

REVIEW ARTICLE

Integrated plant nutrient system – with special emphasis on mineral nutrition and biofertilizers for Black pepper and cardamom – A reviewSangeeth K P¹ and Suseela Bhai R²¹*School of Biological Sciences, Central University of Kerala, Kasaragod, Kerala, India* and ²*Indian Institute of Spices Research, Kozhikode, Kerala, India***Abstract**

Integrated Plant Nutrition System (IPNS) as a concept and farm management strategy embraces and transcends from single season crop fertilization efforts to planning and management of plant nutrients in crop rotations and farming systems on a long-term basis for enhanced productivity, profitability and sustainability. It is estimated that about two-thirds of the required increase in crop production in developing countries will have to come from yield increases from lands already under cultivation. IPNS enhances soil productivity through a balanced use of soil nutrients, chemical fertilizers, combined with organic sources of plant nutrients, including bio-inoculants and nutrient transfer through agro-forestry systems and has adaptation to farming systems in both irrigated and rainfed agriculture. Horticultural crops, mainly plantation crops, management practices include application of fertilizers and pesticides which become inevitable due to the depletion of soil organic matter and incidence of pests and diseases. The extensive use of chemical fertilizers in these crops deteriorated soil health that in turn affected the productivity. To revitalize soil health and to enhance productivity, it is inexorable to enrich the soil using microorganisms. The lacunae observed here is the lack of exploitation of indigenous microbes having the potential to fix atmospheric nitrogen (N) and to solubilize Phosphorus (P) and Potassium (K). The concept of biofertilizer application appears to be technically simple and financially feasible, but the task of developing biofertilizers with efficient strains in appropriate combinations in a consortia mode is not easier. More than developing consortia, a suitable delivery system to discharge the microbial inoculants warranted much effort. This review focuses on the integrated plant nutrition system incorporating biofertilizer with special emphasis on developing and formulating biofertilizer consortium.

Keywords

Biofertilizer consortium, elettaria
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PGPR, Piper nigrum, P solubilizers

History

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Organic manures

Farmyard manure is a conspicuous organic component of an integrated nutrient supply system, which improves soil health, increases the productivity and releases macro and micronutrients. According to Jayathilake et al. (2006) integrated nutrient supply system with nitrogen fixing biofertilizers (*Azospirillum* or *Azotobacter*) in combination with organic manure (vermicompost and farmyard manure) and chemical fertilizers provided optimum economical yield and improved soil fertility.

The “Vermi-compost” has gained impetus in organic farming to boost agricultural production to its important multifarious features such as being rich in nutrients, vitamins, growth regulators, free from pathogen and containing immobilized microflora (Kale et al., 1992). Vermicompost plays a significant role in improving the fertility of topsoil and in boosting the productivity of the crop. The huge quantity of wastes can be converted into nutrient-rich biofertilizer

(vermicompost) for sustainable land restoration practices (Suthar, 2008). In general, a great proportion of the crop nutrient input during cultivation returned in the form of the plant residues. Suthar (2007) estimated that 30–35% of applied N & P and 70–80% K remained in the crop residues of food crops and that nutrient-rich crop residues must be “prepared” before being used as a fertilizer, and earthworms as suitable candidates for such preparation. The earlier workers have reported the positive effect of vermicompost application on growth and productivity of crops (Atiyeh et al., 2000; Benik & Bhebaruah, 2004; Edwards & Burrows, 1988; Kale et al., 1987; Nethra et al., 1999; Suthar, 2006). Atiyeh et al. (2001) concluded that vermicompost, whether used as soil additives or as components of greenhouse bedding plant container media, have improved seed germination, enhanced seedling growth and development and increased overall plant productivity.

Biofertilizers and its role in soil nutrient availability

To overcome the ecological problems and to minimize the use of chemical fertilizers, we are now directing ourselves towards the potential use of ecofriendly approaches such as

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the use of biofertilizers and biopesticides to sustain high production, allow more efficient nutrient utilization and thereby provide solutions for present and future agricultural practices (Bashan & Holguin, 1995; Esitken et al., 2006). Biofertilizers are natural and organic fertilizers. Biofertilizers are preparations containing cells of microorganisms which may be nitrogen fixers, phosphate solubilizers, sulfur oxidizers or organic matter decomposers. Biofertilizers provide biological nitrogen (N) to plants, provide insoluble phosphorus (P) available to the plants, mobilize potassium (P) and enhance nutrient uptake and plant growth (Hedge et al., 1999). They also provide protection from soil borne pathogens and help in the survival of beneficial microorganisms (Vassilev et al., 2006). Besides, they improve soil property and maintain soil fertility which indirectly increases the crop yield. They have less production cost and sustain crop productivity through their metabolic activities independently or in association with the plant root system and are totally pollution free.

The main and direct purposes of applying biofertilizers to soil are to provide nutrient sources and good soil conditions for the growths of crops, to partially substitute and enhance the function of chemical fertilizer and then subdue the application quantities of fertilizers and to lessen the negative effect aroused from applying chemical fertilizers to soil. The indirect purposes of using biofertilizers to soil are to enhance the growth of root system to increase the water and nutrient absorption abilities of crops, extend the life of root, neutralize and degrade harmful materials accumulated in soil, and also to promote survival efficiency of seedling after transplanting and get shorter time for the flower to come out (Chiu, 2005). They are called as bioinoculants bacterium or fungi which on supply to plant improve their growth and yield. These bioinoculants can reside on the surface of the plant or form endophytic association or else interact with other microbes in the rhizosphere or phyllosphere thereby influencing the plant growth. Production of chemical fertilizers require fossil fuel energy while microbes do not, thereby proving to be cost effective ecofriendly with the simple methodology of production and having no hazard to the agro ecosystem. Biofertilizers thus assume a special significance towards development of strategies for improving productivity and economizing the production cost, minimizing our dependence on synthetic chemical fertilizers. Inoculation of plants with beneficial bacteria and fungi (N-fixing, P- and K-solubilizing bacteria) are important in plant nutrition, increasing NPK uptake by the plants and playing a significant role as PGPR in the biofertilization of crops (Ghosh, 2005).

Plant growth promoting rhizobacteria (PGPR)

Bacteria inhabiting the rhizosphere and beneficial to plants are termed PGPR (Kloepper et al., 1980). In general, about 2–5% of rhizosphere bacteria are PGPR (Antoun & Prevost, 2005). Bacteria identified to be PGPR could be members of several genera such as *Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Pseudomonas* and *Bacillus* (Arshad & Frankenberger, 1998; Bashan & Levany, 1990; Kloepper et al., 1989; Probanza et al., 1996; Tang, 1994). Bashan & Holguin (1997) coined the term PGPB (plant

growth promoting bacteria) referred to bacteria exhibiting positive effects on plants and making a distinction between biocontrol PGPB and PGPB. The biocontrol PGPB group encompassed bacteria that suppress plant pathogens by either producing plant pathogen inhibitory substances or by increasing the natural resistance of the plant and the PGPB group encompassed those bacteria that affect plants by means other than suppression of other microorganisms (Bashan & Holguin, 1997).

