ALGINATE BASED CONSORTIAL FORMULATION OF NATIVE MICROBIAL FERTILIZERS

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(2017-11-095)

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ALGINATE BASED CONSORTIAL FORMULATION OF NATIVE MICROBIAL FERTILIZERS

by

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KERALA, INDIA

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DECLARATION

I, hereby declare that this thesis entitled **'ALGINATE BASED CONSORTIAL FORMULATION OF NATIVE MICROBIAL FERTILIZERS'** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Date: 04.11.2019 (2017-11-095)

CERTIFICATE

Certified that this thesis entitled **'ALGINATE BASED CONSORTIAL FORMULATION OF NATIVE MICROBIAL FERTILIZERS'** is a record of research work done independently by **Ms. Alfiya Beegum A.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LIST OF ABBREVIATIONS

BLASTn	Basic Local Alignment Search Tool
BNF	Biological nitrogen fixation
CD	Critical Difference
cfu g^{-1}	Colony forming unit per gram
cm	Centimeter
COH	College of Horticulture
DAI	Days After Inoculation
DNA	Deoxyribo Nucleic acid
EC	Electrical Conductivity
g	Gram
h	Hour (s)
ha	Hectare
HCL	Hydrochloric acid
H_2SO_4	Sulphuric acid
K	Potassium
kg	Kilogram
m	Meter
ml	Milliliter
mm	Millimeter
mM	Millimolar
N	Nitrogen
NaOH	Sodium hydroxide
NCBI	National Centre for Biotechnology Information
No.	Number
P	Phosphorus

PCR	Polymerase chain reaction
PGP	Plant growth promotion
pH	Hydrogen ion concentration
ppm	Parts per million
rDNA	Ribosomal deoxy nucleic acid
rpm	Revolutions per minute
sp.	Species
TAE	Tris acetate EDTA buffer
Trp	Tryptophan
TV	Titre value
µm	Micrometer
µg ml^{-1}	Micro gram per milli litre

Introduction

1. INTRODUCTION

Agriculture in a broad sense, is not an enterprise which leaves everything to nature without intervention. Relatively, it represents a human activity in which the farmer attempts to integrate various agroecological factors and production inputs for obtaining optimum crop and livestock production. Thus, it is reasonable to assume that farmers should be interested in ways and means of controlling beneficial soil microorganisms as an important component of the agricultural environment. The central paradigm for the biological management of soil fertility is to influence soil microbial populations and processes in such a way as to achieve a beneficial effect on soil productivity. Microbial population influences soil fertility and structure in a variety of ways, each of which has an ameliorating effect on the soil based constraints to productivity. Microflora of a soil can be effectively utilized through the application of mixed cultures of selected beneficial and efficient microorganisms than by the use of single or pure cultures however, the selected isolates should be compatible and mutually complementary.

The use of beneficial soil microorganisms as agricultural inputs for improved crop production requires the selection of rhizosphere-competent microorganisms with plant growth-promoting attributes (Hynes *et al*., 2008). Beneficial microbial interactions are responsible in the regulation of key environmental phenomena such as the mineralization of complex organic matters into simpler available nitrogen, solubilization of phosphorus and potassium and in turn regulate the plant growth and productivity. The increasing need for environment friendly agricultural practices is driving the use of microbial fertilizers.

The term ‘biofertilizer’ or ‘microbial inoculant’ can be generally defined as preparations containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic microorganisms used for application in seed, soil or composting areas with the objective of increasing number of microorganism and accelerate those microbial process which augment the availability of nutrients

that can be easily assimilated by plants (Mahdi, 2010). Biofertilizers are the key components of integrated nutrient management in soil. The idea of monitoring and employing the soil microflora through the use of inoculants, organic amendments, and cultural and management practices to create a more favourable microbiological environment for optimum crop production and protection is not new. The probability that a particular beneficial microorganism will become predominant will depend on the ecosystem and environmental condition.

A microorganism which is functioning optimally under laboratory condition might not be able to produce equivalent results under field conditions. *In situ* formulation must be refined for the sophistication of end user. Creation of new formulation is a challenge in practical microbiology. Improvement in formulations are the key to the development of enhanced high-end formulation. The increased interest in the application of bacterial preparations as plant products has promoted studies aiming at improving their stability and increasing their shelf life. The success of inoculation technology depends on two factors such as the microbial strain and inoculants formulation. Formulation typically consists of establishing viable bacteria in a suitable carrier together with additives that aid in stabilization and protection of microbial cell. The formulation should also be easy to handle and apply so that it is delivered to target in most appropriate manner and form, one that protects bacteria from harmful environmental factors and maintain or enhance the activity of the organisms in the field.

The carrier based inoculants produced in India generally have a short shelf life, poor quality, high contamination and unpredictable field performance. The carrier material used are nearly inert materials and forms clumps upon drying, which leads to significant loss of viability. High quality biofertilizers would be expected to have higher population of desired microorganisms, sufficient viability, and remain uncontaminated for longer period of storage. The encapsulation of microorganisms into a polymer matrix is still experimental in the field of bacterial inoculation technology. Encapsulated bacterial formulations temporarily protect the microorganisms from the soil environment and microbial competition and release

them gradually for the colonization of plant roots. Alginate beads are one of the carrier materials which are biodegradable, provides protection to microbial cells and release bacteria slowly at different stages of plant growth.

Alginate is a natural polymer of D- mannuronic acid and L-glucuronic acid, derived from *Macrocystis pyrifera*, *Sargassum sinicola* and they form beads with the multivalent cation Ca^{2+}. Encapsulation enables slow and controlled release of cells and the beads are biodegradable, non-toxic, and maintains a uniform bacterial population. Formulation and field application of inoculants play an important role in improving the potential yield of crops. A good carrier material with controlled release of microorganisms and one that provides protection to the bacteria under stress condition are the need of the hour. On perusal of literature, it is revealed that the systematic research on alginate based microbial fertilizers in Kerala is scanty. So, there is a need to standardize the alginate based formulation using simple methods which is cost effective. Hence a study was undertaken on "Alginate based consortial formulation of native microbial fertilizers" with the following objectives

1. To standardize the optimum concentration of sodium alginate solution and calcium chloride solution needed for alginate beads formation

2. To assess the solubilization of alginate beads in phosphate buffer

3. To evaluate the size, moisture per cent of beads and release of bacteria from alginate beads

4. To evaluate the effect of alginate and talc based consortia on growth and yield of tomato

Review of literature

2. REVIEW OF LITERATURE

Current agricultural practices emphasize on environmental sustainability by limiting the use of chemical fertilizers and pesticides. Rovira and Bowen (1966) reported that the application of plant growth promoting rhizobacteria into agricultural soils have proven to enhance the crop yield tremendously. Soil amendments offer promising alternatives to minimize the deleterious effects of chemical fertilizers (Bashan, 1998). According to Cattelan *et al*., (1999) plant growth promoting microorganisms must be rhizosphere competent, able to survive and flourish in soil following inoculation. According to Ping and Boland (2004), the ability of microorganism to produce and release various metabolites affecting plant growth is considered as one of the most important factors in soil fertility. The ability of phytohormone production, nitrogen fixation, phytopathogen antagonism, cyanogenesis, phosphate solubilization, potash solubilisation and ACC deaminase activities are the main plant growth promoting related traits beneficial to the plant.

Most of the soils of world are deficient in nitrogen and application of fertilizer nitrogen is essential for good yield. Urea is most commonly applied nitrogen source. But, less than 50% of the applied urea is only utilized by plants (Halvorson *et al*., 2002) and the loss is due to volatilisation, denitrification and leaching. Therefore, eco-friendly and economical alternatives have been increasingly demanded. World food production is based on the extensive use of chemical fertilizers, which not only pollute the environment but also are expensive due to their non-renewable sources like fossil fuels, used in their exploitation, transportation and application (Schoebitz, *et al*., 2013). Among some of these alternatives, plant-growth promoting bacteria (PGPB) are sustainable and low-cost biofertilizers, but need specific formulation when used in agronomical practices (Malusa and Vassilev, 2014).

Biofertilizers as inoculants must have three fundamental characteristics *viz* to promote bacterial growth, to keep the cells viable for a certain period of time and to release a minimum population of bacteria, which will certainly be associated to plants (Bashan *et al*., 2014). Microbial survival after soil inoculation depends on both abiotic and biotic factors.

2.1. Microbial fertilizers and its importance

The term 'biofertilizer' or 'microbial inoculant' can be generally defined as preparations containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic microorganisms used for application in seed, soil or composting areas with the objective of increasing number of microorganism and accelerate those microbial process which augment the availability of nutrients that can be easily assimilated by plants (Mahdi *et al*., 2010). Biofertilizers play a very important role in improving soil fertility by fixing atmospheric nitrogen, both in association with plant roots and without it, solubilise insoluble phosphates and potassium and also produces plant growth substances in soil.

The long-term use of biofertilizers is economical, eco-friendly, more efficient, productive and accessible over chemical fertilizers (Venkataraman and Shanmugasundaran, 1992). Biofertilizers being essential components of organic farming play a vital role in maintaining the long-term soil fertility and sustainability by fixing atmospheric dinitrogen (N=N), mobilizing fixed macro and micro nutrients and convert insoluble P in the soil into forms available to plants, there by increases their efficiency and availability. Currently there is a gap of ten million tonnes of plant nutrients between removal of crops and supply through chemical fertilizers. (Mahdi *et al*., 2010)

Organisms that are commonly used as biofertilizers are nitrogen fixers (N-fixer), phosphorus solubilizer (P-solubilizer) and potassium solubilizer (K-solubilizer), or with the combination of molds or fungi. These potential biological fertilizers would play a key role in improving the productivity and sustainability of soil and also protect the environment as eco-friendly. The most important

biofertilizers which improves the plant growth are nitrogen fixing biofertilizers, phosphatic biofertilizers and potassic biofertilizers. Nitrogen fixing biofertilizers increase the soil nitrogen level by fixing the atmospheric nitrogen and make it available to plants.

Phosphatic biofertilizers can be either phosphorus mobilizing or solubilising microorganisms. Phosphatic biofertilizer bring insoluble phosphate in soil to soluble forms by secreting various organic acids. These acids help in lowering the pH and brings about the dissolution of bound forms of phosphorus (Gupta and Samnotra, 2004). Examples are species of *Pseudomonas, Bacillus etc*. Phosphorus mobilizing biofertilizers mobilize the phosphorus from soil to the root cortex by increasing the surface area such as Arbuscular mycorrhiza. Potassium solubilizing biofertilizers contains microorganisms which solubilize insoluble potassium minerals into soluble forms such as *Bacillus, Acinetobacter etc*.

2.1.1. Nitrogen fixation by microbial fertilizers

Nitrogen is one of the major important nutrients which is very essential for crop growth. Microorganisms have the capability to fix atmospheric nitrogen and make it available to plants. Even though, 78% of nitrogen is present in atmosphere, it is unavailable for plants. The atmospheric nitrogen is converted into plant utilizable form by biological nitrogen fixation (BNF), which converts nitrogen to ammonia using a complex enzyme system called nitrogenase (Kim and Rees, 1994). In fact, BNF accounts for approximately two-third of nitrogen fixed globally while the rest of nitrogen is industrially synthesised by Haber-Bosch process (Rubio and Ludden, 2005). The major part of the elemental nitrogen that finds its way into the soil is entirely due to its fixation by certain specialized group of microorganisms.

Apart from *Rhizobium* sp. which is symbiotically associated with legume plants and fix atmospheric nitrogen, there are associative and free-living nitrogen fixers which are known to fix nitrogen. They are equally important to harness atmospheric nitrogen for non-leguminous plants and helps in improving soil fertility.

Even foliar spray of nitrogen fixing bacteria *Azotobacter chrococcum* increased grain and straw yield rice under field condition (Kannaiyan,1980). *Azotobacter* sp. has the ability to fix atmospheric nitrogen and is known to enhance the photosynthetic potentialities of a plant (Okon and Itzigsohn, 1995). Inoculation of *Azotobacter* sp. had resulted in higher concentration of nitrogen in tissues and increased the yield parameters of crop plants (de Freitas, 2000). Khaleequzzaman and Hossain (2008) showed application of *Azotobacter* increased germination, plant height and yield of bush bean. Under greenhouse conditions, plant height, leaf number per plant, number of primary and secondary branches per plant, fresh and dry weight of whole plant, number of siliqua per plant, seeds per siliqua of brown sarson increased significantly with *Azotobacter* inoculation than no inoculation with seed (Wani *et al*., 2013).

Rovira (1965) reported yield increase in wheat after inoculation with *Azotobacter chrococcum*. Inoculation with *Azotobacter* increased the number of root hairs, tillering ratio, dry matter content and N- uptake in plants (Rai and Gaur, 1982). The genus *Paenibacillus* which contains 32 species include *P. polymyxa* and *P. azotofixans* are frequently associated with plants which helps in fixing atmospheric nitrogen (Berge *et al*., 1991). *P. polymyxa* strain (P2b-2R) colonized the pine rhizosphere with $1.65 \pm 0.5 \times 10^5 cfu^{-1}g$ root tissue. Pine seedlings derived 30-60% of foliar nitrogen from the activity of *P. polymyxa* (Bal and Chanway, 2012). *Paenibacillus polymyxa* successfully colonised the rhizosphere of canola and tomato with nearly 10^6-10^7 cfu^{-1} g dry root (Puri *et al*., 2016).

Strains of *Microbacterium* sp. isolated from the phyllosphere of rice significantly increased the plant height, shoot and root biomass after inoculation (Santosa *et al*., 2003). *Burkholderia* inoculation in sugarcane resulted in an effect greater than increasing the fertilizers from half to the full recommended rate saving the cost of nitrogen fertilizers (Raja *et al*., 2006). *Microbacterium* strains containing nif H gene have been isolated from legume nodules (Zakhia *et al*., 2006). *Microbacterium*, a nitrogen fixing bacterial strain isolated from surface sterilized stem of sugarcane grown in China were able to increase the height and stem

diameter in sugarcane plants after inoculation in sugarcane ratoons (Lin *et al*., 2008).

Pre-treatment of soil with *Microbacterium* sp. showed a maximum increase in growth and biomass in terms of root length (93%), dry root biomass (99%), and dry shoot biomass (99%) in *Pisum sativum* under pot culture experiment (Soni *et al*., 2014). They also reported that inoculation of the same strain of *Microbacterium* sp. improved the growth and yield of maize.

Inoculation with nitrogen fixing bacteria significantly increased the chlorophyll content and the uptake of different macro-micro nutrients in red pepper shoot in comparison with uninoculated control. The highest rate of nitrogen fixation determined by acetylene reduction assay occurred in *Novosphingobium* sp. (Islam *et al*., 2013).

Gluconacetobacter diazotrophicus has been shown to contribute significantly to the nitrogen nutrition of sugarcane under controlled conditions (Sevilla *et al*., 2001). A strain of *Cellulosimicrobium cellulans* promoted the growth of chilli plants (Chatterjee *et al*., 2009). The plant growth promoting ability of *Cellulosimicrobium* sp. is based on the production of phytohormones IAA, biosynthesis of siderophore, the ability to fix atmospheric nitrogen and production of enzymes (Nabti *et al*., 2014). Brassica seeds bacterized with rhizosphere isolates of *Cellulosimicrobium* sp. showed significant increase in shoot length, root length, fresh weight and dry weight over control (Singh *et al*., 2014).

Nitrogen fixation is reported in species of *Paenibacillus* such as *Paenibacillus polymyxa, Paenibacillus azotofixans, Paenibacillus odorifer* (Witz *et al*., 1967; Seldin *et al*., 1984). *Brevundimonas* sp. has been used as a PGPR in enhancing growth of wheat plants (Rana *et al*., 2011). In a pot experiment conducted by Kumar and Gera (2014), inoculation of cotton seeds with *Brevundimonas* sp. enhanced the growth of plants by a significant increase in plant height (68.41%), shoot dry weight (58.44%) and root dry weight (64.81%) over untreated control.

2.1.2. Phosphorus solubilization by microbial fertilizers

Phosphorus is a major macronutrient which is necessary for the growth and development of plants. The phosphorus solubilizing microorganisms mainly bacteria and fungi make insoluble phosphorus available to the plants (Gupta and Samnotra, 2004). Several soil bacteria and fungi possess the ability to bring insoluble phosphate in soil into soluble forms by secreting organic acids. These acids lower the soil pH and bring about the dissolution of bound forms of phosphate.

Peix *et al*. (2001) reported that the strain *Burkholderia cepacia* (SAOCVe) was able to mobilize phosphorus efficiently in the common bean, as a result the phosphorus content increased by 44% with respect to uninoculated plants. According to El-Komy (2005), wheat inoculated with mixed inocula of *Bacillus megaterium* and *Azospirillum lipoferum* exhibited high shoot dry weight and the total nitrogen yield and shoot phosphorus content increased by 37% and 53% respectively compared to un-inoculated ones.

Increased dry matter yield of leguminous crops was reported in cowpea, gram and *Vicia faba* respectively due to phosphorus solubilizing bacteria inoculation (Bajpai 1974; Wani *et al*., 1985; and Khalafallah *et al*.,1982). Gyaneshwar *et al*. (2002) reported that the application of rock phosphate along with phosphobacteria as a possible substitute for super phosphate without any apparent reduction in crop yields. Baig *et al*. (2014) reported that *Bacillus subtilis* strain (KAP5) with dual activity of phosphate solubilization and AC deaminase activity caused the highest increase in grain yield of maize. According to Mehta *et al*. (2014) maximum P solubilisation (957.3 mg l^{-1}) was reported in *Bacillus circulans* with a decrease in pH from 6.92 to 4.69.

The tricalcium phosphate solubilization ranged from 96 to 139 µg ml^{-1} by 13 best bacterial isolates from Korean soils and were clustered under the genera *Enterobacte*r, *Pantoea* and *Klebsiella*. Gulati *et al*., (2009) stated that a few species of the genus *Pseudomonas* such as *P. aeruginosa, P. corrugta, P. lutea, P.*

fluorescence, P. rhizosphareae and *P. stutzeri* are known to be good phosphate solubilizers.

According to Song *et al*. (2008) the amount of released phosphorus showed a gradual increase and reached a concentration of 345.9 mg/L by the isolate *Burkholderia cepacia* (DA23) with tricalcium phosphate as the source. The pH value dropped to 4.23 from an initial value of 7.0, which indicated that the reduction is inversely correlated with the phosphorus concentration.

Co-inoculation of *Pseudomonas striata* and *Bacillus polymyxa* strains showing phosphate solubilizing ability with strain of *Azospirillum brasiliense* resulted in a significant improvement of grain and dry matter yield with a corresponding increase in N and P uptake (Alagawadi and Gaur, 1992). The significant increase in plant height and dry weight of walnut seedlings were due to inoculation with PSB strains *Pseudomoas chlororaphis* and *P. putida*, enabled the greater absorption of phosphorus (Yu *et al*., 2011).

2.1.3. Potassium solubilization by microbial fertilizers

Potassium is the third major essential macronutrient for plant growth. The concentration of soluble potassium in soil is usually low and more than 90% of it exists in the form of insoluble rocks and silicate minerals. Rhizosphere bacteria have been found to dissolve potassium from insoluble K bearing minerals. A wide range of bacteria namely *Pseudomonas*, *Burkholderia*, *Acidothiobacillus ferroxidans*, *Bacillus mucilaginosus*, *Paenibacillus* sp. has been reported to release potassium in accessible form from K bearing minerals in soils. The indigenous rhizosphere microorganisms are effective in releasing potassium from structural K through solubilization and form exchangeable pools of total soil K by acidolysis, chelation and solubilization by microorganism (Uroz *et al*., 2009)

Hu *et al*. (2010) isolated two phosphate and potassium solubilizing bacteria *Paenibacillus mucilaginosus* of different strains from the soil of Zhejcang province, China. Both were efficient solubilizer of phosphorus and potassium. Potassium

solubilization was found maximum when KCl was used as K source followed by K_2SO_4 and less solubilisation was found in mica powder.

Rokhbaksh-Zamin *et al.* (2001) reported that the strains *Acinetobacter* sp. (PUCM1007) and *A. baumanii* (PUCM10029) were able to solubilize phosphorus, potassium, zinc oxide and also promote growth through the production of IAA. *Bacillus mucilaginosus* increased potassium availability in soils and increased mineral content in plants. Integrated application of rock phosphorus and potassium minerals with coinoculation of bacteria that solubilize them provide faster and continuous supply of P and K for the optimum growth of plants (Sheng and He, 2006).

Bacillus edaphicus isolated from rhizosphere soil of cotton was a best K solubilizer, water soluble K in the solution increased due to the production of oxalic acid and tartaric acid concentration. The bacterium *Paenibacillus glucanolyticus* isolated from black pepper rhizosphere showed high capacity to solubilise potassium (Gundala *et al*., 2013).

Sugumaran and Janarthanan (2007) isolated K solubilizing bacteria *Bacillus mucilaginosus* and the maximum solubilization was 4.29 mg l^{-1}. Prajapati *et al*. (2013) isolated potassium solubilizing bacterium *Enterobacter hormaechei* and a fungal strain *Aspergillus terreus* and studied their effects and nutrient uptake on okra (*Abelmoschus esculenta*) in K deficient soil using pot experiment. *E. hormaechei* increased root and shoot growth of plant and both were able to mobilize K in okra plant when feldspar was incorporated into pot soil.

Hassan and Hamad (2010) studied the efficacy of *Bacillus circulans* on Khella (*Ammi visnaga*) along with feldspar which increased the plant growth parameters. Inoculation of tea plants with K solubilizing bacteria *Pseudomonas putida* leads to the improvement in tea productivity and nutrient uptake in plants. Quality parameters such as theaflavin, thearubigin, total liquor colour, briskness, caffeine, flavour indexes were greatly improved in K solubilizing bacteria treated plants (Bhagyalekshmi *et al*., 2012).

The solubilization of feldspar by *Bacillus mucilaginosus* and *Bacillus edaphicus* was by the production of citric, tartaric and oxalic acid (Malinovskaya *et al*., 1990; Sheng and He, 2006). Singh *et al*. (2010) have shown that wheat and maize yields increased with the inoculation of *Bacillus mucilaginosus*, *Azotobacter chrococcum* and *Rhizobium* sp. under phytotron growth chamber. A significant improvement in growth parameters of Capsicum Hungarian yellow was observed when a K solubilizing strain *Frateuria aurantia* enriched phosphor compost was applied (Pindi and Satyanarayana, 2012).

Saha *et al*. (2016) reported two bacterial strains *Bacillus licheniformis* and *Pseudomonas azotoformans* which showed higher potassium solubilization 7.22 and 6.03 μg ml-1 at 30 ^{o}C respectively. A higher zone of solubilization was recorded with *Pseudomonas azotoformans* which is 3.61cm. Bakhshandeh *et al*. (2017) reported that *Pantoea ananatis*, *Rahnella aquatilis* and *Enterobacter* sp. increased the plant height, stem diameter, root length, leaf area, biomass and dry weight of rice in a pot experiment when compared with control. The isolates significantly solubilized potassium from mica in both solid and liquid medium *in vitro*.

Keshavarz-Zarjani *et al*. (2013) reported the isolates *Bacillus megaterium* and *Arthrobacter* sp. can potentially increase the dissolution of K bearing minerals. The results showed that *B. megaterium* was having higher potential than *Arthrobacter* sp. The inverse relationship observed between pH and soluble K concentration indicated that organic acid production by these strains play a significant role in acidification of the medium facilitating K solubilization.

2.1.4. Indole acetic acid (IAA) production by microbial fertilizers

Indole acetic acid comes under the group of phytohormones and is considered as the most important native auxin. IAA regulates plant growth and development. It is responsible for division, enlargement and differentiation of plant cells and tissues, it plays a major role in xylem and root formation. Many bacteria isolated from rhizosphere have the capacity to synthesise IAA *in vitro* in the presence or absence of physiological precursors mainly tryptophan (Davies,1999).

