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Research Review

Role of Micronutrients Based Nano Biofertilizers as Biofortifying Agent for Plant Growth Promotion and Development

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ABSTRACT

In developing countries malnutrition is the major issue. Main cause of malnutrition is low grade production of crops and minimum nutrients contents in foods which leads to diet related chronic diseases and nutrient deficient food. It leads to retardation of physical and mental growth and increase in morbidity rate. So, to overcome these effects farmers use large amount of mineral nutrient and chemical fertilizers that leads to certain harmful effects on soil fertility and crop productivity. The innovative way to suppress the harmful effects cause due to chemical fertilizers is by using biofortification which mainly involves Plant Growth Promoting Rhizobacteria (PGPR). They are very well known to fortify Micro- and Macro- nutrients by various direct and Indirect mechanisms. Direct mechanism promotes plant growth by directly facilitating acquisition of resources and modulating plant hormone level which includes solubilization of a Zine and Manganese like micronutrients and NPK solubilization, Magnesium, Calcium, Sulphur like macronutrients, Biological Nitrogen Fixation and production of phytohormone. Indirect mechanism promotes plant growth by decreasing the inhibitory effects of various pathogens on plant growth and development which includes Siderophore production, Induced systemic resistance and Nano biofertilizers. This mechanism of bacteria is boon for agricultural sectors which accelerates the solubilization rate and nutrient uptake by crops which enhances crop productivity therefore Biofortification using PGPR is novel and innovative way for enhancing plant growth promotion and developments.

Key words**:** Biofortification, PGPR, Direct and indirect mechanism, Micronutrients, Macronutrients, NPK, Induced systemic resistance, Nano biofertilizers

Day by day population is increasing. The increasing population directly affects food availability so to fulfilled the need of global population it is mandatory to increase the yield of crops and food production in agricultural sector. Increase in the productivity is not enough to fulfilled the ever-increasing need for food but our aim is to improve quality of crops. Traditional way to improve the crop productivity is use of chemical fertilizers but excess use of chemical fertilizers causes heavy metal accumulation, it alters the soil by creating too high salt concentration and this shows negative impact on beneficial soil microorganisms. In addition to this effectivity of chemical fertilizers in crop productivity is very short and for minimum duration but its shows harmful effects for a long period of time which increases problems like ion chelation, change in soil pH which makes soil infertile and accumulation of heavy salt leads to killing of essential nutrients solubilizing bacteria which play important role in solubilizing Micro- and Macro- nutrients in soil. Plants being Autotrophic they make their own food with the help of carbon dioxide, water in the presence of sunlight along with three key factors plants do require other essential Nutrients and minerals for their proper growth and development. Even a Moderate Deficiencies may adversely effects growth and development of crops [1]. This association of Microbes and Plants play a vital role in diminishing adverse effects by controlling diseases of crops. Plants having different kinds of mechanisms which includes Direct and Indirect mechanism which help in enhancing growth of plants and crop productivity. First mechanism includes solubilization of micro and macronutrients by PGPR. The most important Micronutrients and Macronutrients which is required by plants are namely a Zinc and Manganese like micronutrients and fixation of Nitrogen as well as solubilizations of Phosphorus, Potassium Magnesium, Calcium, Sulphur like macronutrients, so this are present in insoluble form in the soil which is inaccessible to plants and they can't utilize the source of nutrients though it is present in soil. PGPR are friends of plants which give access of nutrients to plants by converting it into soluble form by various reactions and plants takes up these soluble nutrients and enhance its own growth and development another mechanism is Biological Nitrogen Fixation. In biological nitrogen fixation main role is to convert molecular Nitrogen into ammonia and ammonia is the first important product of nitrogen in all nitrogen fixing organisms and it is

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used by other microorganisms either directly or indirectly or it is further converted into other soluble forms such as nitrites, nitrates or amino acids by two way and are of two types Symbiotic nitrogen fixation and non-symbiotic nitrogen fixation. Symbiotic nitrogen fixation carried out by those nitrogen fixing micro-organisms which are present in symbiotic association with other plants. The examples are root nodules of leguminous plants as well as in non-leguminous plants. The Angio spermic plants which are non-leguminous but still can fix atmospheric nitrogen it includes Elegans, Casuarina, Myrica, Alnus. The second type is NF by Non-symbiotic way which includes Diazotrophs which are prokaryotic bacteria and archaea having capacity to fix atmospheric nitrogen gas into usable form such as ammonia by free living bacteria and different species of cyanobacteria and bacteria having this unique ability are known as diazotrophic bacteria and phenomenon of biological fixation of nitrogen is called diazo trophy. Nitrogen fixation reaction is catalyzed by enzyme complex Nitrogenase. It consists of Mo-Feco-factor and if genes also play important role in Nitrogen Fixation. Some nitrogen fixing species are *Azospirillum*, *Azotobacter*, and *Rhizobia*. Next important direct mechanism is production of Phytohormone like Auxins, Gibberellins and Cytokinin's by PGPR. Auxin is served as Indole-3-acetic acid (IAA) which is important for cell elongation, cell division, tissue differentiation, and aids in apical dominance and it is highly developed roots, uptake more nutrients and 80% of the bacterial flora in the rhizosphere produce IAA. Examples of IAA producing microorganisms are*Azetobacter*, *Azospirillum* [2]. Another phytohormone is Cytokinin's which is responsible for cell division, root development, root hair formation, shoot initiation, inhibition of root elongation and helps in influencing their physiological and developmental processes Examples of Cytokinins producing microorganisms are *Pseudomonas, Azospirillum* and *Bacillus.* Most abundant cytokinin's are Adenine-type. Additional is Gibberellines (GAs) helps in seed germination, stem elongation and flowering and fruit setting there are total 136 GA s are known from plants out of which only 4 from PGPR- GA_1, GA_2, GA_4 and $GA_{20.}$ Effect of PGPR producing GA s is not exactly known but used in seed germination examples are *B. pumilus, B. licheniformis* [2]. Now Siderophores production is comes under the both Direct as well as Indirect mechanism because it releasing iron-chelating molecules to rhizosphere and improve iron nutrition -attract iron towards the rhizosphere and another role is in inhibition of growth of other micro-organisms by hindering the growth of pathogens by limiting the availability of iron for pathogen (fungi) examples are *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* therefore siderophore participates in both direct and indirect manner in growth as well protection from pathogens it is fall under both direct and indirect mechanism. Another indirect mechanism is Induced systemic resistance (ISR) it protects the plant from diseases by increase in level of basal resistance to several pathogens consist of two pathways jasmone or ethylene pathway and Salicylic acid response thus inducing host plants defense response. PGPR has potential of above Direct and Indirect mechanism which enhances plant growth and productivity by replacing traditional chemical fertilizers and suppress its toxic effects. Exploring PGPR in agriculture is boon for agricultural sector and some PGPR fertilizers includes preparing consortia of various PGPR strains which apply directly to seeds or in soils for improving productivity by Direct or Indirect mechanisms. But transport of this consortia from Laboratory to field is difficult because of loss of nutritional level and bacterial count. It may hinders the productivity of applied agricultural field therefore there is need to developed innovative method for removing the disadvantage which arises by using bacterial consortia thus Nano biofertilizers is one of nanotechnological advances which is one of the innovative method and plays effective role in promoting plant growth and they are rapidly absorbed by the plants due to their nanoscale size hence nano-materials such as nano-fibers, nano-fertilizers and nano-pesticides may produce revolutionary effects in agricultural sectors. Therefore, PGPR along with nanomaterials has favorable strategy for managing growth and development of plants. And it is achieved by synthesizing nanoparticles by green methods which includes Zinc oxide nanoparticles and Manganese dioxide nanoparticles due to these kinds of important positive effects of PGPR along with nanoparticles thus it serves as pillar of agriculture.

