepper et al. 1989; Okon and Labandera-Gonzalez 1994; Glick 1995; Joseph et al. 2007). These bacteria have been commercialized by the production of their inoculants. They promote plant growth by different mechanisms that include suppression of plant disease (biopesticides), biofertilizers, or phytohormone production (biostimulants). The biopesticides protect the plant system by different mechanisms: induction of systemic (ISR), resistance synthesis of antibiotics, and production of siderophores. The microorganisms which produce siderophores chelate iron, thus making it unavailable to plant pathogen and thus suppress growth of plant pathogen. Induced systemic resistance is effective against a broad spectrum of plant pathogen (Pieterse et al. 2003).

Different strains of *Pseudomonas* serve as effective PGPRs due to their wide range of properties, viz., production of phytohormones (Timmusk et al. 1999; Verma et al. 2001; Bottini et al. 2004; Spaepen et al. 2008); phosphate solubilization (Vyas and Gulati 2009); sideropores production; production of antibiotics like 2,4-diacetylphoroglucinol (2,4-DAPG), phenazines, pyrrolnitrin, pyoluteorin, and surface-active antibiotics; and production of hydrogen cyanide (HCN) (Raaijmakers et al. 2002) and lytic enzymes like chitinases and proteoses (Haas and Defago 2005; Yadav et al. 2007). *Pseudomonas* also produces enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase which regulates ethylene level in plants helpful in protection from plant pathogens (Glick et al. 1998; Penrose and Glick 2003).

The soil bacterium, *Bacillus thuringiensis* (*Bt*), is currently being used worldwide, mainly for management of lepidopterous, coleopterous, and dipterous pests. The insecticidal activity of Bt is primarily due to the presence of proteinaceous crystals (delta endotoxins) pro-

[1] Composting culture –1 kg for 2–3 metric ton of agricultural waste.

Table 11.8 Doses of various biofertilizers for different crops

Target crops	Seed treatment (g/kg)		Soil application (kg/ha)	
	Trichoderma	*Acetobacter*	*Trichoderma*	*Acetobacter*
All pulses crops soybean, groundnut, mung, urd, lentil, pea, gram, etc.	4–5	–	2.5	–
A. Cereals, millets oilseed, wheat, jowar, bajra, mustard, and sunflower etc.	4–5	–	2.5	–
B. Cash crops (sugarcane, potato) vegetables and fruit crops	4–5	–	2.5	–
Sugarcane and sweet potato	–	2.5 kg	2.5 kg	2.5 kg

Table 11.9 Doses of various biofertilizers for different crops

Target crops	Seed treatment			Soil application		
	Rhizobium	*Azotobacter*	PSB	*Rhizobium*	*Azotobacter*	PSB
All pulses crops like soybean, groundnut mung, urd, lentil, pea gram, etc.	50 ml/10 kg seed	–	50 ml/10 kg seed	1.5 l	–	
A. Cereals, millets oilseed, wheat, jowar, bajra, mustard, and sunflower etc.	–	50 ml/10 kg seed For large seed crop and 50 ml per acre for small seed crop	Do	–	2 l liquid culture	2 l liquid culture
B. Cash crops {sugarcane, potato, vegetables, and fruit crops}	–	1.5 l	1.5 l liquid culture	–	2 l	3 l

Urea-coating agent (UCA)[b] is a balanced blend of certain herbs and minerals which inhibits the process of nitrification, resulting in slow release and more assimilation of urea by the plants. It is estimated that 40–50 % saving of urea can be achieved by coating the urea granules before application[b] UCA –1 kg/50 kg urea bag

duced during stationary and sporulating phases. In commercial production, the spores and crystals obtained from fermentation broth are concentrated and formulated variously. Upon ingestion of spores, crystals dissolve in the alkaline pH of the midgut larvae and protoxins of size 120–135 Kda are released which are further acted upon by the midgut proteolytic enzymes, and toxin fragments of size 60–70 Kda are released. These toxin fragments negotiate the receptors found in the columnar epithelial cells and cause pore formation, resulting in osmotic imbalance and eventually death of the insects.

11.3.1.6 Mode of Action of PGPRs

- Production of auxins
- Production of vitamins
- Production of siderophores
- Production of antibiotic substances
- Promoting plant defense mechanism by inducing flavonoids and phytoalexins

- *Acetobacter, Arthrobacter, Alcaligenes, Azospirillum, Bacillus, Pseudomonas, Flavobacterium*

11.3.1.7 Fungi

Fungi play an important role in the recycling of organic matter. These include nonpathogenic soil inhibiting saprophytes. They degrade cellulose, lignin, and hemicelluloses and thus mineralize the organic matter and help in soil aggregation. They also solubilize organic phosphates, e.g., *Alternaria*, *Aspergillus*, *Cladosporium*, *Dematium*, *Gliocladium*, *Helminthosporium*, *Humicola*, and *Metarhizium*. Some fungi promote plant growth by root colonization and are designated as plant growth-promoting fungi (PGPF). These include mycorrhiza (endomycorrhiza and ectomycorrhiza). Mycorrhiza increases the surface area of plant root system and thus helps in absorption of minerals, solubilization of phosphorus, and conversion of moisture. Due to abovementioned properties, it has been commercialized as inoculants.

Over 400 species of fungi infect insects and mites. *Deuteromycetes* and *Phycomycetes* contain most of the useful species for insect control. The entomopathogenic fungi have relatively broad host range and are amenable for mass production. The fungi penetrate through the insect cuticle and sporulate on the dried insects, which provide the way for epizootics. However, fungi are fairly fastidious with respect to humidity and temperature. In order to make effective use of a fungus, applying it at the right time and optimum amount is important for the successful management of insect pest on crops.

Trichoderma is a specific fungus having characteristic capability of inhibiting the growth of a broad range of pathogenic fungal species. Due to being biological, this bio-fungicide has got no adverse effect on the environment. Application of *Trichoderma* is known to prevent various diseases like stem and root rot, damping off, wilt, blight, and other diseases of leaves.

11.3.1.8 Viruses

Many of the commercial bioinsecticides are based on nuclear polyhedrosis viruses (NPVs) and to a lesser extent of granulosis viruses (GVS) and non-occluded viruses (NOVs). These viruses are highly host specific and safer to nontarget organisms including humans.

Upon ingestion of the viral particles, the polyhedron dissolves in the alkaline pH of the midgut, releasing virions. The virions enter the columnar epithelial cells through endocytosis and cause primary infection. Here the secondary infection takes place, ultimately causing death of the insect.

11.4 Constraints in Popularization of Biofertilizer Technology

- The quality of inoculants
- The lack of knowledge about the inoculation technology for the extension personnel and the farmers
- Ineffective inoculant delivery system
- Nonavailability of formulations to the farmers

11.5 Conclusion

The indiscriminate use of chemical fertilizers and pesticides has caused serious damage to the ecosystem; hence, it becomes imperative to turn to more ecofriendly methods of pest and nutrient management. Biofertilizers and biopesticides which are microbial in origin can become viable alternative to sustainable agriculture, although biofertilizers can't complement to chemical fertilizers but can become supplementary to them for maintaining soil health and crop productivity. Therefore, development of newer ecofriendly technology for pest and nutrient management is need of the hour. It is equally important to maintain the quality of biofertilizers and biopesticides. Timely delivery of these organic amendments and awareness to the farmers will help in the improvement of quality and quantity of food products. Biofertilizers and biopesticides are our tools to achieve the goals of not only higher yield but also a cleaner environment. Hence, an integrated approach of scientists and extension workers should be followed for their success.

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