Using PGPR as inoculants in soil, besides altering the structure of the communities, will also influence microbial activity and this could be related to the survival of the PGPR in the environment. Some of the factors influencing the survival and activity of bacteria in the rhizosphere are physical (texture, temperature and humidity), while others are chemical, such as pH, nutrient availability, organic matter content and above all, interactions with other rhizosphere microorganisms. The interaction with the biotic factor is very important because PGPR must occupy a new niche, adhering to the plant roots and the inoculums must compete for available nutrients released essentially by the root exudates, maintaining a minimum population able to exert its biological effect (Ramos et al., 2003).

PGPR action mechanisms have been grouped traditionally into direct and indirect mechanisms. Direct mechanisms include those that affect the balance of plant growth regulators, as either the microorganisms themselves release growth regulators that are integrated into the plant or the microorganisms act as a sink of plant released hormones, and those that induce the plants metabolism leading to an improvement in its adaptive capacity (Kloepper, 1997). Two important mechanisms are included in this group: induction of systemic resistance to plant pathogens and protection against high salinity conditions (Cartieaux et al., 2003). Indirect mechanisms include those that improve nutrient availability to the plant, inhibition of microorganisms that have a negative effect on the plant (niche exclusion) and also free nitrogen fixation in the rhizosphere which improves nitrogen availability (Lucy et al., 2004).

More than 80% of soil bacteria in the rhizosphere are capable of producing auxin. Thus, the potential of these microorganisms to affect the endogenous levels of this regulator and its effects on plant growth are remarkable. The production of gibberellins by PGPR is rare, with only two strains being documented that produce gibberellins, *Bacillus pumilus* and *Bacillus licheniformis* (Gutierrez-Manero et al., 2001). Using PGPR capable of reducing ethylene levels in the plant could be an interesting method to improve certain plant physiological processes (Glick et al., 1994).

Diazotrophic PGPR

Free-living prokaryotes with the ability to fix atmospheric dinitrogen called diazotrophs are ubiquitous in soil. The great diversity of diazotrophs also extends to their physiological characteristics such as N fixation is performed by chemotrophs and phototrophs and by autotrophs as well as heterotrophs (Hill, 1992). In natural ecosystems, biological N fixation (by free-living, associated and symbiotic diazotrophs) is the most important source of N. The estimated

contribution of free-living N-fixing prokaryotes to the nitrogen input of soils ranges from 0 to 60 kg ha⁻¹ year⁻¹ (Cleveland et al., 1999). Due to the direct link of diazotroph populations to the carbon/nitrogen balance of a soil and their high diversity associated with different physiological properties, they are of interest as potential bioindicators for the nitrogen status of soils. Environmental variables that can influence diazotrophy includes host primary production, root exudation and edaphic physicochemical parameters (Piceno & Lovell, 2000).

Free nitrogen-fixing bacteria were probably the first rhizobacteria used to promote plant growth. *Azospirillum* strains have been isolated and used ever since the 1970s when it was first used. This genus has been studied widely. The study by Bashan et al. (2004) being the most important one reporting the advances in physiology, molecular characteristics and agricultural applications of this genus. Other bacterial genera capable of nitrogen fixation that is probably responsible for growth promotion effect are *Azoarcus* sp., *Burkholderia* sp., *Gluconacetobacter diazotrophicus*, *Herbaspirillum* sp., *Azotobacter* sp and *Paenibacillus (Bacillus) polymyxa*. These strains have been isolated from a number of plant species, such as rice, sugarcane, corn, sorghum, other cereals, pineapple and coffee bean (Graham, 1999; Saxena & Tilak, 1994; Zuberer, 1999).

Non-symbiotic nitrogen fixation is known to be of great agronomic significance. Some important non-symbiotic nitrogen-fixing bacteria include *Achromobacter*, *Acetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Azomonas*, *Bacillus*, *Beijerinckia*, *Clostridium*, *Corynebacterium*, *Derxia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, *Rhodospirillum*, *Rhodopseudomonas* and *Xanthobacter* (Saxena & Tilak, 1998). Various diazotrophic bacteria including species of *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia* and *Clostridium* have been commonly associated with higher plants. The widespread distribution of dinitrogen-fixing associative symbiosis has led to interest in determining their relative importance in agricultural systems (Rennie, 1981). The contribution of asymbiotic and symbiotic nitrogen fixation varies greatly but in some terrestrial ecosystems asymbiotic nitrogen fixation may be the dominant source (Paul & Clark, 1989). Several new nitrogen-fixing bacteria associated with grasses and cereals, including sugarcane, have been described by many workers, namely, *Pseudomonas* sp. (Barraquio et al., 2000), *Enterobacter*, *Klebsiella*, *Pseudomonas* sp., *Azospirillum*, *Campylobacter* sp., *Bacillus azotofixans* and *Herbaspirillum seropedicae* (Baldani et al., 1986).

Biological nitrogen fixation is a high cost process in terms of energy. Bacterial strains capable of performing this process do so to fulfill their physiological needs and thus little nitrogen is left for the plants use. However, growth promotion caused by nitrogen-fixing PGPR was attributed to nitrogen fixation for many years, until the use of nitrogen isotopes showed additional effects. This technique showed that the benefits of free nitrogen-fixing bacteria are due to the production of plant growth regulators and nitrogen fixation (Bedmar et al., 2006).

Ninety-five percent of Gram-positive soil bacilli belong to the genus *Bacillus*. The remaining 5% are confirmed to be

Arthrobacter and *Frankia* (Garbeva et al., 2003). Members of *Bacillus* species are able to form endospores and hence survive under adverse conditions; some species are diazotrophs such as *Bacillus subtilis* (Timmusk et al., 1999), whereas others have different PGPR capacities (Probanza et al., 2002). Among Gram-negative soil bacteria, antibiotics, siderophores or hydrogen cyanide are among the metabolites generally released by these strains (Charest et al., 2005) and these metabolites strongly affect the environment, as both they inhibit growth of other deleterious microorganisms and they increase nutrient availability for the plant. Two groups of nitrogen-fixing bacteria, that are rhizobia and *Frankia*, have been studied extensively. *Frankia* forms root nodules on species of *Alnus* and *Casuarina*. Symbiotic nitrogen-fixing rhizobia are now classified into 36 species distributed among seven genera (*Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Methylobacterium*, *Rhizobium* and *Sinorhizobium*; Sahgal & Johri, 2003).

Biological N fixation

Microbial transformations in the N cycle affect N bioavailability in soil by conditioning plant growth and N storage in biosphere. According to Newton (2000), 65% of total nitrogen fixed is provided by biological N fixation (BNF), 25% by industrial fertilizer production and 10% by abiotic, natural processes (lightning, combustion and volcanic activity). BNF in the terrestrial ecosystem is in the order of 90–130 Tg N year⁻¹ (Galloway, 1998). BNF by free bacteria in cultivated soil from temperate areas does not exceed several kg N ha⁻¹ year⁻¹. BNF by endophytic diazotroph bacteria colonizing sugarcane rhizosphere can reach 170 kg N ha⁻¹ year⁻¹ in Brazil (Baldani et al., 2000). In water-submerged rice fields, BNF is due to free cyanobacteria and diazotrophic bacteria colonizing rice roots. Fixed nitrogen can vary from several tens to 150 kg N ha⁻¹ year⁻¹ (Atlas & Bartha, 1993) and could provide the equivalent to 20% of the total nitrogen incorporated into the plant (Baldani et al., 2000). An estimation of BNF by human-induced cultivation of rice and legumes ranged from 30 to 50 Ton N year⁻¹ (Galloway, 1998). Furthermore, BNF efficiency depends on mineral nitrogen availability and is tremendously diminished by N fertilizer application (Vance, 1998; Waterer et al., 1994).

