

## Review Article

# Sustainable Agriculture: Biofertilizers withstanding Environmental Stress

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**Received:** 01 November 2020; **Accepted:** 14 November 2020; **Published:** 25 November 2020

**Citation:** Sunita Kumari Singh. Sustainable Agriculture: Biofertilizers withstanding Environmental Stress. International Journal of Plant, Animal and Environmental Sciences 10 (2020): 158-178.

### Abstract

World's mounting population food demand is totally dependent on the chemical fertilizers which destroy the environment and negatively influencing the health of humans. The useful microorganisms inhabit the plant systems and plays substantial role in nutrient uptake from the ecosystems of plant. Biofertilizers augment the plant development by various direct and indirect plant growth promoting mechanisms such as biological nitrogen fixation, production of several plant growth hormones, siderophores, innumerable hydrolytic enzymes and solubilization and mobilization of potassium, zinc, and phosphorus, therefore, they are the finest substitute of chemical fertilizers as they are eco-friendly for plant growth and soil fertility. Despite these utilities, biofertilizers itself are subjected to the extreme environmental conditions and they developed different mechanisms to cope up with them. Extensive work on the biofertilizers has been done, which divulges that

these microorganisms have the potential of providing the vital nutrients to the crops in optimum amount for the amelioration of harvest without distressing the ecosystem.

**Keywords:** Biofertilizers; Biological nitrogen fixation; Plant growth hormones; Hydrolytic enzymes; Soil fertility

### 1. Introduction

Nowadays increasing population lead to increasing demand of food supply consequently depletion of soil, which is replenished with chemical fertilizers leads to the biomagnifications of chemicals in the food chain, although, biofertilizers retain the soil fertility without any harmful effects. Biofertilizers are microorganisms that accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in

a form easily assimilated by plants and help plants uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil. In other words, biofertilizers are living microorganisms which when applied to seed, plant surfaces, or soil colonizes the rhizosphere or the interior of the plant and promotes growth by escalating the accessibility of primary nutrients to the host plant [1]. According to Mishra et al. [2], biofertilizer is a mixture of live or latent cells encouraging nitrogen fixing, phosphate solubilizing etc. inoculated to soil, seed, roots, or composting areas with the purpose of increasing the quantity of mutualistic beneficial microorganisms and accelerating their microbial processes, which enhance the availability of nutrients that can then be easily assimilated and absorbed by the plants. They include nitrogen fixers, potassium and phosphorus solubilizers, growth promoting rhizobacteria (PGPRs), endo and ecto mycorrhizal fungi, cyanobacteria and other useful microorganisms [3, 4] and establish a vivacious module of sustainable agriculture, playing crucial role in sustaining the well-being of the plants against pathogens as well as supporting the growth by making the accessibility of several nutrients, phytohormones and remain viable simultaneously enhancing the productivity as well as the fertility of the soil [5] (Table 1). Biofertilizers can be applied to crops by seed treatment, seedling root dip, soil treatment etc. *Rhizobium* with phosphotika as seed treatment are recommended for pulses such as pigeon pea, green gram, black gram, cowpea etc. groundnut and soybean, similarly, *Azotobacter* with phosphotika as seed treatment are useful for wheat, sorghum, maize, cotton, mustard etc. and for transplanted rice, the recommendation is to dip the roots of seedlings in a solution of *Azospirillum* with phosphotika. Cortivo et al. [6] suggested that seed-applied biofertilizers may be effectually exploited in sustainable wheat cultivation without altering the biodiversity of the inhabitant

microbiome. They not only fix atmospheric nitrogen in the soil and make it available to the plant but also solubilize the insoluble forms of phosphates like tricalcium, iron and aluminium phosphates into available forms, scavenge phosphate from soil layers, produce hormones and anti-metabolites which promote root growth, decompose organic matter and help in mineralization in soil and increase the availability of nutrients and improve the yield by 10 to 25% without unfavorably affecting the soil environment. In tropical countries, rice production mainly depends on biofertilizers [7]. Latent cells of competent strains of  $N_2$ -fixing, phosphate solubilizing or mobilizing microorganisms are used for application to seed or soil with the intent to increase the number of such microorganisms and hasten those microbial processes which enhance the accessibility of nutrients that can be assimilated by plants [8]. They improve the soil structure, restores soil nutrient, build soil organic matter, water uptake, plant growth and plant tolerance to abiotic and biotic factors [9] (Figure 1). These potential microbes would play a key role in productivity and sustainability of soil and also in protecting the environment as eco-friendly and cost-effective inputs for the farmers [3, 10]. Biopesticides (bacteria that promote plant growth by control of lethal organisms) and bioenhancers (bacteria can augment plant growth by producing phytohormones) should not be included in biofertilizers [11]. Green revolution brought striking increase in food production but with unsatisfactory concern for sustainability. Dependence on chemical fertilizers for forthcoming farming intensification would mean more loss in soil quality, water contamination and unsustainable burden on the economic system [12]. Incorporated use of mineral fertilizers, organic manures, biofertilizers, etc. is the only alternate for improving soil fertility [13]. Organic farming ensures food safety, adds to the biodiversity of soil [14] and additional advantages include longer shelf life causing no adverse effects to

