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ZN AS A VITAL MICRONUTRIENT IN PLANTS

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ABSTRACT

Macro and micronutrients are vital for the growth and productivity of the plants. Zinc (Zn) is considered to be one of the essential micronutrients for the growth and development of cereals as well as fodder crops. It is also a regulatory cofactor for all those enzymes which are required for the synthesis of chlorophyll, proteins and carbohydrates. The functioning of these enzymes is affected significantly due to Zn deficiency and there will be a retarded growth and productivity of plants. Deficiency of Zn is a universal problem among cereal crops. The concentration of Zn varies from 6-1.2 mg/kg in various soils, whereas its concentration reaches 20-300 ppm in plants. Zn deficiency leads to chlorosis in the leaves of plants. Various reasons affect the availability of Zn in the plants, which include soil type, pH of the soil and availability of other nutrients that work antagonistically for the absorption of Zn. Zn applied as the fertilizer gets converted into unavailable form by making insoluble complexes and thus not available for plants. Hence the best alternative to this issue is the use of Zn solubilising bacteria (ZSB). These ZSB will accumulate in the rhizosphere zone of the plants and will reduce the requirement of the applied Zn fertilizer. It will prevent Zn toxicity in the soil and will enhance the uptake of other macronutrients like phosphorus to the plants.

Keywords: Bio fertilizer, Zn, PGPR, Zn Solubilising Bacteria

INTRODUCTION

Appropriate nutrition for plants is one of the vital factors for improving the quality and quantity of product obtained from them. Macronutrients and micronutrients are the essential elements needed by the plants from the germination of the seed up to the fully developed plant. Elements that are required in large quantity are categorized as macronutrients, and those that needed in trace amount are micronutrients (**Sharma *et al.*, 2013**). Micronutrients are very much essential for the growth of plants and their metabolism. The tremendous use of micronutrients in the soil for agricultural purposes cannot be denied. Nutrients like boron (B), chlorine (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and Zinc (Zn) are classified as micronutrients. These metals are considered very significant for plants even if they are taken up in comparatively lesser amounts by the plants (**Grusak *et al.*, 1999**). These micronutrients protect plants from various biotic and abiotic stresses by engaging in numerous roles. However, micronutrient deficiency may lead to multiple diseases in plants, which adversely affects the quantity and quality of the plant's products (**Huber and Wilhelm, 1988**).

Zn (Zn) plays a phenomenal role as a micronutrient in plants. Even if it is required in small quantities by most of the plants, the correct amount of Zn is necessary for the proper functioning of several plant physiological pathways that plays a salient role in their growth and development (**Mousavi *et al.*, 2011; Yosefi *et al.*, 2011; Cabot *et al.*, 2019).** Zn is necessary for various biochemical processes such as synthesis of cytochromes, nucleic acid and activation of enzymes. It is an integral factor of different enzymes playing a significant role in carbohydrates metabolism, oxidation reactions in plants, and various revival mechanisms (**Alloway, 2001).** Zn is an essential cofactor for enzymes required for specific protein biosynthesis and prominent in chlorophyll formation. Zn is necessary for the activity of the enzyme rubisco. Therefore, it has a significant role in regulating the photosynthesis rate in higher plants (**Das *et al.*, 2018**). Zn is necessary to maintain membrane activity and maturation of seed and stalk of plants with an increased rate. Zn also has an intrinsic role in auxin formation, which assists the growth of plants (**Barman *et al.*, 2018**). Hence Zn has an indispensable role in plant development and its productivity.

Cakmak. (2000) note that around 50% of the land utilized for growing crops across the world has a low concentration of accessible Zn, which is needed to support crops' yields and hence increase the nutritional value of the grains. Zn deficit in the soils as well as in the plants has become an issue in most of the countries. The main reasons responsible for the occurrence of this widespread deficiency of Zn are Zn's solubility in the soils is very low despite enough availability of the total amount of Zn. The deficiency of Zn in plants is a significant widespread deficiency compared to other micronutrients, which affects the rate of photosynthesis in various cereal crops (**Barman *et al.*, 2018).** Zn deficiency leads to discolouration of leaf, and this condition is termed chlorosis (**Sharma *et al.*, 2013).** Zn becomes a prooxidant by generating reactive oxygen species when it is in excess or even deficient in plant cells, leading to damaging the plant cell (**Cabot *et al.*, 2019).**

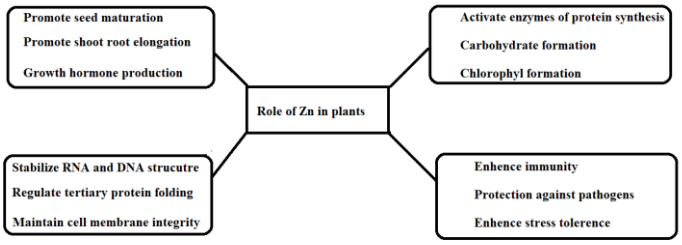

Figure 1 Application of Zn in Human, Plants, Animals and Microbes

Uptake of zinc chemical fertilizer

Plants uptake Zn as a divalent cation (Zn^{2+}); however, only a very minute amount of Zn is readily available as soluble Zn for plant uptake (**Marschner, 1993; Gao *et al.*, 2009).** When soluble forms of zinc are applied to the soil in chemical fertilizer, it gets converted into insoluble complexes. It becomes unavailable to the plants leading to the deficiency of Zn in plants. This results in Zn deficiency, and there are various methods to assuage this critical problem. The application of Zn fertilizer is one way to combat Zn deficiency (**Kamran *et al.*, 2017**). Zn fertilizers are generally used to facilitate crop yields in regions of the Zn deficit. The utilization of fertilizers containing Zn increases the productivity of crops (**Efe and Yarpuz, 2011**). However, the extensive use of fertilizers can cause a myriad of problems like acidification of the soil. When

Zn is applied as zinc sulphate, it gets converted to insoluble complexes within seven days. Overuse of zinc sulphate leads to the accumulation of insoluble Zn in the soil. It causes Zn toxicity which later inhibits the absorption of other macronutrients, thus behaving antagonistically the absorption of other required nutrients. Under such conditions, further application of Zn in the next crop resulting in accumulation of Zn in the soil in a chelated state. When the concentration of total Zn increases to 100-1000 ppm in soil, it leads to Zn toxicity. Zn toxicity then becomes a significant issue its deficiency **(Marten *et al*., 2013)**. Hence the overuse of chemical Zn fertilizer will generate the problem of heavy metal toxicity.