Members of the genus *Azospirillum* fix nitrogen under microaerophilic conditions and are frequently associated with the roots and rhizosphere of a large number of agriculturally important crops and cereals. Plant beneficial effects of *Azospirillum* have mainly been attributed to the production of phytohormones, nitrate reduction and N fixation, which have been the subject of extensive research (Bashan & de Bashan, 2005; Somers & Vanderleyden, 2004). Despite their nitrogen-fixing capability, the increase in yield is mainly attributed to improved root development due to the production of growth promoting substances and consequently increased rates of water and mineral uptake (Fallik et al., 1994). *Azospirillum* proliferate in the rhizosphere of numerous plant species and the genus *Azospirillum* now contains seven species: *A. brasilense*, *A. lipoferum*, *A. amazonense*, *A. halopraeferens*, *A. irakense*, *A. dobereineriae* and *A. largimobile*.

The effect of *Azospirillum* on the total yield increase of field-grown plants generally ranged from 10% to 30% (Kapulnik et al., 1987; Watanabe & Lin, 1984). *Azospirillum* strains had no preference for crop plants or weeds, or for annual or perennial plants, and can be successfully applied to plants that have no previous history of *Azospirillum* in their roots. It appears that *Azospirillum* is a general root colonizer and is not a plant specific bacterium (Bashan & Levanony, 1990). Inoculation of plants with *Azospirillum* can result in a significant change in various plant growth parameters, which may or may not affect crop yield. Most studies of the *Azospirillum*-plant association have been conducted on cereals and grasses and only a few other plant families have been investigated (Bashan et al., 1989b,c; Crossman & Hill, 1987; Kolb & Martin, 1985; Saha et al., 1985;).

In addition to increasing (Kapulnik et al., 1985) or decreasing (Kucey, 1988) many root parameters, plant inoculation with *Azospirillum* affected many foliage parameters. These changes were directly attributed to positive bacterial effects on mineral uptake by the plant. Enhancement in uptake of NO_3^- , NH_4^+ , PO_4^{2-} , K^+ , Rb^+ and Fe^{2+} by *Azospirillum* (Barton et al., 1986; Jain & Patriquin, 1984; Lin et al., 1983; Morgenstern & Okon, 1987; Murty & Ladha, 1988; Sarig et al., 1988) was proposed to cause an increase in foliar dry matter and accumulation of minerals in stems and leaves. It has been further suggested that *Azospirillum* inoculation may promote availability of ions in the soil by helping the plant scavenge limiting nutrients (Lin et al., 1983). The plant may take up N more efficiently from the limited supply in the soil, resulting in a lower requirement of N fertilization to attain a certain yield. Supporting evidence for increased mineral uptake by inoculated roots is provided by enhancement in proton efflux activity of wheat roots inoculated with *Azospirillum* (Bashan, 1990; Bashan et al., 1989a).

Two basic variables that contribute to the complexity of plant yield response to inoculation are the plant cultivars, which often show differential response to inoculation (Millet et al., 1986), and the level of N fertilization. The highest yield increases were obtained when the levels of N fertilization were suboptimal for maximum yield (Lau-Wong, 1987; O'Hara et al., 1987). Therefore, *Azospirillum* inoculation was considered as a partial substitute for N fertilization. However, contradictory data (Bashan et al., 1989b; Millet & Feldman, 1986) showed that yield was increased by inoculation even under high levels of N fertilization and that N can influence the number of bacteria in the rhizosphere. However, the use of nitrogen in combinations with *Azospirillum* produced significantly higher green and dry matter yields than those from inoculation or fertilization alone (Chela et al., 1993). Zaady et al. (1994) based on the above results suggested that inoculation with *Azospirillum brasilense* on a commercial scale may offer means of increasing rangeland production without resorting to costly and ecologically unfavorable fertilizer application.

Azospirillum-inoculated sugarcane seed pieces fertilized with a low level of nitrogen gave higher yields than those obtained from seed pieces inoculated, non-inoculated or fully fertilized. *Azospirillum* inoculated seed pieces replaced about 60% of the required N fertilizer (Macalintal & Urgel, 1992).

Triticale plots fully fertilized and plots inoculated with *Azospirillum* and enriched with low rates of fertilizer of 120 kg N/ha gave a yield increase of about 53% over non-inoculated plants (Del Gallo et al., 1991). Inoculation of wheat with various strains of *Azospirillum* caused significant increases over controls in grain yield, ranging from 23% to 63% (Caballero et al., 1992). Finally, inoculation of sunflower with *Azospirillum brasilense* Cd and *Azospirillum lipoferum* positively affected plant growth, especially under irrigation (Itzigsohn et al., 1995).

As an outcome of the inconsistency in field inoculation of *Azospirillum*, a new research subfield has emerged, namely, co-inoculation of *Azospirillum* with other microorganisms. This promising new trend in the field of microbial inoculants represents the largest single topic of literature on *Azospirillum* in recent years. Co-inoculation is based on mixed inoculants, combinations of microorganisms that interact synergistically or when *Azospirillum* is functioning as a “helper” bacterium to enhance the performance of other beneficial microorganisms. Co-inoculation allows the plants to have a more balanced nutrition and the absorption of N, P and other mineral nutrients is improved. The arbuscular mycorrhiza is a universal symbiosis which is established with more than 80% of plant species, including almost all major agricultural crops and herbaceous and shrub species in natural ecosystems (Barea et al., 1997). The mycorrhizosphere (Barea et al., 2002a,b) interactions related to nutrient cycling concern three types of soil bacteria: (1) plant symbiotic N-fixing rhizobial bacteria; (2) phosphate solubilizing bacteria; and (3) phyto-stimulators *Azospirillum*. By linking the biotic and geochemical portions of the soil ecosystem, the mycorrhizal fungi cannot only contribute to P capture and supply, but also affect P cycling rates and patterns in both agro- and natural ecosystems (Jeffries & Barea, 2001).

Positive interactions between mycorrhizal fungi and *Azospirillum* were reported by many researchers. Volpin & Kapulnik (1994) and Barea (1997) showed that *Azospirillum* enhances mycorrhizal formation and, conversely, mycorrhizal fungi improve *Azospirillum* establishment. These results were corroborated by Vazquez et al. (2000a) who further described an improvement in biomass and N accumulation in dually inoculated plants. Veeraswamy et al. (1992) reported that mixed inoculation of *Azospirillum* and the vesicular arbuscular mycorrhizae (VAM) fungus *Glomus intraradices* in sorghum created a synergistic interaction resulting in a significant increase in many plant-growth parameters including mycorrhizal infection with a concomitant increase in levels of root phosphatases (both alkaline and acid), an increase in the phosphorus content in plants, and enhanced uptake of nitrogen, zinc, copper and iron and this double inoculation could replace the application of N and P fertilizers. Similarly, dual inoculation of wheat plants with *Azospirillum brasilense* and *Glomus* sp. or *Azospirillum brasilense* and an undefined VAM fungus, or dual inoculation of *Glomus macrocarpum* and *Azospirillum brasilense* in *Corchorus ollitorius* plants increased fresh and dry weights of shoots and roots (Al-Nahidh & Gomah, 1991; Bali & Mukerji, 1991; Gori & Favilli, 1995).