IAA has been implicated in a wide range of developmental process some of them include elongation, growth, phototropism and gravitropism, apical dominance, lateral root initiation, differentiation of vascular tissues, embryogenesis, fruit setting, ripening and senescence (Macdonald,1997). IAA produced by several bacteria can stimulate the development and proliferation of roots, with increases in uptake of water and nutrients (Bashan and de-Bashan, 2005).

Tien *et al*. (1979) reported that *Azospirillum brasiliense* produced both indole acetic acid and indole lactic acid. The production of IAA increased with the concentration of tryptophan from 1 to 100 μg ml^{-1}. IAA concentration increased with the age of culture until the bacteria reached the stationary phase. When canola seeds were inoculated with *Pseudomonas putida* (GR12-2), which produced low levels of IAA resulted in three times increase in length of seedling roots (Glick *et al*.,1986; Caron *et al*., 1995).

Most root promoting bacteria synthesise IAA and stimulate the formation of lateral and adventitious roots (Barbieri and Galli, 1993). The tryptophan increased the production of IAA in *Bacillus amyloliquefaciens* (Idriss *et al*., 2002). Previous studies on biological nitrogen fixation by Chaiharn and Lumyong (2011) revealed the role of IAA produced by rhizobacteria in increasing the absorption of nutrients by increasing the production of root hairs in plant.

Pedraza *et al*. (2010) found that *Azospirillum* strains produced highest concentrations of IAA (16.5-38 μg IAA/ mg protein) whereas *Gluconoacetobacter* and *Pseudomonas stutzeri* produced lower amount of IAA (1-2.9 μg IAA/ mg protein). Gravel *et al*. (2007) stated that the production of IAA by *Pseudomonas putida* and *Trichoderma atroviridae* were stimulated *in vitro* with the addition of tryptophan and tryptamine in the culture medium. *P. putida* and *T. atroviridae* increased the fresh weight of both shoot and root of tomato grown in presence of increasing L- tryptophan.

Ahmad *et al*. (2008) reported that the production of IAA in fluorescent *Pseudomonas* isolates increased with an increase in tryptophan concentration from

1-5 mg ml^{-1}. The effect of auxin on plant seedlings are concentration dependent and low concentration will stimulate the growth while high concentration may be inhibitory. The bacterium *Ochrobactrum* sp. produced 6.68 μg ml^{-1} indole acetic acid in the presence of tryptophan and played an important role in plant growth, pathogen control, biodegradation and rhizosphere signalling (Imran *et al*., 2014).

IAA is involved in tomato fruit development especially during fruit setting and in the final phase of development (Srivastava and Handa, 2005). Maximum increase in shoot length and fresh weight were observed with *Bacillus* sp. (11.30%) and *Pseudomonas* sp. (40%) over control. Shoot length and fresh weight recorded highly significant correlation with auxin production for GC-MS analysis.

Acinetobacter rhizosphaerae produced 7.9-8.3 μg ml^{-1} IAA. The root exudates of plants contain rich supplies of tryptophan which is used by microorganism for synthesis and release of auxin as secondary metabolites in rhizosphere (Gulati *et al*., 2009). Mohite (2013) reported that IAA production by *Bacillus* sp. was highest at pH 6 and it decreased by 62 % at pH 5. In the case of *Paenibacillus* sp., IAA production was highest at pH 5 and decreased by 42% at pH 7, which implied that the effect of pH on IAA production varied for different microorganism. *Azospirillum brasiliense* immobilized in alginate beads produced 4-10 μg ml^{-1} IAA during a cycle of 48 hours from which *Chlorella vulgaris* absorbed 0.5-1 μg ml^{-1}.

Ahmad *et al*. (2008) reported that more than 80% of the isolates such as *Azotobacter*, fluorescent *Pseudomonas* and *Mesorhizobium ciceri* produced IAA whereas only 20% of *Bacillus* isolates were IAA producer. Karnwal (2009) stated that *Pseudomonas aeruginosa* was less effective in production of IAA than *P. fluorescens*. Bal *et al*. (2013) revealed that among the six isolates, *Bacillus* sp. produced highest amount of IAA and the lowest was produced by *Corynebacterium* sp. All the isolates produced high quantity of IAA during their stationary phase of growth.

Seed inoculation with two rhizobacterial strain (*Pseudomonas syringae*) which produced higher amount of IAA reduced the primary root length and

increased the shoot: root ratio of sugar beet seedlings compared with control (Loper and Schroth, 1986). Chatterjee *et al*. (2009) stated that bio inoculation with *Cellulosimicrobium cellulans* improved the growth parameters of plants through its IAA production and phosphate mineralization in pot experiment.

Azotobacter diazotrophicus produced 0.14 to 2.42 µg ml^{-1} IAA in the culture medium. As it was found within the plant tissue, the biosynthesis of IAA suggest that bacteria could promote rooting. It improves sugarcane growth by direct effects on metabolic process in addition to nitrogen fixation (Fuentes-Ramirez *et al*., 1993). Production of IAA in the presence of suitable precursor such as tryptophan has been reported for several PGPR belonging to *Azospirillum*, *Azotobacter*, *Burkholderia*, *Enterobacter*, *Pantoea*, *Pseudomonas* and *Serratia* (Tsavkelova *et al*., 2007).

2.2. Carrier materials used for microbial inoculant production

A suitable carrier material is required for successful field application of any biofertilizer. A carrier material acts as the delivery vehicle of live microorganisms from the factory to the field (Bashan, 1998). It provides a protective niche to microbial inoculants in soil, either physically, via the provision of a protective surface of pore space or nutritionally, via the provision of a specific substrate (Arora *et al*., 2008).

The carrier should be free of toxic materials, biodegradable, and non-polluting with minimum environmental risks. A good carrier material should assure a sufficient shelf life, adhere well and should be able to survive on seeds, allow a rapid and controlled release of the microorganisms into the soil near the roots of the host. Kandasamy and Prasad (1971) reported that *Rhizobium* sp. population in lignite based carrier material increased from 13.14 x 10^8 to 19.15x10^8 when it was amended with one per cent soybean powder. Potential life of inoculum in carrier material may be increased by supplementing it with some amendments.

Tilak and Subba Rao (1978) reported that the addition of charcoal to peat soil, FYM and press mud helped to retain viability of *Rhizobium* sp. up to three months storage at 30°C. Tilak *et al*. (1988) tested several materials including peat as carrier for *Azospirillum* soil and farm yard manure mixed in the ratio of 1:1. They also conducted studies on survival of *A. brasilense* in different carriers at room temperature, which revealed that the combination of soil and FYM helped to retain high population (40.0 x 10^8 cfu g^{-1} dry material) up to six months.

Sadasivam *et al*., (1986) reported maximum survival of *Azospirillum* and *Azotobacter* in a mixture of 75:25 of peat and black ash compared to peat or lignite alone. Survival of *Rhizobium* strains in different inoculant formulations such as peat, perlite vermiculite (sterile and unsterile) under different storage conditions were tested. Perlite was as effective as peat in maintaining a high population of *Rhizobium* after 6 months of storage (Temprano *et al*., 2002).

Pandher *et al*. (1993) studied the growth and survival of *Rhizobium* isolates in different carriers (charcoal, lignite, soil, lignite + charcoal, soil + charcoal). Lignite and charcoal favoured higher growth and survival of *Rhizobium* compared to soil and soil+ charcoal which proved to be poor carriers. Roy *et al*. (2010) studied the survival of *A. chroococcum* in different carriers such as charcoal, lignite cured compost and vermicompost population. Crop response to *A. chroococcum* with vermicompost as inoculant carrier was found to be higher when compared with other carriers.

Smith (1992) stated that the raw materials chosen for use in conventional, seed applied, inoculants are determined by availability, consistency of quality and cost. The carrier must display two fundamental properties; it must support growth of the target organism and should maintain the desired populations of inoculant strains.Inocula tion with carrier based *Azotobacter* sp. improved the soil available nitrogen content, growth and yield of onion when compared with the uninoculated control (Balemi *et al*., 2007). Sarma *et al*. (2011) used vermiculite formulations of

fluorescent Pseudomonads strains R62 and R81. The combined inoculation of these two formulations resulted in significant increase in dry root weight, dry shoot weight, and fruit yield of tomato.

The sawdust-based inoculant formulations of *Rhizobium leguminosarum* and *Pseudomonas fluorescens* were better than any other carrier-based inoculant in enhancing seedling biomass and nodule number of *Trifolium repense* (Arora *et al.*, 2008). Gulati *et al*. (2009) prepared bacterial suspension of phosphate-solubilizing PGPR, *Acinetobacter* sp. and mixed it with sterilized activated charcoal (4:6 v/w) to obtain a population density of 10^7/g charcoal. Significant increase in growth of pea, chickpea, maize, and barley were noticed due to inoculation of charcoal based formulation under controlled as well as field conditions.

Tittabutr *et al.* (2007) determined the effectiveness of *Bradyrhizobium* liquid inoculant formulations with gum arabic, polyvinyl pyroledine (PVP), polyethylene glycol (PEG), polyvinyl alcohol (PVA) and cassava starch under field conditions and found that the effectiveness of liquid inoculant was as good as peat based inoculant. According to Biswas and Bhowmmick (2007), the basal application of inorganic nitrogen at the rate of 20 kg/ ha gave the highest seed yield for urd bean and it was closely followed by seed treatment with liquid *Rhizobium* inoculant.

Liquid formulations typically are aqueous, oil, or polymer-based products. They are formulation containing not only the desired microorganisms and their nutrients but also special cell protectant and additives that promote cell survival in storage and after application to seed or soil (Brahmaprakash and Sahu, 2012). Trehalose (15 mM) and PVP (2.5%) were the most suitable chemical additives for enhancing the shelf life of *Azospirillum* sp. and PSB respectively upto 9 months with a population of 108 cfu/ml. These results indicated that the shelf-life of *Azospirillum* sp. and PSB could be enhanced upto 9 months at room temperature when compared to shelf-life of carrier based inoculant (Gopal and Baby, 2016)

2.3. Microbial consortium for plant growth promotion

Soil being a heterogeneous, unpredictable environment, the inoculated bacteria finds it often difficult to establish a niche for survival amongst the competitors and predators. The immediate response will vary depending upon bacteria, plant species, soil type, inoculant density and environmental conditions resulting in a progressive decline in the inoculated bacterial density. Thus, it fails to elicit the intended plant response.

The combination of various groups of beneficial soil microorganisms such as N-fixers, P solubilizers, K solubilizers, plant growth promoters have been exploited for improving fertility status of soil, combination of these beneficial microorganisms is well adopted in agriculture to enhance yields. A combination of different microorganisms is also known as "microbial consortium", which is much more efficient than single strains of organisms with a diversity of metabolic capabilities (Sarma *et al*., 2015).

Combined biofertilizers are popular because nitrogen, phosphorus and potassium is provided in a single formulation and it reduces operational cost and increases growth and yield of crop plants. It is important to identify the best strain of beneficial microbes for planting situation, verify their compatibility and combined efficacy both *in vitro* and *in vivo* and employ this combined multiple inoculum as an essential biofertilizers for production practices. Higa and Wididana (1991) stated that the introduction of mixed cultures of microorganisms to soils and plants would likely be more effective and for a longer period of time than pure culture.

2.3.1. Alginate based formulations

Formulation of inoculant carrier is an industrial art of converting a promising laboratory proven bacterium to a commercial field product. Immobilization of microbial cells into a polymer matrix is more advantageous over direct soil inoculation. (Cassidy *et al*., 1997). The main aim of encapsulation of plant growth promoting rhizobacteria is to protect them from harsh soil environment, reduce microbial competition and release them gradually to facilitate colonization of plant roots (Vassilev *et al*., 2001; Bashan *et al*., 2002).

The alginate polymer is made up of two monomeric units: b-(1,4) linked D-mannuronic acid (M) residues and a-(1,4)-linked L-guluronic acid (G) residues. The basic structure of alginates consists of linear unbranched units of polymers made up of monomers arranged in blocks of M and G residues interspersed with regions containing alternating M-G sequence within the structure (Donati and Paoletti, 2009; Draget, 2009).

Alginate is one of the most commonly used polymers for microbial encapsulation. It is commercially extracted from sea weeds like *Macrocystis pyrifera, Ascophyllum nodosum, Laminaria etc* (Yabur *et al*., 2007). The size (diameter) of alginate gel particles typically range from > 1 mm (macro), 0.2 to 1000 mm (micro) and < 0.2 mm (nano). These gel particles, which typically hold high water content, have adjustable chemical and mechanical properties that are dependent on the type of crosslinking agent used. As a natural ingredient, alginate gel particles are attractive for biological applications because they are biocompatible, nontoxic, biodegradable, and relatively cheap (Andersen *et al*., 2014).

Alginate bead encapsulation protect the inoculants from stress factors and release them gradually thus serving as viable inoculum source for a longer period

to facilitate their establishment in rhizosphere. Alginate beads are also capable of entrapping sufficient number of bacterial cells (Zohar-Perez *et al.,* 2002).

Park and Chang (2000) revealed that gel like matrix allows the cell to remain viable and with its catalytic ability for longer duration. Young *et al*. (2006) reported significant increase in the root and shoot lengths of lettuce with an increase of 28% and 33% respectively following the inoculation with 2.0 x 10^7 suspension of *B. subtilis*. The humic acid enriched beads were spherical in shape with a diameter between 2 mm and 3 mm. Drying of beads resulted in uneven surface of bead and decreased the volume. The porous alginate gel matrix protects the cell against mechanical stress, facilitates the survival for prolonged storage period as well as helps in cell release from the beads. Maximum total nitrogen and phosphorus uptake in green gram was recorded in triple inoculants followed by dual inoculants and single inoculants in which *Bacillus megterium*, *Rhizobium* sp. and *Pseudomonas fluorescens* were used in this study (Gangaraddi and Brahmaprakash, 2018).

Rhizobium sp. was entrapped in sodium alginate and a mixture of xanthan and carob gum as legume inoculants, this was successfully stored for over 90 days (Jung *et al*., 1982). *Azospirillum brasiliense* and *Pseudomonas fluorescens* were encapsulated in alginate beads and they were found alive even after 14 years of ambient temperature storage. A significant number of cells 10^5-10^6 cfu g^{-1} beads survived even after 14 years of storage (Bashan and Gonzalez, 1999).

The survivability of microorganisms in any formulation depend upon the water availability in the product. Water availability is a better representative of moisture available for living organisms. Mugnier and Jung (1985) studied the effect of water activity on the survival of bacteria, fungi and yeast in polymer gels. They found that survival remains constant for more than three years when the water activity is kept below 0.069. Survival decreases when the water activity rises above 0.069 proving that less moisture in the polymer gel gives more protective effect to the inoculum.

Devi *et al*., (2012) reported that the survival of *Bradyrhizobium* sp. and *Bacillus megaterium* was higher in alginate beads when compared with liquid lignite inoculant formulations. Alginate composite inoculant formulations recorded maximum number of cells at the end of 240 days of storage. The viability of microorganism is highly influenced by the shelf life which ultimately depends on the bacterial species, culture medium, physiological condition, storage temperature and water concentration in the inoculum (Schoebitz *et al*., 2013; Sivasakthivelan & Saranraj, 2013).

Kundu and Gaur (1984) reported a higher grain yield, increased phosphorus and nitrogen content as a result of co-inoculation with *Azotobacter chroccocum* and phosphate solubilizers *Pseudomonas striata, Bacillus polymyxa* in the rhizosphere soil than the application of a single inoculant in wheat. Nethravathi and Brahmaprakash (2005) worked on the development of alginate based biofertilizer of *Bradyrhizobium japonicum* and *Bacillus megaterium* for soybean. Root and shoot phosphorus content, plant height and other plant growth parameters were high with respect to dual inoculants when compared with individual inoculants. Ivanova *et al*. (2005) studied encapsulation of nitrogen fixing *Azospirillum* in alginate and found that their application resulted in better yield than application of liquid and powdered formulations in field conditions.

Archana (2011) carried out a study on the survival of *Azotobacter chrococcum*, *Acinetobacter* sp. and *Pseudomonas fluorescens* in alginate based formulation. Results showed that consortium containing triple inoculants gave higher biomass, number of leaves, plant height, stem girth and nutrient uptake in sorghum when compared to single and dual inoculants.

Swapna *et al*., (2015) reported that the consortium of microorganisms has increased the maximum number of leaves, plant height, chlorophyll content, root and shoot nitrogen, phosphorus and total biomass in finger millet when compared

to individual inoculant. *Azotobacter chrococcum*, *Pseudomonas fluorescens* and *Bacillus megaterium* were used as inoculants.

Trivedi *et al*. (2005) studied on carrier based preparations of two plant growth promoting rhizobacteria (*Bacillus subtilis* and *Pseudomonas corrugata*) developed in five different formulations. They were alginate beads, alginate beads supplemented with skim milk, alginate coated seed, charcoal based and broth based ones. Among the formulations, alginate based formulations were more effective followed by charcoal and broth based formulations respectively. Viability of the bacterial inoculants was maximum in alginate beads and alginate supplemented with skim milk formulations after 180 days of storage at 4 ^{o}C.

According to Trevors *et al*. (1993), *Pseudomonas fluorescens* encapsulated in alginate beads amended with skim milk and bentonite clay survived extremely well in the soil and showed optimal colonization of wheat rhizoplane. The encapsulated cells showed good survival rate in both dried and undried beads stored at 15 ^{o} C. Yabur *et al*. (2007) inoculated tomato plants with *Azospirillum brasiliense* immobilized in *Sargassum sinicola* or *Macrocystis pyrifera* alginate and it significantly increased the length of roots in tomato plant.

A microorganism may function optimally under laboratory conditions, but formulating that organism into an end-user affordable product that is consistently able to bring about equivalent results under field conditions is a difficult step. Too much dependence on chemical fertilizers and pesticides to quench the huge demand of food has encouraged the industries to produce life-threatening chemicals as a form of pesticides or fertilizers. Microorganisms are considered as the important tool in solving problems associated with the excessive use of chemical fertilizers and pesticides (Bardi and Malusa, 2012; Bashan *et al*., 2014). Growth and development of plant cannot be properly determined, described and planned without understanding the characteristics of beneficial microorganisms and their interaction with plant and between microorganisms (Vassilev *et al*., 2015).

2.4. Effect of microbial consortia on growth and yield of tomato

Tomato (*Solanum lycopersicum*. L) is one of the important and widely consumed vegetable crops belonging to the family Solanaceae. India is a major tomato growing country and ranks second in production after China. Tomato is used by food industries as a raw material for the production of purees and ketchup. Tomatoes contain higher levels of fructose and glucose than sucrose. Organic acids, soluble sugars, amino acids, pigments and over 400 aroma compounds contribute to the taste, flavour and aroma volatile profile of tomatoes. It is a crop known to harbour several microorganisms with root colonizing activity, which will directly or indirectly influence the soil health though their beneficial activities.

Growth was increased in tomato seedlings inoculated with *Bacillus circulans*. The bacterized seedlings recorded higher root (30.24%) and shoot (16.91%) lengths, increase in dry matter content compared to uninoculated control (Mehta *et al*., 2014). Turan *et al*. (2007) studied the effect of phosphorus solubilizing bacterium (PSB) on the growth performance of tomato under greenhouse condition. The phosphorus availability in soil increased with the application of phosphate solubilizing bacterium (*Bacillus* FS-3). In all the fertilizer types, bacterial application converted 20% of less available phosphorus into labile forms. The highest shoot root dry weight and phosphorus uptake of plant were greater in triple super phosphate (TSP) along with PSB application.

According to Premasekhar and Rajashree (2009), the application of biofertilizers resulted in significantly taller plants and contributed better yield in tomato. *Azospirillum* application has recorded maximum fruit size including a greater number of fruits. The highest yield was recorded with the application of *Azospirillum* + 75% N +100% PK due to high yield contributing characters like number of fruits per plant and average fruit weight. The increased uptake of

available nitrogen influenced the growth characters as nitrogen is the chief constituent of protein, essential for the formation of protoplasm, cell division, enlargement and finally resulting in increased plant growth and yield.

Based on the review of literature it is clear that encapsulation of living cells in polymeric gel is a well-established technology. Alginate gel supports sufficient number of bacteria and also protects from biotic stresses. The viability can be improved by addition of nutrients such as skimmed milk, humic acid *etc*. Alginate formulation is dry, synthetic, simple to use, non-toxic and biodegradable in nature and ensures slow release of bacteria for a longer period.

The key to achieving successful, reproducible results following the introduction of beneficial microbes into soil relies on the survival rate of the inoculated bacteria in a heterogeneous soil environment. Hence, encapsulation in alginate beads can be effectively used to protect the bacterial inoculum from adverse conditions of the soil for their successful establishment in the rhizosphere. The main advantages of alginate preparations are their non-toxic nature, degradation in the soil and their slow release of microorganisms into the soil.

Materials and methods

3. MATERIALS AND METHODS

The present studies on "Alginate based consortial formulation of native microbial fertilizers" were conducted in the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara, during 2017- 2019. Details of materials used and the methods followed are presented below.

3.1. MATERIALS USED

3.1.1. Chemicals, glass wares and plastic wares

The chemicals used for the study were Analytical Grade (AR) and purchased from the agencies like HIMEDIA, Merck India Ltd., Sisco Research Laboratory (SRL). Molecular biology reagents and buffers needed for the experiment were obtained from HIMEDIA (Mumbai) and Sigma-Aldrich India Ltd. (Bangalore). The plastic wares were purchased from Tarson India Ltd. (Kolkata). Jensen's agar media, Pikovskaya's agar media and Aleksandrov's agar media were used for isolation of nitrogen fixers, phosphorus solubilizers and potassium solubilizers respectively.

3.1.2. Equipments and machinery

The equipment's required for the study were available at the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara. Culture media were sterilized in an autoclave (Equitron-SLEFA of Eutech Instruments, Mumbai). pH meter (Eutech pH Tutor, Singapore) was used for checking the pH of culture media. Laminar air flow chamber (Rotek, Mumbai), was used for inoculation and streaking of microorganisms under sterile conditions. Water bath (Rotek, Mumbai) was used for maintaining the temperature of sodium alginate solution at 95^{o}C. Pure cultures of microbes were preserved in glycerol, in ultra-low temperature deep freezer (Haier DW-86L90, Haier International Co. Ltd., China). *In vitro* DNA amplification was carried out in Eppendorf Mastercycler (Eppendorf, Germany). Table top high speed refrigerated centrifuge (Eppendorf-5804R, Eppendorf,

Germany) was used for centrifugation. Visualization of DNA on agarose gel was carried out using UV transilluminator (UVP-Benchtop Transilluminator from UVP, USA).

3.2. METHODOLOGY

3.2.1. Source of isolates

Five nitrogen fixers, five phosphorus solubilizers and five potash solubilizers were obtained from the repository maintained in the Dept. of Agricultural Microbiology, College of Horticulture, Vellanikkara. The bacterial isolates were obtained from Wayanad district of Kerala. The details are given in Table 2.

3.2.2. Purification and maintenance of isolates

The bacterial colonies were repeatedly streaked on a suitable agar media and single discreet colonies were obtained. Pure cultures obtained were maintained on agar slants and also as glycerol stock (broth culture containing 40% glycerol) at -80 ^{o}C in deep freezer.

