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Plant growth promoting rhizobacteria and *Rhizobium* combinations are the key to reduce dependence on phosphorus fertilizers in lentil - A review

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ABSTRACT

Improved agricultural technologies are helping in meeting the foodgrain needs of growing global population while the arable land is shrinking. So, the excessive and continuous use of chemical fertilizers has ensured the high crop production and productivity of foodgrains. However, continuous application of inorganic fertilizers has created many problems related to biosphere i.e. environmental, soil and human being. In recent years, the scientists are concerned about biosphere hazardous; thus, reducing the extra burden of inorganic fertilizers by using microbial inoculants for integrated nutrient management. The microbial inoculations supplement the plant growth and yield by direct ways (increase in supply of nutrients i.e. nitrogen, production of metabolites such as auxins, cytokinins and gibberellins as well as through the solubilization of phosphate and other minerals) and indirect ways (biofertilizers eliminate the pathogens by the production of cyanide, siderophores, chitinase etc.). The microbial inoculation stimulates the plant to use applied as well as fixed nutrients i.e. phosphate efficiently, thus helps to reduce the fertilizers input. Secondly, biofertilizers are considered as economically feasible and environmentally safe.

Key words: Biofertilizer, Eco-friendly, Environmental hazardous, Phosphorus, Plant growth promoting rhizobacteria.

In India, the population has increased manifold and the food production has to be increased to feed the huge population. To meet the needs of growing population several methods are followed for increasing food production i.e. better crop varieties, tremendous use of chemical fertilizers etc (Tilman, 1998; Choudhary, 2016; Noorzai *et al*, 2017). These days increasing use of inorganic fertilizers in agriculture make the country self-dependent in food production, while it had created many problems related to environment and living beings (Ayala and Rao, 2002; Choudhary and Suri, 2014). Some of the applied fertilizer is used by the plant while rest of the fertilizer is fixed in the soil. Thus, excessive use of inorganic fertilizers are costly and also have various adverse impacts on soil i.e. depletion of water holding capacity, soil fertility and reach into water bodies through rain water which cause eutrophication in water bodies (Youssef and Eissa, 2014; Kumar *et al*, 2016a). Hence, unremitting application of inorganic phosphorus get fixed in the soils by immobilized process as a result it becomes unavailable to the plant (Choudhary *et al*, 2014).

Now-a-days researchers felt to develop some low cost, effective and eco-friendly fertilizers such as compost, vermicompost, biofertilizers etc. Among these, eco-friendly organic fertilizers (i.e. biofertilizers) are gaining more importance as these are cheap and renewable source of nutrients thus do not cause any pollution (Suri and Choudhary, 2013a; Ahemad and Kibret, 2014). At the same time when these microorganisms are used as an inoculant then these help to increase the efficiency of applied fertilizers (Singh *et al*, 2005; Verma *et al,* 2017). These play an important role to enrich the soil with nutrients by different processes i.e. mineralization, solubilization, biological nitrogen fixation (BNF), hormonal secretion etc (Sinha *et al*, 2010; Bai *et al*, 2017). They convert the soil fixed P in the soluble form by acidification, chelation, exchange reaction and production of gluconic acid and thus make the P available to the plants (Chen *et al,* 2006; Kumar *et al*, 2014).

This article puts the light for better use of applied fertilizers by improving their efficiency through microbial inoculations. As of their eco-friendly nature some species of microorganisms are replacing supplementary inorganic fertilizers. Biofertilizers i.e. *Rhizobium*, plant growth promoting rhizobacteria (PGPR) play an important role to maintain the soil fertility and have the potential to increase the crop yield when used along with inorganic fertilizers. Thus, this review will guide how to reduce the fertilizers use and highlight the role of biofertilizers in agriculture production system.

Environmental impacts of chemical fertilizers: Increased use of fertilizers played a significant role to increase the

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food production and productivity. However, continuous and overuse use of fertilizers has created detrimental effects related to biosphere i.e. environmental, soil and human beings (Dass *et al*, 2014). Over the Americans, the Indians consume 40 times more pesticides through the food (Kumari *et al*, 2014). It has been estimated that the consumption of phosphatic fertilizers (P_2O_5 total nutrients) in India has increased 37.4 per cent during the 2014 (6367271 tonnes) as compared to 2004 (4633145 tonnes) (Anonymous, 2017). This 37.4 per cent increment in fertilizers consumption is an alarming situation in India as it had created many detrimental effects on biotic as well as abiotic ecosystem. The detrimental effects include nitrate leaching, phosphorus run-off, groundwater pollution, eutrophication and death of aquatic life, production of greenhouse gases, global warming, acid rain etc (Anthony *et al*, 2009; Kumar *et al*., 2016b). Most of the applied N fertilizer is lost as N_2, trace gases or leached as nitrate which causes pollution. Similarly, when P is applied in high amount then it is precipitated by soil metal complexes (Gyaneshwar *et al*, 2002). The applied phosphate fertilizers flow with surface water, leading to increased level in rivers. The high dose of fertilizer application and low uptake efficiency of fertilizers are the main factors responsible for negative environmental effects (Kumar *et al*, 2017). Recently, many researches on the use of organic manures, biofertilizers etc. have been conducted to get rid of these harmful chemical fertilizers. Among those researches, biofertilizers gained the importance as of their eco-friendly behaviour (Suri and Choudhary, 2013b). Secondly, global warming is a great challenge for the whole nation which has mostly been created by the over consumption of fertilizers. To minimize these troubles the government, scientific community, industries as well as farmers them self have been implementing different policies; separately as well as in collaboration for creating an eco-friendly and sustainable environment.