Azospirillum is not yet known as a biocontrol agent of soil borne plant pathogens. However, some evidence shows that

this activity has been overlooked. *Azospirillum lipoferum* M produced catechol type siderophores under iron-starved conditions that exhibited antimicrobial activity against various bacterial and fungal isolates (Shah et al., 1992). Twenty-seven *Azospirillum* isolates produced bacteriocins that inhibited the growth of several indicator bacteria (Tapia-Hernandez et al., 1990). The effect of *Azospirillum brasilense* on crown gall formation in dicotyledonous plants was studied after inoculating them with virulent strains of *Agrobacterium tumefaciens*. When the wounded tissues of grapevines and carrot disks were pre-inoculated with the live cells of *Azospirillum brasilense* 94-3 or Sp-7, the development of the typical bacterial galls was inhibited and the protective effect of *Azospirillum* lasted over a 24 h period (Bakanchikova et al., 1993). When *Azospirillum brasilense* Cd was mixed in a culture with the mangrove rhizosphere bacterium *Staphylococcus* sp., the population of the latter was significantly reduced (Holguin & Bashan, 1996).

P solubilization

Phosphorus is considered to be a major growth-limiting nutrient and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available (Ezawa et al., 2002). It is essential in both cellular energetic (ATP) and cellular structures (DNA, RNA and phospholipids). Indian soil contains on an average 0.05% P which constitutes 0.2% dry weight (Shrivastav et al., 2004). The problem of P supply is more acute in India as the country has to mainly depend on imported raw materials. However, there are substantial deposits of low grade rock phosphate in the country, which cannot be used for direct application in neutral to alkaline soils. Integrated use of suitable microbial cultures along with low grade rock phosphate can supply about 30 kg P₂O₅/ha (Gaur, 1990). Plants are only able to absorb the soluble forms, i.e. mono- and di-basic phosphate. Besides inorganic forms of phosphorous in soil, the phosphorous present in organic matter is of considerable importance. The organic forms of phosphorous are estimated to comprise between 30 and 50% of total soil phosphorous. This reservoir can be mineralized by microorganisms, making it available to the plant as soluble phosphates (Gaur, 1990).

Phosphorus deficiency is a widespread nutrient constraint to crop production on tropical and subtropical soils and it affects an area estimated at over 2 × 10⁹ hectares. However, for most resource-poor farmers in developing countries, correcting soil P deficiency with large applications of P fertilizer is not a viable option. Furthermore, the inexpensive rock phosphate reserves remaining in the world could be depleted in as little as 60–80 years. Therefore, sustainable P management in agriculture requires additional information on the mechanisms in plants that enhance P acquisition to make plants more efficient at acquiring P, development of P efficient germplasm and advanced crop management schemes that increase soil P availability (Rao et al., 1999; Vance et al., 2003). Interest has been focused on the inoculation of phosphate-solubilizing microorganisms into the soil so as to increase the availability of native, fixed P and to reduce the use of fertilizers. The alternative approach is to use these PSM

along with other beneficial rhizospheric microflora to enhance crop productivity.

P solubilizing microorganisms

Phosphate-dissolving soil microorganisms play a profound role. Many bacteria from different genera are capable of solubilizing phosphate and include *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aerobacter*, *Flavobacterium*, *Chryseobacterium* and *Erwinia* (Schachtman et al., 1998). Bacteria use two mechanisms to solubilize phosphate: (i) releasing organic acids that mobilize phosphorous by means of ionic interactions with the cations of the phosphate salt and (ii) by releasing phosphatases responsible for releasing phosphate groups bound to organic matter. Most of these bacteria are able to solubilize the Ca–P complex, and there are others that operate on the Fe–P, Mn–P and Al–P complexes. In general, these mechanisms are more efficient in basic soils. Results with PGPR capable of solubilizing phosphate are sometimes erratic, probably due to soil composition, and to demonstrate their effect they have to be inoculated in soils with a phosphorous deficit and stored in insoluble forms. Although many authors report a growth-promoting effect of phosphorous solubilizing microorganisms (Narula et al., 2000; Sundara et al., 2002), results in the field are highly variable.

Phosphate-solubilizing bacteria are common in rhizospheres (Nautiyal et al., 2000; Vazquez et al., 2000b). However, the ability to solubilize P by no means indicates that a rhizospheric bacterium will constitute a PGPR. Cattelan et al. (1999) found only two of five rhizospheric isolates positive for P solubilization actually had a positive effect on soybean seedling growth. Not all P solubilizing PGPR increase plant growth by increasing P availability to the hosts. de Freitas et al. (1997) found a number of P-solubilizing *Bacillus* sp. isolates and a *Xanthomonas maltophilia* isolate from canola (*Brassica napus* L.) rhizosphere which had positive effects on plant growth, but no effects on P content of the host plants.

A wealth of articles describes the exudation of phosphorous-mobilizing substances by plant roots (Gaume et al., 2001; Ishikawa et al., 2002; Johnson et al., 1996; Neumann & Romheld, 1999; Zhang et al., 1997). However, neutral sugars that sparingly affect P availability occupy the largest part of water-soluble root exudates of annual plants (Gransee & Wittenmayer, 2000; Merbach et al., 1999). In addition, such easily decomposable substances are used by microorganisms within a short time. Microbial decomposition of root exudates leads to a smaller increase in phosphorous solubility in comparison to the sterile treatment. In addition, microbial colonization increases the exudation rate of plants (Meharg & Killham, 1995).

Several mechanisms have been proposed to explain the P solubilization by phosphate solubilizing rhizobacteria (PSRB); they are associated with the release of organic and inorganic acids, and the excretion of protons that accompanies to the NH₄⁺ assimilation (Abd-Alla, 1994; Whitelaw, 2000). In addition, the release of phosphatases enzymes that mineralize organic P compounds has been also suggested as another mechanism involved. Memon (1996) affirm that

Nitrosomonas and *Thiobacillus* mobilized inorganic phosphates by producing nitric and sulfuric acid. Equally, phosphates may be released from solid compounds by carbonic acid formed as a result of the decomposition of organic residues (Memon, 1996). Many organic acids are effective in solubilizing soil phosphates, these acids are produced by rhizosphere microorganisms (Marschner, 1997). Bolan et al. (1991) studied the influence of the addition of organic acids on high P-fixing soils. These acids decreased the P sorption on the clay surfaces, favored the solubilization of rock phosphate, and increased dry matter of ryegrass (*Lolium rigidum*) and plant P uptake. Hue (1991) found similar results in the availability of P when added organic acids on tropical soils in Hawaii and concluded that the efficiency of P fertilizers might be enhanced if these are added with organic acids or, more practically with green manures or animal wastes.