Table 1. Media used for purification of microorganisms

Microorganism	**Media**
Total bacteria	Nutrient agar
Total bacteria	Luria Bertani agar
Nitrogen fixing bacteria	Jensen’s agar
Phosphorus solubilizing bacteria	Pikovskaya’s agar
Potash solubilizing bacteria	Aleksandrov’s agar

Table 2. List of bacterial isolates from Wayanad district of Kerala

Sl. No.	Isolates	Location	Host	Geographical position		
				Latitude (°N)	Longitude (°E)	Altitude (ft)
	Nitrogen fixers					
1	*Cellulosimicrobium* sp.	Pazhaya Vythiri	Pepper	11.53868	076.30516	20575
2	*Paenibacillus* sp.	Thannivayal	Pepper	11.81235	075.96528	26351
3	*Microbacterium* (D) sp.	Kochangod	Pepper	11.61738	076.22922	29514
4	*Nguyenibacter vanlangensis*	Thannivayal	Rice	11.81235	075.96528	26351
5	*Microbacterium* (F) sp.	Pazhaya Vythiri	Pepper	11.53868	076.30516	20575
	Phosphorus solubilizers					
6	P1 (*Burkholderia cepacia)*	Thannivayal	Rice	11.81235	075.96528	26351
7	*Bacillus subtilis* strain (KASB5)	Kochangod	Rice	11.61738	076.22922	29514
8	*Bukholderia vietnamiensis*	Thannivayal	Rice	11.81235	075.96528	26351
9	*Bacillus subtilis* strain (H4)	Kolagapara	Rice	11.65072	076.19145	12563
10	*Pseudomonas putida*	Thannivayal	Rice	11.81235	075.96528	26351
	Potassium solubilizers					
11	*Acinetobacter calcoaceticus*	Kolagapara	Rice	11.65072	076.19145	12563
12	*Pseudochrobactrum* sp.	Kolagapara	Rice	11.65072	076.19145	12563
13	*Burkholderia* sp.	Kolagapara	Rice	11.65072	076.19145	12563
14	*Stenotrophomonas maltophila*	Kolagapara	Rice	11.65072	076.19145	12563
15	*Brevibacterium* sp	Kolagapara	Rice	11.65072	076.19145	12563

3.2.3. *In vitro* screening for plant growth promoting (PGP) activities

All the bacterial isolates were screened for plant growth promoting activities like indole acetic acid (IAA) production, nitrogen fixation, phosphate solubilization and potash solubilization under *in vitro* conditions.

3.2.3.1. Screening of bacterial isolates for nitrogen fixation

The five bacterial isolates were streaked on N-free Jensen's agar. The inoculated plates were then incubated at 28 ± 2 ^{o}C for three days. Isolates which were able to grow on N free media were considered as nitrogen fixers. Based on the growth rate, nitrogen fixers were rated as high (+++), medium (++), poor (+) and no growth (-).

3.2.3.2. Nitrogen fixation by bacterial isolates

Nitrogen fixation by the five bacterial isolates were quantified by micro-Kjeldahl method of Jackson (1973) and Bremner (1960). A loopful of 48-h old culture was inoculated in 5 ml Jensen's broth in a glass tube and incubated for 48 h. One millilitre of this culture was inoculated in 50 ml of Jensen's broth in 250 ml conical flasks and three replicates were maintained for each isolate.

After 15 days of incubation, the cultures were homogenized by shaking. Ten millilitre of the homogenized culture was drawn and mixed with 10 ml of concentrated H_2SO_4 and one gram of digestion mixture (copper sulphate : selenium in the ratio 20:1) in the ratio 10:1. The mixture was kept for digestion overnight at room temperature and then in a block digester for 2 hours at 300 ^{0}C till it became clear.

The clear digest was cooled and transferred to Kjeldahl's distillation unit. Ten millilitre of NaOH (40%) was added and condensed NH_3 was trapped in 10 ml boric acid indicator mixture (4% boric acid solution was prepared in hot water). Four millilitre of mixed indicator solution (0.2% bromocresol green + 0.2% methyl red in alcohol with 5:1 ratio) was added to 1000 ml of 4% boric acid solution. The colour changed from reddish pink to bluish green as the NH_3 was trapped. After this process was over, it was titrated against 0.01N until the solution turned back to reddish pink. A blank was also used for titration. Total nitrogen content of the cultures was determined and the results were expressed as mg N fixed per gram of carbon source utilized.

$$\text{mg of N/ g of C source} = \frac{\text{TV-BV x N x 0.014 x 1000}}{\text{Y}}$$

where,

TV = Titre value

BV = Blank value

N = Normality of H_2SO_4

Y = Weight of carbon source

3.2.3.3. Screening of bacterial isolates for phosphate solubilization

The five isolates obtained were initially screened for phosphate solubilization on Pikovskaya's agar media (Nguyen *et al*., 1992). Ten microlitres of 24 h old bacterial isolates were uniformally spotted on Pikovskaya's agar plate. Inoculated plates were incubated for seven days at 28 ± 2°C. The halo zone and colony diameter were measured after seven days of incubation. The ability to solubilize insoluble phosphorus was expressed as per cent solubilization efficiency (SE)

$$\text{Solubilizing efficiency (\% SE)} = \frac{\text{SD}}{\text{CD}} \text{ x } 100$$

SD - Solubilization diameter (mm)

CD - Colony diameter (mm)

3.2.3.4. Quantitative estimation of phosphate solubilization by bacterial isolates

The bacterial isolates which showed positive reaction for phosphorus solubilization on Pikovskaya's agar in the preliminary screening were further subjected to quantification of phosphorus solubilization (Olsen *et al*., 1962). Flasks containing 50 ml Pikovskaya's broth were inoculated with 500 µl of overnight grown culture of each isolate and incubated for 14 days at 28 ± 2 ^{o} C. The amount of inorganic phosphorus released in broth was estimated after 14 days of incubation along with the uninoculated control by using phospho-molybdic blue colour method

Pikovskaya's broth cultures were centrifuged at 10,000 rpm for 10 min to separate the cells and insoluble phosphate. Five millilitre of the supernatant was taken in a test tube and the volume was made upto 8.6 ml with distilled water. One millilitre of ammonium molybdate reagent was added followed by 0.4 ml of ANSA reagent. The contents were mixed for 10 min for colour development. Intensity of the blue colour was read in a spectrophotometer at 660 nm. The amount of available phosphorus present in the broth was calculated using standard graph of different known concentration of phosphorus using KH_2PO_4. The pH of the supernatant was also recorded after 14 days of incubation to assess the reduction in pH from the initial value of 7.2.

3.2.3.5. Screening of bacterial isolates for potash solubilization

The five isolates obtained were initially screened for potash solubilization on Aleksandrov's agar media (Nguyen *et al*., 1992). Ten microlitres of 24 h old bacterial isolates were uniformally spotted on Aleksandrov's agar plate. Inoculated plates were then sealed properly and incubated for seven days at 28 ± 2^{o}C. The halo

zone and colony diameter were measured after seven days of incubation. The ability to solubilize insoluble phosphorus was expressed as per cent solubilization efficiency (SE)

$$\text{Solubilizing efficiency (\% SE)} = \frac{SD}{CD} \times 100$$

SD - Solubilization diameter (mm)

CD - Colony diameter (mm)

3.2.3.6. Quantitative estimation of potash solubilization by bacterial isolates

One millilitre each of overnight culture was inoculated to 20 ml of Aleksandrov's broth with three replications (Sugumaran and Janarthanam, 2007). All the inoculated tubes were kept for 15 days incubation at room temperature. After incubation, the broth cultures were centrifuged at 10,000 rpm for 10 min to separate the supernatant from cell growth and insoluble potassium. The available K content in the supernatant was determined by flame photometry.

3.2.3.7. Screening of bacterial isolates for IAA production

The bacterial isolates were inoculated in sterile Luria-Bertani supplemented with tryptophan at the rate of 1 mg ml^{-1} (Ahmed *et al*., 2008). The tubes were incubated in the dark for 7 days. After incubation, the cultures were centrifuged at 3000 rpm for 30 min and the supernatant was collected. To the supernatant, 4 ml Salkowski reagent was added. The development of pink colour indicated positive for IAA production.

3.2.3.8. Quantification of IAA production by bacterial isolates

The bacterial isolates were inoculated in sterile Luria-Bertani supplemented with tryptophan at the rate of 1 mg ml^{-1} (Ahmed *et al*., 2008). The tubes were incubated in the dark for 7 days. After incubation, the cultures were centrifuged at 3000 rpm for 30 minutes and the supernatant was collected. To the supernatant, 4 ml Salkowski reagent was added. The tubes that showed pink colour were measured for optical density at 530 nm using a spectrophotometer. The OD values were plotted on a standard graph to obtain the quantity of IAA produced by the isolates and expressed as microgram per millilitre of broth.

3.2.4. Compatibility among selected bacterial isolates

The selected bacterial isolates were tested for compatibility using cross streaking technique (Burlage *et al*., 1998). Two different bacterial isolates were streaked vertically and horizontally in a Petri plate containing nutrient agar medium using a sterile loop. Control plates without inoculum were maintained for each isolate. The cross streaked plates were incubated for 48 h at 28 ± 2 ^{o}C and observed for growth or inhibition.

Dual culture technique was also done for the confirmation of compatibility among the bacterial isolates. The test was done on nutrient agar medium. In order to check the compatibility, one of the culture suspensions was spread and the other bacterial culture was spotted at the center of the Petri dish by making a well at the centre. Twenty microlitre of the overnight grown culture was inoculated into it and incubated at 30 ± 2 ^{o}C for 24 h. Inhibition of bacterial growth was recorded between two to four days after incubation by measuring the size of the inhibition zone (mm). The following formula was used to find the per cent growth inhibition (PGI).

$$PGI\ (\%) = \frac{(C - T)}{C} \times 100$$

C - Diameter of bacterial growth in control plate

T - Diameter of bacterial growth in treated plate

3.3. Standardization of contaminant free alginate beads preparation

3.3.1. Standardization of optimum concentration of sodium alginate and calcium chloride solution

An experiment was conducted to determine the optimum concentration of sodium alginate and calcium chloride solution for the preparation of good quality and contamination free alginate beads (Bashan, 1986). Different concentrations of sodium alginate and calcium chloride solution (0.5%, 1%, 1.5%, 2%, 3%, 4%, 5%) were evaluated to prepare the alginate beads. The weight and diameter of alginate beads formed were recorded.

3.3.2. Dissolution of alginate beads using phosphate buffer solution

An experiment was conducted to determine the time taken for dissolution of alginate beads for the release of bacteria using phosphate buffer (Bashan,1986). Beads were immersed in various concentration of potassium phosphate buffer (0.06, 0.2, 0.4 M). It was incubated for 30 to 60 min (depending on gel type) at 30 ± 2 ^{o}C. To facilitate the solubility, the beads were vigorously shaken on a vortex mixer. The time taken for the dissolution of beads were recorded after specific time period in hours.

3.3.3. Optimization of temperature and time to obtain contamination free alginate beads

Alginate beads consisting of nitrogen fixer, phosphorus solubilizer and potash solubilizer were prepared. Sodium alginate solution (3%) was maintained at different temperatures such as 80 ^{o}C for 10 min, 85 ^{o}C for 10 min and 95^{o}C for 15 min. Three millilitre of bacterial strains of nitrogen fixer, phosphorus solubilizer and potash solubilizer were incubated in nutrient broth and it was inoculated into 90 ml of sodium alginate solution (3%). It was incubated for 24 h for the bacterial

cells to multiply in the sodium alginate solution. The alginate beads were prepared by using sterilized calcium chloride solution (3%).

3.3.4. Optimization of cultural conditions for the growth of bacteria

Bacterial isolates of nitrogen fixer, phosphorus solubilizer and potash solubilizer were grown individually in 250 ml flask containing 100 ml broth each of Jensen's broth, Pikovskaya's broth and Aleksandrov's broth respectively (Bashan *et al*.,2002). It was incubated at 30 ^{o}C for 24 h in an orbital shaker at 180 rpm.

After growth, 3.3 ml from each of the broth was added to 90 ml of boiled and cooled sodium alginate solution (3%). The solution was incubated at 30^{o}C for 24 h in an orbital shaker at 180 rpm. Later, the alginate beads were prepared by using sterilized calcium chloride solution (3%).

3.3.5. Encapsulation of bacterial cells in alginate beads

The sodium alginate solution (3%) containing the bacterial suspension was added dropwise from a separating funnel into a pre-cooled sterile calcium chloride solution (3%) with mild agitation (Bashan, 1986). The water-soluble sodium alginate was converted into water insoluble calcium alginate through cross linkage. The resulting alginate beads were encapsulated with the bacterial cells and these beads were maintained at room temperature for an additional one hour to obtain intact and solid beads.

The calcium chloride (3%) solution was then pumped out and the beads were washed twice with sterile water. After washings, the beads were incubated in fresh nutrient broth medium for an additional 24 h to allow the bacteria to multiply inside the alginate beads. Then, the beads were washed twice with sterile water and air dried.

3.3.6. Enumeration of bacterial population in alginate based inoculants

The alginate beads were first dissolved in phosphate buffer of pH 6.8 and incubated overnight. Population of bacteria in the consortia were estimated at 15 days interval. The enumeration was done by serial dilution and plate count technique using respective selective media (Jensen's agar media, Pikokvskaya's agar media and Aleksandrov's agar media). The population of each isolate was recorded and expressed as cfu g^{-1}.

3.4. Molecular characterization

3.4.1. 16S rRNA gene sequence analysis of selected isolates

The unknown bacterial isolate (P1) was subjected to molecular characterization by 16S rRNA gene sequencing to identify the bacterial isolate.

3.4.1.2. Amplification of 16S rRNA gene

Colony PCR was used for amplification of 16S rRNA gene (Woodman, 2008). Single isolated colony was taken using a micropipette and mixed with 10µl of sterile water. For amplification of 16S rRNA gene, 2µl of the suspension was used as template. The details about the primers (Siddapura *et al.,* 2010) used are given in Table 3.

Polymerase chain reaction was carried out in Eppendorf Master Cycler (Gradient) using 'Emerald Amp GT PCR' PCR master mix. The details of the composition of PCR reaction mixture are given in Table 4. The reagents were mixed by a momentary spin and the PCR reaction set in master cycler. The details of master cycler programme are given in Table 5.

Table 3. Primers used for 16S rRNA gene amplification

Primer details	Sequence 5'- 3'	Length in bp
8 F	AGAGTTTGATCCTGGCTCAG	20
1522R	AAG GAG GTG ATC CAG CCG CA	20

Table 4. Composition of PCR reaction mixture

Component	Per reaction volume required (µl)
Master mix	12.5
Template	2.0
Forward primer	0.5
Reverse primer	0.5
dH_2O	9.5
Total	25.0

Table 5. Details of master cycle programme

No.	Step	Temperature (^{0}C)	Time (min)
1	Initial denaturation	95	3.00
2	Denaturation	94	1.30
3	Annealing	55	0.40
4	Primer extension	72	1.30
5	Steps 2-4	34 cycles	-
6	Final extension	72	20.00
7	Final hold	4	10.00

3.4.1.3. Agarose gel electrophoresis

Assessment of quality of isolated DNA was carried out by agarose gel electrophoresis (Sambrook *et al*., 1989). Hundred ml of 1X TAE buffer was prepared from 50X TAE stock solution (pH 8.0). Agarose gel was prepared by dissolving 1.0 g agarose (Genei, Low EEO) in 100 ml 1X TAE buffer and 0.5 µg ml^{-1} ethidium bromide prepared from a stock of 10 mg ml^{-1} was added to the cooled solution. The gel casting tray was set up with properly placed comb and agarose was poured into the tray with care and allowed to solidify. The comb was carefully removed to obtain wells and1X TAE buffer was poured into the tank. The gel loading dye and 2 µl PCR product were mixed and loaded gently into the wells. The electrodes were connected and a constant voltage of 80 V was applied till the tracking dye reached at about 3 cm away from the anode end.

3.4.1.4. Gel documentation

After the separation of DNA bands using electrophoresis, they were visualized in the UV illuminator and photographed using gel documentation system.

3.4.1.5. Purification and sequencing of PCR production

The PCR product was then purified and sequenced at AgriGenome Labs Pvt Ltd., Kochi, using the primer 8F and 1522r. The sequencing of the PCR product was done using the sequencing machine ABI 3730 XL DNA analyser.

3.4.1.6. Nucleotide sequence analysis

Sequence analysis and nucleotide homology of each isolate were analyzed through the BLASTn (basic local alignment search tool) programme of NCBI (National Centre for Biotechnology Information) (http://www.ncbi.nlm.nih.gov).

The accession sharing maximum homology with the query sequence was considered for the identification of the test organism.

3.5. Evaluation of alginate based consortia for growth enhancement under pot culture studies (Sterile condition)

The three most efficient and compatible isolates of alginate and talc based consortia were evaluated under sterile conditions for growth promotion using tomato as the test crop. The soil was sterilized using formaldehyde solution (5%) and it was covered with a plastic sheet, sealed and kept for 15 days. The experiment was conducted during April to July 2019 in the net house of Department of Agricultural Microbiology, Vellanikkara.

The treatment details of experiment were as follows:

Design :CRD

Replications :3

Treatments :10

Variety :Anagha

T_1: Bacterial consortia-1 (alginate based)

T_2: Bacterial consortia-2 (alginate based)

T_3: Bacterial consortia-3(alginate based)

T_4: $T_1+T_2+T_3$

T_5: Bacterial consortia-1(talc based)

T_6: Bacterial consortia-2 (talc based)

T_7: Bacterial consortia-3 (talc based)

T_8: Package of Practices Recommendation of KAU (2016)

(Fertilizer dose- 75: 40:25 Kg N: P_2O_5: K_2O ha^{-1})

T_9: Organic Package (Ad hoc) of practices Recommendation of KAU (2017)

(Application of vermicompost at the rate of 1t ha^{-1})

T_{10}: Absolute control

Treatment dosage :1g/ plant

3.5.1. Preparation of potting mixture and planting

The potting mixture was prepared with sand: soil: cowdung (1:1:1) and was sterilized by adding formaldehyde solution (5%). The mixture was then covered with a polythene film to retain the vapors in potting mixture. After 15 days, the mixture was raked thoroughly and left open for seven days. The sterile potting mixture was filled in pots. The pots were also sterilized by using formaldehyde solution (5%). Disease free seeds of tomato variety "Anagha" were obtained from Olericulture Department, College of Horticulture, Vellanikkara.

3.6. Evaluation of alginate based bacterial consortia for growth enhancement under pot culture studies (Unsterile condition)

The three most efficient and compatible isolates of alginate and talc based consortia were evaluated under unsterile conditions for growth promotion using tomato as the test crop. The soil was kept unsterilized. The experiment was conducted during April to July 2019 in the net house of Department of Agricultural Microbiology, Vellanikkara.

The treatment details of experiment were as follows:

Design :CRD

Replications :3

Treatments :10

Variety :Anagha

T_1: Bacterial consortia-1 (alginate based)

T_2: Bacterial consortia-2 (alginate based)

T_3: Bacterial consortia-3(alginate based)

T_4: T_1+T_2+T_3

T_5: Bacterial consortia-1(talc based)

T_6: Bacterial consortia-2(talc based)

T_7: Bacterial consortia-3(talc based)

T_8: Package of Practices Recommendation of KAU (2016)

(Fertilizer dose- 75: 40:25 Kg N: P_2O_5: K_2O ha^{-1})

T_9: Organic Package (Ad hoc) of practices Recommendation of KAU (2017)

(Application of vermicompost at the rate of 1t ha^{-1})

T_{10}: Absolute control

Treatment dosage :1g/ plant

3.6.1. Preparation of potting mixture and planting

The potting mixture was prepared with sand: soil: cowdung (1:1:1) and it was unsterilized. Disease free seeds of tomato variety "Anagha" were obtained from Olericulture Department, College of Horticulture, Vellanikkara.

3.7. Enumeration of inoculated bacterial isolates in potting mixture

Population of inoculated bacterial isolates in potting mixture (sterile and unsterile soil) were enumerated by serial dilution and plating technique using selective media (Jensen agar media, Pikovskaya's agar media and Aleksandrov's agar media).

3.8. Soil analysis during planting time, at flowering and at harvest

Table 6. Analysis of the physio chemical properties of soil

Particulars	Method	Reference
Soil pH	Soil – water suspension of 1:2.5 read in pH meter	Jackson (1958)
Electrical conductivity	The electrical conductivity of supernatant solution was measured using conductivity meter	Jackson (1958)
Organic carbon (%)	Walkley – Black method	Walkley and Black (1934)
Available N (kg/ha)	Alkaline permanganate method	Subbiah and Asija (1956)
Available P (kg/ha)	Ascorbic acid reduced molybdo phosphoric blue colour method	Bray and Kurtz (1945); Watanable and Olsen (1965)
Available K (kg/ha)	Neutral normal ammonium acetate extract method using flame photometer	Jackson (1958)

3.9. Observations recorded

3.9.1. Alginate beads character

3.9.1.1. Moisture per cent of beads

The moisture per cent of the alginate beads of different concentrations (3%, 4%, 5%) were analyzed by using a moisture meter.

3.9.1.2. Size of beads

Diameter of the alginate beads prepared from sodium alginate solution maintained at 95^{o}C were measured using a stereomicroscope and expressed in

millimeter. The beads prepared from sterilized sodium alginate solution was measured using an Image Analyzer.

3.9.1.3. Weight of beads

The weight of beads was recorded in mg.

3.9.2. Plant biometric characters

Biometric characters like plant height, number of branches, total dry matter production was recorded. Yield parameters like number of days taken for flowering, number of fruits per plant and yield per plant were recorded. Observations on incidence of pest and disease were also made.

3.9.2.1. Plant height

Height of plants was measured from base of the plant to the tip of the topmost leaf at the harvesting stage and expressed in centimeters.

3.9.2.2. Number of branches

The number of branches per each plant were counted at the final stage of harvest and the mean number was obtained.

3.9.2.3. Days to flowering

Number of days taken from transplanting to opening of first flower were recorded in all the plants and the mean was found out.

3.9.2.4. Fresh and dry weight of plants

Plants were uprooted, cleaned and the fresh weight was taken. Dry weight was also recorded after drying in an oven at $60 \pm 5\ ^{o}C$.

3.10. Number of fruits per plant

Total number of fruits harvested from each plant at different stages of maturity were counted and the mean was obtained.

3.11. Yield per plant

Fruit yield were recorded from each plant and expressed as gram per plant.

3.12. Pest and disease incidence

Incidence of pest and diseases on plants were recorded throughout the period of study.

3.12.1. Incidence of mealy bug

Incidence of mealy bug was recorded during the growth period of crop.

3.13. Statistical analysis

Analysis of variance was done on the data collected by using the statistical package WASP 2.0.(Gomez and Gomez, 1984).

Result

4. RESULTS

The results of the study on "Alginate based consortial formulation of native microbial fertilizers" conducted during the period 2017-2019 at Department of Agricultural Microbiology, College of Horticulture, Vellanikkara are presented in this chapter.

4.1. *IN VITRO* SCREENING OF BACTERIAL ISOLATES FOR PLANT GROWTH PROMOTING (PGP) ACTIVITIES

4.1.1. *In vitro* screening of nitrogen fixers

4.1.1.1. Qualitative screening for nitrogen fixation

Nitrogen fixers were screened for their nitrogen fixation ability based on their growth in nitrogen free medium (Table 7 and Plate 1). Among these, *Microbacterium arborescence* and *Microbacterium testaceum* recorded the highest growth on nitrogen free medium. Moderate growth rate was observed in the case of *Cellulosimicrobium* sp. and growth rate was poor in *Nguyenibacter vanlangensi*s.

4.1.1.2. Indole acetic acid (IAA) production by nitrogen fixers

IAA production by the bacterial isolates were quantified and the data are presented in Table 7 and Plate 6 a. The amount of IAA production ranged from 3.67 to 6.00 µg ml^{-1}. The highest production of IAA was recorded in *Microbacterium arborescence* (6.00 µg ml^{-1}) which was significantly different from all other isolates. *Microbacterium testaceum* (5.20 µg ml^{-1}) and *Nguyenibacter vanlangensi*s (5.33 µg ml^{-1}) were statistically on par with each other. The lowest production of IAA was recorded in *Cellulosimicrobium* sp. (3.70 µg ml^{-1}) which was on par with *Paenibacillus* sp. (3.67 µg ml^{-1}).