2. *Mechanism of PGPR in biofortification*

1. *Direct mechanism*

A. *Solubilization of nutrients*

One of the obstacles to plant growth is lack of availability of nutrients elements in many agricultural environments. By using different mechanisms of actions, PGPR participates in geochemical nutrition cycle and give access to different nutrients to plants and microbial community. Bacteria serves as bio-inoculants which will increase the availability of nutrients elements in soil, help to minimize the chemical fertilizers, reduce environmental pollution and promote sustainable agriculture. Different types of micronutrients like manganese and zinc as well as macronutrients like nitrogen, phosphorous, potassium, magnesium, calcium, sulfur. Plant associated microorganisms on the other hand regulate growth and morphogenesis of plants or activates plants immunity by releasing small molecules and phytohormone (Ortiz et al.,2009). PGPR belong to genera *Frankia, Acinetobacter, Arthrobacter, Azotobacter, Azospirillum Streptomyces spp., Bacillus, Enterobacter, Burkholderia, Bradyrhizobium, Rhizobium, Serratia, Thiobacillus, and Pseudomonas* [3-5]. PGPR benefits plant growth and development by different beneficial mechanisms such as direct and indirect mechanisms. The mechanism benefits the plant by reducing or protecting plant against infections such as pathogens, biological and abiotic stress or help plant growth and promotion. Thus, direct mechanisms include solubilization of nutrients, growth regulations, increasing nutrients availability and also enhances plants access towards soil nutrients. Beneficial nutrients are unavailable to plant roots because nutrients in soil are generally bound to organic and inorganic soil constituents or present in insoluble precipitates thus, mechanisms are important for enhancing solubility of phosphorous and iron and PGPR derived ion concentration would the help uptake of nutrients by roots by diffusion movement due to concentration gradient.

Manganese

Manganese is vital Micro-nutrients essential for growth and development. It helps plant to sustains and maintain homoeostatic. Manganese is important micronutrients require by plant for photosynthesis process in which plant make their own food with the help of carbon dioxide, water in the presence of sunlight in leaf due to presence of chlorophyll pigment. It is very essential pigment in plants. Manganese play role in leaf development and has metabolic roles in different cell compartments and important cofactor for oxygen-evolving complex (OEC) of the photosynthetic machinery, catalyzing the water splitting reaction in photosystem II (PSII). Manganese dependent metabolic processes like glycosylation, ROS scavenging and photosynthesis mediated by multitude of transport proteins from diverse families [6]. Manganese helps in breakdown of carbohydrates and involved in effective metabolism of nitrogen and soil is known reservoir of manganese.

Deficiency of manganese

Manganese deficiency is serious, widespread plant nutritional disorder in dry, well-aerated and calcareous soils as well as in soils containing high amounts of organic matter where bio-availability of manganese can decrease far below the level that required for normal growth. Deficiency leads to light chlorosis in between leaf veins throughout the plants, increase plant stress, leaves tend to show metallic gloomy shine with necrosis and darker leaf spots. Purplish coloration on leaf is also an indication of Mn deficiency. Many barleys, wheat and oats are victims of Mn deficiency impaired growth and decrease biomass, lower number of chloroplasts and lower photosynthetic efficiency decrease chlorophyll content which leads to disintegration of PSII complex and lowers net photosynthetic rate [7] and also damages thylakoid structure and promote chlorophyll degradation leading to development of characteristics necrosis [8]. Mn starred Arabidopsis seedling show decrease net photosynthetic rate which there was no loss of Mn SOD activity [9].

Toxicity of manganese

Ion conversion playing important role in number of oxidation reduction processes such as electron transport chain in photosynthesis. Furthermore, manganese acts as potential activator of several enzymes that are involved in citric acid cycle, phosphorous reactions, carbohydrates metabolism, carboxylation processes and oxidation reaction protein manganese enzyme of photosystem-II are two important enzymes [10]. Mn toxicity leads to spatial callose formation in areas characterized with brown spots.