Kim et al. (1997) point out that the production of organic acid was the major mechanism involved in the solubilization of hydroxyapatite (rock phosphate) by the PSRB *Enterobacter agglomerans*, but other mechanisms might be involved. Under *in vitro* conditions, the pH of the growth medium has decreased as a result of the release of organic acids by PSRB. Some of the organic acids commonly found are gluconic acid (Bar-Yosef et al., 1999; Di-Simine et al., 1998), oxalic acid, citric acid (Kim et al., 1997), lactic acid, tartaric acid and aspartic acid. These acids are the product of the microbial metabolism, mostly by oxidative respiration or by fermentation of organic carbon sources (Atlas & Bartha, 1997; Prescott et al., 1999). Such biological reactions occur in the rhizosphere where carbonaceous compounds are used by PSRB and the phosphate released is taken up by the roots or mycorrhiza symbiosis. When PSRB are inoculated to neutral or alkaline soils, the acid production decreases the rhizosphere pH, favoring thus the solubility of calcium phosphates and apatites. In acid soils, the minerals variscite and strengite control the solubility of phosphate.

A great interest in the use of phosphate solubilizers as biofertilizers exists especially in areas with a low P availability as a result of an unfavorable soil pH. In addition, inoculates are used to improve the fertilizer efficiency of rock phosphate (Goenadi et al., 2000; Reddy et al., 2002). Improvement of phosphate uptake by plants is typically carried out by mycorrhizal fungi (Smith & Read, 1997). The mycorrhizal fungi did not mineralize organic P, however, they contribute to a closer P cycling and avoid P fixation upon mineralization by associate saprophytic microorganisms. For bacterial solubilization of phosphates in soil to be effective, several factors must operate. First of all, phosphate-solubilizing bacteria inoculants must be established in the root associated soil habitats. In addition, the role of such inoculated bacteria for the P supply seems limited, because of the transient nature of the released compounds and their possible re-fixation on their way to the root surface (Kucey et al., 1989).

K Solubilization

Ghosh & Hassan (1980) documented the state wise available potassium status in India and categorized that 21% of districts

are low, 51% are medium and 28% are high. According to Buchholz & Brown (1993) more than 98% of the potassium in soil exists in the form of silicate minerals. Early in this century, scientists found that some microorganisms in soil could damage the silicate crystal and release the Si from quartz. With the rapid development of world agriculture, available soils K levels have dropped due to crop removal, leaching, runoff and erosion. Total soil K reserves are generally large although the distribution of K forms differs from soil to soil as a function of the dominant soil minerals present (McLean & Watson, 1985). For optimal nutrition of a crop, the replenishment of a K depleted soil solution is affected predominantly by the release of exchangeable K from clay minerals. Consequently, for maximal crop growth, soil solution and exchangeable K need to be replenished continually with K through the release of non-exchangeable K through the weathering of K reserves (i.e. micas and feldspars; Sparks & Huang, 1985) or the addition of K fertilizers.

K solubilizing bacteria

Many microorganisms in the soil are able to solubilize ‘‘unavailable’’ forms of K-bearing minerals, such as micas, illite and orthoclases, by excreting organic acids which either directly dissolves rock K or chelate silicon ions to bring the K into solution (Bennett et al., 1998; Ullman et al., 1996). Therefore, the application of K solubilizing microorganisms (KSM; Barker et al., 1998; Vandevivere et al., 1994) is a promising approach for increasing K availability in KSM amended soils. Lin et al. (2002) also demonstrated that bacterial inoculation could result in growth promotion and higher K contents of plant components. The use of plant growth promoting rhizobacteria (PGPR), including phosphate and potassium solubilizing bacteria (PSB and KSB) as biofertilizers, was suggested as a sustainable solution to improve plant nutrient and production (Vessey, 2003). Increasing the bioavailability of P and K in soils with inoculation of PGPR or with combined inoculation and rock materials, which may lead to increased P uptake and plant growth, was reported by many researchers (Lin et al., 2002; Omer, 1998; Sahin et al., 2004; Wahid & Mehana, 2000).

Potassium solubilizer mediated increase in potassium uptake has been recorded by Chandra & Singh (1999). Similarly, Nayak (2001) studied the effect of potash solubilizer on brinjal and recorded increased potash uptake and increased plant biomass in potash mobilize-treated plants as compared to control.

Integrated plant nutrient management

A promising trend for increasing nitrogen, phosphorus and potassium availability to plants has been increased using combined inoculation of nitrogen fixing, phosphorus dissolving and potassium mobilizing organisms. There have been many successful attempts to improve plant development using mixtures of microbial inoculants. To access the feasibility and compatibility of different biofertilizers, co-cultured multiple inoculants is an alternative. Inoculation and introduction of more than one bacteria/fungus into target crops was referred as multiple inoculations. The combined inoculation of

Azospirillum and P-solubilizing bacteria was successfully used for N and P nutrition of wheat plants (Hesham, 2005). Namdeo & Gupta (1999) using inoculants of *Rhizobium*, Phosphate solubilizing bacteria and *Rhizobium* and PSB with 100, 75 and 50% levels of recommended dose of fertilizer (RDF), found that *Rhizobium*, PSB and *Rhizobium*+PSB with 100% level of RDF produced higher grain yield of pigeon pea than 100% of RDF alone. The treatment *Rhizobium*+PSB along with 75% level of RDF indicated a saving of 25% of the chemical fertilizer by way of producing yield equivalent to that of 100% alone. Similar results were obtained by Kumar et al. (1999) on sorghum and Panwar et al. (2000) using wheat under field condition. It is obvious that yield, quality and nutrient content of Khasi mandarin could be sustained to its maximum by integrated use of biofertilizers, organic manures and inorganic NPK fertilization (Medhi et al., 2007).

Inoculation of pigeon pea and mungbean with multiple co-inoculants (*Azospirillum*, *Azotobacter*, *Bacillus* and *Pseudomonas*) produced maximum nodule biomass, plant biomass and total soil nitrogen (Pooja et al., 2007). Mahantesh et al. (2002) studied the effect of nitrogen fixers and phosphorus solubilizing biofertilizers along with different levels of N and P on growth and yield of chilies. Application of biofertilizers along with reduced levels of chemical fertilizers showed beneficial effect when compared to application of chemical fertilizers or biofertilizers alone. Nirmala Devi et al. (1995) reported that application of *Azospirillum* and phosphobacteria as seed treatment significantly increased seedling vigour in chilli. Chellamuthu (2002) reported that in bajra, soil application of *Azospirillum* and phosphobacteria as biofertilizer mixture along with 75% recommended dose of N and P recorded more number of tillers and economically higher green and dry fodder yield. Phosphorus uptake and growth promotion of chickpea was significantly increased by co-inoculation of mineral phosphate solubilizing bacteria and a mixed rhizobial culture (Gull et al., 2004). The combination of *Azospirillum sp.* with *B. cepacea* significantly increased sugarcane yield (Shivakrishnaswamy et al., 2003). Biofertilizers containing N-fixer, P and K-solubilizers and AM fungi significantly increased the nutritional assimilation of *Zea mays* (total N, P and K) and also improved soil properties such as organic matter content and total N in soil (Wu et al., 2005).