Phosphorus use: After nitrogen, phosphorus (P) is one of the most important plant growth limiting nutrients, which is available in large reservoir of the soil in organic as well as inorganic forms i.e. rocks, organic matter and soil extracts (uracil, cytosine, xanthine, guanine, hypoxanthine and adenine). The rocks are considered as the biggest reservoir and in India there are approximately 40 million tonnes of phosphatic rock deposits. Despite of large reservoir of P in soil, the amount of available forms to plants is usually low. Under such conditions, farmers applied phosphatic fertilizers in bulk to the crop to overcome the deficiency of P (Anthony *et al*, 2009) as well as to get the better output (Bai *et al*. 2016). Lentil responds to phosphorus application (Sekhon *et al*, 2008; Singh and Sekhon, 2006; Singh *et al*, 2001, 2006, 2008) and integrated nutrient management (Singh *et al*, 2011). Generally plants absorb very less amount of applied phosphatic fertilizers, while rest of the P becomes immobile by forming complexes in soil (Choudhary *et al*, 2010). Thus, this frequent application of phosphatic fertilizers leads to be immobilized with the surfaces of hydrated oxides of Fe, Al and Mn (Suri and Choudhary, 2013c). The soil type and pH are the most important factors responsible for the immobilization of applied P (Kumar *et al*, 2016b). In acidic soils, P is fixed in the form of free oxides and hydroxides, however, in alkaline soils it is fixed by calcium (Pooniya *et al,* 2015; Kumar *et al*, 2016b). The regular application of phosphate fertilizers is not only costly but is also environmentally undesirable (Pooniya *et al,* 2015; Jahish *et al*, 2017). Under such conditions the need of biofertilizers had been realized as of their eco-friendly behaviour.

Importance of biofertilizers in phosphorus solubilisation: India has so diversified flora and fauna, consisting millions of fauna i.e. bacteria, fungi etc present in the soil. From centuries these microorganisms are known to be exerting beneficial effects on growth and development of the crop (Bakker *et al*, 2007). Thus, different genera of these microorganisms have been commercialized in agricultural uses as biofertilizers which are commonly called as plant growth promoting rhizobacteria (PGPR). These inoculations not only reduce the negative environmental impacts but at the same time also increase the efficiency of the applied inorganic fertilizers. These biofertilizers help to increase the availability of phosphorus by solubilization as well as mineralization. Solubilization of inorganic phosphorus occurs in the presence of low molecular weight organic acids which are synthesized by these bacteria (Zaidi *et al*, 2009; Kumar *et al,* 2016b). While in mineralization, phosphatises are released by the bacteria which boost the hydrolysis of phosphoric esters and convert the organic phosphorus into available form (Glick, 2012). PGPR increases the availability of nutrients by direct and indirect ways. Several researches concluded that single strain inoculations are effective; however, co-inoculations are better than the single inoculations (Singh *et al*, 2016). Thus interactions of two strains (*Rhizobium* + PGPR) and combination with inorganic fertilizers (NPK) are effectively increasing the nutrient use effcicincy and also make the plant healthy. This, option is an ecologically safe and economically reasonable for improving crop production. Bhattacharyya and Jha (2012) reported that *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* are the most significant phosphate solubilizing genera.