Role of microorganisms in bioavailability of manganese

Mn biogeochemistry is present in soil and exists in three oxidation states Mn(I), Mn(II), and Mn(III) which are insoluble forms. Mn (IV) is highly insoluble and precipitates in soil. Mn bioavailability to plants is greatly influenced by soil pH and redox conditions as increase in soil pH causes reduction in its availability by forming MnO_2complex. Nutrients availability increases with reduced oxygen conditions. Agronomic effectiveness of various organic and inorganic Mn fertilizers is greatly influenced by solubility in water, $MnSO_4$ is mostly applied to soil and foliar sprays to Mn deficient and efficiency lower alkaline and dry soils [11] Mn (IV) is reduced to Mn (II) through biological or chemical processes due to presence of protons and electrons carrying reducing agents produced by plants roots, microorganisms or through organic matter decomposition. Mn reducing microorganisms *Arthrobacter, Bacillus, Clostridium, Enterobacter, Lysinibacillus, Micrococcus, Pseudomonas, Styphylococcus* [12]. Mn reducing bacteria is called MSB means Manganese solubilizing bacteria that promote Mn dissolution through protonation of metals anions.

Mechanism solubilization of manganese

Manganese solubilizing bacteria is mainly work by solubilizing nutrients and convert into accessible form and make them available to plants. Mn availability mainly affects plant growth and root exudates and electrons and H^+ ions are reducers of rhizosphere activities and insoluble manganese is mainly present in alkaline soils and its inaccessible to plants thus roots and PGPR produces chelating agents like phenolic compounds and organic acids which plays important role in solubilizing nutrients and it is available to plants accelerate the growth and development of plants.

Zinc

Zinc is essential plant nutrient. Plant takes the zinc from soil solution in form of divalent cation more specifically in case of calcareous soil having higher pH values. Inside the xylem, zinc is either bonded with organic acid or transmitted to divalent cations via chemical modifications while in phloem sap [11]. Zinc forms organic complex having low molecular weight substances and present in relatively higher concentrations. Iron is the fourth abundant soil element on earth that is not readily available for microorganisms and plants because of lower mineral solubility, especially in arid regions having alkaline soil conditions. Zinc is essential plant nutrient contributes in many types of life processes and it is second most copious transition metal after iron. It is mandatory to maintain Zn levels in soil otherwise it leads to shortage of commercial problems in plant growth and promotion, for maintenance all physiochemical tend to regulate balance of Zn in plants [13]. Zinc is activator of various enzymes which are responsible for synthesis of certain proteins and it is important in formation of chlorophyll and some carbohydrates. It converts starches to sugar and its presence also withstand cold temperature [14]. Zn is important in formation of "Auxin" which are essential plant growth promoting phytohormone important for stem elongation.

Deficiency of zinc

Zinc is essential Micronutrients and deficiency symptoms occurs in new leaves, so chlorosis of leaf is major problem and varying pattern of chlorosis of leaves and necrotic spots may form on marginal tips of leaves. Shortened internodes which is space between two nodes gives plant rosette appearance and bud is also affected and leads to poor bud development and reduced flowering and branching so they also affect essential plant parts.

Toxicity of zinc

Excess of zinc can compete with other essential nutrients like Iron, Manganese, Copper and cause deficiencies in plant tissue because these metals have similar ionic radii [15]. It also indication of soil toxicity because Zn is more available for plant when pH of growing medium is low so if Zinc toxicity is occurring it is always preferred to check pH. It decreases moisture content of plant tissues and it also changes concentration of other nutrients like phosphorous and magnesium in plants.

Role of microorganisms in bioavailability of zinc

Biofortification is solution for micronutrient-associated malnutrition by augmenting particular or all required micronutrients in edible parts of the crop. The biofortification of a crop is achieved by agronomic methods, plant breeding and biotechnological or genetic engineering approaches [16-18]. Therefore, by adopting organic agriculture which supports the concept of use of zinc solubilizing bacteria, can be a fruitful aspect to increase zinc levels in plants [19-20]. It has been documented that many zinc solubilizing bacteria increase the zinc content in several edible crops and thus provides a novel scenario of microbial-assisted biofortification for plants [21]. Use of bioinoculants is an economically suitable method to increase plant growth and increased yield in a sustainable manner [22-23]. Some zinc solubilizing bacteria includes *Psudomonas aeruginosa, Gluconacetobacter diazotrophicus, Bacillus species, Acinetobacter, Azospirillum.*

Mechanism of zinc solubilization

Zinc solubilizing bacteria solubilize zinc by various mechanisms. One of very important mechanism is the acidification, by producing organic acids in soil and leads to decrease in soil pH [24] which helps in chelation of ions mainly zinc and enhance zinc solubility [25]. Another mechanism of zinc solubilization includes siderophore production [26] and proton, oxido-reductive systems on cell membranes and chelated ligands. Great efforts are being made to generate new and more efficient sources of fertilizer to alleviate nutrient deficiencies. Use of layered double hydroxides (LDH) as a novel structure to improve the anion (nitrate and phosphate) exchange properties of problematic soils while minimizing negative environmental effects of sequestration of these nutrients on non-agricultural ecosystems [27].

Nitrogen

Nitrogen is important for plant overall development and physiological functions. Nitrogen is very essential plant nutrients and it is supplemented to soil in form of nitrogenase fertilizers [28]. Nitrogen is present in 78% atmosphere but it is inaccessible to plants therefore it is converted into soluble forms ammonia with help of microorganisms which are symbiotic and non-symbiotic nitrogen fixing bacteria and this is called as nitrogen fixation. It is of two types symbiotic and non-symbiotic which consist of nodule formation by leguminous rhizobia and non-leguminous *Frankia* and Diazotrophs bacteria are *Azospirillum, Azotobacter, Bacillus* respectively. After fixation of nitrogen pH of soil is change at soil-root interface while ammonium ions in rhizosphere which create acidified environment. Nitrogen is important for enzymes, proteins and structural components required for proper growth and it also helps to fight against diseases.

Deficiency of nitrogen

Nitrogen is an essential element of all amino acids in plant. Amino acids are building blocks of plants proteins and important for growth and development of vital plants tissues and cells like cell membranes and chlorophyll and deficiency leads to chlorosis of leaves. In Chlorosis leaves become yellow in appearance due to halt in carbohydrate formation process by photosynthesis and substances that impart green colour to leaves. Nitrogen enhances this process and help in plant growth and promotion. Deficiency of Nitrogen leads to diseases like *Alternaria solani* early blight diseases and excess of nitrogen leads to weaker vegetative growth [10].