Patidar & Mali (2004) showed that the application of biofertilizers *Azospirillum* and PSB with 10 tonnes of FYM ha⁻¹ significantly increased grain yield of sorghum over their individual effect. Application of biofertilizer, *Azospirillum* and phosphobacterium both as seed treatment and soil application along with recommended dose of chemical fertilizer (60:90:60 NPK kg ha⁻¹) increase the seed quality and yield in sunflower (Renugadevi & Balamurugan, 2002). Combined application of nitrogen fixers (*Azotobacter* and *Azospirillum*) and phosphorus solubilizing bacteria through root dipping and soil application recorded significantly higher NPK over treatments where only one biofertilizer was applied (Sharma et al., 2008). Significant increase in leaf yield and N and P uptake of mulberry was recorded with dual inoculations of *Bacillus megaterium* and *Azospirillum brasilense* as

compared to the uninoculated control and individually inoculated plants (Sukumar et al., 2003).

Formulation of biofertilizers

Researches on the formulation of biofertilizers are limited. Somasegaran (1985) demonstrated the potential of using diluted cultures of *Rhizobium sp.* with autoclaved and gamma irradiated peat for inoculants preparation. Bahme et al. (1988) developed two new delivery systems viz. granule application and low pressure drip irrigation delivery for applying rhizobacteria to potato. Fly ash and its different combinations with soil as a carrier and showed maximum viability for diazotrophs and phosphobacteria (Gand, 2004). New forms of dehydrated biofertilizers (big capsules) with *Azospirillum brasilense* have been developed for application in cereal fields (Ivanova et al., 2002). Preininger et al. (2003) developed biolistic method for the direct introduction of nitrogen fixing bacteria (*Azotobacter vinelandii*) into the strawberry tissues. Viveganandgan & Jauhri (2000) reported the superiority of alginate-based formulations over charcoal-based ones in maintaining the population of two phosphate solubilizing bacteria during storage. A method using alginate microbeads as a substrate and *Azospirillum brasilense* as the model PGPB was developed by Bashan et al. (2002). Rekha et al. (2007) demonstrated that inoculation of encapsulated bacterial isolates promoted plant growth similar to their respective free cells and could be a novel and feasible technique for application in agriculture. Sodium alginate based formulations of two plant growth promoting bacteria, *Bacillus subtilis* and *Pseudomonas corrugata* retained plant growth promoting ability, root colonization, antifungal and enzyme activities after 3 years (Pankaj & Anita, 2008).

Efficiency of biocontrol agents could be increased by the development of compatible strain mixtures (Nikoo et al., 2013). Mixed inoculants (combinations of microorganisms) that interact synergistically are currently being devised. Microbial studies performed without plants indicate that some mixtures allow the bacteria to interact with each other synergistically providing nutrients, removing inhibitory products and stimulating each other through physical or biochemical activities that may enhance some beneficial aspects of their physiology like nitrogen fixation. Plant studies have shown that the beneficial effects of *Azospirillum* on plants can be enhanced by co-inoculation with other microorganisms. Co-inoculation, frequently increased growth and yield, compared to single inoculation, provided the plants with more balanced nutrition, and improved absorption of nitrogen, phosphorus and mineral nutrients. Charpentier et al. (1999) released *P. fluorescens-putida* from the microencapsulated pellets containing 15 PGPR formulations after 261 min immersion in aqueous buffer and showed that water served as triggering material for the bacterial release. Development of cocktail formulation with compatible isolates will improve disease control through synergy in crosstalk between the isolates that lead to increased production of antibiotics at the site of colonization and thereby could suppress the establishment of pathogenic microbes. Advantages of strain mixtures include, broad spectrum of action, enhanced efficacy, reliability and also allow

combination of various traits without genetic engineering. Application of mixed PGPR strains based formulations to field might ensure at least one of the mechanism to operate under variable environment that exist under field conditions (Duffy et al., 1996).

Viveganandan & Jauhari (2000) used Calcium alginate and found it as superior to conventional charcoal–soil (3:1) carrier for phosphate-solubilizing bacteria (PSB). High populations of *Pseudomonas striata* (27) and *Bacillus polymyxa* (H-5) could be maintained in this polymer during storage. Incorporation of charcoal–soil (3:1) adversely affected the initial loading of these organisms in alginate gel. Alginate alone supported maximum survival of these organisms at elevated storage temperature (40 °C). Till date, only a few different methods are used to inoculate with *Azospirillum*. Fallik et al. (1988), Millet & Feldman (1986) and Smith et al. (1984b) developed the simplest inoculation method by application of bacteria in liquid suspension either directly to the soil or to the seeds. This technique was used in numerous greenhouse and field experiments. More reliable procedures were used with various organic carriers (Okon, 1985; Sadasivam et al., 1986). Okon & Hadar (1987) found that peat suspensions dripped into the sowing furrow or by spreading granular peat inoculants at the time of sowing is best. These practical inoculants cannot provide some of the requirements of good inoculants owing to uncontrolled bacterial release and several technical difficulties, and they thus probably result in inconsistent yield results. Bashan (1986b) and Bashan et al. (1987) used a different approach using encapsulated freeze dried bacteria in dry alginate beads which could overcome some of the problems of liquid and peat inoculants and fulfils many of the requirements for a good practical inoculants because it is dry, synthetic, simple to use, uniform, biodegradable by soil microorganisms, non-toxic in nature, contains a large uniform bacterial population, provides for the slow release of the bacteria for long periods and may be produced on a large scale. Development of advanced inoculants is a most important task in future application of *Azospirillum*.

In India, spices are grown in an area of 2.571 million hectare with a production of 3.817 million tonnes. India commands a formidable position in the worlds spice trade with 47% share in volume and 40% in value. However, about 90% of spices production in the country is used to meet the domestic demand and only 10% is exported. As per the latest statistics, black pepper in India is cultivated in an area of 197 hectares with 47.1 tonnes production and an average yield of 239 kg ha⁻¹ and cardamom in 81.8 hectares with 13.4 tonnes production and average yield 164 kg ha⁻¹. The overall scenario of spices production in the country is not encouraging. The probability of occurrence of yield of all spices above the trend-line was less than 50%. The World Spice Congress held in New Delhi, India during February 2010 expressed concern over the declining productivity of major spices in developing countries. As per latest data available, global pepper output is expected to increase to 2.95 lakh tonnes in 2010 against 2.84 lakh tonnes produced last year. Pepper productivity in India has declined mainly due to senile vines, poor soil fertility and disease incidence (International Pepper Community, 2010).

Black Pepper (*Piper nigrum* Linn.), called as the ‘‘King of Spices’’ is one of the oldest and best-known spice in the world. The humid tropical evergreen forests bordering the Malabar Coast (The Western Ghats) is the centre of origin and diversity and from there pepper was taken to Indonesia, Malaysia and subsequently to other pepper growing countries. Currently pepper is grown in 26 countries.