What are biofertilizers: Microbial inoculants or commonly known as biofertilizers are the formulations with microorganisms which when applied to the seeds of leguminous crops increase the availability and uptake of mineral nutrients (Vessey, 2003). Biofertilizers help to keep the soil rich in all kinds of micro and macro-nutrients (Ahmad *et al*, 2008) through biological nitrogen fixation (BNF), phosphate and potassium solubilization or mineralization,

production of plant growth regulating substances and release of antibiotics in the soil (Vessey, 2003). The plant-microbial interaction influences the soil ecosystem and produces supplementary substances for the plants. It has also been reported that dual inoculation (consortium) i.e. *Rhizobium* and plant growth promoting rhizobacteria (PGPR) is more beneficial over the single inoculation in terms of increase in yield potential (Khanna and Sharma, 2011). However, specific strain of microorganism of biofertilizers is used for inoculation of specific pulse crop. For phosphorus, there are two types of biofertilizers i.e Phosphorus solubilizing bacteria (PSB) and Phosphorus solubilizing fungi (PSF). In rhizospheric soil, the bacteria population is higher than that of the fungi (Chen *et al*, 2006); Alam *et al* (2002) revealed that these bacteria are more effective than the fungi for phosphorus solubilization. Among the rhizospheric soil bacteria *Pseudomonas*, *Rhizobium* and *Bacillus*; and among the fungi *Penicillium* and *Aspergillus* are reported to be the most effective phosphate solubilizers. The soil properties i.e. physical and chemical properties, organic matter, P content and cultural activities affect the population of PSB. *Bacillus* is one of the most important and abundant genera present in the rhizosphere. This genus mainly consists of *Bacillus megaterium, B. circulans, B. subtilis, B. polymyxa and B. sircalmous* species (Subbarao, 1988). These organisms increase nutrient availability to the plants by releasing different metabolites. *Bacillus megaterium* is regarded as one of the most important bacteria. Their inoculation leads to increase the availability of nutrients and the plant growth. The *Pseudomonas* sp. is present everywhere in agricultural soils. Mycorrhiza also increases the nutrient uptake in plants especially P.

Biofertilizers and phosphorus availability: Phosphorus is the least mobile in soil and is the second most important growth limiting element for the plant. The organic phosphates (inositol phosphatases, phosphoesters, phosphodiesters and phosphotriesters), highly insoluble in the soil, are the prime source of P for agricultural crops (Zimmermann, 2003; Ehsan *et al*, 2017). Among these sources of P, inositol phosphatases/ phytate is considered as one of the most important source of P for plant nutrition. It has been reported that Arbuscular mycorrhizal Fungi (AMF) present in the soil are responsible for the mobilization of soil insoluble P (Julia *et al*, 2010). However, this P is not sufficient for the plant nutrition; under such conditions farmers supply external/organic P to fulfil the need of the crop (Ohno *et al*, 2005). Phosphorus application is essential for proper growth and high productivity of legumes (Singh and Singh, 2016; Noorzai and Choudhary, 2017). Merely low concentration of externally applied inorganic P is available to plants while rest of them becomes unavailable because of highly reactive nature of P element; which forms complexes with elements i.e. Fe, Al, and Ca by forming precipitations in the soil (Hao *et al*, 2002). Hence, unremitting application of inorganic phosphorus get fixed in the soils by immobilized process as a result it becomes unavailable to the plant. However, it has been reported that external inoculations of rhizospheric microorganisms (*Rhizobium* and/or PGPR) play an important role in soil P dynamics and result in increased availability of phosphorus to plants (Richardson, 2001).

The principal mechanism in soil for mineral phosphate solubilization is lowering of soil pH by microbial production of organic acids and mineralization of organic P by acid phosphatases (Stevenson, 2005). Soil fixed P is solubilized by the release of microbial metabolites such as organic acids in which hydroxyl and carboxyl chelate with cations (Al, Fe and Ca) afterward convert it to soluble form (Kpomblekou and Tabatabai, 1994). At the same time, plant-microbes interaction in the rhizosphere area promotes the plant growth activity and also improves the fertility of the soil (Ahmad *et al*, 2008). Thus, microbes have an ability to stimulate the plant growth and yield by direct and indirect mechanisms. Direct promotion of plant productivity by microbes occur, when rhizobacteria improve the supply of nutrients i.e. nitrogen, production of metabolites such as auxins, cytikinins and gibberellins as well as through the solubilization of phosphate and other minerals. Under indirect plant growth promotion, microbes eliminate the pathogens by the production of cyanide, siderophores, chitinase etc. (Ahemad and Kibret, 2014).

Crop response to inoculations: Lentil requires high phosphorus for the symbiotic nitrogen fixation (Gahoonia *et al*, 2006), thus phosphorus application and ability of inoculant to solubilize phosphate to plant available from affect the nodule formation positively. Application of 20 kg P_2O_5 ha^{-1} to conjointly inoculated (*Rhizobium* + *Bacillus* sp.) lentil significantly increased symbiotic parameters and leghaemoglobin content over the dual inoculation or phosphorus application alone (Jindal *et al*, 2008). Higher number of nodules with the inoculations of biofertilizers i.e. *Rhizobium* and PGPR attributed to the ability of biofertilizers to produce indole acetic acid (IAA), consequently the endogenous IAA level in plant regulates the formation of nodules and nitrogenase activity (Saini and Khanna, 2012) consequently it also affect the nodular leghaemoglobin content (Dutta and Bandyopadhyay, 2009).