Toxicity of nitrogen

Toxicity arises when a different form of nitrogen causes consequent effects of some nutrients and their rate of application on plants diseases is documented. Change in pH during nitrogen fixation which leads to deleterious effects on plant growth and development. Existence of ammonium ions at pH greater than 8 while nitrous acid and nitrites are usually generated in presence of ammonium ions. At pH less than 6 infections decreased by acidifying irrigation water owing to specific pH solution and reduces chances of diseases [28].

Role of microorganism in bioavailability of Nitrogen

Biological nitrogen fixation (BNF) is the process by which nitrogen is reduced to ammonia by a specialized group of microorganisms. Nitrogen fixation is characterized as symbiotic and non-symbiotic nitrogen fixation. Symbiotic nitrogen fixation includes fixation of nitrogen by symbiotic association of microorganisms with plants and fixes atmospheric nitrogen. In symbiotic, free living, and associative mechanism root/legume-associated symbiotic bacteria possess the specificity and infect the roots to produce nodule. Several types of symbiotic biological N_2-fixing associations are known. The most prominent leguminous bacteria is Rhizobium. All nitrogen fixing organisms are prokaryotes. Another type includes non-symbiotic nitrogen fixation includes Diazotrophic bacteria possessing the trait of N_2 fixation are classified into three subgroups. The legume host plant provides the bacteria with their necessary carbohydrates and possibly all the other nutrients they require in the exchange for the fixed N_2 by the bacteria. In this biological process, nodule-forming rhizobia inhabit the roots of leguminous plants live in symbiotic relationship with plants and convert atmospheric N_2 to a usable form and that the plant can use. Non-symbiotic nitrogen-fixing bacteria are a wide variety of the diazotrophs that form associative relationships with a wide variety of plant roots including those of cereals and colonize the root surface of non-leguminous plants. Availability ranges of nutrient elements depending on soil pH 9, Plant Growth-Promoting Rhizobacteria (PGPR) and their action mechanisms. Due to a very close association established between associative NFB and plants, the fixed N_2 by these bacteria can also be taken up by the plant and the microbes can utilize plant-derived carbon compounds to fuel the nitrogen fixation reaction. Furthermore, plants may provide suitable conditions for protecting the nitrogenase complex from exposure to oxygen. Because nitrogenase can only function in anoxic conditions. Generally, these bacteria can make only a small contribution to the nitrogen nutrition of the plant because nitrogen fixation is an energy-expensive process, and large amounts of organic nutrients are not continuously available to microbes in the rhizosphere. Bacterial genera such as *Klebsiella, Azotobacter, Azoarcus, Bacillus, Enterobacter, Xanthobacter, Beijerinckia, Achromobacter spp., Arthrobacter spp., Clostridium spp., Corynebacterium spp., Herbaspirillum spp., Pseudomonas spp., Rhodopseudomonas, Rhodospirillum, Azomonas, and Derxia* [29] are examples of the NFB that live independently of other organisms. These bacteria are also named as free-living nitrogen fixing bacteria. Almost all of the nitrogen fixed by free-living NFB is used by these bacteria.

Mechanism of nitrogen solubilization

Many associated symbiotic nitrogen fixing bacteria can fix N_2 so that they could provide N to the plant. N_2- fixing PGPR can increase rate of uptake of nitrogen by different processes. The N cycle is biologically influenced by PGPR and has a central role in almost all aspects of N availability. In terms of availability of N to plants, some bacteria such as diazotrophs can convert N_2 into ammonia by the process termed biological nitrogen fixation (BNF) and using a complex enzyme system known as nitrogenase in case of non-symbiotic nitrogen fixation. Nitrogenase enzyme consist of two subunit larger and smaller and larger subunit called molybdenum ferrous protein or nitrogenase reductase and smaller is known as ferrous protein and nitrogenase can reduce a wide variety of substrates such as N_2, N_3, N_3O, HCN, C_3H_3, $2H^+$, acetylene, cyclopropane. Mechanism of BNF has been well known and documented [30]. In addition to rhizobia bacteria associated with legume plants, numerous symbiotic nitrogen fixing species have also been identified that are able to colonize the root surface and, in some cases, the root interior of a variety of pasture grasses and cereal crops (non-leguminous plants) [30].

Phosphorous

It is important plant constituent as it serves as important component of major cell proteins and nucleotides that are mainly responsible for development of different plant structures. It is known to be vital supplement of plants as phosphorous deficiency results in loss of several plant generations. Phosphorous plays structural role as being a major component of ribonucleic acid of plant cell and being needed for essential physiochemical processes of plants as protein digestion system, vitality exchange and different capacities [31]. Accessibility of phosphorous to plants present in soil is related to various activities like pH, [32] carboxylates and organization of root clusters [33] which is important for development of root clusters. Phosphorous is important for cell proteins, nucleotides and responsible for plant structures and ribonucleic acids of plant cells and it also decreases development of rust.

Deficiency of phosphorous

Deficiency of phosphorous leads to incomplete or gradual plant growth along with irregularities in younger plants and leads to stunted growth.

Toxicity of phosphorous

Change in pH of soil and leaf decolouration leads to turning of leaf into yellow or black colour which is followed by necrosis and chlorosis of leaves at top of plant. Such plants wilt easily and lead to stunted growth.