Two strategies are employed to overcome Zn deficiency 1) genetic biofortification 2) agronomic biofortification. Genetic biofortification involves the use of breeding practices and transgenic approaches. In inbreeding practices, the genetic morphology of plants is transformed to create suitable characters. However, the developed variety poses specific issues like instability of newly integrated traits. Further, it is a prolonged, labour-intensive technique that may take many years to build and perfected a biofortified variant.

Moreover, the complex laboratory procedures and the high cost involved also present challenges in the practical implementation **(Hafeez *et al*., 2013)**. The transgenic approach includes augmenting biofortified crops. **Abaid-Ullah *et al*. (2015)** showed that the expression of transcription factors such as bZIP19 and bZIP23 resulted in increased bioavailability of Zn in the plants. Transport protein of plasma membranes is the main target of Zn concentration alteration in various regions of plants. In the agronomic approach, Zn fertilization is given to plants which ensure Zn translocation for a limited duration. Zn sulphate, which is transportable, can amplify the supply of Zn concentration within the plants **(Kamalakannan *et al*., 2019).**

Rhizospheric bacteria

The soil is the home of microscopic life forms like fungi, bacteria, algae, and protozoa, found in abundance. Some of them are pathogenic to the plants, some do not interact with them and while others are beneficial. Bacterial cells are found in abundance as compared to others. The type and concentration of bacteria depend upon the pH of the soil, type of soil, concentration of moisture in it, types of plants grown there, and the nutrients present there. Likewise, the presence and abundance of bacteria are not homogenous in the same soil. The concentration is higher around the roots of the plant. These potential bacteria present in the narrow region of soil around the bases (rhizosphere soil) of most of the plant are called rhizosphere bacteria **(Vacheron *et al*., 2013)**. This narrow zone of root-soil is very high in nutrient concentration than the overall surface or subsurface soils because of the accumulation of several root exudates, sugar and various amino acids, and the primary nutrients the energy source for microorganisms. This is why the colonization of bacteria in the ecological niches of the root makes their habitat in the root zone **(Abaid et al., 2015; Mumtaz *et al*., 2017)**.

The way these rhizospheric bacteria will behave depends upon many factors. They can enhance metal absorption and promote plant growth, or they may decrease the absorption of specific macro or micronutrient because of other metal nutrients. **)**. Depending upon their mode of action, bacteria can be categorized as growth-promoting or growth-inhibiting bacteria **(Sirohi *et al*., 2015).** Bacteria that promote plant growth are called plant growth-promoting bacteria or PGPR. All those bacteria that improve plant health and its productivity come under the category of PGPR; they support the development even when the competing microflora is present in the rhizosphere. In General, around 2-5% of rhizospheric bacteria are in the category of PGPR. Other factors that influence the mechanism of PGPR are the concentration of the nutrients, applied fertilizers or the type of crop grown. One such example is when *Pseudomonas fluorescein* used to cut blackcurrant stimulates root development whereas works opposite in cherry **(Dubeikovsky *et al*., 1993)**. These PGPR can be free-living, live in rhizosphere soil in a symbiotic relationship with the plant, or be entophytic bacteria that live within plant tissue in colonized form. These PGPRs have two mechanisms: the direct one and the indirect one in promoting the plant's growth. Either they provide the plants with the nutrients they take from the soil, or they synthesize the growth hormones, and the indirect way is by inhibiting the attack of pathogens **(Glick, 1995).** Zn Solubilising bacteria, or we can say Zn solubilizing PGPR finds their role as bio inoculants as they solubilize the insoluble Zn forms present in soil by various mechanisms like production of organic acids, decreasing the pH of the soil, by synthesizing siderophores and by chelating through the output of anions and makes the Zn available to the plant. The application of PGPR to make the already present zinc available to the plants leads to reduced chemical fertilizers **(Liu *et al*., 2015)**. As Zn has gained much importance in recent years due to its increased significance in obtaining higher yield and productivity in cereal crops **(Dell *et al*., 1985)** and research related to the role played by Zn in plant growth and human health has generated a great curiosity, and it has become a significantly important topic of study. This review focuses on the role of Zn in plant growth, its significance as a micronutrient and the application of Zn Solubilising PGPRs to promote sustainable development and reduce the use of chemical Zn fertilizer.

Distribution and bioavailability of Zn

The natural sources of Zn in soil include weathering of rocks into the soil and atmospheric contributions (fires, dust). In soils, the quantity of total Zn is distributed in five fractions including the Zn found in soil solutions, Zn bound to particles of soil in the form of ions, Zn complexed with organic ligands, adsorbed Zn^{2+}, Zn adsorbed on to clay minerals and insoluble metallic oxides **(Alloway, 2001)**. Zn is available in the soil as Zones (sphalerite), and sometimes in small quantities as smithsonite ($ZnCO_3$), Znite (ZnO), zinkosite ($ZnSO_4$); these are Zn containing mineral ores **(Hafeez *et al*., 2013).**Aggregate Zn profile of soil is mainly dependent on the geographical composition of rocks. The total Zn content in earth crust is 78 mg of Zn per kg of soil **(Noulas *et al*., 2018)**. **Salah *et al*. (2015)** reported that the concentration of Zn which is present in soluble form and available to plants is approximately 4 to 270 μg L-1 (ppb) in most of the soils. This concentration is too low when it is compared to the total Zn concentrations that range from 50 to 80 ppm. In highly acidic soils, the concentration of soluble Zn is about 7137 μg L-1 which indicates that solubility is strongly linked to and is inversely proportional to pH of soil. It is reported that concentration. Of Zn ranges from 10-300 μg Zn g-1 of soil in the majority of arable soils **(Gupta *et al*., 2016).** Although Zn is present in abundance in such soils, a large amount exists in an insoluble form (zinc sulphate, zinc oxide, zinc chloride, zinc phosphate and zinc carbonate) and hence unavailable to the plant. Only the Zn that can be desorbed easily and is available in soluble fractions is used by the plants, but that fraction of available Zn is very low. Moreover, the accessibility of Zn is determined by the physicochemical properties of soils, plant root activity and presence of micro flora in the rhizosphere zone of the plant **(Broadley *et al*., 2007)**. Zn interacts with chloride, sulphate, phosphate, and nitrate ions to form soluble complexes. Out of these, nitrates, chlorides and sulphates are soluble in water and pose no hindrance in Zn availability to plants **(Prasad *et al*., 2016)**. The neutral Zn phosphate ($Zn_3(PO_4)_2$) and Zn sulphate ($ZnSO_4$), species are the very crucial ones as they have been recognized as the prime ones for contributing Zn in soil solution. Addition of Zn sulphate leads to increase in the solubility of Zn^{2+} ions within the soils, Therefore, the presence of acidifying fertilizers, such as ammonium sulphate ($NH_4(SO_4)_2$), which reduces the pH of the soil and makes the soil acidic and hence increase the solubility which leads to the increased availability of Zn to the plants**(Alloway, 2002)**. The Zn which was earlier in organically complexed form, after acidification of the soil becomes increasingly mobile. Now when the Zn exists in its soluble structure in soil then it is easily accessible to plants **(Shambhavi *et al*., 2019).**