ecosystem [15]. Sharma and Upadhyay [16] described the present status of supply demand, marketing strategies, networks and governmental interventions in the pricing policies of biofertilizers in India. The international market for biofertilizers was predictable to be value about five billion USD in 2011 and is doubled in 2017, actively in Latin America, India and China [17-19]. Progress of quality standards of manufacture and a legal framework that guarantees both manufacturers and farmers are needed to maintain such prospective fiscal

improvement [20]. Out of total applied fertilizer, only 30-50% of applied nitrogen fertilizers and 10-45% of phosphate fertilizers are taken up by crops [21, 22]. Several biotic, abiotic and anthropogenic factors cause challenges in booming application of commercial biofertilizer [20]. The 50-60% nitrogen requisite is fulfilled through the amalgamation of mineralization of soil organic nitrogen and biological nitrogen fixation by free living and rice plant associated bacteria [23].

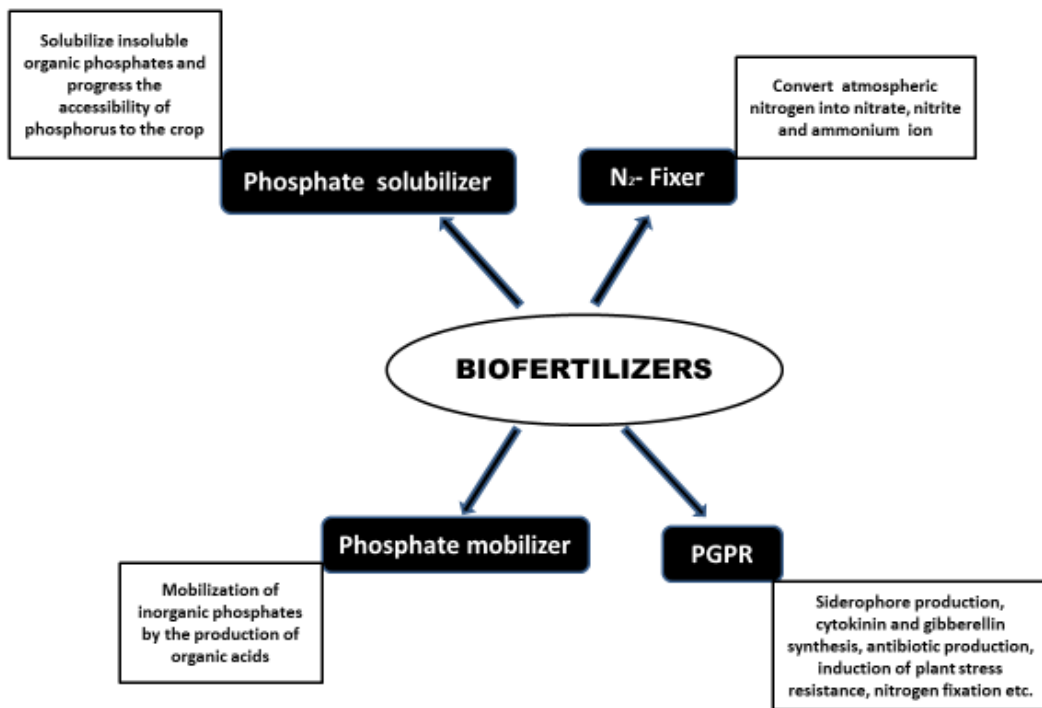


Figure 1: Role of biofertilizers in promoting plants growth by innumerable approaches.