Role of microorganism in bioavailability of phosphorous

Phosphate-solubilizing bacteria (PSB) provide accessible forms of phosphorus for plants which is present in soils. The conversion of insoluble phosphate compounds (both organic and inorganic) in a form accessible or soluble to the plant is an important trait of Phosphate solubilizing bacteria. Solubilization of insoluble phosphate by microorganisms was reported by Pikovskaya [34]. In soil, phosphate-solubilizing bacteria constitute 1–50% of the total soil population. phosphate-solubilizing bacteria have been isolated, using serial plate dilution method or by enrichment culture technique by providing selective media for proper growth of bacteria from almost all areas, including from rhizosphere and non-rhizosphere soils, rhizoplane. The ability to solubilize insoluble inorganic phosphate compounds such as hydroxyapatite, tricalcium phosphate, rock phosphate and dicalcium phosphate has been reported in PGPR strains belonging to various genera [35]. A significant number of microbial species show the capacity of solubilizing P; these include actinobacteria, bacteria, fungi, and even algae. The solubilization of insoluble phosphates has been reported in most known bacterial genera (e.g., *Streptomyces sp., Agrobacterium sp., Azospirillum brasilense, Bacillus sp., B. circulans, B. cereus, B. fusiformis, B. pumilus, B. megaterium, B. mycoides, B. polymyxa, B. coagulans, B. subtilis, Rhodococcus, Klebsiella, Vibrio proteolyticus, Alcaligenes sp., Aerobacter aerogenes, Achromobacter sp., Enterobacter, Thiobacillus ferrooxidans, T. thiooxidans, Xanthomonas sp., Actinomaduraoligospora, Brevibacterium sp., Citrobacter sp., Arthrobacter, Serratia, Chryseobacterium, Gordonia, Phyllobacterium, Xanthobacter agilis, Delftia sp., Azotobacter, Xanthomonas, Pantoea, Pseudomonas sp., P putida, P. striata, P. fluorescens, P. calcis, Flavobacterium sp., Nitrosomonas sp., Erwinia sp., Micrococcus sp.*, and *Nitrobacter sp.*) [36]. By mobilizing inorganic and organic P and thus help in uptake it by plants.

Mechanism of phosphate solubilization

Solubilization and mineralization of phosphate in rhizosphere are the most common modes of action implicated in PGPR that increase the nutrient availability to the host plant [37]. PGPR play an important role in all three major components of the soil phosphate cycle includes dissolution–precipitation, sorption–desorption, and mineralization–immobilization of phosphorous. An example is the phosphate solubilizing bacteria, which dissolve various sparingly soluble phosphate sources such as $Ca_3(PO_4)_2$ [38] and $Zn_3(PO_4)_2$ [39] and lowering pH of the rhizosphere soil and making phosphate available for plant uptake. By solubilizing and mineralizing reactions, and immobilizing phosphate into microbial biomass or forming sparingly available forms of inorganic and organic soil P, phosphate-solubilizing PGPRs can either convert these insoluble phosphates into available forms through acidification (reducing the pH), chelation, exchange reactions, release of complexing or mineral dissolving compounds. Though it is difficult to pinpoint a single mechanism, production of organic acids and consequent pH reduction appear to be of great importance.

Potassium

The potassium is extremely mobile and essential macronutrient of plant which is abundantly found in all younger parts of plants it is alkaline in nature and it is basic cellular component and plants involves in proper nourishment, development and growth it helps in yield of seeds and rate of oil in growing plants [40] supplement for vegetative growth and stem assumes several vital parts in nourishment of plants.

Deficiency of potassium

Deficiency of potassium may lead to Chlorotic and indicate exploitation at the edges of leaves of plants [41] and discouraged root advancements and excess supply may lead to soil borne diseases. Potassium has maximum ability to create tolerance in plants and enhance quality and quantity to satisfy current food requirements [42]. Low potassium to nitrogen ratio indicates higher rate of infection and high potassium to nitrogen ratio indicates low infection rate.

Toxicity of potassium

Excess of potassium is not absorbed by the plants and may leads to leaching problems and soil toxicity is increased and that potassium may run off in water bodies and causes toxic effects in lives [43].

Role of microorganism in bioavailability of potassium

Potassium is again important nutrients required by plants thus soil bacteria, fungi, and actinobacteria are important in the cycling of mineral elements. Among these microbes bacteria play important role in this system. The bacteria involved in the solubilization of potassium from potassium-bearing minerals are called KSB (potassium-solubilizing bacteria). Potassium-solubilizing PGPRs have the ability to convert insoluble/mineral potassium into available potassium in soil making them available to the plants [44-46]. KSB play an important role in the natural potassium cycle [47-49]. A wide range of bacteria including *Pseudomonas, Burkholderia, Acidithiobacillus ferrooxidans, Enterobacter hormaechei, Paenibacillus glucanolyticus, Arthrobacter spp., Paenibacillus mucilaginosus, P. glucanolyticus, Bacillus mucilaginosus, B. edaphicus and B. circulans* have been reported to release K from K-bearing minerals [50-52].

Mechanism of potassium solubilization

The potassium-bearing minerals are a major source of insoluble potassium in soils [53]. The minerals are biotite, feldspar, mica, vermiculite, muscovite etc. These minerals make available potassium to plants slowly. Clay minerals are selective for potassium ions and release potassium slowly from the lattice wedge sites [53]. It has been known that KSB by converting mineral K into available K have a significant role in providing potassium to plants. At this time, there is little information on the mechanisms used by KSB to solubilize potassium. The potassium solubilization is a complex phenomenon affected by many factors, e.g., amount of mineral, microorganisms involved, nutritional status of soil, soil mineral type, size of mineral, and environmental factors [49]. Like the basic mechanism of PGPR for solubilizing phosphate, potassium-solubilizing PGPR also solubilize potassium through the production of organic acids. Extracellular polysaccharides, production of capsular polysaccharides, hydroxyl anion, siderophores, organic ligands, extracellular enzymes. In general, some of the direct mechanisms used by KSB include acidolysis, chelation, oxidation and production of carbon dioxide (CO_2).

Magnesium

Magnesium is the essential plant nutrient that has a wide range of roles in many plant functions. It is important for photosynthesis process which is important property of all autotrophs entities. Magnesium is a macronutrient and its availability in soil depends on multiple factors such as weather, local climate and also depends on specific agricultural system and its management practices. Therefore, magnesium is a central compound of photosynthesis. It supports absorption of sunlight during photosynthesis in which carbon dioxide and water in presence of sunlight in this process magnesium acts as catalyst which gives carbohydrates and at the time oxygen is released. It is also acting as phosphorous carrier and help in phosphorous metabolism and it is also important in cell division and protein formation as well as play important role in activation of several enzymes systems and important for plant respiration [54].