Adsorption of Zn by soil constituents

Adsorption and precipitation are two methods which are responsible for regulating the concentration of various ions in the soil. Adsorption of Zn cations which are exchangeable with other metal cations present in soils is expressed in the simplest way as:

$$Zn^{2+} + X\text{-}Soil \leftrightarrows Zn\text{-}Soil + X^{2+}$$

Where X is any of the divalent cations.

Cations get attracted to negatively charge solid surfaces in preference and selectivity, which varies based on the various kinds of adsorbents such as hydrous oxides, clay minerals, and sticky substances depending upon the chemical composition of the soil. The cation exchange capacity of soil will determine how efficiently the soil will supply nutrients to plant **(Diatta and Kocialkowski, 1998).** **Speir *et al*. (2003)** studied the adsorption of Zn on calcareous soil in which severe Zn deficiency problems in crops is being found. They found that some concentration of total Zn had been irreversibly fixed to the soil, making the reaction irreversible. According to **Alloway (2002),** there are two non-identical methods required for Zn adsorption by the soil and organic matter present in it. One is the cation exchange method, which is primarily applicable for acidic soil conditions and other alkaline soils, mostly involving chemisorptions and complex formation by organic ligands.

Factors affecting the Zn availability to plants

Many factors are responsible for affecting the Zn availability to plants, such as the total content of Zn present in the soil. The solubility of Zn in the soil is very low; hence many times, despite sufficient concentration in the soil, the Zn availability to plant remains low. As Zn is a trace element, it occurs in the soil in various chemical forms that affect its availability to the plants. It remains surrounded by various metals; it does not get accumulated on the soil surface; instead, it makes complexes with other heavy metals and gets precipitated. This matrix of Zn with aluminium, iron and manganese oxides, and other silicates and carbonates, impose their control over the availability of Zn to the plants (**Alloway, 2008**). The pH of the soil is also one of these factors. In highly acidic soils, the mean value of available Zn is around 7.12 ppm, indicating that the concentration of soluble Zn is strongly associated with the pH and is inversely proportional to the pH of soil. It was reported that the concentration of total Zn ranges from 10-300 ppm of the majority of agricultural soils **(Gupta *et al*., 2016).** At the same time, the total concentration of available Zn decreases by 100 folds for every unit of pH increase (**Moreno Jiménez *et al*., 2019**). Another reason affecting Zn availability to crops is the profile of the soil, geology and erosive processes, clay consistency, calcium carbonate available in the soil, redox conditions of the land, moisture status of soil, the concentration of various other micronutrients, macro-nutrients availability importantly phosphorus and climate conditions of the area control the sorption-desorption process of Zn ions in the soil and cumulatively affect the availability of Zn to plants **(Rutkowska *et al*., 2015)**. Organic matter present in the soil also affects its availability. Such calcareous soil has low organic matter; hence, Zn remains in an inactivated form (**Barman *et al*., 2018**).Microbial activity present in the rhizosphere zone of the plant controls the amount of Zn that will be made available to the crop. It is also affected by various anthropical activities, such as the application of fertilizers, over-irrigation, and industrialization near the agricultural and urban sewage discards. (**Dos Santos *et al*., 2013**). These factors cumulatively hinder or facilitate Zn availability to plants.

Uptake and translocation of Zn in plants

Assimilation of Zn micronutrient from the soil surface to the rhizosphere of a plant is the principal approach for its accretion inside the plant precedent to its transportation to seeds **(Hafeez *et al*., 2013)**. Although the Zn uptake changes amid different species, it is usually calculated via the composition and concentrations of the media used for growth **(Tsonev and Lindon, 2012)**. The assimilation of Zn varies among the grains of different crops and their species **(Wu *et al*., 2010)**. Zn concentrations in different cultivars of rice are found to be very different **(Yang *et al*., 1998; Graham *et al*., 1999)**. The mechanisms through which Zn gets transferred from soil to plant are much explored and understood. The basic physiology of varied Zn uptake efficiency is well documented **(Wheal *et al*., 1997; Hacisalihoglu *et al*., 2003; Genc *et al*., 2009; Widodo *et al*., 2010)**. However, the translocation of Zn and its redistribution after entering the transpiration stream is very meagre. **Jiang *et al*. (2007)** reported that Zn accumulation is related to many factors amongst which its uptake by the root system plays a crucial role. Zn status within the plant is further dictated by the redistribution and remobilization of stored Zn. Zn uptake by the plant occurs when Zn complexes with organic ligands or is available in its divalent ion form. Uptake of zinc follows a linear relationship with its concentration in soil or solution of nutrients **(Tsonev and Lindon, 2012)**. The Zn is transported from the roots to the shoot tissues through the xylem. Later, apoplast help in the translocation of Zn to xylem of roots **(Broadley et *al*., 2007)**. Moreover, it is found that the phloem also had high levels of Zn, it indicates that Zn is translocated to the plants leave by both phloem and xylem tissues **(Haslett *et al*., 2001).**