Roles of biofertilizers	Plants affected	Microbes	References
Increases the photosynthetic rate	Rice	<i>Rhizobia</i>	Mia and Shamsuddin [24]
	Basil	<i>Pseudomonas</i> sp., <i>Bacillus lentus</i> , and <i>Azospirillum brasilense</i> <i>Pseudomonas</i> sp., <i>Bacillus lentus</i> , and <i>Azospirillum brasilense</i>	Heidari and Golpayegani [25]
	Potato	<i>Bacillus</i> sp.	Gururani et al. [26]
	<i>Arabidopsis thaliana</i>	<i>Azospirillum brasilense</i> sp 245 strain	Cohen et al. [27]
Bioremediation	-	<i>Achromobacter</i> , <i>Azotobacter</i> , <i>Bacillus</i> , <i>Bradyrhizobium</i> , <i>Brevibacillus</i> , <i>Kluyvera</i> , <i>Mesorhizobium</i> , <i>Ochrobactrum</i> , <i>Pseudomonas</i> , <i>Psycrobacter</i> , <i>Ralstonia</i> , <i>Rhizobium</i> , <i>Sinorhizobium</i> , <i>Variovox</i> , and <i>Xanthomonas</i>	Shinwari et al. [28]
Pesticides remediation	-	<i>Azospirillum</i> , <i>Azotobacter</i> , <i>Bacillus</i> , <i>Enterobacter</i> , <i>Gordonia</i> , <i>Klebsiella</i> , <i>Paenibacillus</i> , <i>Pseudomonas</i> and <i>Serratia</i> ,	Shaheen and Sundari [29]
To overcome abiotic stresses	-	<i>Pseudomonas alcaligenes</i> , <i>P. aurantiaca</i> , <i>P. aureofaciens</i> and <i>P. chlororaphis</i>	Verma et al. [30], Yadav et al. [31]
	<i>Platyclusus orientalis</i>	<i>Bacillus subtilis</i>	Liu et al. [32]

**Table 1:** Plausible biotechnological protagonist of biofertilizers [5].

## 2. Nitrogen fixing biofertilizers

Nitrogen is one of the primary macronutrients which is essential for the formation of base pair for DNA, RNA, phosphate group of protein and hormones such as cytokines, metal uptake, transport in xylem and phloem, as osmoregulator, alkaloids etc. [33], which is absorbed by plants in form of nitrates, ammonium and sometimes urea [34] and is added to the soil through fertilizer, biological nitrogen fixation, rainfall and thunder, and decomposition of organic matter [3]. Symbiotic and free-living eubacteria, including cyanobacteria, are two groups of nitrogen-fixing organisms. Heterocystous cyanobacteria such as *Nostoc*, *Anabaena*, *Nodularia*, *Scytonema*, *Cylindrospermum*, *Mastigocladus*, *Calothrix*, *Anabaenopsis*, *Aulosira*, *Tolypothrix*, *Haplosiphon*, *Stigonema*, *Fischerella*, *Gloeotrichia*, *Rivularia*, *Nostochopsis*, *Westiellopsis*, *Westiella*, *Chlorogloea*, etc. and non-heterocystous cyanobacteria such as *Lyngbya*, *Oscillatoria*, *Schizothrix*, *Plectonema*, *Trichodesmium* etc. are proficient nitrogen fixers [35, 36]. The free-living cyanobacteria fix more than 10 kg of nitrogen per hectare per year; however, dense mats of cyanobacteria fix annually approx. 10-30 kg of nitrogen per hectare [37] as well as fix about 20-30 kg nitrogen per hectare along with organic matter to the paddy fields [38]. It also makes symbiotic associations with different photosynthetic and non-photosynthetic organisms such as algae, fungi, diatoms, bryophytes, hornworts, liverworts, mosses, pteridophytes, gymnosperms, and angiosperms [39, 40]. Successful growth and survival of cyanobacteria in the nitrogen deprived habitats due to its nitrogen fixing ability makes them agronomically and economically significant as biofertilizers [7, 41]. According to Dubey and Rai [42] *Anabaena fertilissima* and *A. doliolum* quantitatively and qualitatively increases the yield of rice without chemical fertilizer. Wheat crops are also benefitted by cyanobacterial biofertilizers [43, 44]. It fixes approx. 200 Mt of nitrogen annually [45]. Abdel-Raouf et al. [46]

suggested that the humus content generated after death and decay of cyanobacteria, improves the soil structure and fertility.