4.1.1.3. Quantitative screening for nitrogen fixation

Quantity of nitrogen fixed by the bacterial isolates were estimated by micro-Kjeldahl method. All the isolates performed equally well under *in vitro* conditions. There were no significant differences among the isolates for the fixation of nitrogen (Table 7). Amount of nitrogen fixed varied from 20.00 to 22.63 mg of N g^{-1} sucrose utilized. The highest amount of nitrogen fixed was recorded in the case of *Microbacterium arborescence* (22.63 mg N g^{-1} sucrose utilized) followed by *Microbacterium testaceum* (21.25 mg N g^{-1} sucrose utilized). The lowest nitrogen fixation was recorded by *Paenibacillus* sp. (20.0 mg N g^{-1} sucrose utilized).

4.1.2. *In vitro* screening of phosphorus solubilizers

4.1.2.1. Indole acetic acid (IAA) production by phosphorus solubilizers

IAA production by the isolates were quantified and the data are presented in Table 8 and Plate 6 b. Amount of IAA produced ranged from 0.46 to 8.67 µg ml^{-1}. The highest production of IAA was recorded in *Burkholderia cepacia* (8.67 µg ml^{-1}) which was significantly different from all other isolates. *Bacillus subtilis* (KASB5) produced IAA (3.43 µg ml^{-1}) which was statistically on par with *Burkholderia vietnamiensis* (3.20 µg ml^{-1}). The lowest production of IAA was recorded in *Pseudomonas putida* (0.46 µg ml^{-1}).

4.1.2.2. Qualitative screening for phosphate solubilization

Isolates were screened for phosphate solubilization on Pikovsakaya's agar media (Table 8 and Plate 2). All the five isolates tested were positive for phosphorus solubilization. *Burkholderia cepacia* isolate recorded highest solubilization index (117.85) followed by *Bacillus subtilis* (KASB5) with a solubilization index of 95.60.

4.1.2.3. Quantification of phosphorus solubilization by bacterial isolates

Solubilization index and the amount of phosphorus solubilized are presented in Table 8 and Plate 3. Amount of phosphorus solubilized varied between 47.46 - 64.83 μg ml^{-1}. The highest amount of phosphorus solubilized was recorded in the case of *Bukholderia cepacia* (64.83 μg ml^{-1}) followed by *Bacillus subtilis* strain (KASB5) with 60.03 μg ml^{-1}. The lowest amount of phosphorus was solubilized by the isolate *Burkholderia vietnamiensis* (47.46 μg ml^{-1}).

4.1.3. *In vitro* screening of potassium solubilizers

4.1.3.1. Indole acetic acid (IAA) production by potassium solubilizers

IAA production by the isolates were quantified and the data are presented in Table 9 and Plate 6 c. Amount of IAA produced ranged from 1.06 to 4.00 μg ml^{-1}. The highest production of IAA was recorded in *Acinetobacter calcoaceticus* (4.00 μg ml^{-1}) followed by *Burkholderia* sp. (2.70 μg ml^{-1}). The lowest production of IAA was recorded in the case of *Stenotrophomonas maltophila* (1.7 μg ml^{-1}).

4.1.3.2. Qualitative screening of isolates for potash solubilization

The isolates were screened for potash solubilization on Aleksandrov's agar media (Table 9 and Plate 4). All the five isolates tested were positive for potash solubilization. *Acinetobacter calcoaceticus* isolate recorded highest solubilization index (60.0) followed by *Burkholderia* sp. (36.0).

4.1.3.3. Quantitative screening of potassium solubilization by bacterial isolates

The quantity of potassium solubilized are given in Table 9 and Plate 5. Amount of potassium solubilized varied between 13.77 - 41.63 μg ml^{-1}. The highest amount of potassium solubilized was recorded in *Acinetobacter calcoaceticus* (41.63 μg ml^{-1}) followed by *Burkholderia* sp. (31.26 μg ml^{-1}). The lowest amount of potassium was solubilized by *Pseudochrobactrum* sp. (13.77 μg ml^{-1}).

Table 7. Screening of bacterial isolates for growth rate, IAA production and nitrogen fixation

Sl. No.	Isolates	Growth on N free medium* (3 DAI)	Concentration of IAA (µg ml^{-1})	Amount of nitrogen fixed (mg of N g^{-1} sucrose utilized)
1	*Cellulosimicrobium* sp.	+++	3.70^{c}	20.06
2	*Paenibacillus* sp.	++	3.67^{c}	20.00
3	*Microbacterium testaceum*	++++	5.20^{b}	21.25
4	*Nguyenibacter vanlangensis*	+	5.33^{b}	20.63
5	*Microbacterium arborescence*	++++	6.00^{a}	22.63

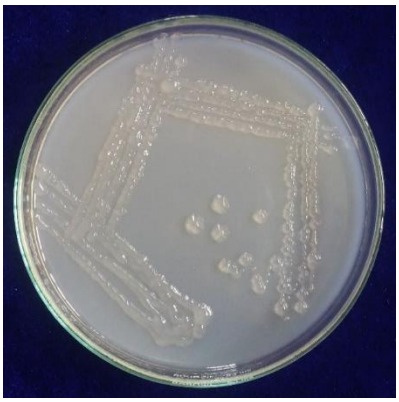
*DAI- Days after incubation

++++ High
+++ Moderate
++ Low
+ Poor
- No growth

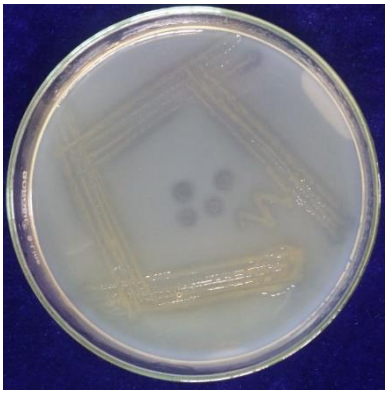
Cellulosimicrobium sp.

Microbacterium testaceum

Paenibacillus sp.



Microbacterium arborescence

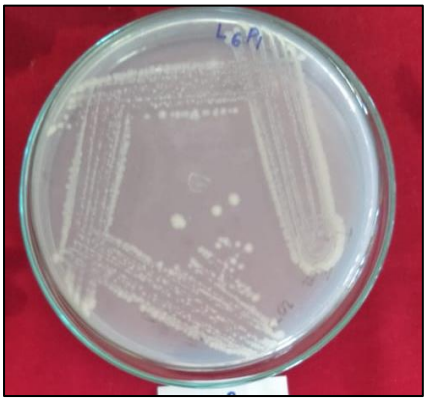


Nguyenibacter vanlangensis

Plate 1. Bacterial colonies of nitrogen fixers

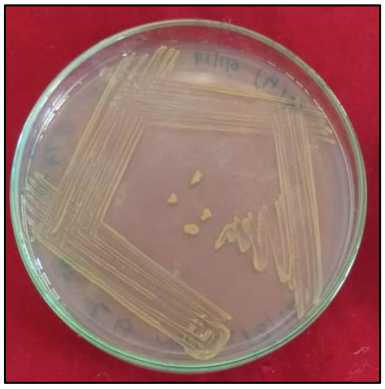
Table 8. Screening of bacterial isolates for IAA production and phosphate solubilization

Sl. No	Isolates	Concentration of IAA ($\mu g\ ml^{-1}$)	Solubilization index	Quantity of P solubilized ($\mu g\ ml^{-1}$)	Reduction in pH
1	*Pseudomonas putida*	0.46^{c}	56.82	51.56^{c}	5.2
2	*Bacillus subtilis* strain (KASB5)	3.43^{ab}	95.60	60.03^{b}	3.6
3	*Burkholderia vietnamiensis*	3.20^{ab}	73.90	47.46^{d}	5.6
4	*Bacillus subtilis* strain (H4)	2.33^{b}	82.40	58.73^{b}	4.8
5	*Burkholderia cepacia*	8.67^{a}	117.85	64.83^{a}	3.4

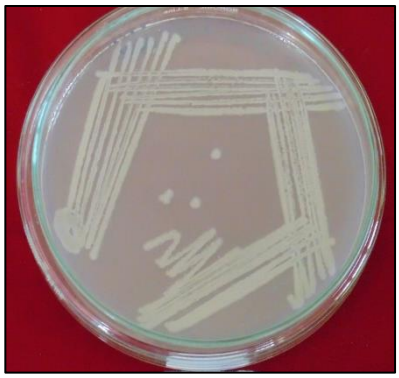


Pseudomonas putida

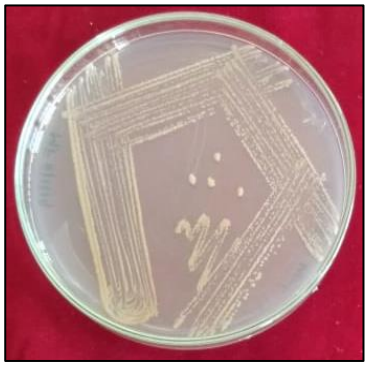
Bacillus subtilis (KASB5)



Burkholderia vietnamiensis

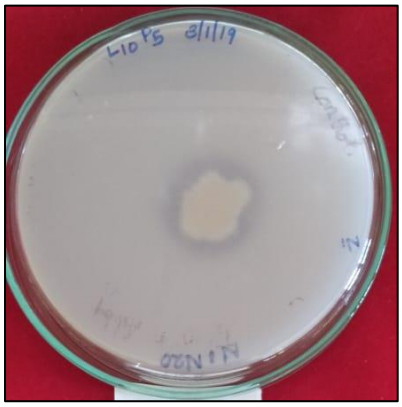


Bacillus subtilis (H4)

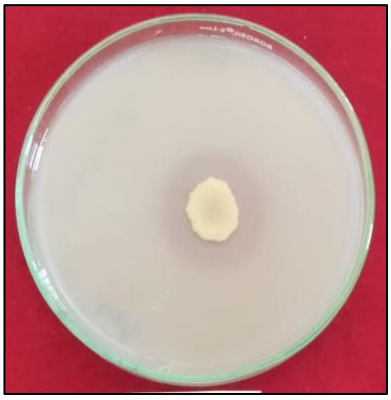


Burkholderia cepacia

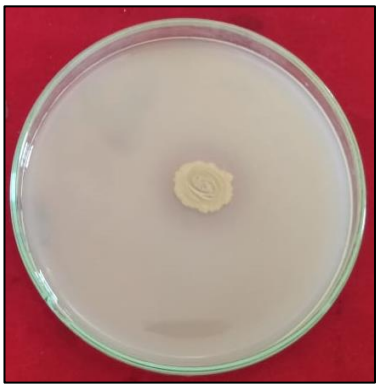
Plate 2. Bacterial colonies of phosphorus solubilizers



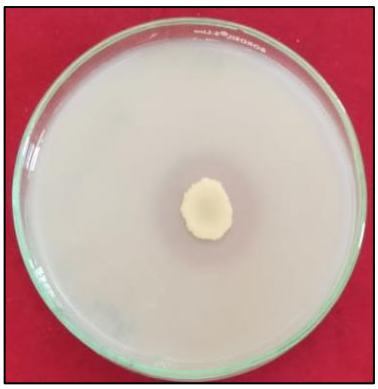
Pseudomonas putida



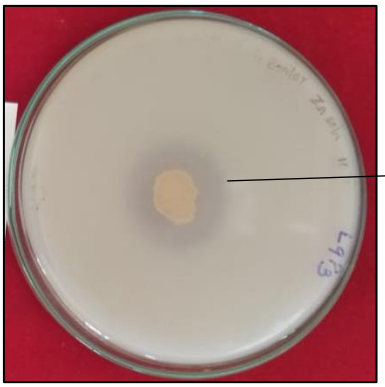
Bacillus subtilis (KASB5)



Burkholderia vietnamiensis



Bacillus subtilis (H4)



Solubilization zone

Burkholderia cepacia

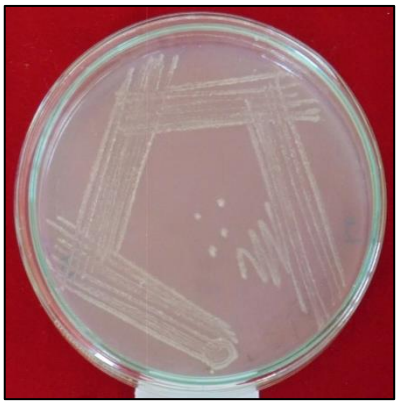
Plate 3. Phosphate solubilization by bacterial isolates

Table 9. Screening of bacterial isolates for IAA production and potassium solubilization

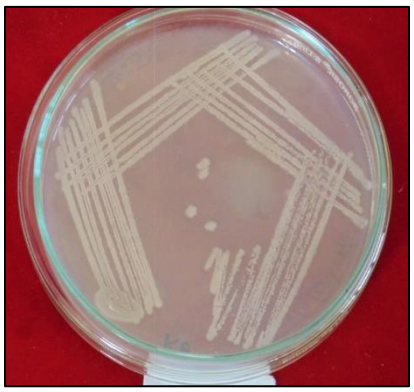
Sl. No	Isolates	Concentration of IAA (µg ml^{-1})	Solubilization Index	Quantity of K solubilized (µg ml^{-1})
1	*Acinetobacter calcoaceticus*	4.00^{a}	60	41.63^{a}
2	*Pseudochrobactrum* sp.	1.73^{b}	20	13.77^{d}
3	*Burkholderia* sp.	2.70^{c}	36	31.63^{b}
4	*Stenotrophomonas maltophila*	1.70^{c}	18	18.73^{c}
5	*Brevibacterium* sp.	1.67^{d}	26	29.78^{b}

Acinetobacter calcoaceticus

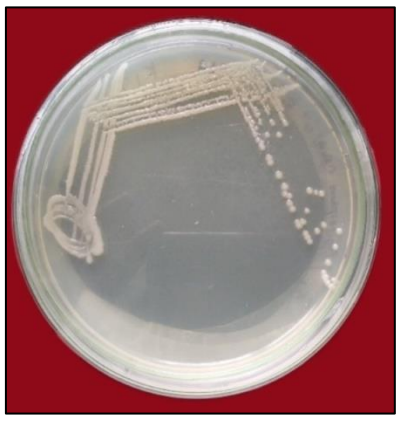
Pseudochrobactrum sp.



Burkholderia sp.

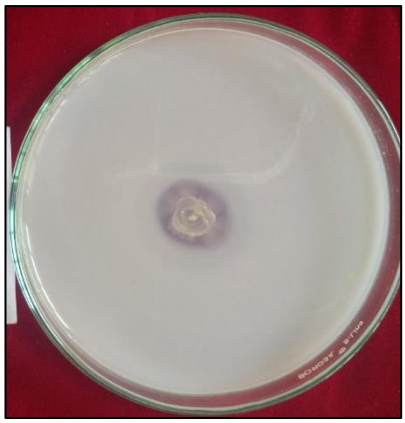


Stenotrophomonas maltophila

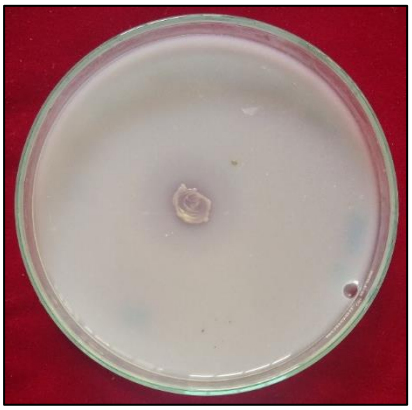


Brevibacterium sp.

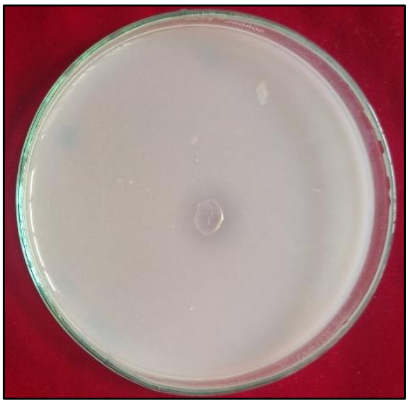
Plate 4. Bacterial colonies of potassium solubilizers



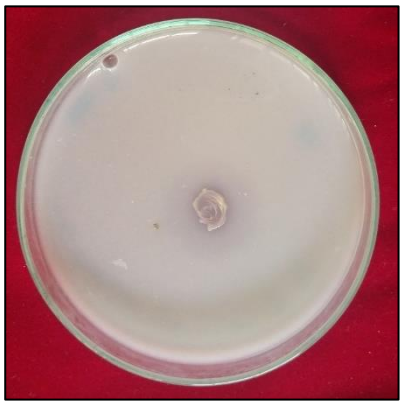
Acinetobacter calcoaceticus



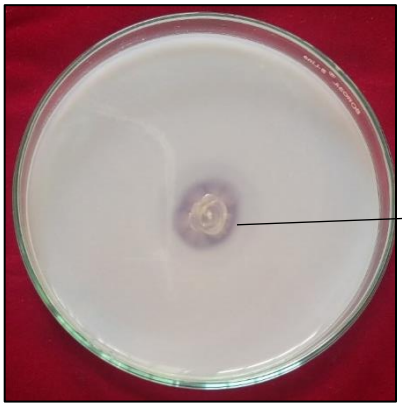
Brevibacterium sp.



Pseudochrobactrum sp.

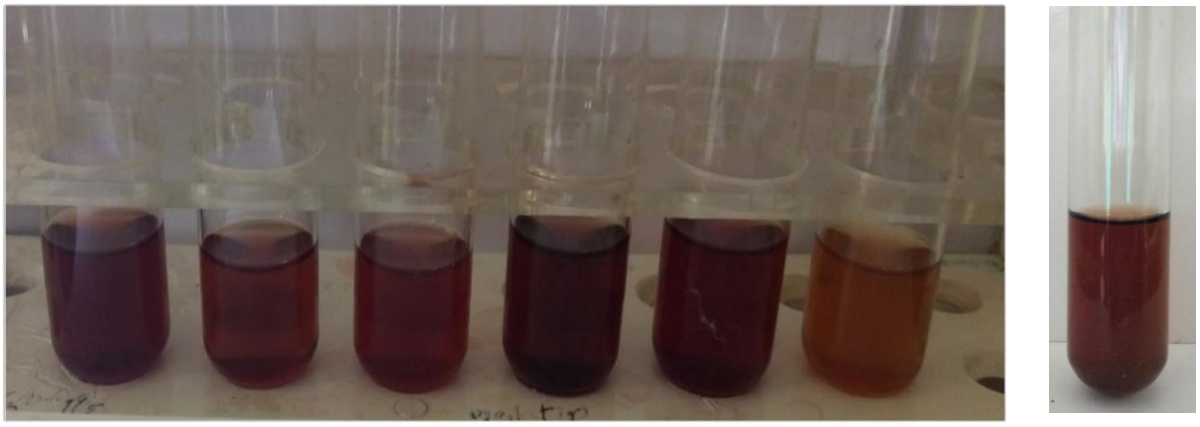


Stenotrophomonas maltophila



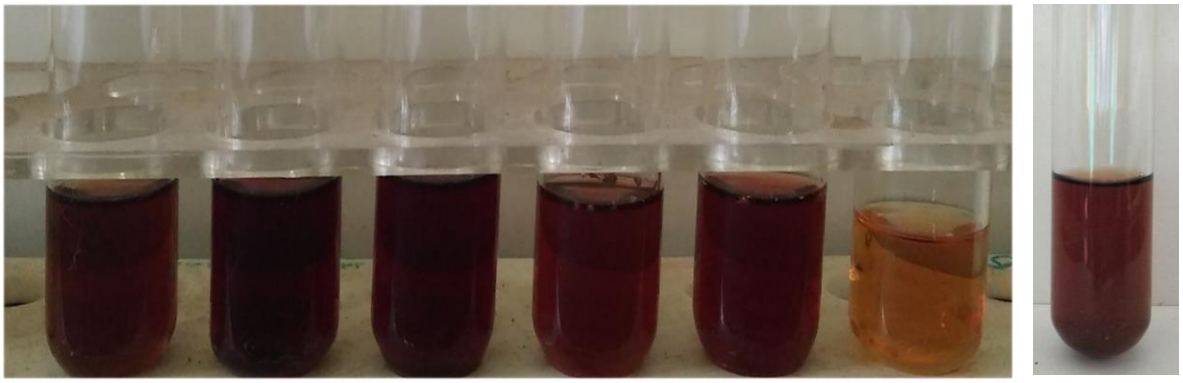
Burkholderia sp.

Plate 5. Potassium solubilization by bacterial isolates



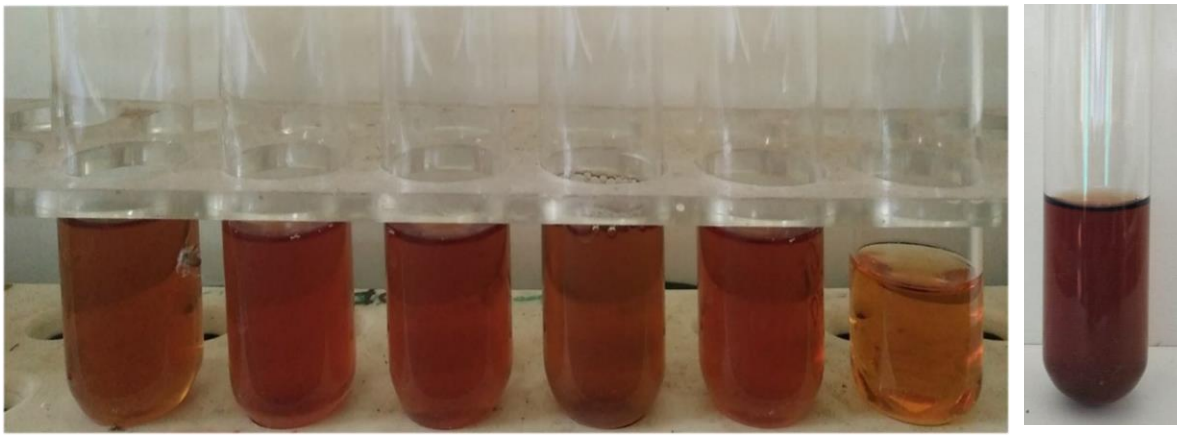
N5 N4 N3 N2 N1 Control Positive control

a) Indole acetic acid production by nitrogen fixers



P5 P4 P3 P2 P1 Control Positive control

b) Indole acetic acid production by phosphorus solubilizers



K5 K4 K3 K2 K1 Control Positive control

c) Indole acetic acid production by potassium solubilizers

Plate 6. Indole acetic acid production by nitrogen fixers, phosphorus and potassium solubilizers

4.2. Selection of efficient isolates of nitrogen fixers, phosphorus solubilizers and potassium solubilizers for compatibility studies

Based on the efficient nitrogen fixers, phosphorus and potash solubilizers, the three most efficient isolates under each nitrogen fixers, phosphorus and potash solubilizers were selected (Table 10).

Table 10. Three most efficient isolates of nitrogen fixers, phosphorus solubilizers and potash solubilizers

Nitrogen fixers	Phosphorus solubilizers	Potash solubilizers
Microbacterium arborescence	*Burkholderia cepacia*	*Acinetobacter calcoaceticus*
Microbacterium testaceum	*Bacillus subtilis* (KASB5)	*Burkholderia* sp.
Nguyenibacter vanlangenssis	*Bacillus subtilis* (H4)	*Brevibacterium* sp.

4.3. Identification of the unknown culture (P1)

The unknown culture was identified by 16S rDNA sequence analysis. Homology search of nucleotide sequences obtained from the isolates with other reported sequences are given in Plate 7. The isolate has shown maximum homology with *Burkholderia cepacia*. Phylogenetic tree of *Burkholderia cepacia* as constructed by using MEGA7 software (Plate 8) and it was deposited in NCBI.