Zn is primarily absorbed by roots as Zn^{2+} from the soil solution or as Zn $(OH)_2$ when the pH of the soil is high. The process is found to be regulated by proteins having high affinity for Zn. Wheal ***et al***. (1997) proposed that Zn is transported inside the plant cell towards larger negative electrical potential and this process is thermodynamically passive. It is also found that metabolic inhibitors do not have any impact on Zn^{2+} uptake and therefore the process of Zn uptake is independent metabolically. Irrespective of the kinetics of Zn uptake, its mobility in plants occurs via making a bond with light organic compounds in xylem fluids (Salah ***et al***., 2015). Bowen ***et al***. (1974) studied the impact of temperature on adsorption of Zn in *Pinus radiate* roots and demonstrated that Zn absorption by plant roots is inhibited by low temperatures

Biochemical and physiological functions of Zn in plants

The metabolic attributes of Zn are assigned to its capacity of forming complexes with N, O and S inside the plant cells. Intracellular Zn remains inactivated by forming nexus with organic ligands **(Tsonev and Lindon, 2012)**. Zn affects the uptake of water and its transportation capacity inside the plants **(Kasim, 2007; Disante *et al*., 2010)**. Zn is also found to decrease the negative consequences of heat and salt stress **(Peck and McDonald, 2010; Tavallali*et al*., 2010)**. It is actively involved in the synthesis of auxin, which is an essential growth hormone. Zn is needed to synthesize tryptophan, which leads to IAA (a heteroauxin) synthesis by activating tryptophan synthetase **(Brown *et al*., 1993; Castillo-González *et al*., 2018)**. Zn has an active role in the transduction of the signals **(Hänsch and Mendel, 2009)**. Zn also acts as the prosthetic group of several enzymes such as RNA and DNA polymerases, aldolases, dehydrogenases, isomerases, and Trans phosphorylases **(López-Millán *et al*., 2005)**. Zn is a catalytic as well as structural protein cofactor in thousands of proteins. There are three Zn ligands binding sites, namely structural, catalytic, and catalytical. Structural Zn sites make sure proper protein folding by Zn. In catalytic sites, Zn is directly included in the catalytic functioning of enzymes. In a catalytical site, Zn is present in close propinquity and is connected via glutamic acid (Glu), Aspartic acid (Asp) or histidine (His) and bound by water **(Broadley *et al*., 2007)**. Protein synthesis and energy production are dependent on Zn. Zn is involved in regulating various biochemical reactions in the process of photosynthesis and is needed for repairing the photosynthetic apparatus, as it turns over the D1 protein that gets photo-damaged **(Bailey *et al*., 2002; Hänsch and Mendel, 2009).**

Zn finger protein arbitrates protein-protein interactions **(Cabot *et al*., 2019)**. Zn helps preserve the structure of macromolecules and has a primary role in the maintenance of cellular membrane integrity by making interaction with sulfhydryl groups and phosphor lipids of membrane proteins. It also aids in maintaining the transportation of ions within plant cells **(Disante *et al*., 2010; Salah *et al*., 2015)**. The plant cell membranes destabilized due to Zn deficiency could not be reversed quickly. They could lead to the accumulation of boron and phosphorus due to their non-selective entry of boron in the Zn deficient roots and leaves **(Holloway, 1996).** Zn is needed for synthesizing nucleic acids; it also maintains the stability of nucleic acids by forming complexes **(Coleman, 1992)**. It is also involved in the metabolism of lipids **(Marschner, 1993)**. It is observed that Zn deficient plant is also deficient in its protein content, which occurs due to the deformation of ribosomes. A significant reduction in the synthesis of both RNA and ribosomes in the plant occurs due to Zn deficiency. This shows that Zn is also required for protein synthesis **(Brown *et al*., 1993)**. Zn is needed for carbohydrate fixation. It regulates the formation of reactive bicarbonate species from carbon dioxide, especially in C4 plants and maintains a high CA activity. Zn aids in the active functioning of PEP carboxylase by shifting the equilibrium in favour of its substrate HCO_3 and maintains enough supply of HCO_3 in guard cells where Zn also determines the K^+ ion influx **(Brennan, 2005; Sharma *et al*., 2013).** Zn plays a vital role in carbonic anhydrase activity. Zn is also needed for the respiratory enzyme, quinone assimilation, and altering amino acids and protein **(Castillo-González *et al*., 2018)**.

In addition to this, Zn also prevents damage of membrane, which is caused by Reactive Oxygen Species (ROS) and is needed for the synthesis of an antioxidant enzyme that is H2O2 scavenging ascorbate peroxidase which is synthesized in response to the oxidative stresses **(Alscher *et al*., 1997; Cakmak, 2000)**. One of the primary functions of Zn is the regulation and expression of genes as Zn finger transcription factors. These are involved in the regulation mechanism of various biological processes like flowering, pathogen responses and photomorphogenesis **(Castillo-González *et al*., 2018)**. Zn is also found to be vital for flowering and seed production. **Brown *et al*. (1993)** demonstrated that when Zn deficient plants are treated, it has increased the number of inflorescences and yield of seeds compared to the production of dry matter or the seed size in subterranean clover. Metal response element-binding transcription factor 1, a Zn discerning molecule, responds to free levels of Zn by regulation of gene expression to sustain homeostasis of Zn. Zn acts on intracellular signalling molecules by imitating the actions of hormones, growth factors and cytokines. It also inhibits the protein tyrosine phosphatase **(Yamasaki *et al*., 2007).**

Zn and its interaction with metalloenzyme in plants

Zn is a substantial part of an enzymatic cofactor. A cofactor is a component present in the enzymes and enhances their catalytic activity. An enzyme needs a metal ion to initiate the catalytic activity; therefore, metal ions are a significant part of most of the cofactors. Most of the metal ions have a close interaction with the respective enzymes. The trace metal ions link the substrate to their respective enzyme. Hence the ions accept electrons; they stabilize the structure of enzymes and regulate the speed of biochemical reaction **(Eide, 2011).**