Deficiency of magnesium

Deficiency may arise due to intensively used agricultural soil also by weathering of rocks. Deficiency of magnesium can be first recognized by seeing defects in leaves first because magnesium is important for photosynthesis. It affects mainly outer edges of leaves. Leaves started becoming yellow because of interveinal chlorosis, newly growing leaves also yellow with dark spots and sunlight also affects the structure so is important to maintain magnesium amount in soil to avoids barriers in photosynthesis process.

Toxicity of magnesium

Excess used of magnesium may leads to leaching problems and during rainy season heavy rains it leads to influenced soil acidity and it badly affects growth and leads to stunted growth [55].

Role of microorganism in bioavailability of magnesium

Magnesium solubilizing bacteria is beneficial in converting vital insoluble nutrients into soluble forms such as silicon and help in tolerating biotic and abiotic stress. Silica again presents in polymeric forms in soil and it get converted into the soluble forms due to weathering which release monosilicic acid into soil solution. This is bioavailable form of silicon absorbed by plants and this soluble magnesium promotes plant growth and promotion. *Rhizobium sp.* has the potential to be employed as a plant beneficial bacterium for accelerating the weathering process and it increases concentration of silica in rhizosphere. Some magnesium solubilizing bacteria includes *Psudomonas aeruginosa*, *Gluconacetobacter diazotrophicus*, *Bacillus species*, *Acinetobacter*, *Azospirillum* etc.

Mechanism of magnesium solubilization

Some magnesium solubilizing bacteria helps the plants growth and development by providing nutrients in the soluble form which is easily acquired by plants and leads to plants growth and mechanism includes chelation, acidolysis and leads to magnesium in the usable form and easily metabolized by plant for growth and promotion.

Calcium

Calcium plays very important role in producing plant tissues and enables plants to grow better. It is building block for cell wall and which important for providing structural rigidity due to pectin polysaccharide matrix also provides stem strengthening which is important for holding fruits and flowers on branches and maintained quality of fruit. It also protect plant by synthesizing calcium in higher concentration when bacterial or fungal infections arises then they harm by invading leaf lamella by synthesizing proteolytic enzymes and damage cells so Antagonistic activity performed by increasing concentration of calcium my lead to suppression of proteolytic enzymes and save plant from poison attack by bacteria and fungus and some known fungal diseases includes Crown rot and wilt in tomatoes and it also play important role in conversion of nitrate-nitrogen into forms needed for protein production. It also improves the absorption of other nutrients by roots and their translocation within the plants. Calcium is an important constituent for growth and development of plants that also involves in enzyme activation including salt balance and water movements in cells of plants thereby activating potassium in order to control opening and closing of small pores called stomata [10].

Deficiency of calcium

First sign of deficiency is observed on younger leaves leads to the leaves necrosis due to failure of mobility of calcium ions examples is blossom end rot in tomatoes, tips burn of cabbage and brown black heart of escarole and celery and new leaves has distorted curved hook shaped and growing tips [56]. Poor translocation is because of root tips die and roots grow slow and xylem and phloem tissues are main translocating system damages and affects badly due to low concentration of calcium and calcium deficiencies affects roots, shoots and leaves by affecting absorption of water and minerals, stem elongation and photosynthesis rate respectively [57].

Toxicity of calcium

Excess use of calcium may lead to leaching problems and during rainy season heavy rains it leads to influenced soil acidity and it badly affects growth and leads to stunted growth [55].

Role of microorganism in bioavailability of calcium

Calcium is present in insoluble form and which is not accessible to plants and thus plant may suffer from nutrient deficiency and leads to stunted growth and different deficiency symptoms. Some PGPR performs the solubilization of nutrients by some calcium solubilizing bacteria and leads to different mechanism like abiotic leaching and solubilize insoluble forms of calcium like aluminium phosphate and aluminium silicate. It includes *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Chromobacterium*, *Bacillus*, *Micrococcus*.

Mechanism of calcium solubilization

Some calcium solubilizing bacteria helps in plants growth and development by providing nutrients in the soluble form which is easily acquired by plants and leads to plants growth. The mechanism includes chelation, acidolysis which leads to conversion of calcium in the usable form and easily metabolized by plant for growth and promotion.

Sulphur

Sulphur is essential element in forming proteins, vitamins and chlorophyll in plants and important for nodule development and efficiently increases ability in Nitrogen Fixation in legumes like Alfalfa, Clover, Soyabean. Sulphur plays very important role in formation of oils within the seeds and it's a constitutes of several amino acids and vitamins. Sulphur is the most beneficial plant element for almost all living organisms as it is known to perform number of dynamic roles essential for proper development, growth and survival of plant life. For maximum production sulphur is regarded as essential nutrient for all crops [58].

Deficiency of Sulphur

Sulphur is mobile within plants and deficiency symptoms appears on younger leaves and leads to chlorosis and petioles strains highly red colour and also may badly affects nitrogen level in plants because sulphur is of essential for nitrogen fixation in leguminous plants.

Toxicity of Sulphur

Excessive use of sulphur containing fertilizers often decreases pH of soil and uptake of sulphur is found to decrease with lowering pH of soil.