4.4. Compatibility among the selected bacterial isolates

Compatibility among the selected microbial isolates were determined by cross-streak method. There was no inhibition among the selected microbial isolates (Table 11 and Plate 9) which indicated that all the isolates were compatible.

4.4.1. Selection of efficient isolates for consortium preparation

The three most efficient and compatible isolates selected based on the indole acetic acid (IAA) production, nitrogen fixation, phosphorus and potassium solubilization are presented in Table 12.

ATGGGGGTCTCGCTAGAGATTGTCCTGGGTCTCGTGCGCGGGTGCCCCCAGCCGGATTAATACCGCATAC
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ACACCATGGGAGTGGGTTTTACCAGAAGTGGCTAGTCTAACCGCAAGGACTCTTGAGATTCACACTGGGA
CTCAGTGCGGCGGGTCATCTCGTAGAGTACGACGT

A. Sequence of 16S rDNA amplicon

Color key for alignment scores

B. Blastn output

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Burkholderia cepacia strain HSC-48S18 (P1 isolate)	2145	88	MK672877.1	97.05	0.0
Burkholderia sp. strain Beta-58	2145	88	MH698908.1	97.05	0.0
Burkholderia cepacia BAS-52	2145	88	MG846092.1	97.05	0.0
Burkholderia sp. strain C25	2145	88	MF784500.1	97.05	0.0

Plate 7. Sequence analysis of the P1 isolate

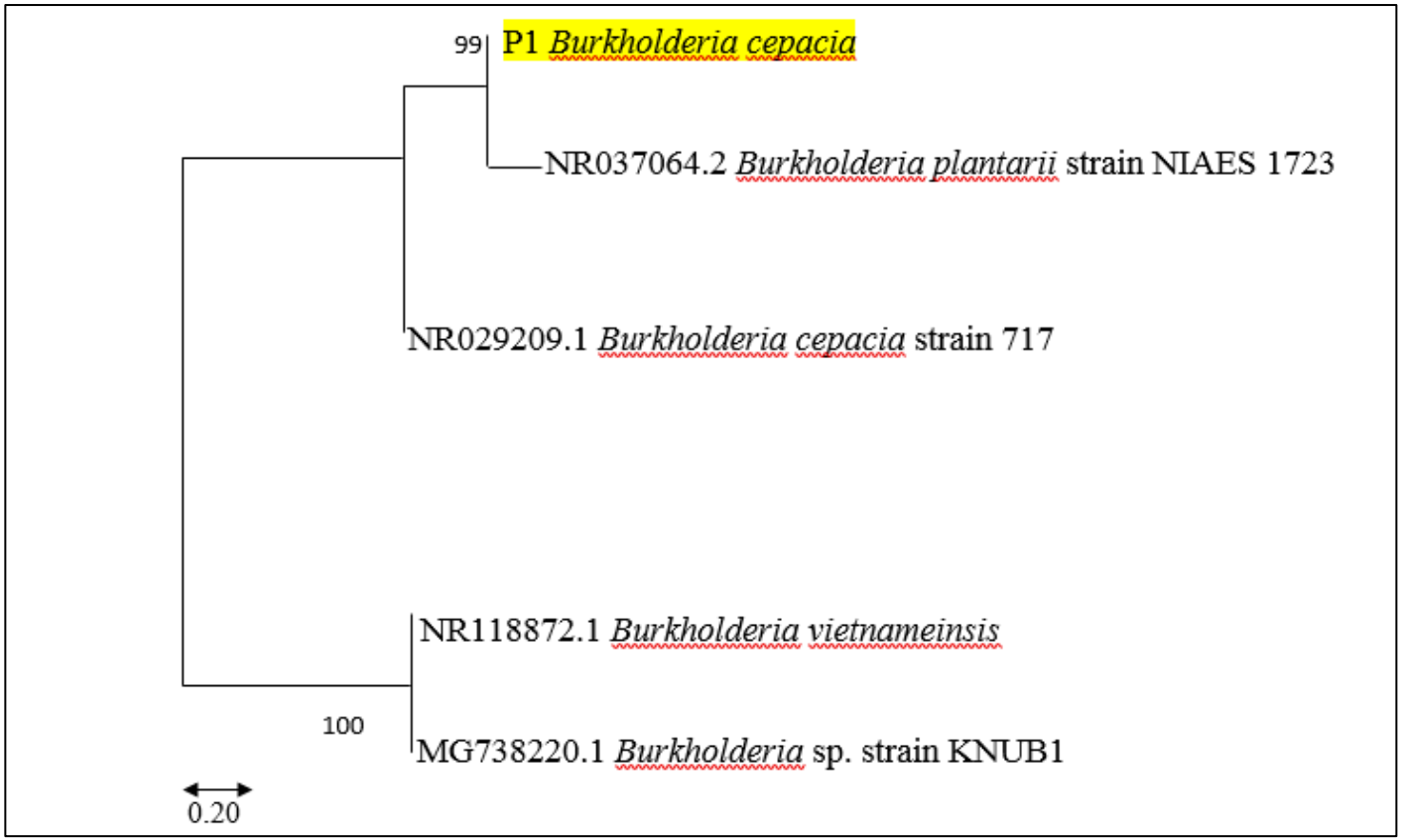


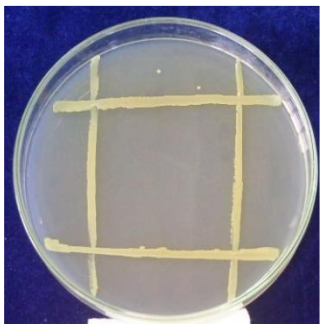
Plate 8. Phylogenetic tree of *Burkholderia cepacia* isolate P1 with other members of *Burkholderia* sp. on the basis of 16S rRNA gene sequences

Isolate with maximum bootstrap confidence value is highlighted

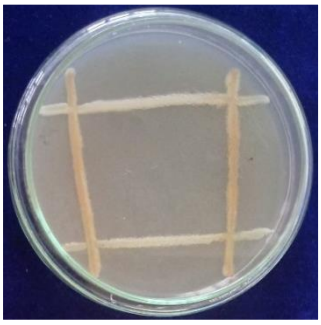
Table 11. Compatibility among different isolates of nitrogen fixers, phosphorus solubilizers and potash solubilizers

Sl. No	Combination of isolates	Compatible or incompatible
1	*Microbacterium arborescence* x *Burkholderia cepacia*	Compatible
2	*Microbacterium arborescence* x *Bacillus subtilis* (KASB5)	Compatible
3	*Microbacterium arborescence* x *Bacillus subtilis* (H4)	Compatible
4	*Microbacterium testaceum* x *Burkholderia cepacia*	Compatible
5	*Microbacterium testaceum* x *Bacillus subtilis* (KASB5)	Compatible
6	*Microbacterium testaceum* x *Bacillus subtilis* (H4)	Compatible
7	*Nguyenibacter vanlangenssis* x *Burkholderia cepacia*	Compatible
8	*Nguyenibacter vanlangenssis* x *Bacillus subtilis* (KASB5)	Compatible
9	*Nguyenibacter vanlangenssis* x *Bacillus subtilis* (H4)	Compatible
10	*Burkholderia cepacia* x *Acinetobacter calcoaceticus*	Compatible
11	*Burkholderia cepacia* x *Brevibacterium sp.*	Compatible
12	*Burkholderia cepacia* x *Burkholderia* sp.	Compatible
13	*Bacillus subtilis* (KASB5) x *Acinetobacter calcoaceticus*	Compatible
14	*Bacillus subtilis* (KASB5) x *Burkholderia* sp.	Compatible

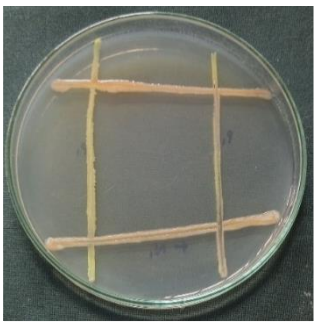
15	*Bacillus subtilis* (KASB5)	x	*Brevibacterium* sp.	Compatible
16	*Bacillus subtilis* (H4)	x	*Acinetobacter calcoaceticus*	Compatible
17	*Bacillus subtilis* (H4)	x	*Burkholderia* sp.	Compatible
18	*Bacillus subtilis* (H4)	x	*Brevibacterium* sp.	Compatible
19	*Microbacterium arborescence*	x	*Acinetobacter calcoaceticus*	Compatible
20	*Microbacterium arborescence*	x	*Burkholderia* sp.	Compatible
21	*Microbacterium arborescence*	x	*Brevibacterium* sp.	Compatible
22	*Microbacterium testaceum*	x	*Acinetobacter calcoaceticus*	Compatible
23	*Microbacterium testaceum*	x	*Burkholderia* sp.	Compatible
24	*Microbacterium testaceum*	x	*Brevibacterium* sp.	Compatible
25	*Nguyenibacter vanlangenssis*	x	*Acinetobacter calcoaceticus*	Compatible
26	*Nguyenibacter vanlangenssis*	x	*Burkholderia* sp.	Compatible
27	*Nguyenibacter vanlangenssis*	x	*Brevibacterium* sp.	Compatible



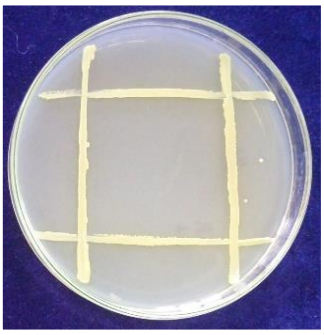
Microbacterium arborescence x *Burkholderia cepacia*



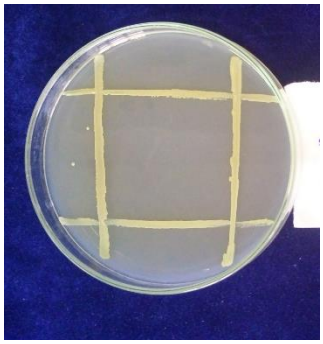
Microbacterium arborescence x *Bacillus subtilis* (KASB5)



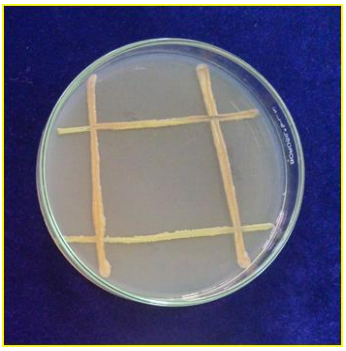
Microbacterium testaceum x *Burkholderia cepacia*



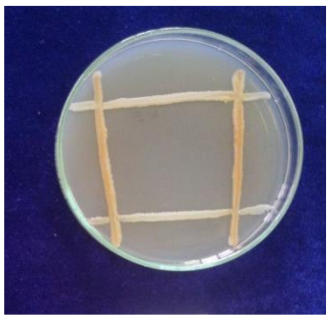
Microbacterium arborescence x *Brevibacterium* sp.



Nguyenibacter vanlangenssis x *Burkholderia cepacia*

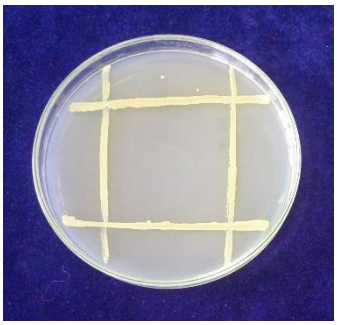


Microbacterium testaceum x *Acinetobacter calcoaceticus*



Bacillus subtilis (KASB5) x *Acinetobacter calcoaceticus*

Nguyenibacter vanlangensis x *Burkholderia* sp.



Bacillus subtilis (H4) x *Burkholderia* sp

Plate 9. Compatibility test among nitrogen fixers, phosphorus and potassium solubilizers

Table 12. Efficient and compatible bacterial isolates selected for consortium preparation

Consortium	Nitrogen fixer + Phosphorus solubilizer + Potash solubilizer
$N_1P_1K_1$ Consortium 1	*Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*
$N_2P_2K_2$ Consortium 2	*Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*
$N_3P_3K_3$ Consortium 3	*Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

4.5. Standardization of protocol for sterile alginate beads

4.5.1. Optimum concentrations of sodium alginate and calcium chloride for alginate beads

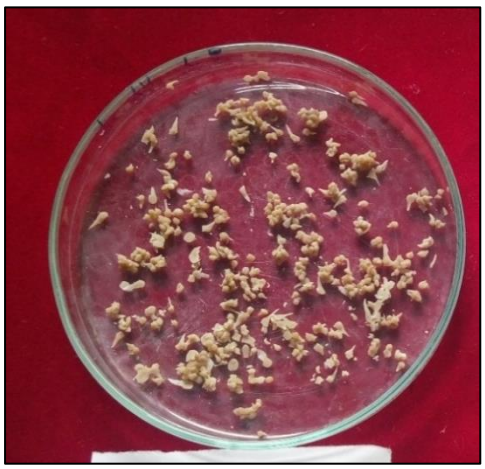
Eight different concentrations of sodium alginate and calcium chloride solution were used to standardise the alginate bead formation (Table 13 and Plate 10). Lower concentration (0.5, 1.0,1.5, 2) of sodium alginate and calcium chloride solution resulted in distorted and shape less beads. Uniform and spherical beads were obtained when the concentration of sodium alginate and calcium chloride was 3% and 4% respectively. Large and rigid beads were formed when the concentration of sodium alginate and calcium chloride solution was 5%, which was not a desirable character and hence, it was not used for further studies. Therefore, alginate beads formed at 3% concentration each of sodium alginate and calcium chloride solution were selected for further studies.

Table 13. Optimum concentration of sodium alginate and calcium chloride for alginate beads formation

Concentration of Sodium alginate (%)	Concentration of Calcium chloride (%)	Formation of beads	Characteristics of the beads formed
0.5	0.5	-	-
1.0	1.0	-	-
1.5	0.5	-	-
1.5	1.5	-	-
2.0	2.0	+	Flat, irregular beads
3.0	3.0	+	Uniform, spherical beads
4.0	4.0	+	Uniform, spherical beads
5.0	5.0	+	Uniform, rigid beads

+ Beads were formed

- Beads were not formed



Dry and distorted beads

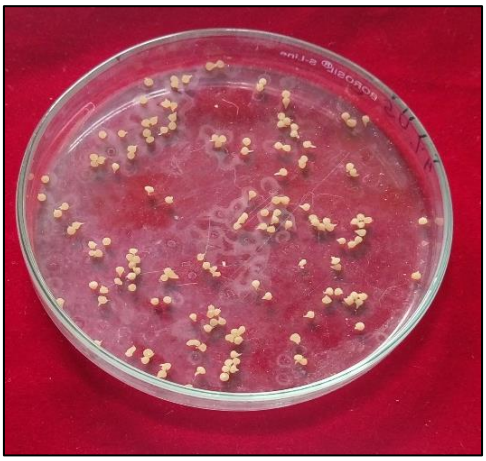
Uniform and spherical beads

Alginate beads (3%)

Dry and distorted beads

Uniform and spherical beads

Alginate beads (4%)



Dry and distorted beads

Rigid and spherical beads

Alginate beads (5%)

Plate 10. Alginate beads at different concentrations

4.5.2. Time taken for the solubilization of beads in phosphate buffer

Beads were solubilized by immersing them in phosphate buffer (0.06, 0.2 and 0.4 M; pH 6.8 ± 0.1), known for its ability to dissolve alginate gels. As the concentration of the sodium alginate solution increased, the time taken for the solubilization of alginate beads also increased (Table 14). The sodium alginate beads (3%) were dissolved in 0.4 M phosphate buffer in one hour while sodium alginate beads (5%) recorded dissolution after two hours of incubation. Hence, the phosphate buffer concentration of 0.4 M reduced the time required for solubilization of alginate beads.

Table 14. Solubilization of alginate beads in phosphate buffer

Concentration of sodium alginate beads	Time taken to dissolve alginate beads (h)		
	0.06 M	0.2 M	0.4 M
3%	1.5	1.5	1.0
4%	2	1.5	1.15
5%	3	2.5	2.0

4.5.3. Moisture per cent of beads

The moisture per cent of beads are presented in Table 15. Moisture content was high for the beads prepared from sodium alginate solution heated at 95 ^{o}C than those prepared from sodium alginate solution sterilized at 121^{o}C. As the concentration of the sodium alginate solution increased, a corresponding increase in moisture content was noticed. The ideal moisture per cent of the alginate beads (3%) selected in the present study was 15 per cent and more moisture is retained in the beads obtained from sodium alginate solution heated at 95 ^{o}C.

Table 15. Moisture per cent of alginate beads

Concentration of alginate beads	Moisture per cent of beads (%)	
	Sodium alginate solution heated at 95 ^{o}C	Sodium alginate solution sterilized at 121^{o}C
3%	15.0	9.4
4%	18.0	13.0
5%	22.4	19.0

4.5.4. Size of alginate beads

Diameter of alginate beads was determined through stereomicroscope (Table 16 and Table 17). As the concentration of sodium alginate solution increased, the mean diameter of the alginate beads also increased gradually. The beads obtained from sterilized sodium alginate solution at 121^{o}C had a diameter from 2.0 mm to 2.6 mm whereas, the beads obtained from sodium alginate solution heated at 95 ^{o}C had a diameter of 2.2 mm to 2.7 mm. The mean diameter of beads obtained from sodium alginate solution heated at 95 ^{o}C had the highest diameter (2.7 mm). The lowest diameter (2.0 mm) was for the beads obtained from sodium alginate solution (3%) sterilized at 121 ^{o}C. As the concentration of sodium alginate solution increased, the beads became more and more rigid when compared with the beads obtained at lower concentration. A total of 1866 beads were obtained from 100 ml of the sodium alginate solution (3%).

Table 16. Size of alginate beads at different concentration from sterilized sodium alginate solution (121ºC)

Sl. No	Alginate bead diameter (3%) (mm)	Alginate bead diameter (4%) (mm)	Alginate bead diameter (5%) (mm)
1	2.04	2.31	2.63
2	1.93	2.45	2.53
3	2.31	2.54	2.66
4	2.19	2.64	2.70
5	2.43	2.45	2.62
6	1.81	2.40	2.53
7	1.86	2.60	2.60
8	1.95	2.43	2.63
9	1.85	2.31	2.62
10	2.03	2.45	2.58
Mean value	**2.0**	**2.4**	**2.6**

Table 17. Size of alginate beads at different concentrations from sodium alginate solution heated at 95ºC for15 min.

Sl. No	Alginate bead diameter 3% (mm)	Alginate bead diameter 4% (mm)	Alginate bead diameter 5% (mm)
1	1.97	2.52	2.85
2	2.08	2.55	2.78
3	2.32	2.53	2.77
4	2.21	2.51	2.74
5	2.32	2.50	2.69
6	2.43	2.49	2.87
7	2.14	2.51	2.81
8	2.52	2.50	2.76
9	2.41	2.45	2.75
10	2.31	2.56	2.80
Mean value	**2.2**	**2.5**	**2.7**

4.5.5. Optimization of number and weight of beads

The number and weight of alginate beads obtained from sterile and heated sodium alginate solution are presented in Table 18. Higher number of beads were taken to obtain one gram of beads in the case of sterilized sodium alginate solution maintained at 121 ^{o}C. For the 5% concentration of sterilized sodium alginate solution (121 ^{o}C), 103 number of beads constituted one gram of beads while 24 beads constituted one gram of beads for the solution maintained at 95 ^{o}C. For 5% concentration of sterilized sodium alginate solution, the weight of an individual bead corresponds to 4.3 mg while for solution maintained at 95 ^{o}C, the weight was 34.8 mg.

Table 18. Characteristics of beads obtained from sterile and heated sodium alginate solution

Concentration of sodium alginate solution	Number of beads/g		Weight of an individual bead (mg)	
	Sodium alginate solution sterilized at 121^{o}C	Sodium alginate solution heated at 95 ^{o}C	Sodium alginate solution sterilized at 121^{o}C	Sodium alginate solution heated at 95 ^{o}C
3%	252	34	2.2	18.2
4%	115	28	3.1	24.6
5%	103	24	4.3	34.8

4.5.6. Optimization of temperature and time for contaminant free alginate beads

Sodium alginate solution (3%) were maintained at different temperatures such as 80 ^{o}C for 10 min, 85 ^{o}C for 10 min. and 95 ^{o}C for 15 min. The optimum temperature and time required for obtaining contamination free alginate beads was fund to be 95 ^{o}C for 15 min. Based on the parameters standardised, beads formed at 3% concentration of sodium alginate solution and calcium chloride, a moisture per cent of 15% and an optimum temperature of 95 ^{o}C for 15 min. were found to be the optimum condition for obtaining contaminant free alginate beads.

4.6. Rate of bacterial release from alginate based consortium at 15 days interval

Based on the optimum conditions standardized for contaminant free alginate beads, the consortium was prepared and the rate of release of bacteria from the beads were studied. The rate of bacterial release from alginate based consortium at 15 days interval are presented in Table 19, 20, 21. The plate count technique was followed on respective media; Jensen's agar media for nitrogen fixers, Pikovskaya's agar media for phosphorus solubilizers and Aleksandrov's agar media for potassium solubilizers. The initial population of bacteria in the alginate beads were significantly higher when compared with the population of microorganisms at 90^{th} day. The initial population of nitrogen fixers in consortium 1 [*Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*] (41.67 x 10^6 cfu g^{-1}), consortium 2 [*Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*] (39.33 x 10^6 cfu g^{-1}) and consortium 3 [*Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.] (40.67x 10^6 cfu g^{-1}) were statistically on par with each other. The population declined in consortium 1 (21.0 x 10^6 cfu g^{-1}), consortium 2 (23.67 x 10^6 cfu g^{-1}) and in consortium 3 (23.0 x 10^6 cfu g^{-1}) at 90^{th} day respectively.

The initial population of phosphorus solubilizers in consortium 1 (13.67 x 10^6 cfu g^{-1}), consortium 2 (12.33 x 10^6 cfu g^{-1}) and consortium 3 (11.33 x 10^6 cfu g^{-1}) were statistically on par with each other. The population decreased gradually and at 90^{th} day, the population of consortium 1 (7.67 x 10^6 cfu g^{-1}) was on par with the population in consortium 3 (9.0 x 10^6 cfu g^{-1}). Lower population of bacteria was recorded in consortium 2 (5.33 x 10^6 cfu g^{-1}) at 90^{th} day.The initial population of potassium solubilizers in consortium 1 (8.33 x 10^6 cfu/g) was higher which was also statistically on par with consortium 3 (7.00 x 10^6 cfu /g). The population of bacteria in consortium 1 (3.67 x 10^6 cfu g^{-1}), consortium 2 (4.67 x 10^6 cfu g^{-1}) and in consortium 3 (4.30 x 10^6 cfu g^{-1}) decreased at 90^{th} day.