Zn is very crucial for various plant enzyme systems, including Carbonic anhydrase, several dehydrogenases: alcohol dehydrogenase, glutamic dehydrogenase, L-lactic dehydrogenase, malic dehydrogenase, D-glyceraldehyde-3- phosphate dehydrogenase, and D-lactate dehydrogenase, Aldolase Carboxypeptidase, Alkaline phosphatase, Superoxide dismutase, RNA polymerase, Ribulose bi-phosphate carboxylase, and Phospholipase **(Sharma *et al*., 2013).** Unlike other micronutrients, Zn is present as a transition element and does not undergo a change in valency and exists only as Zn (II) in plants **((Patel *et al*., 2007)**. In plants, nearly 70 Zn containing metalloenzyme have been reported to date **(Zastrow and Pecoraro, 2014).** Zn is a structural, functional and regulatory cofactor of the enzymes belonging to all the enzyme classes. Zn is omnipresent of all metallic cofactors, and more than 300 enzymes have Zn as a cofactor. As Zn exists in the Zn^{2+} form, therefore; it does not have any redox properties **(McCall *et al*., 2000)**. It makes substantial nexus with free radicals of the polar groups that contain N, O and S **(Alloway, 2001; Lin *et al*., 2005)**. Atoms of Zn that are tightly bound to the apoenzyme are difficult to remove even if they are chemically treated. Zn usually bound through imidazole and cysteine rings in enzymes requiring Zn for their activity. The binding of a water molecule to the cooperative is required for the catalytic activity of Zn. Therefore, catalytic Zn makes the bond with one molecule of water and three protein ligands. On the other hand, in all those enzymes where Zn has a regulatory or structural role, Zn bound with four ligands **(Auld and Bergman, 2008).**

Alcohol dehydrogenase catalyzes reverse oxidation; the enzyme has two Zn atoms. The first one is for catalytic function and the second one has a structural function. Alcohol produced under anoxic condition because of waterlogging is metabolized by alcohol dehydrogenase. The anoxic condition leads to oxygen reduction in soil. This can result in the production of toxic substances and increase the concentration of various natural compounds like carbon dioxide, organic acids, sulphides, and hydrocarbons (gaseous). During Zn deficit, alcohol dehydrogenase activity subsides to a low limit. Moreover, this limitation is linked to reducing root binding capacity **(Hafeez *et al*., 2013)**.

Carbonic anhydrase, a metalloenzyme that requires a Zn cofactor, catalyzes instant change of carbon dioxide and water to bicarbonate ion (HCO^{3-}) and a proton. Zn ions activate the catalytic site of the enzyme by reacting with water and thus catalyzing carbonic anhydrase. The enzyme is responsible for numerous operations like pH regulation, photosynthetic CO_2 fixation, respiration, CO_2 transfer, ion exchange and stomata closure. Carbonic anhydrase is required in numerous physiological processes. Any alterations in Carbonic anhydrase activity promptly impact the photosynthetic fixation of carbon dioxide under the carbon dioxide limiting conditions. Carbonic anhydrase activity is thus dependent on atmospheric carbon dioxide levels. Zn deficit will eventually affect Carbonic anhydrase and influence all the processes linked to the enzyme **(Castillo-González *et al*., 2018).**

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide and hydrogen peroxide. Therefore, it has a role in plant defence against antioxidant. At the active site of an enzyme, Zn links with copper to form Cu-Zn SOD. During Zn deficiency, SOD activity decreases. There is a surge in oxygen production as Cu-Zn SOD is responsible for controlling the generation of toxic oxygen radicals. As toxic oxygen radicals rise, the plasma membrane permeability also surges. This elevation results in depletion in sugar, amino acid, and potassium (**López-Millán *et al*., 2005)**. Zn controls the inception of toxic oxygen. It impairs the NADPH oxidation as harmful oxygen-free radicals disintegrate the polyunsaturated fatty acids and phospholipid membrane bonds. Zn is required to perpetuate membrane integrity as it can bond membrane phospholipid groups and configure tetrahedral groups with cysteine residues **(McCracken *et al*., 2013)**.

Zn interaction with other nutrients present in the soil

Interaction with macronutrients

Interaction of Zn with other macronutrients and micronutrient is a very crucial factor in plant production. For example, in some soils, nitrogen promotes the growth of plants and changes the pH of the root area and thus affects the Zn status of crops, whereas, in many other soils, nitrogen is found to be the significant factor in inhibiting the growth and yield of crops. Thus, nitrogen and Zn fertilizers, when applied in combination, significantly improved crop yields due to the positive interactions among the two nutrients (Shri *et al*., 2017). At the same time, Kirk *et al.* (1995) reported that when nitrogen fertilizers are applied alone, they led to a deficiency of Zn. Nitrogen fertilizer enhances plant growth, but it also shows negative interaction with various other micronutrients, such as copper. Plant growth which is promoted by the application of nitrogen fertilizer, causes reduces copper concentration in the plant, which is further made worse by applying Zn fertilizer. The application of nitrogen fertilizers exacerbates Zn-Cu interaction. In solution culture experiments, many micronutrients such as magnesium, calcium, sodium and potassium are amongst the one that inhibits the Zn absorption by the plant root system. Within the soil, they affect the pH of the soil. CaSO4 application decreased the pH of the soil, so it enhanced the mobility of Zn ions and expanded uptake by the plants. In contrast, application of the same concentration of $CaCO_3$ led to an increase in pH, which leads to a decrease in free ions and therefore retards the uptake of Zn. (Alloway, 2002; Golubovic et al., 2012).