Role of microorganism in bioavailability of sulphur and its mechanism

Like other nutrients, sulphur is also important nutrients required by the plants and it also present in insoluble form which is inaccessible to plants, thus transformation of insoluble form to soluble form is important for uptake of nutrients and its utilization by plants for growth and promotion. Some important process involved mineralization of organic sulphur to inorganic form like H_2S, the basic process is to interconversion sulphate ester-S and carbon-bonded S to inorganic sulphate by rhizobacteria and also includes some aerobic and anaerobic bacteria which includes *Pseudomonas* and *Clostridium* which releases S from sulphate esters using sulfatases, reaction is further catalyzed by bacterial multicomponent monooxygenase and bacteria which mobilizes sulphate esters includes *Pseudomonas, Klebsiella, Salmonella, Enterobacter, Serratia,* and *Comamonas* complex. Sulphide can be produced by various anaerobic bacteria which leads to breakdown of proteins to amino acids and further degradation leads to formation of sulphide and name of process is desulfurization [59-60]. Another aspect is to immobilize and assimilate sulphur into organic compounds by plants or microorganisms which leads to microbial conversion of inorganic sulphur into soluble forms or organic sulphur compounds. This sulphur is used for biosynthesis of amino acids and this sulphur is available for plants and fulfilled the nutrients need of plants. Immobilization is mainly occurring when cells die [61] and also by oxidation of sulphur and inorganic compounds of Sulphur. Oxidation is the process in which microorganisms converts hydrogen sulphide into elemental sulphur or into sulphate which is taken up the plants and also increases alkaline nature of soils. Some important rhizobacteria includes *Thiobacillus, Thiothrix, Sulfolobus*this are known as sulfur-oxidizing bacteria are important chemolithotrophs and play important role in sulphur cycle and improves soil fertility by decreasing pH of soil and increases the availability of nutrients by converting it into sulphate. Some important sulphur solubilizing bacteria includes *Chlorobium, Prosthecochloris, pelodictyon* and *Alcalochloris* [62].

Table 1 List of essential micro and macro nutrients of plants and their importance

Nutrients	Deficiency	Toxicity
Manganese	Chlorosis, decrease in photosynthetic rate	Brown spots on leaf
Zinc	Chlorosis, shortened internodes, affects protein and phytohormones synthesis	Compete with other nutrients leads to nutrients deficiency
Nitrogen	Weaker vegetative growth	Leads to early blight diseases
Phosphorous	Stunted growth	Decrease in pH
Potassium	Chlorosis and damages edges of leaves	Leaching problem in soil
Magnesium	Decrease in photosynthetic rate	Spot on leaves and Leaching
Calcium	Change in orientation of leaves, transportation and absorption rate affected	Damage of vascular tissues
Sulphur	Affects rate of nitrogen fixation	Decrease in pH of soil

Phytohormone production

Phytohormone (IAA) producing PGPR can enhance indirectly the availability of micronutrients by improving root development and growth. The exudates released by roots of plant also attract the wide range of PGPR with other plant growth-promoting traits such as siderophore production, phosphate solubilizing and ACC deaminase production. PGPR plays very important role in increasing the availability of micronutrients to plant by different mechanisms. This direct Mechanism enhance the availability of micronutrients like siderophore as a chelating agent, production of acid, and decrease of ethylene and subsequent increase of root system. Root exudates can also act as binding material agents of soil thus improve soil structure and regulate and maintain the microbial population near the root surface. Microbial activity near the root surface plays an important role in the development and rooting pattern of the plant. In addition, attracted PGPR themselves also produce many exudates, which are very helpful in plant nutrition and growth. The presence of various composites of amino acids, organic acids, sugars, vitamins, purines, adenine, guanine, cytidine, uridine, enzymes (e.g., phosphatase) and some gaseous molecules (e.g., CO_2) in root exudates [63] enhances the availability of micronutrients. A fraction of these exudates are further metabolized by PGPR in the vicinity as carbon and nitrogen sources, and some bacterium-oriented molecules are subsequently retaken up by plants for growth and development [64]. Another nutritional effect that organic acids have in root exudates is acidification of the rhizosphere, which enhances the availability of micronutrients. In general, PGPR, especially IAA producing bacteria, can increase the availability of micronutrients in the soil directly by the production of different compounds such as carboxylates, phenolic compounds, etc. or indirectly through affecting plant growth and hence the production of root exudates [65]. Chelating agents produced by PGPR play important role in increasing nutrient availability to plants by different ways mechanism of PGPR in the availability of micronutrients to plant.

Indirect mechanism

Siderophore production

Most plant-associated bacteria produce iron chelators and are known as siderophores in response to low iron levels in rhizosphere region. Siderophores are low-molecular weight organic compounds, which has affinity to bind some elements such as Fe^{3+}as well as other metal ions and increases their bioavailability. There is different mechanism by which plants make insoluble ions into soluble forms and which is then accessible to plants by different ways for plant growth and development of plants. Microbial siderophores are mainly used as Fe source for plants growth and for different strategies by plants. According to previous studies it is most probable that bacterial siderophores is not absorbed by plants and iron is obtained through reduction-based mechanism [66]. Ferric siderophores are transported into cells via specific siderophore membrane receptors allowing siderophores release for subsequent reuse [67]. The ability of siderophores to supply Fe^{3+} to roots surfaces and intracellular spaces of root cells. It is most important function of chelating compounds in plant nutrition. Higher concentration of Fe^{3+} ions which are available for root phyto siderophores in ligand exchange and is one of the important mechanisms of ion chelation and availability of nutrients and enhance their subsequent absorption by plants. Plants are also capable of using hydroxamates, ferrichrome, rhodotorulic acid, agrobactin and catecholate-hydroxamate as iron source and utilized it for plant growth and promotion. Most of siderophores form complex with other types of ions and like phosphate and it is assimilated by plants for growth and development [68].