Table 19. Population of nitrogen fixing bacteria released from alginate beads at fortnightly intervals

Consortium	Population of nitrogen fixers (x 10^6 cfu g^{-1})						
	Initial	15 days	30 days	45 days	60 days	75 days	90 days
Consortium 1	41.67 (1.61)	39.00 (1.59)	35.00 (1.54)	33.00 (1.51)	28.33 (1.45)[a]	23.33 (1.36)	21.00 (1.31)
Consortium 2	39.33 (1.59)	39.33 1.56)	33.00 (1.52)	29.33 (1.46)	23.33 (1.36)[b]	21.33 (1.32)	23.67 (1.37)
Consortium 3	40.67 (1.60)	37.00 (1.56)	35.00 (1.54)	31.67 (1.50)	24.67 (1.39)[ab]	26.00 (1.40)	23.00 (1.36)
CD (0.05)	NS	NS	NS	NS	-	-	NS

NS : Non significant

Log transformed values given in parantheses

Table 20. Population of phosphate solubilizing bacteria released from alginate beads at fortnightly intervals

Consortium	Population of phosphorus solubilizers (x 10^6 cfu g^{-1})						
	Initial	15 days	30 days	45 days	60 days	75 days	90 days
Consortium 1	13.67 (1.13)	10.67 (1.02)	9.00 (0.95)	8.67 (0.93)	8.33 (0.91)[b]	8.00 (0.90)	7.67 (0.87)[a]
Consortium 2	12.33 (1.69)	11.67 (1.06)	9.33 (0.96)	10.00 (0.99)	7.67 (0.88)[b]	7.00 (0.83)	5.33 (0.72)[b]
Consortium 3	11.33 (1.05)	10.33 (1.01)	10.33 (1.00)	9.00 (0.95)	12.00 (1.07)[a]	8.33 (0.92)	9.00 (0.95)[a]
CD (0.05)	NS	NS	NS	NS	-	NS	-

NS : Non significant

Log transformed values given in parantheses

Table 21. Population of potassium solubilizing bacteria released from alginate beads at fortnightly intervals

Consortium	Population of potash solubilizers (x 10^6 cfu g^{-1})						
	Initial	15 days	30 days	45 days	60 days	75 days	90 days
Consortium 1	8.33 (0.92)[a]	7.33 (0.86)	5.33 (0.71)	4.33 (0.63)	4.00 (0.59)	3.00 (0.46)	3.67 (0.53)
Consortium 2	6.33 (0.80)[b]	7.00 (0.84)	5.67 (0.74)	5.67 (0.75)	4.67 (0.66)	4.33 (0.62)	4.67 (0.66)
Consortium 3	7.00 (0.84)[ab]	6.67 (0.82)	7.00 (0.84)	6.00 (0.77)	4.33 (0.63)	4.00 (0.59)	4.33 (0.63)
CD (0.05)	-	NS	NS	NS	NS	NS	NS

NS : Non significant

Log transformed values given in parentheses

Consortium 1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

Consortium 2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

Consortium 3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

4.7. Population of inoculated bacterial isolates in sterile and unsterile potting mixture

A study was conducted to evaluate the alginate based consortium for growth enhancement and survivability in soil under pot culture studies.

4.7.1. Population of bacterial isolates in sterile potting mixture at fortnightly intervals

The population of nitrogen fixers, phosphorus solubilizers and potash solubilizers were assessed at fortnightly intervals under sterile potting mixture done by serial dilution and plating technique (Table 22, 23 and 24).

In sterile soil, the population of nitrogen fixing bacteria were higher for T_1, T_2, T_3, T_4, T_5, T_6, and T_7 treatments during the initial 15 days after planting. Treatment T_{10} (Absolute control) showed the lowest population of nitrogen fixers (2 x 10^5 cfu g^{-1}). In sterile soil, the population of phosphorus solubilizing microorganisms after 15 days were higher for the treatment T_1 and T_3, with a population of 13 x 104 cfu g^{-1} and 12.0 x 10^4 cfu g^{-1} respectively, which gradually decreased to 7.67 x 10^4 cfu g^{-1} and 9.0 x 10^4 cfu g^{-1}. In sterile soil, the population of potassium solubilizing bacteria were statistically on par with each other for the treatments T_4 (9.33 x 10^4 cfu g-^{1}), T_5 (9.00 x 10^4 cfu g^{-1}), T_3 (8.33x 10^4 cfu g^{-1}), T_2 (7.67 x 10^4 cfu g^{-1}) and T_1 (7.33 x 10^4 cfu g^{-1}). T_{10} (Absolute control) showed the lowest population (1.33 x 10^4 cfu g^{-1}).

4.7.2. Population of bacterial isolates in unsterile potting mixture at fortnightly intervals

The population of nitrogen fixers, phosphorus solubilizers and potash solubilizers at fortnightly intervals under unsterile potting mixture were enumerated (Table 25, 26 and 27). In unsterile soil, the population of nitrogen fixers were high in T_1, T_2, T_3, T_4, T_5, T_6 and T_7 during the initial 15 days after planting. The population of nitrogen fixers were comparatively higher in unsterile soil for the treatment T_{10} (13.33 x 10^5 cfu g^{-1}) when compared with sterile soil.

In unsterile potting mixture, at 15 days after planting, the population of phosphorus solubilizing bacteria in the treatments T_1 (10.0 x 10^4 cfu g^{-1}), T_2 (10.0 x 10^4 cfu g^{-1}), T_3 (9.33 x 10^4 cfu g^{-1}), T_4 (9.0 x 10^4 cfu g^{-1}), T_5 (8.0 x 10^4 cfu g^{-1}) and T_6 (7.0 x 10^4 cfu g^{-1}) were statistically on par with each other. The population of potassium solubilizers were high in T_5 (9.0 x 10^4 cfu g^{-1}), T_4 (8.67 x 10^4 cfu g^{-1}) and T_3 (8.33 x 10^4 cfu g^{-1}) which were also statistically on par with each other. The population of potassium solubilizers decreased (4.0 x 10^4 cfu g^{-1}) at 120^{th} day after planting.

Table 22. Population of nitrogen fixing microorganisms in sterile potting mixture ($x10^5$cfu g^{-1})

Treatments	Population of nitrogen fixing microorganisms in sterile potting mixture							
	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP	120 DAP
T_1 : Bacterial consortium 1 (Alginate based)	47.33 (1.67)^{a}	40.33 (1.60)^{ab}	32.66 (1.51)^{abc}	24.67 (1.39)^{cd}	(26.6 (1.42)^{a}	23.33 (1.41)^{a}	23.33 (1.36)^{a}	24.67 (1.39)^{a}
T_2 : Bacterial consortium 2 (Alginate based)	46.67 (1.69)^{ab}	36.33 (1.56)^{b}	25.00 (1.39)^{d}	25.33 (1.40)^{bcd}	24.67 (1.39)^{a}	23.67 (1.38)^{a}	23.67 (1.37)^{a}	21.00 (1.32)^{b}
T_3 : Bacterial consortium 3 (Alginate based)	44.67 (1.65)^{b}	40.33 (1.60)^{ab}	31.66 (1.49)^{bc}	25.67 (1.40)^{bcd}	27.00 (1.43)^{a}	24.00 (1.36)^{a}	24.00 (1.37)^{a}	22.67 (1.35)^{ab}
T_4 : T_1+ T_2 + T_3	46.00 (1.63)^{ab}	40.67 (1.61)^{ab}	35.67 (1.55)^{ab}	29.00 (1.46)^{a}	25.33 (1.39)^{a}	23.67 (1.39)^{a}	23.67 (1.37)^{a}	22.33 (1.34)^{ab}
T_5 : Bacterial consortium 1 (Talc based)	44.67 (1.65)^{b}	44.00 (1.64)^{a}	31.00 (1.49)^{c}	23.33 (1.36)^{d}	25.00 (1.39)^{a}	21.67 (1.30)^{a}	21.67 (1.33)^{a}	20.33 (1.30)^{b}
T_6 : Bacterial consortium 2 (Talc based)	46.00 (1.63)^{ab}	41.33 (1.61)^{a}	36.67 (1.56)^{a}	28.00 (1.45)^{ab}	26.00 (1.41)^{a}	24.33 (1.36)^{a}	24.33 (1.38)^{a}	22.67 (1.35)^{ab}
T_7 : Bacterial consortium 3 (Talc based)	45.00 (1.65)^{ab}	42.00 (1.62)^{a}	36.67 (1.56)^{a}	27.33 (1.44)^{abc}	24.00 (1.37)^{a}	23.00 (1.31)^{a}	(23.00) 1.36^{a}	20.67 (1.31)^{b}
T_8 : Package of Practices KAU, 2016	5.67 (0.74)^{d}	5.33 (0.72)^{cd}	5.00 (0.69)^{ef}	3.33 (0.52)^{f}	4.33 (0.63)^{bc}	2.67 (0.36)^{b}	2.67 (0.36)^{b}	2.33 (0.30)^{c}
T_9 : Organic POP, 2017	8.33 (0.92)^{c}	8.00 (0.90)^{c}	8.67 (0.93)^{e}	8.33 (0.92)^{e}	7.33 (0.85)^{b}	3.67 (0.70)^{b}	3.67 (0.53)^{b}	3.67 (0.51)^{c}
T_{10} : Absolute control	2.00 (0.30)^{e}	2.00 (0.20)^{d}	2.00 (0.26)^{f}	1.67 (0.20)^{f}	2.33 (0.36)^{c}	2.00 (0.20)^{b}	2.00 (0.25)^{b}	2.67 (0.40)^{c}

Log transformed values given in parantheses

Table 23. Population of phosphorus solubilizing microorganisms in sterile potting mixture (x10^5 cfu g^{-1})

Treatments	Population of phosphorus solubilizing microorganisms in sterile potting mixture							
	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP	120 DAP
T_1 : Bacterial consortium 1 (Alginate based)	13.00 (1.14)^{a}	10.33 (1.01)^{b}	9.33 (0.96)^{b}	8.67 (0.93)^{ab}	9.00 (0.99)^{a}	8.67 (0.93)^{b}	8.67 (0.98)^{ab}	7.67 (0.93)^{ab}
T_2 : Bacterial consortium 2 (Alginate based)	11.33 (1.09)^{b}	10.33 (1.01)^{b}	8.67 (0.93)^{bc}	9.33 (0.96)^{a}	6.67 (0.87)^{bc}	5.00 (0.69)^{c}	6.67 (0.86)^{bc}	5.00 (0.77)^{c}
T_3 : Bacterial consortium 3 (Alginate based)	12.00 (1.11)^{ab}	13.66 (1.13)^{a}	11.33 (1.05)^{a}	9.33 (0.96)^{a}	8.67 (0.98)^{a}	8.67 (0.93)^{b}	7.67 (0.93)^{abc}	9.00 (0.99)^{a}
T_4 : T_1+ T_2 + T_3	10.67 1.06^{b}	11.00 (1.04)^{b}	8.33 (0.91)^{bc}	10.00 (0.99)^{a}	7.67 (0.93)^{ab}	10.33 (1.01)^{a}	9.33 (1.01)^{a}	8.00 (0.95)^{a}
T_5 : Bacterial consortium 1 (Talc based)	9.00 (0.99)^{c}	8.00 (0.90)^{c}	6.33 (0.79)^{d}	7.00 (0.84)^{bc}	6.67 (0.88)^{bc}	5.67 (0.74)^{c}	6.33 (0.86)^{bcd}	5.67 (0.81)^{bc}
T_6 : Bacterial consortium 2 (Talc based)	8.67 (0.98)^{c}	6.33 (0.80)^{de}	7.00 (0.84)^{cd}	5.00 (0.69)^{cd}	5.67 (0.82)^{c}	6.00 (0.77)^{c}	5.33 (0.79)^{cd}	5.67 (0.82)^{bc}
T_7 : Bacterial consortium 3 (Talc based)	6.67 (0.88)^{d}	7.33 (0.86)cd	5.33 (0.72)^{de}	6.67 (0.81)^{bc}	4.00 (0.69)^{d}	4.67 (0.66)^{c}	4.00 (0.69)^{de}	3.67 (0.64)^{cd}
T_8 : Package of Practices KAU, 2016	5.00 (0.77)^{e}	5.33 (0.72)^{e}	4.33 (0.63)^{e}	3.33 (0.49)^{de}	3.33 (0.63)^{de}	2.00 (0.25)^{d}	4.00 (0.693)^{de}	3.67 (0.65)^{cd}
T_9 : Organic POP, 2017	4.00 (0.69)^{e}	5.00 (0.69)^{e}	3.67 (0.53)^{ef}	4.00 (0.59)^{de}	2.00 (0.46)^{ef}	2.67 (0.40)^{d}	2.67 0.53^{ef}	1.67 (0.36)^{de}
T_{10} : Absolute control	1.33 (0.30)^{f}	2.33 (0.36)^{f}	2.33 (0.36)^{f}	2.00 (0.25)^{e}	1.00 (0.25)^{f}	2.00 (0.25)^{d}	0.67 (0.20)^{f}	1.33 (0.36)^{e}

Log transformed values given in parantheses

Table 24. Population of potassium solubilizing microorganisms in sterile potting mixture ($x10^5$ cfu g^{-1})

Treatments	Population of potassium solubilizing microorganisms in sterile potting mixture							
	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP	120 DAP
T_1 : Bacterial consortium 1 (Alginate based)	7.33 (0.86)^{bc}	6.67 (0.82)^{bc}	7.00 (0.90)^{abc}	7.33 (0.86)^{a}	5.67 (0.81)^{ab}	5.33 (0.71)^{ab}	3.67 (0.51)^{bc}	4.33 (0.62)^{a}
T_2 : Bacterial consortium 2 (Alginate based)	7.67 (0.88)^{bc}	5.67 (0.75)^{c}	5.00 (0.77)^{cd}	3.67 (0.56)^{c}	4.67 (0.75)^{ab}	4.67 (0.66)^{abc}	5.00 (0.69)^{ab}	5.00 (0.69)^{a}
T_3 : Bacterial consortium 3 (Alginate based)	8.33 (0.92)^{ab}	7.00 (0.84)^{ab}	6.33 (0.86)^{bc}	5.67 (0.75)^{b}	4.67 (0.75)^{ab}	5.67 (0.75)^{ab}	5.67 (0.74)^{a}	4.67 (0.66)^{a}
T_4 : T_1+ T_2 + T_3	9.33 (0.96)^{a}	8.00 (0.90)^{a}	8.67 (0.97)^{a}	6.00 (0.77)^{b}	6.33 (0.85)^{a}	6.33 (0.78)^{a}	5.67 (0.75)^{a}	5.33 (0.72)^{a}
T_5 : Bacterial consortium 1 (Talc based)	9.00 (0.95)^{a}	8.00 (0.90)^{a}	7.00 (0.90)^{abc}	5.67 (0.75)^{b}	4.00 (0.69)^{bc}	4.00 (0.60)^{bc}	3.33 (0.51)^{bc}	3.67 (0.55)^{ab}
T_6 : Bacterial consortium 2 (Talc based)	8.33 (0.92)^{ab}	7.33 (0.86)^{ab}	8.00 (0.95)^{ab}	5.67 (0.75)^{b}	5.67 (0.82)^{ab}	5.33 (0.72)^{ab}	6.33 (0.80)^{a}	4.67 (0.66)^{a}
T_7 : Bacterial consortium 3 (Talc based)	6.67 (0.82)^{c}	7.67 (0.88)^{ab}	7.00 (0.89)^{abc}	5.67 (0.75)^{b}	5.00 (0.77)^{ab}	6.00 (0.77)^{a}	4.67 (0.66)^{ab}	3.67 (0.53)^{ab}
T_8 : Package of Practices KAU, 2016	2.67 (0.41)^{d}	2.00 (0.25)^{de}	2.33 (0.49)^{ef}	2.00 (0.25)^{d}	2.33 (0.51)^{cd}	3.00 (0.46)^{cd}	2.33 (0.36)^{cd}	2.00 (0.25)^{b}
T_9 : Organic POP, 2017	2.33 (0.36)^{de}	2.67 (0.40)^{d}	3.00 (0.56)^{de}	2.67 (0.41)^{cd}	1.33 (0.36)^{d}	2.00 (0.25)^{d}	2.00 (0.20)^{cd}	2.00 (0.20)^{b}
T_{10} : Absolute control	1.33 (0.10)^{e}	1.33 (0.10)^{e}	0.67 (0.20)^{f}	2.00 (0.25)^{d}	1.00 (0.25)^{d}	1.67 (0.20)^{d}	1.33 (0.10)^{d}	2.00 (0.25)^{b}

Log transformed values given in parantheses

Table 25. Population of nitrogen fixing microorganisms in unsterile potting mixture ($x10^5$ cfu g^{-1})

Treatments	Population of nitrogen fixing microorganisms in unsterile potting mixture							
	15 DAP	**30 DAP**	**45 DAP**	**60 DAP**	**75 DAP**	**90 DAP**	**105 DAP**	**120 DAP**
T_1 : Bacterial consortium 1 (Alginate based)	44.33 (1.64)^{a}	35.00 (1.54)^{c}	33.00 (1.51)^{abc}	23.67 (1.37)^{d}	26.67 (1.42)^{a}	26.33 (1.41)^{a}	21.33 (1.32)^{ab}	20.67 (1.31)^{ab}
T_2 : Bacterial consortium 2 (Alginate based)	45.00 (1.65)^{a}	34.67 (1.53)^{c}	23.00 (1.36)^{d}	24.00 (1.38)^{cd}	23.00 (1.35)^{a}	20.33 (1.30)^{b}	23.00 (1.36)^{a}	20.00 (1.30)^{b}
T_3 : Bacterial consortium 3 (Alginate based)	43.67 (1.63)^{a}	38.33 (1.58)^{bc}	30.33 (1.48)^{bc}	25.33 (1.40)^{bcd}	23.67 (1.37)^{a}	22.67 (1.35)^{ab}	21.00 (1.32)^{ab}	19.33 (1.28)^{b}
T_4 : T_1+ T_2 + T_3	45.33 (1.65)^{a}	40.67 (1.60)^{ab}	35.67 (1.55)^{ab}	29.00 (1.46)^{a}	25.33 (1.39)^{a}	25.00 (1.39)^{a}	21.00 (1.31)^{ab}	21.00 (1.32)^{ab}
T_5 : Bacterial consortium 1 (Talc based)	45.00 (1.65)^{a}	44.00 (1.64)^{a}	28.00 (1.43)^{cd}	23.33 (1.36)^{d}	24.00 (1.37)^{a}	20.00 (1.30)^{b}	21.33 (1.32)^{ab}	19.00 (1.27)^{b}
T_6 : Bacterial consortium 2 (Talc based)	43.67 (1.64)^{a}	41.33 (1.61)^{ab}	36.67 (1.56)^{a}	28.00 (1.44)^{ab}	26.00 (1.41)^{a}	23.00 (1.36)^{ab}	18.67 (1.27)^{b}	22.67 (1.35)^{a}
T_7 : Bacterial consortium 3 (Talc based)	44.33 (1.64)^{a}	41.33 (1.61)^{ab}	35.33 (1.54)^{ab}	27.00 (1.43)^{abc}	24.00 (1.37)^{a}	20.00 (1.30)^{b}	21.33 (1.32)^{ab}	19.67 (1.3)^{b}
T_8 : Package of Practices KAU, 2016	18.67 (1.27)^{b}	16.00 (1.20)^{d}	16.00 (1.20)^{e}	13.00 (1.11)^{e}	12.67 (1.10)^{b}	12.33 (1.08)^{c}	13.67 (1.13)^{c}	11.00 (1.04)^{c}
T_9 : Organic POP, 2017	19.33 (1.28)^{b}	16.67 (1.22)^{d}	13.00 (1.11)^{e}	10.67 (1.02)^{e}	11.33 (1.04)^{b}	10.00 (1.02)^{c}	13.33 (1.12)^{c}	11.00 (1.04)^{c}
T_{10} : Absolute control	13.33 (1.11)^{c}	13.00 1.10^{d}	11.33 (1.04)^{e}	13.00 (1.11)^{e}	10.33 (1.01)^{b}	10.67 (0.99)^{c}	12.33 (1.08)^{c}	11.00 (1.04)^{c}

Log transformed values given in parantheses

Table 26. Population of phosphorus solubilizing microorganisms in unsterile potting mixture ($x10^5$ cfu g^{-1})

Treatments	Population of phosphorus solubilizing microorganisms in unsterile potting mixture							
	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP	120 DAP
T_1 : Bacterial consortium 1 (Alginate based)	10.00 (0.99)^{a}	9.33 (0.96)^{a}	9.00 (0.95)^{a}	(8.67) 0.93^{ab}	(9.00) 0.99^{a}	8.67 (0.93)^{a}	7.00 (0.90)^{abc}	7.00 (0.84)^{ab}
T_2 : Bacterial consortium 2 (Alginate based)	10.00 (0.99)^{a}	9.33 (0.96)^{a}	8.67 (0.93)^{a}	8.00 (0.88)^{ab}	5.67 (0.81)^{bcd}	5.33 (0.72)^{bc}	6.67 (0.86)$^{abcd`}$	5.00 (0.69)^{bcd}
T_3 : Bacterial consortium 3 (Alginate based)	9.33 (0.96)^{a}	9.33 (0.96)^{a}	8.67 (0.92)^{a}	9.00 (0.95)^{ab}	6.33 (0.85)^{bc}	8.00 (0.90)^{a}	7.67 (0.93)^{ab}	7.67 (0.87)^{a}
T_4 : T_1+ T_2 + T_3	9.00 (0.95)^{ab}	9.67 (0.98)^{a}	8.00 (0.90)^{ab}	9.33 (0.96)^{a}	7.00 (0.90)^{ab}	9.67 (0.98)^{a}	8.67 (0.97)^{a}	6.33 (0.79)^{abc}
T_5 : Bacterial consortium 1 (Talc based)	8.00 (0.89)^{abc}	8.00 (0.90)^{ab}	6.33 (0.79)^{bc}	7.00 (0.84)^{abc}	6.67 (0.88)^{b}	4.67 (0.66)^{bc}	5.67 (0.82)^{bcd}	5.00 (0.69)^{bcd}
T_6 : Bacterial consortium 2 (Talc based)	7.00 (0.84)^{bc}	(5.6 (0.75)^{cd}	7.00 (0.84)^{abc}	5.00 (0.69)^{cd}	5.33 (0.80)^{bcd}	6.00 (0.77)^{b}	4.67 (0.74)^{cdef}	4.33 (0.63)^{cd}
T_7 : Bacterial consortium 3 (Talc based)	6.67 (0.82)^{cd}	6.67 (0.82)^{bcd}	5.00 (0.69)^{cd}	6.67 (0.81)^{bc}	4.33 (0.72)^{cd}	4.67 (0.66)^{c}	4.00 (0.69)^{def}	3.67 (0.53)^{de}
T_8 : Package of Practices KAU, 2016	4.00 (0.59)^{e}	7.00 (0.84)^{bc}	3.67 (0.53)^{d}	3.67 (0.51)^{de}	4.33 (0.71)^{cd}	4.00 (0.54)^{cd}	5.33 (0.80)^{bcde}	3.67 (0.53)^{de}
T_9 : Organic POP, 2017	6.00 (0.77)^{cde}	5.00 (0.69)^{d}	5.33 (0.71)^{cd}	4.00 (0.59)^{de}	4.00 (0.69)^{d}	3.67 (0.53)^{cd}	2.67 (0.53)^{ef}	3.00 (0.46)^{de}
T_{10} : Absolute control	4.67 (0.65)^{de}	2.33 (0.36)^{e}	3.33 (0.50)^{d}	2.00 (0.25)^{e}	1.33 (0.31)^{e}	2.67 (0.41)^{d}	2.33 (0.43)^{f}	1.67 (0.15)^{e}

Log transformed values given in parantheses

Table 27. Population of potassium solubilizing microorganisms in unsterile potting mixture (x10^5 cfu g^{-1})

Treatments	Population of potassium solubilizing microorganisms in unsterile potting mixture							
	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP	120 DAP
T_1 : Bacterial consortium 1 (Alginate based)	6.67 (0.82)^{cd}	6.00 (0.77)^{bc}	7.00 (0.84)^{abc}	7.33 (0.86)^{a}	7.00 (0.84)^{a}	7.33 (0.84)^{a}	3.67 (0.55)^{c}	4.33 (0.61)^{a}
T_2 : Bacterial consortium 2 (Alginate based)	6.33 (0.79)^{cde}	5.33 (0.72)^{cd}	5.00 (0.69)^{cde}	3.33 (0.51)^{d}	3.33 (0.51)^{de}	4.33 (0.62)^{bc}	4.33 (0.63)^{abc}	4.00 (0.59)^{ab}
T_3 : Bacterial consortium 3 (Alginate based)	8.33 (0.92)^{ab}	6.00 (0.76)^{bc}	6.00 (0.77)^{bcd}	5.67 (0.75)^{bc}	5.33 (0.72)^{abc}	5.33 (0.71)^{ab}	5.33 (0.72)^{ab}	5.33 (0.72)^{a}
T_4 : T_1+ T_2 + T_3	8.67 (0.93)^{ab}	8.00 (0.90)^{a}	8.67 (0.93)^{a}	6.00 (0.77)^{ab}	6.00 (0.77)^{ab}	6.33 (0.78)^{ab}	5.67 (0.75)^{a}	5.67 (0.75)^{a}
T_5 : Bacterial consortium 1 (Talc based)	9.00 (0.95)^{a}	7.33 (0.86)^{ab}	6.67 (0.81)^{abc}	5.67 (0.75)^{bc}	5.00 (0.69)^{cd}	4.67 (0.66)^{bc}	3.33 (0.51)^{c}	4.00 (0.59)^{ab}
T_6 : Bacterial consortium 2 (Talc based)	7.33 (0.85)^{bc}	7.33 (0.86)^{ab}	8.00 (0.90)^{ab}	4.33 (0.62)^{cd}	4.33 (0.61)^{bcde}	4.67 (0.67)^{bc}	4.33 (0.61)^{abc}	4.33 (0.61)^{a}
T_7 : Bacterial consortium 3 (Talc based)	6.33 (0.80)^{cde}	7.67 (0.88)^{ab}	6.00 (0.76)^{bcd}	5.33 (0.72)^{bc}	5.00 (0.69)^{bcd}	6.33 (0.80)^{ab}	4.33 (0.63)^{abc}	4.33 (0.63)^{a}
T_8 : Package of Practices KAU, 2016	5.66 (0.75)^{de}	5.00 (0.69)^{cd}	4.67 (0.65)^{cde}	3.67 (0.56)^{d}	3.67 (0.55)^{cde}	4.00 (0.59)^{bc}	3.00 (0.46)^{cd}	2.33 (0.36)^{bc}
T_9 : Organic POP, 2017	5.00 (0.69)^{ef}	4.00 (0.56)^{de}	4.00 (0.59)^{de}	3.67 (0.55)^{d}	2.67 (0.36)^{e}	2.67 (0.41)^{c}	4.00 (0.59)^{bc}	4.00 (0.59)^{ab}
T_{10} : Absolute control	4.00 (0.59)^{f}	3.00 (0.46)^{e}	3.33 (0.49)^{e}	3.67 (0.53)^{d}	2.67 (0.41)^{e}	2.33 (0.30)^{c}	1.67 (0.20)^{d}	2.00 (0.26)^{c}

Log transformed values given in parantheses

4.8. Soil analysis of tomato grown under sterile soil at flowering and harvesting stage

4.8.1 pH

There were no significant differences among soil pH during flowering and harvesting stage (Table 28).