Interaction with micronutrients

Zn interacts with micronutrients such as boron, iron, copper, and manganese and affects the concentrations of these micronutrients in plants. Interaction of Zn with these metal ions has shown varied responses in plants. **Loneragan and Webb (1993)** reported that when Zn is applied with iron, it increases the supply of Zn to the plants. However, iron concentrations were found to increase in some plants, decreases in some, and even affect Fe concentrations. According to **Imtiaz *et al*. (2003)**, the use of Zn fertilizers had adversely affected the concentration and intake of Fe in plants. The Roots of the Zn deficient plants have shown more iron mobilization from Fe^{2+} hydroxides compared to the plant roots that have adequate Zn. Zn and copper, both micronutrients, share the same site on the root for absorption. Therefore, the application of Cu does affect the absorption of Zn in plants. Interaction of both Manganese and boron with Zn might have both positive and negative responses in plants regarding the availability, uptake, and assignment of Zn in the plant. Zn-deficient plants absorb more boron due to decreased membrane function in the plant's root than plants having sufficient Zn **(Mousavi *et al*., 2012).**

Role of Zn in plant defense

Zn plays a substantial role in plant growth. It has an equally important role in plants defence mechanisms in response to insects and pathogens **(Yamasaki *et al*., 2007)**. According to **Li *et al*. (2016)** application of Zn fertilizers ensure a remarkable decline in disease symptoms in plants. Moreover, **Helfenstein *et al*. (2015)** observed that sometimes a particular concentration of Zn used against a particular pathogen could make that plant susceptible to other pathogens. It shows that the Zn proteins, which are involved in the plant defence, play a dual role, and they can support as well inhibit the plant growth and the invaders simultaneously. The response of plant and the pathogen towards the application of Zn is greatly dependent on the ability of pathogens to survive the plant defence mechanisms and whether the applied zinc is inhibiting or combating the pathogen attack. It may also depend on the conditions of the surroundings favouring either the plant or the pathogen **(Grewal *et al.*, 2001).**

Cabot *et al*. (2019) described Zn mediated protein-based mechanisms for plant defence. The mechanism studies address effective plant defence with increased gene expression or enhanced activity of the referred protein function by enhancing Zn availability to the pant. However, it must be made clear that Zn proteins alone are not involved in these defence mechanisms; other responses related to Zn are also combined. **Machado *et al*. (2018)** documented that Zn is involved in two broad-spectrum responses in plant–pest/ pathogen interactions. These include oxidative stress and regulation of Zn finger proteins. In the case of Zn deficiency, reactive oxygen species (ROS) are contemplated to be the chief factor accountable for hindrance to plant growth. The plant immune system causes oxidative damage or triggers non-oxidative mechanisms via ROS to combat pathogen attack. The oxygen radicals are triggered by plant defence mechanisms post identification of attack by pathogens on plants. The defence mechanisms mainly are hypersensitive responses and systemic acquired resistance (SAR). The Superoxide dismutase, an antioxidant enzyme that controls the activity of oxygen radicals, is increased during pathogen attack **(Gupta *et al*., 2012)**. Plant response to toxic Zn concentration is via regulating nitric oxide (NO), and systemic defence of plant response against stress is by regulating salicylic acid and jasmonates (JA) **(Helfenstein *et al*., 2015)**. Response of plant and pathogen towards the application of Zn also depends on the conditions of the surroundings that can favour either the plant or the pathogen **(Cabot *et al*., 2019).**

Deficiency of zinc and appearance of symptoms in crops

Inadequate supply of Zn to plants is called Zn deficiency. Zn deficiency in crops is a severe problem that predominantly affects food production **(Alloway, 2001; Welch and Graham, 2002)**. Zn deficiency leads to impairment of many essential Zn dependent physiological functions and has adverse effects on plant growth **(Sadeghzadeh, 2013).** However, almost all Zn deficient crops positively responded to Zn application **(Welch and Graham, 2002)**. The deficiency of Zn in plants is because of many factors like low Zn availability, low total soil Zn concentration, and high levels of nitrogen, calcium carbonate, bicarbonates, organic matter and high pH soil. Zn deficiency is very dominant in sandy soils, calcareous soils, peat soils, clayey soil and the soils that have increased silicon and phosphorus concentration **(Alloway, 2001).** Soil formed from gneiss and granite is also low in Zn **(Sadeghzadeh, 2013)**. Soils rich in phosphates also tends to make Zn unavailable to the plant **(Imtiaz, 2003). Spirit *al.* (2003)** also investigated the concentration of Zn in different types of soil and observed that Spodosols (28 ppm) and luvisols (35 ppm) are Zn deficient while fluvisols and histosols were found to have higher Zn concentrations corresponding to (60 ppm) and 58ppm respectively. The submerged soils are predominantly Zn deficient as submergence and flooding lead to a reduction in available Zn by forming insoluble Zn complexes and change in pH. **Sajwan (1988)** reported that rice cultivation under the submerged conditions requires the transformation of Zn into amorphous sesquioxide precipitates or franklinite; ZnFe2O4.Another contributing factor reported was restricted root exploration in highly compacted soils, including high water table. This is particularly true for soils with a marginal Zn status **(Alloway, 2002)**.

Zn deficiency may also result from seasonal changes and becoming prominent in cold and wet weather conditions. The condition can be accounted for the reduced microbial activity under cold conditions. The low release of Zn which is complexed with organic matter leads to limited root growth. Zn deficiency may also vary with Crop type **(Alam *et al*., 2010)**. Different crops have been found to have different comparative sensitivities to the deficiency of Zn. The Food and Agriculture Organization mentioned that approximately 30% of the agricultural soil worldwide has a deficient level of soluble Zn accessible to plants **(Sillanpaa, 1990)**.

Zn deficiency might lead to multiple symptoms in plants. **Hafeez *et al.* (2013)** reported that in rice seedlings, symptoms of Zn deficiency usually appear three weeks after transplantation. The symptoms may vary from brown blotches to streaks that cover the entire surface of the older leaves, resulting in stunted growth. In severe cases, the plant may die. Moreover, the plants that recover manifest a delay in maturity and a substantial reduction in yield. Symptoms of zinc deficiency start occurring at the earliest on the new leaves as Zn cannot be transferred to new tissues from the older ones. The areas between nerves start appearing yellow in the leaves **(Vitosh *et al.*, 1998)**. **Mousavi (2011)** described that in dicots, internode distance and leaf size is shortened due to Zn deficiency. In monocots (especially corn), bands start appearing in the central nerve on both sides of monocot leaves.