Cardamom (*Elettaria cardamomum* Maton.) with its pleasant aroma and taste often referred as the ‘‘Queen of Spices’’ belongs to the family *Zingiberaceae* and is the third most expensive spice in the world, after Saffron and Vanilla. It is grown extensively in the hilly regions of south India at elevations of 800–1300 m as an under crop in forest lands but today it is grown in Sri Lanka, Guatemala, Indo-China and Tanzania. It is grown on a commercial scale in Guatemala, which incidentally is also the largest producer of cardamom. Among the several factors responsible for low yield in cardamom, inadequate application of fertilizers or manures and dry spells are the major ones. Korikanthimath (2000) emphasized that application of organic and inorganic nutrients is essential as a part of scientific management that helps in maintenance of soil health and production of the crop. Maintaining soil fertility status at optimum levels should be one of the prime concerns of any cardamom planter. In India, soils of major cardamom growing areas come under the order Alfisols, formed under alternate wet and dry conditions and the suborder ustalfs derived from schists, granite and gneiss and are lateritic in nature (Sadanandan et al., 1990). The clay fraction is predominantly kaolinitic and hence there is some fixation of potassium in these soils.

The management practices in these crops include application of chemical fertilizers and plant protection chemicals which become inevitable to the crop due to the depletion of soil organic matter and increased incidence of pests and diseases. However, the extensive use of inorganic chemicals deteriorated the soil health which in turn affected the productivity of the crops. Therefore, in order to revitalize the health of the soil and to increase the productivity, it is inevitable to enrich the soil through the use of microorganisms. Moreover, these spices are highly export oriented crops and it is mandatory that the produce as well as the products from them should be in pesticide free condition as a biosafety measure. The production can be increased considerably through integrated nutrient management. However, as the extent of cultivation is increased, the amount of inorganic fertilizers and pesticides reaching the soil strata are found to be beyond the residue limit. In order to reduce the chemical pollution and to satisfy the increasing demands of organic produce, extensive research is required to replace the inorganic fertilizers with organic/biofertilizers as well as chemical pesticides to bio-protectants.

Integrated use of organic manures and biofertilizers with optimal levels of NPK fertilizer is the need of the hour, as it will not only improve the nutrient status and soil health, but also prove to be a boon in stabilizing the crop yields over a period of time. Integrated plant nutrient management (IPNM) is a wise move that has yet to gather momentum and realize its full potential. The development of integrated plant nutrient system to suit different farming systems is a major challenge for all stakeholders in agriculture to ensure sustainable

food security. A number of experiments have been conducted to study the effect of biofertilizers including N-fixers and phosphate solubilizers in black pepper and cardamom.

Nutrient management in spice crops such as black pepper and cardamom is a matter of great concern to the farmers. In early days these spices were grown with only organic manures. The increase in area under cultivation as well as the advances made in agriculture due to green revolution has made the nutrient management practices of these spices oriented towards high production technology resulting in indiscriminate use of chemical/inorganic fertilizers and plant protection chemicals. Repeated and heavy application of inorganic fertilizers has led to the degradation of soil which in turn has affected the productivity of spices especially black pepper (Pillai et al., 1979, 1987). Conversely, cardamom has responded positively to chemical fertilization but organic cultivation has proved to be a failure due to increased incidence of pests for which suitable biocontrol strategies were not available (Krishnakumar & Potty, 2002). Therefore, in order to revitalize the quality of the soil and to increase the productivity and to maintain the biological equilibrium, it is inevitable to enrich the soil through the use of beneficial microorganisms especially bacteria in black pepper and cardamom. Such bacteria have been applied to a wide range of plants for the purpose of plant growth enhancement and disease control (Barka et al., 2000). They promote plant growth by several mechanisms including N fixation, P solubilization, K solubilization, hormone secretion and suppression of soil borne plant pathogens (Chakraborty et al., 2005). Beneficial effect of *Azospirillum* inoculation has been reported in black pepper (Bashan & Levanony, 1989; Bopaiah & Khader, 1989; Govindan & Chandy, 1985; Kandiannan et al., 2000). Application of *Azospirillum* and chemical fertilizers NPK in addition to FYM resulted in high dry berry yield (Kanthaswamy et al., 1996). Higher values of all the soil nutrients of black pepper were exhibited with the application of organics and biofertilizers with inorganic combinations (Nybe & Filitte, 2003). However, very little effort has been taken so far to revitalize soil fertility using beneficial microorganisms.

Sangeeth et al. (2008, 2012) studied the performance of 36 indigenous bacterial isolates both *in vitro* and *in vivo* and shortlisted 10 highly efficient biofertilizer isolates having the potential of N fixation and P and K solubilization in black pepper and cardamom. The shortlisted isolates consisted of both root endophytes and rhizospheric inhabitants and were identified as *Azospirillum lipoferum*, *Azospirillum brasiliense*, *Agrobacterium tumefaciens*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Bacillus cereus*, *Acinetobacter baumannii* and *Paenibacillus glucanolyticus*. The amount of N fixed ranged from 3.20 to 8.7 mg N g⁻¹ of malic acid, indicating higher N fixing potential of *Azospirillum*. Though the isolates recorded growth in N-free condition, significant variation was observed among the isolates.

In the same study, they also indicated that the amount of Pi released from TCP by PSB isolates ranged from 57.0 to 80.0 mg PL⁻¹ after 15 days of incubation. Among the nine PSB isolates evaluated, five were strong P solubilizers and four were moderate solubilizers. Significant differences existed between the isolates with respect to the amount of Pi released

from TCP and were ascribed to genetic make of the strains (Deepa, 2000; Kundu *et al.* 2002; Narsian & Patel, 2006; Son et al. 2003). Potassium solubilization rate showed a positive dependence on pH. Many microorganisms in the soil are able to solubilize “unavailable” forms of K-bearing minerals, such as micas, illite and orthoclases, by excreting organic acids which either directly dissolves rock K or chelate silicon ions to bring the K into solution (Bennett et al., 1998; Ullman et al., 1996). Mbah & Nkpaji (2009) showed that when synthetic fertilizers were not applied, the use of wood ash produced significant effects on the growth and yield of many crops, particularly maize.

In planta testing of the N fixers showed significant increase in shoot and root growth, dry matter accumulation and nutrient content over uninoculated control under nursery conditions (Sangeeth, 2011). The increased dry matter in *A. lipoferum* isolates inoculation could be attributed to the increased plant height, which is one of the main contributing factors for shoot biomass. The increased height and biomass is the resultant of increased nutrient uptake by different parts of the plant system as a result of enhanced microbial activity. Higher nutrient uptake in can be related to the inoculation effects by *A. tumefaciens* and *A. brasiliense* which stimulated root growth and development of the host plants. There are also reports that inoculation of wild cardon cactus seeds with *Azospirillum brasiliense* Sp-245 significantly enhanced seed germination and seedling growth (Puente & Bashan, 1993).