4.8.2. Electrical conductivity

There were no significant differences among electrical conductivity during flowering and harvesting stage (Table 28 and 29).

4.8.3. Organic carbon (%)

A slight increase in organic carbon was noticed at flowering stage in T_9 (3.92%) when compared with the initial value (3.48%). At harvesting stage, the organic carbon content reduced (Table 28 and 29).

4.8.4. Available Nitrogen

Soil analysis showed that at flowering stage, there was an increase in available nitrogen when compared with the pre-analysis value (388 kg ha^{-1}) (Table 28 and 29). The available nitrogen increased in T_1 (542.96 kg ha^{-1}) and T_8 (551.27 kg ha^{-1}) followed by T_2 (511.44 kg ha^{-1}). Lowest amount of available nitrogen was present in T_{10} (406.07 kg ha^{-1}). At harvesting stage, there was a slight reduction in available nitrogen when compared with the flowering stage.

4.8.5. Available Phosphorus

Soil analysis showed that at the flowering stage, there was an increase in available phosphorus when compared with the pre analysis value (35 kg ha^{-1}). The treatments T_1 (159.26 kg ha^{-1}), T_7 (156.27 kg ha^{-1}) and T_8 (158.87 kg ha^{-1}) had higher amount of available phosphorus. At harvesting stage, the available phosphorus decreased in all treatments (Table 28 and 29).

4.8.6. Available Potassium

Soil analysis showed that at the flowering stage, there was an increase in available potassium when compared with the pre-analysis value (380.22 kg ha^{-1}). The treatments T_1 (521.22 kg ha^{-1}), T_8 (523.87 kg ha^{-1}) and T_9 (518.24 kg ha^{-1}) were having higher amount of potassium content (Table 28 and 29).

Table 28. Effect of treatments on soil pH, EC and nutrient status at flowering stage (Sterile potting mixture)

Treatments	pH	EC (dSm^{-1})	Organic carbon (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
T_1 : Bacterial consortium 1 (Alginate based)	5.23	0.07	3.20^{b}	542.96^{a}	59.26^{a}	521.22^{a}
T_2 : Bacterial consortium 2 (Alginate based)	5.43	0.08	3.13^{b}	511.44^{b}	53.61^{b}	486.67^{b}
T_3 : Bacterial consortium 3 (Alginate based)	5.40	0.07	3.26^{b}	494.33^{c}	42.93^{de}	486.04^{b}
T_4 : T_1+ T_2 + T_3	5.46	0.07	3.33^{b}	474.56^{e}	45.40^{cde}	489.89^{b}
T_5 : Bacterial consortium 1 (Talc based)	5.26	0.07	3.10^{b}	494.19^{c}	42.00^{e}	490.55^{b}
T_6 : Bacterial consortium 2 (Talc based)	5.33	0.06	3.36^{b}	495.22^{c}	46.60^{cd}	479.81^{b}
T_7 : Bacterial consortium 3 (Talc based)	5.43	0.07	3.23^{b}	488.64^{cd}	56.27^{ab}	491.59^{b}
T_8 : Package of Practices KAU, 2016	5.46	0.06	3.16^{b}	551.27^{a}	58.87^{a}	523.87^{a}
T_9 : Organic POP, 2017	5.66	0.07	3.92^{a}	478.47^{de}	47.27^{c}	498.24^{a}
T_{10}: Absolute control	5.16	0.06	2.36^{c}	406.07^{f}	29.07^{f}	365.68^{c}
CD (0.05)	NS	NS	-	-	-	-
Initial soil testing value	5.41	0.07	3.48	388	35.00	380.22

NS: Non significant

Consortium 1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

Consortium 2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

Consortium 3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

Table 29. Effect of treatments on soil pH, EC and nutrient status at harvesting stage (Sterile potting mixture)

Treatments	pH	EC (dSm^{-1})	Organic carbon (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
T_1: Bacterial consortium 1 (Alginate based)	5.30	0.07	2.43^{bc}	450.05^{a}	52.59^{ab}	432.53
T_2: Bacterial consortium 2 (Alginate based)	5.13	0.07	2.21^{d}	416.04^{bc}	49.52^{bc}	417.04
T_3: Bacterial consortium 3 (Alginate based)	5.23	0.06	2.26^{d}	425.29^{ab}	38.49^{fg}	415.52
T_4: T_1+ T_2 + T_3	5.33	0.08	2.18^{d}	420.36^{bc}	36.18^{g}	404.61
T_5: Bacterial consortium 1 (Talc based)	5.16	0.07	2.28^{cd}	395.64^{cde}	40.17^{ef}	426.33
T_6: Bacterial consortium 2 (Talc based)	5.23	0.07	2.53^{b}	397.64^{cd}	43.41^{cd}	421.09
T_7: Bacterial consortium 3 (Talc based)	5.16	0.07	2.25^{d}	433.08^{ab}	45.33^{de}	421.09
T_8: Package of Practices KAU, 2016	5.26	0.08	2.25^{d}	387.37^{de}	54.20^{a}	414.32
T_9: Organic POP, 2017	5.20	0.07	2.89^{a}	407.99^{bcd}	42.68^{e}	398.54
T_{10}: Absolute control	5.30	0.06	2.01^{e}	369.65^{e}	18.73^{bcd}	325.90
CD (0.05)	NS	NS	-	-	-	NS

NS : Non significant

Consortium 1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

Consortium 2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

Consortium 3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

4.9. Soil analysis of tomato grown under unsterile soil at flowering and harvesting stage

4.9.1. pH

There were no significant differences among soil pH during the flowering and harvesting stage (Table 30 and 31).

4.9.2. Electrical conductivity

There were no significant differences among the electrical conductivity during flowering and harvesting stage (Table 30 and 31).

4.9.3. Organic carbon (%)

A slight increase in organic carbon was noticed in the flowering stage in T_9 (4.08%) when compared with the initial value (3.62%). At harvesting stage, the organic carbon content reduced (Table 30 and 31).

4.9.4. Available Nitrogen

Soil analysis showed that at flowering stage, there was an increase in available nitrogen when compared with the pre-analysis value (356.25 kg ha^{-1}) (Table 30 and 31). The available nitrogen increased in T_1 (540.75 kg ha^{-1}) and T_8 (545.38 kg ha^{-1}). Lowest amount of available nitrogen was present in T_{10} (436.05 kg ha^{-1}). At harvest, there was a slight reduction in available nitrogen when compared with the flowering stage.

4.9.5. Available Phosphorus

Soil analysis showed that at flowering stage, there was an increase in available phosphorus when compared with the pre-analysis value (37.52 kg ha^{-1}) (Table 30 and 31).

4.9.6. Available Potassium

Soil analysis showed that at flowering stage, there was an increase in available potassium when compared with the pre-analysis value (389.58 kg ha^{-1}). The treatments T_2 (540.42 kg ha^{-1}), T_3 (539.52 kg ha^{-1}) and T_8 (527.03 kg ha^{-1}) had higher amount of potassium content (Table 30 and 31).

Table 30. Effect of treatments on soil pH, EC and nutrient status at flowering stage (Unsterile potting mixture)

Treatments	pH	EC (dSm^{-1})	Organic carbon (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
T_1 : Bacterial consortium 1 (Alginate based)	5.20^{d}	0.07	3.33^{b}	540.75^{a}	62.89	503.98^{bc}
T_2 : Bacterial consortium 2 (Alginate based)	5.36^{cd}	0.07	3.26^{b}	484.60^{b}	56.65	540.42^{a}
T_3 : Bacterial consortium 3 (Alginate based)	5.40^{bcd}	0.07	3.36^{b}	494.26^{b}	48.73	539.52^{a}
T_4 : T_1+ T_2 + T_3	5.63^{ab}	0.06	3.33^{b}	495.82^{b}	55.08	490.64^{cd}
T_5 : Bacterial consortium 1 (Talc based)	5.43^{bcd}	0.06	3.30^{b}	493.97^{b}	63.62	486.91^{cd}
T_6 : Bacterial consortium 2 (Talc based)	5.26^{cd}	0.07	3.40^{b}	487.23^{b}	57.67	490.10^{cd}
T_7 : Bacterial consortium 3 (Talc based)	5.50^{abc}	0.08	3.30^{b}	494.38^{b}	57.10	508.23^{bc}
T_8 : Package of Practices KAU, 2016	5.46^{abc}	0.06	3.20^{bc}	545.38^{a}	75.34	527.03^{ab}
T_9 : Organic POP, 2017	5.70^{a}	0.07	4.08^{a}	495.34^{b}	68.47	466.06^{d}
T_{10} : Absolute control	5.30^{cd}	0.07	3.03^{c}	436.05^{c}	35.25	430.47^{e}
	NS	NS	-	-	NS	-
Initial soil testing values	5.2	0.08	3.62	356.25	37.52	389.58

NS: Non significant

Consortium 1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

Consortium 2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

Consortium 3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

Table 31. Effect of treatments on soil pH, EC and nutrient status at harvesting stage (Unsterile potting mixture)

Treatments	pH	EC (dSm^{-1})	Organic carbon (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
T_1 : Bacterial consortium 1 (Alginate based)	5.13	0.08	2.50^{b}	421.76^{a}	61.71	482.20^{a}
T_2 : Bacterial consortium 2 (Alginate based)	5.30	0.07	2.52^{b}	415.47^{a}	58.20	483.44^{a}
T_3 : Bacterial consortium 3 (Alginate based)	5.23	0.07	2.15^{e}	397.78^{a}	57.04	424.03^{bc}
T_4 : T_1+ T_2 + T_3	5.26	0.07	2.31^{cd}	402.24^{a}	48.53	411.63^{c}
T_5 : Bacterial consortium 1 (Talc based)	5.26	0.08	2.39^{bc}	392.27^{a}	52.69	430.02^{bc}
T_6 : Bacterial consortium 2 (Talc based)	5.20	0.07	2.28^{cde}	415.87^{a}	53.13	420.37^{bc}
T_7 : Bacterial consortium 3 (Talc based)	5.16	0.06	2.32^{cd}	411.11^{a}	57.21	444.31^{b}
T_8 : Package of Practices KAU, 2016	5.23	0.07	2.26^{cde}	407.37^{a}	56.22	423.95^{bc}
T_9 : Organic POP, 2017	5.26	0.07	2.92^{a}	350.33^{b}	58.99	411.26^{c}
T_{10} : Absolute control	5.23	0.06	2.23^{de}	351.04^{b}	61.07	356.77^{c}
CD (0.05)	NS	NS	-	-	NS	-

NS: Non significant

Consortium 1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

Consortium 2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

Consortium 3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

4.10. Biometric characters of tomato grown under sterile and unsterile potting mixture

4.10.1. Plant height

Data on plant height of tomato grown under sterile and unsterile potting mixture at harvesting stage are presented in Table 32. In sterile soil, higher plant height was recorded for T_1 (Alginate based consortium 1) and T_2 (Alginate based consortium 2) with 129.17 cm and 126.07 cm respectively. It was followed by T_3 (Alginate based consortium 3) and T_4 (T_1 + T_2+ T_3) with 119.37cm and 119.63 cm which were statistically on par with each other. Lowest plant height was recorded in the treatment T_{10} (Absolute control) with a height of 95.56 cm.

In unsterile soil, greater plant height was recorded for T_1 (Alginate based consortium 1) and T_2 (Alginate based consortium 2) with 128.56 cm and 124.60 cm respectively. It was followed by treatment T_4 (T_1 +T_2+ T_3) and T_3 (Alginate based consortium 3) with 119.37 cm and 117.37 cm. The treatment T_{10} (Absolute control) recorded the lowest height (93.90 cm).

4.10.2. Number of branches

The data on number of branches of tomato grown under sterile and unsterile soil are given in Table 32. In sterile soil, higher number of branches were obtained for T_1 (3.33), T_2 (3.00), T_3 (3.00), T_4 (2.67), T_5 (3.33), T_6 (3.00) and T_7 (2.67) treatments respectively. The lowest number of branches was recorded for T_{10} (Absolute control) with one branch per plant. In unsterile soil, higher number of branches were recorded for T_1, T_3, T_6, T_8 which were on par with the treatments T_2, T_4, T_5 and T_7. The lowest number of branches was recorded for T_{10} (Absolute control) with one branch per plant.

4.10.3. Days to flowering

The data on days taken for first flowering of tomato plants grown under both sterile and unsterile soil are presented on the Table 33. In sterile soil, the number of days taken for flowering ranged from 52 to 67 days. Minimum number of days for

first blooming was recorded in T_1 (Alginate based consortium 1) and T_3 (Alginate based consortium 3) with 53.0 days and 55.67 days respectively. Maximum number of days to first blooming was recorded in T_{10} (Absolute control) and T_9 (Organic POP 2017) with 66.67 days and 65.0 days respectively. In unsterile soil, the number of days taken for flowering ranged from 56 to 68 days. Minimum number of days for first blooming was recorded in T_1 (Alginate based consortium 1) with 57.33 days. The treatments T_2, T_3, T_4, T_5, T_6, T_7, T_8 and T_9 were statistically on par with each other. Maximum number of days to first blooming was recorded in T_{10} with 67.0 days.

4.10.4. Fresh weight and dry weight of plants

The treatment effects on plant fresh weight and dry weight grown under sterile and unsterile soil is represented in the Table 34. In sterile soil, the fresh weight of plant was highest for the treatment T_1 (Alginate based consortium 1) with 173.46 g which was followed by T_4 (T_1 +T_2 +T_3) with 163.35 g. Lowest fresh weight of 98.48 g was recorded for T_{10} (Absolute control). Similarly, the dry weight was found to be highest in T_1 (Alginate based consortium 1) with 44.60 g. The treatments T_4 (35.26 g), T_6 (35.26 g), and T_2 (31.4 g) were statistically on par with each other.

In unsterile soil, the fresh weight of plant was superior for the treatment T_1 (Alginate based consortium 1) with 164.18 g. The treatments T_2 (156.23 g), T_5 (152.95 g) and T_1 (144.16 g) were statistically on par with each other. Fresh weight was lowest for T_{10} (Absolute control) with 86.0 g. The dry weight was highest for T_1 (Alginate based consortium 1) with 38.76 g. The treatments T_2 (27.86 g) and T_5 (27.33 g) were statistically on par with each other. Lowest dry weight was recorded for T_7 (20.28 g), T_9 (20.25 g) and T_{10} (19.30 g) respectively.

Table 32. Effect of treatments on plant height and number of branches in tomato under sterile and unsterile potting mixture

Treatments	Plant height (cm)		No of branches	
	Sterile	Unsterile	Sterile	Unsterile
T_1 : Bacterial consortium 1 (Alginate based)	129.17^{a}	128.56^{a}	3.33^{a}	3.33^{a}
T_2 : Bacterial consortium 1 (Alginate based)	126.07^{a}	124.60^{a}	3.00^{ab}	2.67^{ab}
T_3 : Bacterial consortium 1 (Alginate based)	119.37^{b}	117.37^{bc}	3.00^{ab}	3.00^{a}
T_4 : $T_1 + T_2 + T_3$	119.63^{b}	119.37^{b}	2.67^{abc}	2.67^{ab}
T_5 : Bacterial consortium 1 (Talc based)	114.33^{cd}	114.03^{cde}	3.33^{a}	2.67^{ab}
T_6 : Bacterial consortium 2 (Talc based)	114.37^{cd}	112.47^{de}	3.00^{ab}	3.00^{a}
T_7 : Bacterial consortium 2 (Talc based)	116.73^{bc}	116.77^{bcd}	2.67^{abc}	2.67^{ab}
T_8 : POP KAU 2016	112.03^{cd}	111.47^{e}	2.00^{bcd}	3.00^{a}
T_9 : Organic POP KAU 2017	111.83^{d}	110.63^{e}	1.67^{cd}	1.67^{bc}
T_{10} : Absolute control	95.56^{e}	93.90^{f}	1.00^{d}	1.00^{c}

Consortium 1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

Consortium 2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

Consortium 3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

Table 33. Effect of different treatments on days taken for flowering in plants grown under sterile and unsterile potting mixture

Treatments	No of days taken for flowering (Sterile)	No of days taken for flowering (Unsterile)
T_1 : Bacterial consortium 1 (Alginate based)	53.00^{e}	57.33^{d}
T_2 : Bacterial consortium 2 (Alginate based)	61.33^{d}	60.67^{c}
T_3 : Bacterial consortium 3 (Alginate based)	55.67^{e}	61.00^{c}
T_4: T_1 + T_2 + T_3	64.33^{abc}	61.33^{bc}
T_5 : Bacterial consortium 1 (Talc based)	62.33^{bcd}	63.33^{bc}
T_6 : Bacterial consortium 2 (Talc based)	62.33^{bcd}	62.67^{bc}
T_7 : Bacterial consortium 3 (Talc based)	63.00^{bcd}	63.00^{bc}
T_8 : POP KAU 2016	62.00^{cd}	64.33^{ab}
T_9 : Organic POP KAU 2017	65.00^{ab}	63.67^{bc}
T_{10} : Absolute control	66.67^{a}	67.00^{a}

Consortium 1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

Consortium 2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

Consortium 3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

Table 34. Effect of treatments on fresh weight and dry weight of plants grown under sterile and unsterile potting mixture

Treatments	Fresh weight (g)		Dry weight (g)	
	Sterile	**Unsterile**	**Sterile**	**Unsterile**
T_1 : Bacterial consortium 1 (Alginate based)	173.46[a]	164.18[a]	44.60[a]	38.76[a]
T_2 : Bacterial consortium 2 (Alginate based)	155.00[c]	156.23[b]	31.40[bc]	27.86[b]
T_3 : Bacterial consortium 3 (Alginate based)	133.46[d]	144.16[b]	24.63[de]	24.83[d]
T_4 : $T_1 + T_2 + T_3$	163.35[b]	131.27[d]	35.26[b]	21.16[e]
T_5 : Bacterial consortium 1 (Talc based)	132.76[d]	152.95[b]	24.20[de]	27.33[bc]
T_6 : Bacterial consortium 2 (Talc based)	151.90[c]	132.00[d]	32.67[b]	25.83[cd]
T_7 : Bacterial consortium 3 (Talc based)	122.00[e]	121.96[e]	21.83[ef]	20.28[ef]
T_8 : POP KAU 2016	108.33[g]	118.50[ef]	21.00[ef]	20.95[e]
T_9 : Organic POP KAU 2017	115.300[f]	117.13[f]	27.46[cd]	20.25[ef]
T_{10} : Absolute control	98.48[h]	86.0[g]	19.00[f]	19.30[f]

Consortium 1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

Consortium 2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

Consortium 3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

4.11. Yield attributes of tomato grown under sterile potting mixture

4.11.1. Average fruit weight

The data on average fruit weight are given in Table 35. Higher fruit weight was recorded for T_1 (30.0 g), T_2 (25.0 g), T_3 (28.0 g), T_5 (30.0 g), T_6 (27.0 g) which were statically on par with each other. Minimum fruit weight was observed for the treatments T_9 (Organic Package of Practices, KAU,2017) and T_{10} (Absolute control) with 18.00 g and 16.00 g respectively.

4.11.2. Number of fruits per plant

The effect of application of different treatments on number of fruits are given in Table 35. Higher number of fruits were produced in treatment T_1 (30.00), T_3 (28.0), T_5 (25.0) and T_8 (27.0) which were statistically on par with each other. Minimum number of fruits were produced in T_9 (Organic Package of Practices, KAU,2017) and T_{10} (Absolute control) with 13.00 and 10.00 fruits respectively.

4.11.3. Yield per plant

The data on effect of treatments on the yield are given in Table 35. The treatments T_1 (897.00 g), T_3 (781.33 g), T_5 (743.33 g) and T_8 (734.33 g) recorded a higher yield. Lowest yield per plant was recorded for T_9 (Organic Package of Practices, KAU,2017) and T_{10} (Absolute control) with 231.00 g and 166.00 g.

4.12. Yield attributes of tomato grown under unsterile potting mixture

4.12.1. Average fruit weight

The data on average fruit weight are given in Table 36. Higher fruit weight was recorded for T_8 (30.0 g), T_1 (26.0 g), and T_6 (28.0 g) which were statistically on par with each other. Minimum fruit weight was observed for the treatment T_{10} (Absolute control) with 16.0 g.

4.12.2. Number of fruits per plant

The effect of application of different treatments on number of fruits are given in Table 36. Higher number of fruits were produced in treatment T_1 (27.33) and T_8 (30.0) which were statistically on par with each other. Minimum number of fruits were produced in T_{10} (Absolute control) with 12.33 number of fruits.

4.12.3 Yield per plant

The data on effect of treatments on the yield are given in Table 36. The treatment T_8 recorded the highest yield of 897.00 g. This was followed by treatment T_1 with 707.33 g. Lowest yield per plant was recorded for T_{10} (Absolute control) with 193.33 g per plant.