Arbuscular mycorrhiza (AM) can take up nitrogen both as inorganic (either ammonium or nitrate) and organic form [47], which is important under arid and semi-arid environment, where water availability confines uptake of inorganic nitrogen [48]. Strains of *Azotobacter*, *Azospirillum*, *Phosphobacter* and *Rhizobacter*, add significant amount of nitrogen to *Helianthus annuus* and also increases the plant height, number of leaves, stem diameter percentage of seed filling and seed dry weight [49], likewise, these microorganisms promote the physiology and improve the root morphology of rice plants [50]. *Azolla anabaenae* is also one of the important biofertilizer in rice fields as *Anabaena* lives in symbiosis with *Azolla*, a small free-floating fresh water fern, fixes nitrogen in rice field in the range of 30-40 up to 70-110 kg N ha<sup>-1</sup> [51]. The important factor in using *Azolla* as biofertilizers for paddy crop is its rapid decomposition in the soil and efficient accessibility of its nitrogen to rice plants. It has symbiotic relation with *Anabaena* and can help rice or other crops through dual cropping or green manuring of soil [52]. *Rhizobium* is one of the important symbiotic nitrogen fixing bacteria while *Azospirillum*, *Azotobacter*, *Clostridium*, *Frankia* etc. are asymbiotic nitrogen fixing bacteria. Rhizobia play a very important role in agriculture by inducing nitrogen fixings nodules on the root of legumes such as peas, beans, clove and alfalfa. They fix up to 90% of the nitrogen requirements of the host [53], but they can also act as plant growth promoting rhizobacteria (PGPR) with non-legumes such as maize, wheat, rice, and canola [54, 55]. *Rhizobium* not only fixes atmospheric nitrogen in symbiotic association with legumes but also with certain non-legumes like *Parasponia* [56]. *Rhizobium* sp. is crop specific as *R. trifoli*, *R. meliloti*, *R. phaseoli*, *R. japonicum*, *R. leguminosorum* and *R.*

lupine for berseem, leucerne, gram, soybean, pea and chickpea respectively [12]. In India, approx. 30 million hectares of land is under pulses (chickpea, red-gram, pea, lentil, black gram etc.), oil-seed legumes (soybean and groundnut) and forage legumes (berseem and lucerne) cultivation which fix 50-100 kg/ha nitrogen by the family Rhizobiaceae which colonizes the roots of specific legumes to form root nodules and produces ammonia.

*Azotobacter* is a non-symbiotic free-living aerobic bacterium which can fix nitrogen approx. 25 kg/ ha under optimum conditions and increase yield up to 50% and they also improve seed germination and plant growth by producing vitamin B, NAA, GA and plant hormones that are inhibitory to certain root pathogens [57]. This biofertilizer have been successfully used in *Triticum aestivum*, *Zea mays*, *Gossypium arboretum*, *Pennisetum glaucum* and *Oryza sativa*. It also protects the roots from other pathogens present in the soil. Similarly, *Azospirillum* (*Bacillus polymixa*) fixes approx 20-40 kg/ha nitrogen, secretes growth promoters as IAA, gibberellins and cytokinins and vicissitudes root morphology, which eventually augments the growth of plant [58]. *Azospirillum* could aid in existence of the plants under distressing conditions by persuading changes in pliability of cell wall, and osmotic regulations [59]. Dipping the roots of rice seedlings in 2% suspension of *Azospirillum* inoculant increased the yield by 100 kg/ ha [60]. *Herbospirillum* lives in symbiotic association with the roots of sugarcane and fixes atmospheric nitrogen [8]. *Acetobacter* can fix approx. 15 kg/ha/year nitrogen as they live endophytically in sugarcane ecosystem [12]. Strains of *Azospirillum* are vended as biofertilizers in different countries as Africa, Argentina, Australia, Belgium, Brazil, Germany, France, India, Italy, Mexico, Pakistan, Uruguay and USA [61].

### 3. Phosphate solubilizing-mobilizing biofertilizer

Phosphorus is needed in relatively large amounts, but in lower quantity as compared to nitrogen and potassium. It promotes legume growth, yield, nodule number and nodule mass. It is important for phospholipids in membrane, phosphor proteins for life functions, improvement of crop yield and quality. Analysis has shown that it forms a substantial component of seeds and fruits [3]. Phosphate either in native or in inorganic form becomes mostly unavailable to crops because of its low levels of mobility and solubility and its propensity to become fixed in soil. The key mechanism for phosphorus solubilization is the lowering of soil pH due to organic acids production by microbes [62-68] and mineralisation by the production of phytase by fungi (*Aspergillus candidus*, *A. fumigatus*, *A. niger*, *A. parasiticus*, *A. rugulosus*, *A. terreus*, *Penicillium rubrum*, *P. simplicissimum*, *Pseudeurotium zonatum*, *Trichoderma harzianum*, and *T. viride*) [62, 63, 67, 69-73]. PSBs enhance plant development by producing phytohormones, such as auxins, gibberellins, cytokinins, or polyamides [68, 73-75]. PSB includes bacteria (*Bacillus megaterium*, *B. circulans*, *Pseudomonas striata*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aereobacter*, *Flavobacterium*, *Erwinia* etc), fungi (*Penicillium sp.*, *Aspergillus awamori*, *Trichoderma viridae* etc) and mycorrhiza which solubilize insoluble inorganic phosphate compounds, such as tri-calcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate. In addition to bacteria and fungi, some cyanobacteria also solubilize the insoluble organic phosphates and progress the accessibility of phosphorus to the crop [76, 77]. *Aspergillus fumigates* and *A. niger* convert cassava wastes to phosphate biofertilizers [78]. *Burkholderia vietnamiensis*, a stress tolerant bacterium, secretes gluconic and 2-ketogluconic acids, which solubilises phosphate [79]. Arbuscular mycorrhiza