Singh *et al* (2010) conducted an experiment for integrated nutrient management (INM) in lentil and reported that application of 40 kg P_2O_5 ha^{-1} to dual inoculated lentil (*Rhizobium* + PGPR) further improved the symbiotic, growth parameters and economics over phosphorus or biofertilizers alone. Biofertilizer inoculation helps to increase the returns by boosting the efficiency of applied fertilizers. When maximum returns per unit of fertilizer are recorded then fertilizers are considered as the efficiently used (Mortvedt *et al*, 2001). The another study was conducted with two levels

of nitrogen (N) + P_2O_5 fertilizers (12+30 and 24+60 kg ha^{-1} respectively) with co-inoculation (*Rhizobium* + PGPR) and it was found that at full dose of N + P_2O_5 fertilizer (24+60 kg ha^{-1}) the co-inoculated lentil significantly enhanced biomass production, nodulation and its dry weight, root length, shoot length, root dry weight and shoot dry weight $plant^{-1}$, available P content in soil, plant N content and uptake of N & P (Akhtar *et al*, 2012). Inoculation of *Rhizobium* alone and with PGPR increases the chlorophyll content (Saini and Khanna, 2012). The improvement in chlorophyll content due to increased level of nitrogen improved the photosynthesis process. Biswas *et al* (2015) reported that application of 60 kg P_2O_5 ha^{-1} to dual inoculated (*Rhizobium* + PGPR) lentil recorded a maximum increment in different parameters *viz*. growth, symbiotic and yield parameters, however it was statistically similar to treatment of *Rhizobium* + PGPR + 45 kg P_2O_5 ha^{-1}. They revealed that extra cost of 15 kg P_2O_5 ha^{-1} can be saved by using biofertilizers along with application of 45 kg P_2O_5 ha^{-1}. Singh *et al* (2016) revealed that consortium should be used for improving the crop growth, which can ultimately help in obtaining high grain yield of lentil.

Advantages: Application of biofertilizers reduces the costs of fertilizers input up to 25 per cent, especially nitrogen and phosphorus fertilizers. Biofertilizers supplement the good effect of chemical fertilizers; at the same time, do not cause problems caused by chemical fertilizers. They also supply micro nutrients. Microbial inoculation increases the crop yield up to 20-30 per cent. Biofertilizers are eco-friendly, sustainable and cheap source of nutrients. Biofertilizers restore the natural soil fertility by activating microbial activities, secretion of growth promoting hormone and counteracting negative impact of chemical fertilizers. Biofertilizers do not encourage salinity of soil (Ghumare *et al*, 2014).

Future perspective of biofertilizers: With increase in population pyramid the food requirement has been increased as well as diversified. It has been reported that by the 2020 approximately 321 million tonnes of foodgrain is required to feed the population; for this purpose the requirement of nutrients will be 28.8 million tonnes, whereas the availability will be only 21.6 million tonnes and this leads to the deficiency of 7.2 million tonnes of nutrients (Mazid and Khan, 2014). Secondly, scientists are concerned about the detrimental effects related to biosphere i.e. environmental, soil and human being, due to overuse of chemical fertilizers. To meet the food production needs of the population on sustainable basis, the Government of India has already implemented National project on development and use of biofertilizers (NPDB) for the production, distribution and promotion of biofertilizers for better agriculture on renewable basis. National Biofertilizers Development Centre, Ghaziabad has taken the responsibility to promote the biofertilizers in agriculture. State Agricultural Universities (SAUs) are working on different aspects of biofertilizers which includes selection of effective and multi-functional biofertilizers for a range of crops, quality control system for the better production of inoculants, better microbial inoculants suitable for soil stressful conditions (i.e saline, alkaline); and biofertilizers also available in seeds centres or Microbiology Departments of SAUs. At the same time, some initiatives have also been taken by the public sector i.e. IFFCO in collaboration with SAUs and research units to disseminate the better production technologies on biofertilizers.

CONCLUSION

Inoculation of *Rhizobium* and/or PGPR alongwith application of phosphorus fertilization enhance the grain yield by improving growth, symbiotic parameters and yield traits of lentil. The dual inoculation helps in increasing crop production and productivity of the crop by increasing BNF, availability and/or uptake of nutrients through solubilization, hormonal secretion or by decomposition of organic residues. Thus, input of phosphorus fertilizers can be reduced by microbial inoculations; and dual inoculation further improves the fertilizers use efficiency by direct and indirect way. Furthermore, dual inoculation reduces the adverse impact of fertilizers on environment and helps to achieve the sustainability in crop production system. It may be concluded that to obtain the higher profit it is necessary to use the fertilizers as efficiently as possible without any wastage or losses which could be possible due to use of biofertilizers which increased the efficiency of applied phosphorus.

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