Phosphate solubilizing bacterial isolates significantly enhanced growth, dry matter content and N and P uptake of black pepper (Sangeeth, 2011). The inoculated organisms established well in the rhizosphere and increased the available P in the soil, thereby increasing its uptake by the crop. Paul et al. (2005) studied the root proliferation and enhanced vigour and significant uptake of N and P of black pepper by inoculation with *P. fluorescens*. The isolate *A. baumannii* solubilized significant amount of TCP and also enhanced P uptake by the host plant. The isolates of *Agrobacterium* and *Azospirillum* also enhanced P uptake. The increase in N uptake due to inoculation may be ascribed to increased P availability to the crop and subsequent increase in biomass. The *in vivo* assay of K solubilizing isolates, *Paenibacillus glucanolyticus* and *Bacillus* sp. showed that these isolates release insoluble K from ash into the soil and enhances the K content of the soil and promote plant growth (Sangeeth, 2011). The use of plant growth promoting rhizobacteria (PGPR), including P and K solubilizing bacteria (PSB and KSB) as biofertilizers, was suggested as a sustainable solution to improve plant nutrient and production (Kavitha et al., 2007; Vessey, 2003). Microbial inoculum not only increased the nutrient assimilation of plant, but also improved soil properties, such as organic matter content and total N in soil.

The *A. lipoferum* isolates showed better colonization in different portions of root as well as in the soil. This property is attributed to the ability of *Azospirillum* to attain significant populations on the host root system which has been shown to be a prerequisite for their beneficial effects on plant growth (Bashan, 1986a). The *Azospirillum* isolates are root endophytes from black pepper. The total population density of the isolates in the inoculated microcosms was higher than those in the uninoculated microcosms, which might promote directly

or indirectly plant growth. *A. tumefaciens* and *A. brasilense* also showed efficient root/rhizosphere colonization ability. *A. tumefaciens* was a root endophyte and *A. brasilense* a rhizospheric inhabitant. The metabolic differences among the strains of *Azospirillum* and their ability to utilize the carbon sources might have influenced the differential colonization of strains as described by Wani (1992).

The increased values of the growth parameters recorded in a study (Sangeeth, 2011) clearly indicated that the inoculation of plants with these indigenous isolates resulted in better growth of black pepper and cardamom under nursery conditions. In this way, for the intensive use of inoculants with associative bacteria, it is needed to have a wide isolation to select the best combination between genotype of the plant and bacterial strain as well as selection of efficient root colonizers and Phytohormone producers that are capable of supplying significant amount of nutrients (Baldani et al. 2002; Vahid & Naser, 2011; Yasar et al., 2010).

The efficiency of the isolates with different levels of inorganic fertilizers clearly indicated that N status of the soil with organisms alone is comparable to the status at 75% NPK level which showed the potential of these organisms in fixing atmospheric N (Sangeeth, 2011). This is indicated from the uptake values which are also more than in treatments without fertilizers. Based on the performance of individual consortia, 75% of recommended dose of NPK with *A. lipoferum* + *B. subtilis* + *P. glucanolyticus* combination was superior in growth promotion, biomass production, NPK uptake, soil and leaf nutrient status and rhizosphere colonization and is at par with same combination at zero NPK. The organisms were selected based on their compatibility; hence, there will be no competition among effective organisms and therefore, *Azospirillum* inoculation was considered as a partial substitute for N fertilization. The use of N in combinations with *Azospirillum* produced significantly higher green and dry matter yields than those from inoculation or fertilization alone (Chela et al., 1993). The combined application of *Azospirillum* and 60% N produced comparable results to that obtained due to the application of the recommended doses of fertilizers and control with regard to germination percentage, plant height, cob length, cob diameter, cob weight and number of rows per cob, number of grains per row and yield of maize (Swati et al., 2011).

There are already reports of combined inoculations with different biofertilizers to replace or reduce the quantity of inorganic NPK under INM. Based on the consortia performance and overall performance, the consortia holding *A. brasilense* + *A. baumannii* + *Bacillus sp.* at 50 and 75% recommended dose of NPK fertilizer showed better growth of cardamom. The combination gives increased plant height, number of tillers, root length, total dry weight, soil and leaf nutrient status and higher uptake of P and K. Since the performance of the consortia with lowest dose of fertilizer holds well, it is advisable to apply the organisms with lowest dose in order to reduce the amount of inorganic fertilizer reaching the soil (Sangeeth, 2011).

The consortia of isolates found to be efficient in *in vivo* experiment were incorporated in different formulations and co-immobilization of isolates in alginate beads was found to be the best for survival of the consortia and individual

isolates. Uniform synthetic beads were developed as carriers for the bacterial inoculation to plants. Of the different formulations tested like talc and broth, alginate bead was found to be a suitable carrier media for delivering the biofertilizers into the rhizosphere (Sangeeth, 2011). There are also reports in accordance with the above (Fallik et al., 1988; Jambhulkar & Sharma, 2014; Millet & Feldman, 1986; Samaneh et al., 2014; Smith et al., 1984b; Sutruedee et al., 2013). This technique was used in several greenhouse and field experiments but was found inadequate because *Azospirillum* survives poorly in soil in the absence of a carrier. The rate of bacterial survival of the inoculants over time was taken as a parameter for determining the capacity of the formulations to support the survival. The black pepper isolates of *Azospirillum lipoferum* (N fixer), *Bacillus subtilis* (P solubilizer) and *Paenibacillus glucanolyticus* (K solubilizer) and cardamom isolates *Azospirillum brasilense*, *Acinetobacter baumannii* and *Bacillus sp.* also showed maximum survival in consortia in encapsulated beads (Sangeeth, 2011). Suitable and viable storage condition for long term storage of the biofertilizer encapsulated bead was also standardized. The beads stored under water showed higher microbial count both inside and outside indicating the release of organisms. The release of *P. fluorescens-putida* from the microencapsulated pellets occurred after 15 PGPR formulations 261 min immersion in aqueous buffer.

Among different organic substrates such as vermicompost (VC), farmyard manure (FYM) and neem cake (NC) tested in comparison with soil; vermicompost supports maximum growth of isolates (Sangeeth, 2011). The characteristics of beads such as disintegration, size and colour varied in different substrates. VC with efficient biofertilizers has added advantage over VC alone which has got an inherent property of improving plant growth. Vermicompost with 20–25 encapsulated alginate beads (1 g) were found optimum to enhance the growth and overall performance of black pepper and cardamom in the nursery level. Integrated nutrient supply system with biofertilizers in combination with organic manure and chemical fertilizers can be used to obtain optimum economical yield and to ensure the improvement of soil fertility with higher plant nutrient contents and higher population as compared to application of recommended dose of chemical fertilizers.

The review has clearly brought out the importance of indigenous biofertilizers in enhancing growth and nutrient uptake through multiple beneficial functions. Studies have shown that the beneficial effects on plants can be enhanced by co-inoculation, frequently; increased growth compared to single inoculation, provided the plants with more balanced nutrition and improved absorption of N, P, K and other nutrients. Therefore, it is important to identify the effective strains of beneficial microorganisms for crops, based on their compatibility and combined efficacy, both *in vitro* and *in vivo* and employ this consortia of microorganisms in real agricultural situations for efficient management and production to promote plant growth and soil health. Further research is required to study the performance of these efficient isolates in the form of consortia in combination with other inputs to improve growth of plants under field condition.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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