enhances the plant phosphorus uptake [80], solubilization of inorganic phosphorus [81] and hydrolyzation of organic phosphorus [82]. Begum et al. [83] suggested that nurturing arbuscular mycorrhizal fungi (AMF) plant symbiosis, can significantly improve nutrients accretion, plant physiology and biomass accrual, root growth promotion and abiotic stress resistance. Several PGPR are also very effective in solubilizing phosphorus from the extremely insoluble phosphate forms as tricalcium phosphate, hydroxyl apatite and rock phosphate [84, 85]. Sub-culturing of most of the phosphorus-solubilizing bacteria results in the loss of the phosphate solubilizing activity [86] while fungi retain their activity to leach phosphorus-containing rocks even after prolonged culturing [87]. Rhizobial strains which solubilize phosphorus promote *Daucus carota* and *Lactuca sativa* development and a *Phyllobacterium* strain improves the quality of strawberries [88, 89]. *Rhizobium leguminosarum* strain PETP01 and *R. leguminosarum* strain TPV08 solubilize phosphate and are PGPR for pepper and tomato plants [90]. PSB also act as a biocontrol against plant pathogens by production of antibiotics, hydrogen cyanate, and antifungal metabolites, therefore, PSBs embody potential alternatives for inorganic phosphate fertilizers to encounter the phosphorus demands of plants, improving yield in sustainable agriculture. Their application is an ecologically and economically comprehensive approach [91].

The insoluble phosphate compounds are mobilized by the production of organic acids, accompanied by acidification of the medium [12]. Phosphate mobilizing biofertilizers are mycorrhiza which includes both ecto- and endo-mycorrhiza such as *Glomus* sp., *Gigaspora* sp., *Acaulospora* sp., *Scutellospora* sp., *Sclerocystis* sp., *Rhizoctonia solani*, *Amanita* sp., *Boletus* sp., *Laccaria* sp. etc. Not only phosphorus, mycorrhiza also mobilizes zinc, boron and other trace elements. They are broad

spectrum biofertilizers. Vesicular arbuscular mycorrhiza facilitates the phosphorus nutrition by not only increasing its availability, but also increasing its mobility. Cyanobacteria contribute to mobilization of inorganic phosphates through excretion of organic acids and extracellular phosphatases [92, 93].

#### **4. Plant growth promoting rhizobacteria (PGPR)**

For sustainable agricultural development, crops produced should be disease resistance, salt tolerance, drought tolerance, heavy metal stress tolerance and better nutritional value. To accomplish the above desired crop properties, soil microorganisms (bacteria, fungi, algae, etc.) are used, which increase the nutrient uptake capacity and water use efficiency [94]. Among these soil microorganisms, bacteria known as plant growth promoting rhizobacteria (PGPR) are the most promising. They may be used to augment plant health and promote plant growth rate without environmental contamination [95]. It has been recommended that endophytic N<sub>2</sub>-fixing bacteria may be more imperative than rhizospheric bacteria in promoting plant growth because they escape competition with rhizosphere microorganisms and achieve close contact with the plant tissues [96, 97]. *Azotobacter* sp., *Azospirillum* sp., *Pseudomonas fluorescens*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella* etc. colonize the plant rhizosphere and enhance the plant productivity by nitrogen-fixation, solubilization of phosphorus and other nutrients, production of phytohormones, antagonism against pathogens and degradation of phytotoxins [98]. Gou et al. [99] demonstrated that WM13-24 biofertilizer containing *Bacillus* sp. and the integrated biofertilizer promoted chili plant growth, fruit yield, quality, the rhizosphere soil nitrogen content and enzyme activities. *Phosphobacterin* could increase plant yield by 10-37% and there are recorded savings approx. US \$ 0.67 × 10<sup>9</sup> by the application of *Azospirillum* biofertilizer to

various cereals [100]. *Pseudomonas putida* controls both potato soft rot and seed decay by siderophore production which chelates iron, consequently iron become unavailable to these pathogens [101-103]. *Rhizobia* also act as PGPR with non-legumes such as maize, wheat, rice, and canola [54, 55]. Interaction of PGPR and AMF (arbuscular mycorrhizal fungi) was better suited to 70% fertilizer for phosphate uptake [22].