Induced systemic resistance

Plants exhibit different types of mechanism which protects plants from attack of pathogens two types of resistance mechanisms. Depending upon resistance mechanisms two types of external stimuli are systemic acquired resistance (SAR) and ISR. SAR is executed upon the pathogen attack over plants, while ISR is switched on through colonization by PGPR [69-70]. SAR is executed through salicylic acid (SA) signal and any kind of difficulties in SA accumulation may impair SAR. On the other hand, in ISR, PGPR colonization does not manifest any symptoms over host plant [71]. PGPR trigger ISR either by strengthens the structural integrity (physical and mechanical strength) of the cell wall or by altering the host physiological and biochemical reactions. This phenomenon leads to the production of defense chemicals signals such as chitinase, peroxidase, and proteinase inhibitors [72]. Diverse root-associated PGPR that excite the plant immune system primarily through Jasmonic acid-Ethylene (JA-ET) signaling pathway in NRP-1 dependent manner [73]. In fact in some cases, PGPR mediated JA-ET dependent signaling pathway overlaps the SA dependent pathways. Under supply of iron, certain PGPR strains reported to produce SA as a siderophore [74]. ISR elicitors comprise cell wall components like lipopolysaccharides and flagellaas as well as metabolites. ISR-eliciting activity against wide array of fungal pathogens. Recently, multiple elicitors have been detected in *B. amyloliquefaciens* that act synergistically to induce ISR against different phytopathogens through different signaling pathway genes [75]. ISR activity was amplified through structural barriers, production of toxic substances (e.g., phenolics and phytoalexin), accumulation of molecules (e.g., chitinase) and hydrolytic enzymes (e.g., β-1,3-glucanases), which contribute in releasing oligosaccharides that results in stimulating other defence reactions. More or less the ISR control the pathogens over the outer region of the plant root cortex and other plant parts by modifying the cell wall integrity [76]. Such type of ISR was regulated by pathogenesis-related (PR) proteins, (chitinase and β-1,3-glucanase) and defence-related proteins, (peroxidase, PPO, phenylalanine ammonia-lyase) and phenolic compounds [77]. Apart from fungal disease management, progressive research in endophytes highlighted ISR activity in plants to cope up viral pathogens.

Nano biofertilizers

PGPR has potential of direct and indirect mechanism which enhances plant growth and productivity by replacing traditional chemical fertilizers and suppress its toxic effects. Exploring PGPR in agriculture is boon for agricultural sector and some PGPR fertilizers includes preparing consortia of various PGPR strains which apply directly to seeds or in soils for improving productivity. But transport of this consortia from Lab to field is difficult because loss of nutritional level and bacterial count may hinders the productivity of applied agricultural field therefore there is need to developed innovative method for removing the disadvantage which arises by using bacterial consortia thus Nano biofertilizers is one of nanotechnological advances which is one of the innovative method and plays effective role in promoting plant growth and they are rapidly absorbed by the plants due to their nanoscale size hence nano-materials such as nano-fibers, nano-fertilizers and nano-pesticides may produce revolutionary effects in agricultural sectors. Nanoparticles can be prepared by using bacterial consortium and consist of various methods sol-gel method and green synthesis method which uses certain type of chemicals as precursors for synthesis of nanoparticles and which can use in solid or in soluble form in field for enhancement of crop productivity. Due to its nanoscale size, it can easily penetrate into roots and plant parts and enhance productivity. Mainly three methods can be used for synthesis are physical (vaporization, radiolysis), chemical (chemical reduction, co-precipitation) and biological methods again classified as biosynthesis and biomimetic method, biosynthesis includes Fungi, yeast, plants, amino acids and biomimetic includes DNA/RNA, proteins. Its characterization is done by UV-VIS spectroscopy, FTIR, XRD. Therefore, PGPR along with nanomaterials has favorable strategy for managing growth and development of plants. It is achieved by synthesizing nanoparticles by green methods which includes zinc oxide nanoparticles and Manganese dioxide nanoparticles due to these kinds of important positive effects of PGPR along with nanoparticles thus it serves as pillar of agriculture

CONCLUSION

PGPR are soil borne bacteria inhabiting around the root surface and are directly and indirectly involved in promoting plant growth and development and also synthesizing various chemicals in vicinity to rhizosphere. Plant growth promotion is facilitated directly by either assisting in resource acquisition of important Micro- and Macro- nutrients like Manganese, Zinc, Nitrogen, Phosphorous, Potassium, Magnesium, Calcium, Sulphur or indirectly by phytohormone production and decreasing inhibitory effects of various pathogens on plants. Various study documented that the increased health and productivity of different plant species by application of PGPR under both normal and stress condition and plant beneficial rhizobacteria may decrease the global dependence on hazardous agricultural chemicals which destabilizes the agro-ecosystems. Therefore, PGPR are important for solubilization of important Micro- and Macro- nutrients and make them available for plants for proper growth and development. This review article focuses on role, deficiency and toxicity of two micro- and six macro-nutrients. It also concludes that role of PGPR in solubilization of the nutrients and act as biofortifying agent by improving root, stem and leaves structures and enhancing and contributing in proper transportation and translocation through xylem and phloem guided by calcium and stem elongation by upregulating the phytohormones synthesis like auxin by zinc ions and accelerates the rate of photosynthesis by regulating PSII complex by manganese's. Sulphur plays important role in fixation of nitrogen by converting insoluble nitrogen into soluble form like ammonia and nitrates. Therefore, integrative plant nutrition is essential component in sustainable agriculture because use of PGPR as biofortifying agent is most cost effective and environmentally friendly method for improvement of plant growth and promotion. Calcium act as biocontrol agent by increasing concentration when there is attack of fungal pathogen and suppress the proteolytic effects and protect plant from poison attack. Siderophores also act as iron chelating agents and give access if iron to plants and also shows certain induced systemic resistance against plant pathogen. This PGPR strains are mainly used in liquid form and known as consortium and it is applied to field for plant growth and biocontrol agents. Because it suppresses harmful effects on plants and promote growth. But transport of this consortium from laboratory to field is quite hazy and may leads to nutrients loss and minimize the bacterial count due to climatic change. Thus, there is need to develop innovative method to win drawn the disadvantages arise due to use of bacterial consortium. So nano biofertilizers is methods which is important and effective methods due to its nanoscale size and easily penetrate into roots and reach all over the plants and enhance crop productivity.

Future aspects

This Article mainly focuses on direct mechanism and indirect mechanism of plant growth and promotion after further study will proceed for actual results gain after implication of nana biofertilizers to field. And observe for proper plant growth and promotion and observed induced systemic resistance shown by PGPR which help the plant to fight against deleterious pathogens and act as biocontrol agents. Its aim is to prove organic farming is best way for crop improvement with no hazardous side effects on plants as well as environment and rules out problems which are associated with excess use of chemical fertilizers in field.

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