Zn and its association with crop productivity

It is evident from the studies that Zn is necessary for the proper functioning of many important plant physiological pathways. Hence, the concentration of Zn available to plants is very critical **(Alloway, 2002)**. An appropriate amount of Zn is necessary for the proper regulation of such pathways **(Mousavi *et al.*, 2011; Yosefi *et al.*, 2011)**. The deficiency of Zn in plants is most widespread throughout the globe compared to the deficiency of other micronutrients **(Alloway, 2001).** As discussed earlier, Zn has a vital role in enzyme activation, protein synthesis, and carbohydrates metabolism and revival reactions. **Efe and Yarpuz. (2011)** reported that the performance of crop and the quality of their products increase by utilizing Zn containing fertilizers. In contrast, there is a sharp decline in photosynthesis and, therefore the productivity due to the shortage of Zn. Zn also acts as a regulatory cofactor of many different enzymes other than those associated with photosynthesis. It is a structural constituent of the proteins involved in critical biochemical pathways related to auxin metabolism, carbohydrate metabolism, protein metabolism, photosynthesis, pollen formation, sugars to starch conversion and plants defence mechanism **(Alloway, 2001). T**herefore plays a significant role in crop production (**Graham *et al*., 1992)**. Chemical fertilizers are most used to deal with Zn deficiencies, but the cost of these fertilizers is usually too high. Therefore, there is a need for suitable alternative methods **(Alloway, 2002).**

PGPR for dealing with Zn deficiency

Preventing the occurrence of Zn deficiency and using appropriate management methods to regulate the Zn concentration in the soil are two ideal ways to deal with the widespread problem of its deficiency **(Alloway, 2002; Mousavi *et al*., 2011)**. Due to an increased demand for animal and human food, there is a need to increase edible plants and fodder crops. Hence, farmers' application of fertilizers in crops is the most preferred method used to increase productivity following the population's demand. The agricultural departments of various countries focus on enhancing biofertilizers in place of chemical ones. Recently, environment-friendly methods are being implemented in agriculture which has led to applying sustainable alternative methods in place of chemical fertilizers **(Sindhu *et al.*, 2019)**. One such alternative used to achieve increased crop production is the inoculation of microorganisms that enhances the growth of plants and maintains the quality of the soil. These microbes are called "microbial inoculants" or plant growth-promoting rhizobacteria (PGPR) **(Abaid *et al*., 2014)**.

"Plant Growth Promoting Rhizobacteria" (PGPR) play a central role in promoting sustainable agriculture. PGPR are bacteria belonging to different groups that live in the rhizospheric region of the plant in association with roots or on the root surfaces **(Maheshwari *et al.*, 2012)**. Bacteria translocate from the soil's surface to the plant rhizosphere and start colonization in the rhizospheric zone of plant roots **(Hafeez *et al.*, 2013*)*.** All those bacteria that enhance the growth and development of plants fall under the category of PGPR **(Hayat *et al*., 2010; Hafeez *et al*., 2013)**. It has been demonstrated by **Noulas *et al.* (2018)** that the enhancement in development and productivity of the PGPR inoculated plants was achieved due to the increased uptake of nutrients and the improved nutrient status in the plant. Some strains of Zn solubilizing bacteria can solubilize nutrients by producing phosphatases and organic acids, enhancing the accessibility of nutrients to the plants. PGPR enhance plant growth by various direct and indirect mechanisms. Directly they promote growth by synthesizing phytohormones and indirectly by getting involved in the accessibility of nutrients or acting as control agents **(Yasmin *et al*., 2004).**

In addition to some quantitative effects and plant growth promotion, PGPR also aids in increasing the concentration of macronutrients and micronutrients (Fe, Zn, P, N and K) in tissues of plants and provides qualitative benefit to the plants **(Imtiaz, 2003)**. Therefore, this is considered the latest and efficient approach to increase Zn in various crops. Some of the Zn solubilizing PGPR includes *Acinetobacter spp., Pseudomonas spp., Trabusiella spp., Bacillus spp., Aeromonas spp., Arthrobacter spp., Gluconacetobacter spp., and Exiquobacterium spp.* **(Sillanpaa, 1990; Imtiaz, 2003)**. These bacteria significantly increase the growth and productivity of the plants. They also improve the overall quality of crops via the synthesis of growth-promoting hormones and various vitamins. The application of Zn solubilizing rhizobacteria as bio inoculants to enhance the yield of various crops such as barley, maize, rice and wheat has been very well documented. **Tariq *et al.* (2007)** reported a significant alleviation from Zn deficiency and a substantial increase in the total biomass, grain yield and harvest index of rice crop. It also increased the zinc concentration in the rice grains. Furthermore, a notably positive impact on root weight, root length, root area, root volume, and shoot weight was determined. The inoculated one exhibited significant Zn mobility as compared to uninoculated and control. PGPR solubilized the Zn with efficiency in the liquid culture as well. The Zn mobilizing strains are isolated from the rhizosphere of various crops. The isolated strains are screened by using a plate assay method. The efficient Zn solubilizers formed clear halo zones over the media contains insoluble zinc forms. **Yasmin (2004)** isolated *Pseudomonas* sp. from the rhizospheric soil of rice crop and determined its Zn mobilizing ability. It enhanced the Zn solubilization and improved yield, and increased Zn concentrations in rice plants. Similarly, **Sirohi *et al.* (2015)** reported Zn biofortification in wheat crops using Pseudomonas strain isolated from the rhizosphere of wheat. **Mumtaz *et al.* (2017)** reported the application of *Bacillus* sp. in maize. *Bacillus aryabhattai* and *Bacillus subtilis* significantly promoted the productivity of the maize crop. Thus, these bacteria are potential inoculants for biofortification to deal with malnutrition in populations where it is grown as a major cereal crop.