*Pseudomonas*, *Burkholderia*, *Acidithiobacillus*, *Bacillus* and *Paenibacillus* are proficient in releasing potassium from minerals such as mica, illite, muscovite, biotite and orthoclases [32, 104], increasing potassium availability up to 15% [105]. Similarly, *Bacillus* sp. are silicate and zinc solubilizer biofertilizer. Although PGPR are very effective at promoting plant growth and

development, but few bacterial species may inhibit growth. However, this negative impact may only occur under certain specific conditions and by some particular traits. Therefore, selection of a particular strain is of the highest importance in obtaining utmost benefits in terms of improved plant growth and development. Table 2 summarizes some PGPR [106]. Gonzalez et al. [107] in their experiment shown that *Azospirillum brasilense* improves the salt tolerance of the jojoba plant during in vitro rooting, as the bacteria reduces the undesirable effects of saline conditions. PGPR can be classified as biofertilizers once they act as a plant nutrition and amelioration basis that would replenish the nutrient cycle between the soil, plant roots and microorganisms present [106].

Function	Microorganisms	Benefitted Crops
Nitrogen Fixation	<i>Burkholderia</i> , <i>Beijerinckia</i> , <i>Frankia</i> , <i>Gluconacetobacter</i> , <i>Herbaspirillum</i> , <i>Rhizobium</i> , <i>Azorhizobium</i> , <i>Azoarcus</i> , <i>Azotobacter</i>	Rice, Alnus, sugarcane, legumes
Siderophore production	<i>Chryseobacterium</i> , <i>Bacillus</i> , <i>Phyllobacterium</i> , <i>Pseudomonas</i> , <i>Rhizobium</i> , <i>Streptomyces</i>	Tomato, strawberries, potato, maize, pepper, lettuce, carrot, Indian lilac
Phosphate solubilization	<i>Phyllobacterium</i>	Strawberries
Potassium solubilization	<i>Paenibacillus</i> , <i>Bacillus</i>	Black pepper
Chitinase and β-glucanases production	<i>Pseudomonas</i> , <i>Sinorhizobium</i>	Several crops
Indole acetic acid synthesis	<i>Streptomyces</i> , <i>Rhizobium</i> , <i>Paenibacillus</i>	Lodgepole pine, pepper, lettuce, carrot, tomato, Indian lilac
Plant stress resistance induction	<i>Mycobacterium</i> , <i>Rhizobia</i> , <i>Bacillus</i>	Maize, peanuts
ACC deaminase synthesis	<i>Pseudomonas</i> , <i>Rhizobium</i>	Mung beans, wheat, pepper, tomato, mung, beans

**Table 2:** Plant growth promoting rhizobacteria and their functions [106].



## 5. Potassium solubilizing-mobilizing biofertilizers

Potassium have effects on water uptake, root growth, maintenance of turgor, transpiration and stomatal regulation of plants [108]. It is present in soil generally as silicate minerals which are inaccessible to plants and is absorbed by plants in the form of potassium ions which is insoluble in water. Microbes as *Bacillus* sp., *Aspergillus niger* etc. solubilize silicates by producing organic acids which cause the decomposition of silicates and helps in the removal of metal ions. They are broad spectrum bio-fertilizers [3].

## 6. Environmental stress on biofertilizers

Environmental stresses have substantial influence on microbial physiology. Mutagenic electromagnetic radiations as ionizing radiation (x-rays or  $\gamma$  radiation) carries enough energy to remove electrons from molecules in a cell consequently free radicals are formed which can damage DNA or RNA by oxidizing them and non-ionizing radiation (ultraviolet radiation) exerts its mutagenic effect by exciting electrons in molecules consequently formation of pyrimidine dimer which often change the shape of the DNA in the cell and can cause tribulations during replication. Since ionizing radiation has high frequency, it can penetrate cells and endospores very easily. The high-energetic ultraviolet radiation has great potential for cell damage moreover by direct effects on biologically pertinent molecules as DNA, proteins and lipids and indirectly by the production of reactive oxygen species, resulting in mutagenesis and impairment of essential cellular physiology [109]. UV radiation affects adversely several life processes of cyanobacteria such as growth, survival, photosynthesis, carbon dioxide uptake, pigmentation, motility, phycobiliprotein composition, and nitrogen metabolism etc. [110-113]. Many workers have suggested that the cellular constituents absorbing radiation between 280-315 nm are destroyed by UV-B

radiation, which may further affect the cellular membrane permeability and protein damage eventually resulting in the death of the cell [114, 115]. Reactive oxygen species (ROS) is an important component in signal transduction pathway, resulting in the inhibition of gene expression of key proteins involved in photosynthesis during UV-B exposure [116]. Chen et al. [117] observed that *Microcoleus vaginatus* after being irradiated with UV- B radiation, showed decreased photosynthetic activity ( $F_v/F_m$ ), and increased reactive oxygen species (ROS) generation. Kós et al. [118] suggested that upon exposure of the cells to  $500 \mu\text{mol m}^{-2}\text{s}^{-1}$  intensity visible light *psbA3* replaces *psbA1* as the dominating *psbA* mRNA species, and *psbD2* increases at the expense of *psbD1*. RuBISCO is the key target involved in UV-induced inhibition [119] might be due to alteration in mRNA level of RuBISCO [120]. Most of the biofertilizers possess an assimilatory enzyme, nitrate reductase. Thapar et al. [121] suggested that the nitrate reductase is bounded to the chlorophyll-containing membrane fractions; inhibition of chlorophyll may directly affect the nitrate reductase activity. Ultraviolet radiation destroys the complex organization within phycobilisomes because their aromatic amino acids absorbs in the range of UV-B. In cyanobacteria, more than 99% of the UV-B is absorbed by chlorophyll-binding proteins and phycobilisomes [110]. Exposure of *Synechocystis* cells (phycoerythrin absent) to moderate intensity of UV-B ( $1.8 \text{ Wm}^{-2}$ ) induces loss of  $\beta$ -phycocyanin, which may be due to the two bilins present in  $\beta$ -phycocyanin whereas the other biliproteins contain single bilin [122]. *Synechococcus* sp. PCC 7942 phycobilisomes when exposed to UV-B radiation, showed photodestruction of both  $\alpha$ - and  $\beta$ -phycocyanin [123]. DNA is one of the most prominent targets of solar UV radiation, in all living organisms [124]. UV-induced lesions may lead to chronic mutations and even death of the cell. In comparison to UV-B, the

wavelength of UV-A has poor efficiency in inducing the DNA damage, since they are not absorbed by native DNA. However, UV-A is able to generate singlet oxygen ( $^1\text{O}_2$ ) or reactive oxygen species (ROS) that can damage DNA via indirect photosensitization reactions [116, 125]. The ability of UV-B radiation to damage a given base is determined by the flexibility of the DNA; the nature of bases play a major role since the distribution of dimeric photoproducts strongly depends on the pyrimidine bases involved. DNA alteration may occur mainly by mispairing of bases during replication, hydrolytic deamination, depurination/depyrimidination, oxidative damage by ionizing radiation (IR) as well as by free radicals or reactive oxygen species (ROS) and by certain alkylating agents [126]. The incidence of UV-B radiation may result in single as well as double DNA strand breaks (DSBs). DSBs may lead to loss of genetic information. The most potent carcinogenic forms of UV-induced DNA lesions are CPDs, 6-4PPs and their Dewar isomers [127] that may impede with normal cellular capability and functional integrity, reduction of RNA synthesis, arrest of cell cycle progression, resulting in mutagenesis, tumorigenesis and apoptosis [124]. It has been found that thymine-thymine (T-T) and thymine-cytosine (T-C) sequences are more photoreactive than C-T and C-C sequences [128]. The yield ratio of CPDs and 6-4PPs mostly depends upon the two adjacent bases involved in the formation of dimer, though, it has been reported that the amount of CPDs and 6-4PPs are about 75 and 25% respectively of the total UV-induced DNA damage product [129]. If unrepaired, a single CPD is sufficient to completely eliminate expression of a transcriptional unit [130]. It seems probable that UV affects the DNA of cyanobacteria and the killing of these microbes might be due to the irreversible damages caused to DNA by the high energy UVR. UV-B-induced formation of thymine dimer has also been reported in *Chroococcus* sp. and *Anabaenopsis* sp. However, cyanobacteria

exhibit photoreactivation and excision repair [131] mechanisms to overcome the deleterious effects of UV-B radiation.