Table 1 Bacterial species isolated from different sources and potential bio inoculants to ameliorate the Zn status in the plants

Bacterium	**Zn Source used in solubilization**	**Crop/location**	**Reference**
Pseudomonas fluorescens	Zn phosphate	Forest soil	**Di Simine *et al.*,1998**
Stenotrophomonas maltophilia, Mycobacterium brisbanense, Enterobacter aerogenes, Pseudomonas aeruginosa and Xanthomonas retroflexus.	Zn oxide and Zn phosphate	banana, chili, field bean, ground nut, maize, sugarcane, sorghum and tomato	**Sunithakumari.k *et al.*,2016**
Pseudomonas Aeruginosa	Zn oxide, Zn phosphate	Air (tannery)	**Fasim *et al.*,2002**
Gluconacetobacter diazotrophicus	Zn Oxide, Zn carbonate	Maize	**Sarathambal *et al.*, 2010**
Acinetobacter sp.	Zn oxide and Zn Carbonate	Rice	**Gandhi *et al.*,2016**
Bacillus aryabhattai	Zn oxide, Zn Carbonate and Zn Phosphate	Soybean and wheat	**Ramesh *et al.*,2014**
Bacillus aerius ,Bacillus xiamenensis, Burkholderia cenocepacia, Burkholderia ambifaria and Sphingobacterium multivorum	Zn oxide, Zn carbonate	Rice	**Nepomuceno *et al.*,2020**
Pseudomonas fragi, Pantoea dispersa, Pantoea agglomerans , E. cloacae, Rhizobium sp.	Zn Carbonate,Zn sulphate, Zn oxide, Zn phosphate	wheat	**Kamran *et al.*,2017**
Serratia liquefaciens, S. marcescens and Bacillus thuringiensis.	Zn oxide, Zn Carbonate, Zn Sulphide and Zn Phosphate	Wheat	**Abaid *et al.*, 2015**

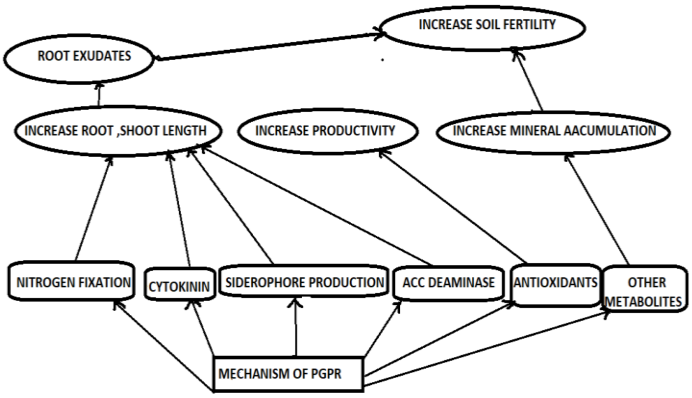

Figure 2 Summarize mechanism of PGPR

Zn solubilizing bacteria in consortium

It is well-conceived that PGPR present around the roots of a specific crop can also be inoculated in the different locality and for the different crop. They are found to promote plant growth significantly. In most of the studies, PGPR has been used as a single culture as biofertilizers. In contrast, some work has been done using more than one bacterial species. They are found to grow well in a similar soil environment. This microbial combination of more than two microbial species working together as a microbial community for promoting plant growth is called consortium. These bacterial isolates can be from the plant's rhizosphere or can be taken from the non-rhizosphere zone to work together as a consortium (**Pandey *et al.*, 2012**).

Consortium formed using bacterial and fungal species has been applied to the sugar cane crop. It increased the sugar level significantly. It also reduced the need to apply Zn sulphate fertilizer by 75% **(Deshmukh D.P *et al.*, 2019)**. Strains of *Bacillus cereus, B. subtilis* and *Serratia* sp. used in consortium reduced the soil-borne disease in sweet pepper. The consortium protected the plant from the pathogens, and hence via an indirect mechanism, it acted as a biocontrol agent. The consortium also acted as biofertilizers by increasing the productivity of the crop (**Raklami *et al.*, 2019**). The consortium of bacterial and mycorrhizae fungi stimulates the germination of spores, root colonization of the bacteria, and multiplies the population of other valuable bacteria in the rhizospheric soil. The synergy of bacteria and mycorrhizae enhances the nitrogenase activity in the bacteria associated with nitrogen fixation in the rhizosphere. This leads to an improvement in atmospheric nitrogen fixation. This consortium has an excellent ability to infect the plant root and enhance the shoot length, root length, biomass and overall productivity of the plants **(Raklami *et al.*, 2019)**. The consortium also improves soil fertility by changing the physicochemical and nutritional characteristic of the soil. **Baslam *et al.* (2014)** reported that the overall fertility of the soil had been improved after the application of consortium. Physicochemical characteristics of soil were found to be improved after the harvest of the crop grown with the consortium. The synergy has increased the organic matter and the concentration of carbon in the soil. It has also enhanced rhizospheric nitrogen and phosphorous. This improvement in the nutritional value of the soil is due to the capability of these bacteria to metabolize various organic compounds that are synthesized by the roots of the plants like various amino acids, carbohydrates and organic acids **(Caravaca *et al.*, 2003).**

CONCLUSION

Micronutrients are required for many cellular and metabolic activities in plants. These nutrients are required in trace amounts but are of greater significance in plants. The adequate supply of these microelements to plants requires proper uptake, accumulation, mobilization, and storage in the plants **(Hänsch and Mendel, 2009).** The inadequate supply of micronutrients dramatically affects the productivity of crops throughout the world. For obtaining optimal productivity in plants, there should be a continuous supply of micronutrients in the maturation phase of plants. Zn is a vital micronutrient in higher plants and has many biochemical, physiological roles in plants. Inorganic Zn applied in the soil readily becomes unavailable after application. This conversion reduces Zn availability to plants. Zn deficiency leads to severe effects in plants and dramatically reduces their productivity.

The application of rhizobacterium is a recent approach used for increasing the availability of essential elements such as Zn and Fe in different crops. Using Zn solubilizing rhizobacteria found in rhizosphere soil has been shown to improve the quality and productivity of crops. These bacteria can also mobilize micronutrients and are excellent alternatives to Chemical fertilizers in dealing with Zn deficiency. The application of PGPR is considered the new technological approach to combat Zn deficiency problems in plants effectively by enhancing the bioavailability of Zn, Fe, and other micro and macronutrients through economic and eco-friendly aspect.

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