## **7. Mitigation strategies against environmental stress by biofertilizers**

Biofertilizers have developed a sequence of strategies to defend against environmental stresses (Figure 2). For example, they sustain the integrity and fluidity of cell membranes by modulating their structure and composition and the permeability and activities of transporters are adjusted to control nutrient transport and ion exchange. Certain transcription factors are activated to augment gene expression and specific signal transduction pathways are induced to acclimatize to environmental changes. They also have repair mechanisms that shield their macromolecules against damages inflicted by environmental stresses [132]. Bacteria have successfully colonised every niche on the planet, as the soil-dwelling Gram-positive *Bacillus* sp. which can survive radiation doses sufficient to kill all other life forms [133]. Cyanobacteria which are exposed to high solar radiation in their habitats have developed a number of mechanisms to cope up with this stress, one of them is avoidance. Wu et al. [134] proposed that decreased helix pitch in the presence of UV-B radiation to obtain a more compact structure of the spirals, which eventually results in self-shading, is an effective protective mechanism against photoinhibition in *Arthrospira platensis*. The synthesis of extracellular glycan in *Nostoc commune* was stimulated by UV-B radiation and was proposed to provide UV resistance by increasing the effective path length for the absorption of radiation [135]. An accumulation of active iron superoxide dismutase (FeSOD) in desiccated field cyanobacterium *N. commune* was found to reverse the effects of oxidative stress imposed by multiple cycles of desiccation and rehydration during the UV-A or UV-B irradiation [136]. The other mechanism to tolerate UV

radiation is synthesis of UV-absorbing or photoprotective compounds in the cyanobacteria. Carotenoid pigments are better known for their function as antioxidants that provide protection against detrimental photoproducts [137]. In the cyanobacterium *N. commune*, changes in the carotenoids pattern in response to UV-B irradiation was reported and myxoxanthophyll and echinenone were suggested to act as outer membrane-bound UV-B photoprotectors [135]. A possible role of pterins as UV-protecting compounds has been suggested for the marine planktonic cyanobacterium *Oscillatoria* sp., since UV-A radiation has been reported to be very effective in eliciting biosynthesis of a biopterin glucoside compound [138]. Revelations to varied abiotic stresses have levied cyanobacteria towards gradual evolution to sustain the cell viability. Process of photosynthesis have exposed cyanobacteria to the wide range of solar radiations including harmful doses of ultraviolet radiations in their

natural habitats which positively forced them to synthesize a number of photon engrossing substances [139]. A number of cyanobacteria synthesize water-soluble colorless, mycosporine-like amino acids (MAA) and the lipid soluble yellow-brown colored sheath pigment, scytonemin [135, 140-142] to provide protection from deleterious effects of UV radiation. MAAs seem well suited for a sunscreen function due to their high molar extinction coefficients and their absorbance spectra peaking within the UV-B and UV-A regions [143]. The presence of other photoprotective compound, scytonemin most probably helped cyanobacteria to survive from lethal effects of UV radiation when there was no stratospheric ozone layer. This assumption is supported by the fact that scytonemin has an *in vivo* absorption maximum at 370 nm whereas purified scytonemin shows maximum absorption at 386 nm, but it also absorbs significantly at 252, 278 and 300 nm.

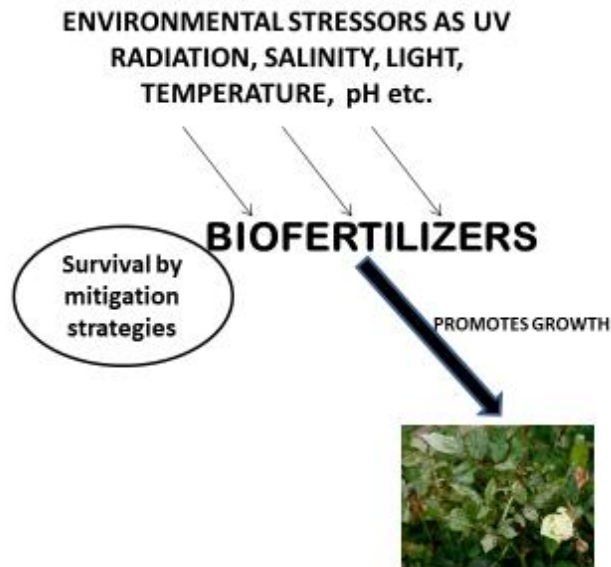


Figure 2: Biofertilizers sustainability after various stresses by various mitigation strategies.

## 8. Conclusion

Dependence on chemical fertilizers lead to the overutilization of the soil ecosystem and environment.

The rising global usage of N fertilizers enhance N<sub>2</sub>O conc. in the atmosphere 300 times more effective than CO<sub>2</sub> that ruins in the atmosphere 100 years or more

[144]. Growing global population food requirements can be unravelled by the use of biofertilizers only. Worthy impact of biofertilizers in biocontrol and bioremediation impose a positive impact on crop yield and ecosystem. Hence, reassurance should be specified to its execution in agronomy. Nanotechnology is required to improve the current biofertilizers to sustenance and elevate agricultural sustainability worldwide.

### **Conflicts of Interest**

The author confirms that this article content has no conflict of interest.

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