Table 35. Effect of treatments on yield of tomato grown under sterile potting mixture

Sl. No	Treatments	Number of fruits per plant	Average fruit weight (g)	Yield per plant (g)
1	T_1 :Bacterial consortium 1 (Alginate based)	30.00^{a}	30.00^{a}	897.00^{a}
2	T_2 :Bacterial consortium 2 (Alginate based)	24.00^{bcd}	25.00^{abcd}	606.00^{bc}
3	T_3: Bacterial consortium 3 (Alginate based)	28.00^{ab}	28.00^{ab}	781.33^{ab}
4	T_4 : T_1+ T_2 + T_3	19.00^{de}	22.00^{cde}	415.33^{d}
5	T_5 : Bacterial consortium 1 (Talc based)	25.00^{abc}	30.00^{a}	743.33^{ab}
6	T_6 : Bacterial consortium 2 (Talc based)	22.00^{cd}	23.00^{bcde}	511.33^{cd}
7	T_7 : Bacterial consortium 3 (Talc based)	16.00^{ef}	21.00^{def}	332.00^{de}
8	T_8 : Package of Practices KAU, 2016	27.00^{abc}	27.00^{abc}	734.33^{ab}
9	T_9 : Organic POP, 2017	13.00^{fg}	18.00^{ef}	231.00^{e}
10	T_{10} : Absolute control	10.00^{g}	16.00^{f}	166.00^{e}

Consortium 1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

Consortium 2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

Consortium 3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

Table 36. Effect of treatments on yield of tomato grown under unsterile potting mixture

Sl. No	Treatments	Number of fruits per plant	Average fruit weight (g)	Yield per plant (g)
1	T_1 :Bacterial consortium 1 (Alginate based)	27.33^{ab}	26.00^{ab}	707.33^{b}
2	T_2 :Bacterial consortium 2 (Alginate based)	24.00^{bcd}	18.67^{cd}	449.33^{def}
3	T_3: Bacterial consortium 3 (Alginate based)	23.00^{cde}	22.00^{bc}	505.33^{de}
4	T_4 : T_1+ T_2 + T_3	20.00^{def}	21.00^{c}	423.33^{ef}
5	T_5 : Bacterial consortium 1 (Talc based)	25.00^{bc}	22.67^{bc}	565.66^{cd}
6	T_6 : Bacterial consortium 2 (Talc based)	24.33^{bc}	28.00^{a}	678.00^{b}
7	T_7 : Bacterial consortium 3 (Talc based)	19.33^{ef}	22.00^{bc}	429.33^{ef}
8	T_8 : Package of Practices KAU, 2016	30.00^{a}	30.00^{a}	897.00^{a}
9	T_9 : Organic POP, 2017	17.00^{f}	20.67^{c}	354.67^{f}
10	T_{10} : Absolute control	12.33^{g}	16.00^{d}	193.33^{g}

Consortium 1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

Consortium 2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

Consortium 3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

At transplanting stage

30 DAP

Flowering stage

Harvesting stage

Plate 11. Stages of plant growth under pot culture

Discussion

5. DISCUSSION

Plant growth promoting rhizobacteria are the soil bacteria inhabiting in and around the root surface and are directly or indirectly involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere (Kloepper and Schroth, 1981). Plant rhizosphere is a versatile and active ecological atmosphere of intense microbe-plant interactions for harnessing essential micro and macronutrients from the limited nutrient pool. Generally, plant growth promoting rhizobacteria facilitate the plant growth directly by either assisting in resource acquisition, fixation of atmospheric nitrogen, solubilization of phosphorus, potassium, zinc, production of siderophores, synthesis of plant growth hormones such as indole acetic acid, gibberellic acid, cytokinins *etc*. Indirect mechanism involves biological control of plant pathogens and deleterious microbes, thereby they act as biocontrol agents. Several factors such as root morphology, the stage of plant growth, root exudates, and the physio-chemical properties of the soil are reported to influence the occurrence and distribution of microbial communities in the soil and rhizosphere.

The term “biofertilizer” or “microbial fertilizers” refers to formulation containing live microbes which helps in enhancing the soil fertility by fixing atmospheric nitrogen, solubilization of phosphorus and other nutrients and augmenting plant growth by producing growth hormones. So, an extensive research is needed to identify more suitable strains, develop better production technologies with increased shelf life and quality control measures for wide commercialization. The development of biofertilizer with multi-crop growth promoting activities is most important for sustainable global agriculture (Barman *et al*., 2017).

In a nutshell, biofertilizer is an "ecofriendly" organic agro-input which has the ability to convert nutritionally important elements from unavailable to available form through biological processes (Vessey, 2003). To increase the crop yield so as to meet the appetite of world population without drastically hurting the

environment, a visionary new approach is required. Combining biofertilizer technology with the conventional fertilizer can help to grow crops in a sustainable way.

The carrier based inoculants produced in India generally have a short shelf life, poor quality, high contamination and unpredictable field performance. The carriers used are nearly inert material and forms clumps upon drying, which leads to significant loss of viability. High quality biofertilizers would be expected to have higher population of desired microorganisms, sufficient viability, and remain uncontaminated for longer period of storage. The encapsulation of microorganisms into a polymer matrix is still experimental in the field of bacterial-inoculation technology. Encapsulated bacterial formulations temporarily protect the encapsulated microorganisms from the soil environment and microbial competition and release them gradually for the colonization of plant roots. In this context, a study was conducted on "Alginate based consortial formulation of native microbial fertilizers". Alginate is a natural polymer of D- mannuronic acid and L-glucuronic acid, derived from *Macrocystis pyrifera*, *Sargassum sinicola* and they form beads with the multivalent cation Ca^{2+}. Encapsulation enables slow and controlled release of cells and the beads are biodegradable, non-toxic, and maintains a uniform bacterial population.

Microbial biosynthesis of the phytohormone auxin (indole-3-acetic acid/ IAA) has been known for a long time. It is being reported that 80 per cent of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release auxins as a secondary metabolite (Patten and Glick, 1996). IAA is the main auxin in plants, controlling many important physiological processes including cell enlargement and division, tissue differentiation, and responses to light (Frey-Klett *et al*., 2011, Gordon and Weber, 1950; Khalid *et al*., 2004, Leveau and Lindow, 2005). Biosynthesis of IAA is considered very critical in plant growth and development (Ali *et al.,* 2009). Therefore, bacteria with the ability of IAA production can be used as a basic criterion for screening effective PGPB in the commercial production of a biofertilizer for crops. Bacteria producing

significant amount of IAA have been extensively studied as bio-fertilizers (Naveed *et al.,* 2015).

In the present study, five nitrogen fixers were screened for the production of indole acetic acid and they were subjected to quantitative estimation under *in vitro*. Quantity of IAA produced varied with the isolates and the isolate *Microbacterium arborescence* recorded highest production of IAA (6.00 µg ml^{-1}) (Table 7). Khalid *et al*. (2004) reported that majority of the rhizobacteria (89%) are active in IAA production and they showed a stimulatory effect on root elongation (up to 233%) and weight (150%) of rice seedlings. Yu *et al*. (2014) also reported that nine species of *Microbacterium* isolated from corn and soybean cultivated in the soil of China produced IAA in a range of 10-20 µg ml^{-1}, which significantly improved the growth of wheat which is in agreement with the present study.

IAA production was reported by Ji *et al*. (2015) in *Microbacterium binotti* (4.1 µg ml^{-1}) and *Microbacterium trichotecenolyticum* (18.0 µg ml^{-1}). IAA production was also reported in *Cellulosimicrobium cellulans* (Chatterjee *et al*., 2009, Nabti *et al*., 2014) which is in agreement with the present study where *Celluosimicrobium* sp. produced 3.7 µg ml^{-1} IAA. In a similar study, Bal *et al*. (2013) reported the production of IAA in *Bacillus* sp. (37.65 µM ml^{-1}) and *Microbacterium* sp. (32.24 µM ml^{-1}). The present study also revealed that all the isolates produced high quantity of IAA during the stationary phase of growth. Earlier studies clearly indicated that, there is a positive correlation between *in vitro* IAA production by the isolates and plant root elongation under controlled condition. The IAA production varied in the present study which might be due to bacterial strains, culture conditions, amount of tryptophan supplemented and method of analysis (Tambalo *et al.,* 2006). Three species of *Microbacterium* isolated from Korean rice cultivars produced IAA in a range of 4 to 19 µg ml^{-1}, which had been tested with abilities of increasing rice height, dry weight and antagonistic effects against fungal pathogens (Ji *et al.,* 2015) which is in agreement with the present study where *Microbacterium arborescence* increased the plant height in T_1 (129.17 cm).

In the present study, phosphorus solubilizers and potassium solubilizers were also screened for IAA production (Table 8 and Table 9). Among the phosphorus solubilizers, *Burkholderia cepacia* (8.67 µg ml^{-1}), *Burkholderia vietnamiensis* (3.20 µg ml^{-1}) and *Bacillus subtilis* KASB5 (3.43 µg mL^{-1}) showed high production of IAA. Among the potassium solubilizers, *Acinetobacter calcoaceticus* produced significant amount of IAA (4.0 µg ml^{-1}) (Table 9).

Hernandez- Rodriguez *et al*. (2010) stated that *Burkholderia cepacia* played an active role as a plant growth promoting bacteria and produced indole acetic acid. Farokh (2011) reported that *Acinetobacter calcoaceticus* produced 13 µg ml^{-1} of IAA and significantly promoted the growth of pearl millet seedlings, increased the shoot height, root length and root dry weight which is in concurrence with the present study where *Acinetobacter calcoaceticus* recorded higher production of IAA (4.00 µg ml^{-1}).

Nitrogen is one of the most vibrant nutrients required for plant growth and productivity. Although, there is about 78 per cent N_2 in the atmosphere, it is unavailable to the growing plants. The atmospheric nitrogen is converted into plant available forms by biological nitrogen fixation (BNF) which changes nitrogen to ammonia by nitrogen fixing microorganisms using a complex enzyme system known as nitrogenase (Kim and Rees, 1994).

In the present study, five nitrogen fixers were screened for nitrogen fixation in N-free medium and quantification of the amount of nitrogen fixed were also assessed (Table 7). The performance of nitrogen fixers was equally well and there were no significant differences among the isolates. *Microbacterium arborescence* fixed maximum amount of nitrogen (22.63 mg of N g^{-1} of sucrose utilized) and their growth rate was also high on N- free medium. Lin *et al*. (2012) reported that *Microbacterium* sp. is a nitrogen fixing endophyte which boosted the growth on its host plant sugarcane. They fix atmospheric nitrogen, forms biofilms on the host surfaces and colonizes the intact plant. A study conducted by Shaheena *et al*., (2016) also stated that *Cellulosimicrobium* sp. and *Microbacterium* sp. are good nitrogen fixers, which is in agreement with the present study.

Phosphorus is the second important plant growth limiting nutrient after nitrogen. It is abundantly available in soils in both organic and inorganic forms. Phosphorus makes up about 0.2% of a plant's dry weight. Despite large reservoir of phosphorus, the amount of available forms to plants is generally low. Since, the majority of soil phosphorus is found in insoluble forms, there is a less availability of phosphorus to plants while the plants absorb it only in two soluble forms, the monobasic ($H_2PO_4^-$) and the dibasic (HPO_4^{2-}) ions (Bhattacharyya and Jha, 2012). The use of phosphorus biofertilizers is a promising approach towards improving food production through enhancing agricultural yield as it is better to use an environmentally friendly approach. Most of the efficient phosphate solubilizer reduces the medium pH by secreting organic acids like gluconic and 2-ketogluconic acids which chelates the metal cations from insoluble phosphates (Khan *et al*., 2014). So, typical phosphate solubilizing kinetics includes a gradual decrease of pH along with the increase of soluble phosphate count over time.

In the present study, all the phosphorus solubilizers were screened for P solubilization (Table 8). The solubilization of phosphorus is dependent on the source of phosphorus and to a greater extent influenced by the culture conditions. Similar studies reviewed by Kucey *et al*. (1989) pointed out that microbial solubilization of phosphate in medium is due to the secretion of organic acids which resulted in a decrease of pH. In the present study, the pH dropped from 7.2 to 3.4 by the isolate *Burkholderia cepacia* which is in accordance with the previous report of Kpomblekou and Tatabai (1994) where the microorganisms reduced the pH of medium during their growth in liquid medium which are considered to be efficient P solubilizers.

Preliminary screening for phosphate solubilization efficiency of the isolates were carried out by the formation of a clear halo zone on agar media and solubilization efficiency were calculated by the quantitative method (Nguyen *et al*., 1992). In the present study, the solubilization index ranged from 56.82 (*Pseudomonas putida*) to 117.85 (*Burkholderia cepacia*). Quantitative estimation

of phosphorus solubilized by phospho blue colour method revealed that *Burkholderia cepacia* was the most efficient one (64.83 μg ml^{-1}) followed by two different strains of *Bacillus subtilis.* The results are in agreement with the earlier studies of Song *et al*. (2008) who reported that the production of soluble phosphorus by *Burkholderia cepacia* (DA23) with tri-calcium phosphate and hydroxyl apatite was higher when compared with aluminium phosphate. *Burkholderia cepacia* strains were found to have a high capacity for the solubilization of calcium phosphate (Peix *et al*., 2001 and Lin *et al*. 2008). Zhao *et al*. (2014) also stated that *Burkholderia cepacia* solubilized 450 μg ml^{-1} of phosphorus.

The potential of phosphate solubilizing bacteria, such as *Bacillus* sp., for increasing crop yields to convert insoluble phosphate in rocks into soluble forms available for plant growth, have been reported (Bojinova *et al.,* 1998). This conversion is through acidification, chelation and exchange reactions and produces strong organic acids in the periplasm which is in agreement with the present study, where the pH was reduced from 7.2 to 3.4 due to the production of organic acids by the phosphorus solubilizers.

Potassium solubilizing bacteria (KSB) are well known for its capability to solubilize rock potassium such as mica, illite, and rock phosphate. Therefore, application of rock P and K minerals with inoculation of bacteria may provide a continuous supply of soluble phosphorus and potassium, which will increase the soil fertility. In the present study, all potassium solubilizers were screened for potassium solubilization (Table 9). The highest amount of potassium was solubilized by *Acinetobacter calcoaceticus* (41.63 μg ml^{-1}) followed by *Burkholderia* sp. (31.26 μg ml^{-1}). Bagyalakshmi *et al*. (2017) studied the efficiency of six potential potassium solubilizing bacteria in different basal medium containing MOP, SOP and Montmorillonite sources under *in vitro*. The results revealed that the KSB strains solubilized K content more in the medium supplemented with MOP than with other potassium sources.

Bhattacharya *et al*. (2016) also stated that *Acinetobacter soli* can solubilize potassium from the feldspar matrix through the release of organic acids such as gluconic acids, acetic acids, α ketogluconic acid and aminocarboxylic acid. Recent literature also indicated that *Acinetobcter calcoaceticus* has a positive role in plant growth enhancement and biologically active metabolites production (Kang *et al.*, 2010) which is in agreement with the present study, where all the isolates solubilized potassium. Khanghahi *et al*. (2018) stated that single potassium solubilising bacterium (KSB) inoculation (without K chemical fertilizer) slightly increased the grain yield in rice as compared to the nitrogen-phosphorus (NP) treatment in the pot experiment. They reported that native KSBs have the potential to be used as bioinoculants to reduce consumption of potassium chemical fertilizer for rice production. In the present study, the yield of tomato was higher in T_1 (alginate based consortium1) with 897.0 g per plant which consisted the consortia *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus* where no addition of chemical fertilizers were done.

Results indicated that *A. calcoaceticus* application resulted in 66.58% and 40.87% higher shoot lengths of cucumber plants when compared with the control. Fresh weight of PGPR treated plants were 22.17 per cent and 39.98 per cent higher than the uninoculated ones. This growth promotion capacity of *A. calcoaceticus* might be attributed to its potential to secrete gibberellins (Kang *et al*., 2014), which is in conformity with the present study where consortia consisting of *A. calcoaceticus* increased the plant height (129.17 cm) and fresh weight (173.46) in T_1 (alginate based consortium1).

The three most efficient isolates producing indole acetic acid, fixing nitrogen, solubilizing phosphorus and potassium under nitrogen fixers, phosphorus and potassium solubilizers were selected (Table 10). The selected nitrogen fixers were *Microbacterium arborescence, Microbacterium testaceum* and *Nguyenibacter vanlangenssis*. The selected phosphorus solubilizers were *Burkholderia cepacia, Bacillus subtilis* (KASB5) and *Bacillus subtilis* (H4) and selected potassium

solubilizers were *Acinetobacter calcoaceticus, Burkholderia* sp. and *Brevibacterium* sp.

Formulating a successful microbial consortium relies on the compatibility among the bacterial isolates. The compatibility among the selected bacterial isolates were done by cross streak method. Two different bacterial isolates were streaked vertically and horizontally in a Petri plate containing nutrient agar medium to know whether they are compatible or not (Table 11). An important prerequisite needed for the successful development of a microbial consortia appears to be the compatibility among the co-inoculated microorganisms.

There was no inhibition among the selected bacterial isolates which indicated that all the isolates were compatible. It is in accordance with the study conducted by James and Mathew (2017), who reported the mutual compatibility between bacterial isolates by cross streak method and found no lysis at the juncture. So, compatibility plays an important role in developing a microbial consortium. The three most efficient and compatible isolates obtained in the present study were compatible with each other which indicated that they could be used for consortia formulation (Table 12).

In order to develop an effective formulation using alginate beads it is important to standardise the protocol for mass production of alginate beads. Therefore, eight different concentrations of sodium alginate and calcium chloride solution were used to standardize the formation of alginate beads. Lower concentration of either sodium alginate (0.5, 1.0, 1.5) or calcium chloride (0.5, 1.0, 1.5) resulted in improper formation of beads (Table 13). Uniform and spherical beads were formed when the concentration of sodium alginate and calcium chloride were 3% and 4%. Large and rigid beads were formed when the concentration of sodium alginate and calcium chloride solution was 5%. Therefore, beads formed from 3% concentration of both sodium alginate and calcium chloride solution was selected. The present study is in conformity with the finding of Archana and Brahmaprakash (2014), who reported that sodium alginate solution of

concentrations 0.1 M, 0.2 M and 0.3 M formed prominent and smooth beads with 0.1 M calcium chloride, whereas the beads formed with 0.2 M calcium chloride were large and rigid. It indicated that the appropriate concentration needs to be standardized for sodium alginate and calcium chloride solution in order to develop alginate formulation.

The solubilization of alginate beads is important because the bacteria have to be released in the rhizosphere on application to the soil. Hence, time taken for the solubilization for alginate beads were determined. Beads were solubilized by immersing them in potassium phosphate buffer of various concentrations. As the concentration of the sodium alginate solution increased, the time taken for the solubilization of alginate beads also increased (Table 14). The sodium alginate beads (3%) dissolved in 0.4 M phosphate buffer within one hour while the 5% concentration beads got dissolved only after two hours of incubation. The phosphate buffer concentration of 0.4 M reduced the time required for solubilization of alginate beads. Bashan (1986) also reported that the time required for solubilization of lyophilized beads were much longer, (18 hours) in phosphate buffer which did not result in total solubilization of alginate beads.

Moisture content of alginate beads is important because as it affects the survivability of bacteria and enables in slow release. Moisture content was high for the beads prepared from sodium alginate solution maintained at 95 ^{o}C than those prepared from sterilized sodium alginate solution (Table 15). As the concentration of the sodium alginate solution was increased, a corresponding increase in moisture content was noticed. Considering the high influence of water on stability when formulations containing active compounds are stored, the analysis of moisture content becomes prime importance. In the present study, a higher moisture content of 15 per cent was recorded for the alginate beads (3%) obtained from sodium alginate solution heated at 95 ^{o}C while the moisture content of beads obtained from sodium alginate solution sterilized at 121 ^{o}C was 9.4 per cent.

As the concentration of sodium alginate solution increased, the mean diameter of the alginate beads also increased gradually (Table 16 and Table 17). The beads obtained from sterilized sodium alginate solution at 121^{o}C had a diameter range of 2.0 mm to 2.6 mm whereas the beads obtained from sodium alginate solution maintained at 95^{o}C had a diameter range of 2.2 mm to 2.7 mm. The mean diameter of beads obtained from sodium alginate solution maintained at 95^{o}C has the highest diameter (2.7 mm). The lowest diameter was 2.0 mm for the beads obtained from sterilized sodium alginate solution of 3% concentration. As the concentration of sodium alginate solution increased, the beads became more and more rigid when compared with the beads obtained from lower concentration. The results were comparable with Huang and Lin (2017) who reported that the size of alginate beads was changed by crosslinkers and temperature. The bead size in Ca^{2+} solution was larger than those prepared from Ba^{2+}. The shrinkage, tightness, release behaviours, and swelling properties of alginate beads are all relating to each other. Keshavaraz *et al*. also (1996) reported the use of different types of equipment for making alginate beads such as pressurized multi nozzle, resonance, rotating nozzle ring, rotating disk and obtained a corresponding bead diameter of 0.5-1.2 mm, 1.0-2.0 mm, 0.5-1.0 mm and 1.6-6.4 mm respectively. Thus, the size of alginate beads can vary according to the instrument being used for the preparation of alginate beads.

Reetha *et al*. (2014) obtained encapsulated particles of smaller size (1.3 to 3.2 mm) and lower weight (0.5 to 10.3 mg) compared to our data which is explained by the type of pipetting instrument used during the extrusion of the inoculum. Ivanova *et al*. (2005) also reported another protocol in which the beads size ranged from 1 to 5 mm and observed that by increasing the sphere size, bacteria survival enhanced by 36%.

Zago *et al*. (2017) reported that weight of alginate beads ranged between 17 mg and 38 mg and diameter of 3.3 mm to 4.3 mm. He also stated that variation in bead weight and diameter can occur with the addition of additives like humic acid and trehalose. In the present study, the weight of beads ranged from 18.2 mg to 34.8 mg for the beads obtained by heating the sodium alginate solution at 95 ^{o}C

(Table.18). The most common experimental formulation for bacterial inoculants is macrobeads with a diameter of 1-4 mm either for agricultural or environmental use (Bashan 1986; Bashan and Gonzalez, 1999) which is in agreement with the present study where a bead size of 2.0-2.7 mm was obtained. The present study is in agreement with the findings of Sankalia *et al*. (2005) who reported that the bead size is influenced by the opening through which the sodium alginate solution is allowed to pass and also depends on the viscosity of the alginate solution. She reported that increased viscosity at a higher concentration of sodium alginate resulted in larger particles and reported a diameter of 660 to 715 mm.

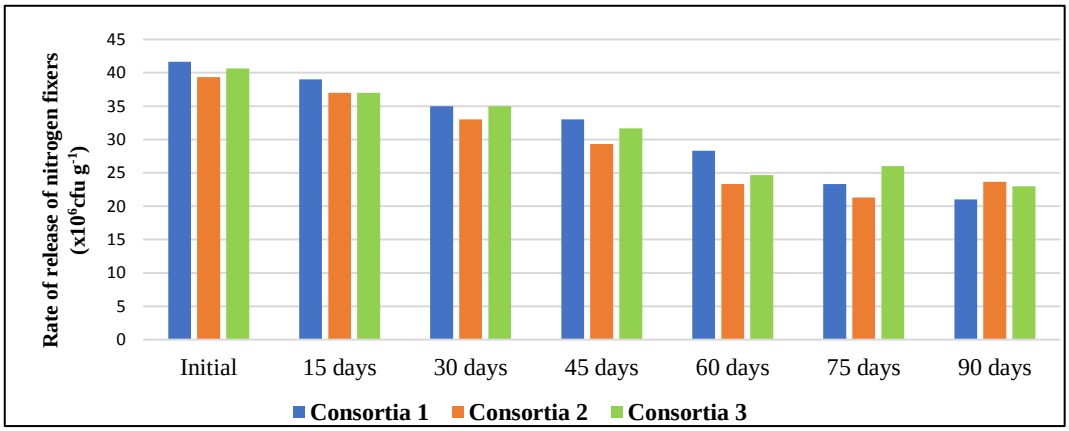
For any formulation, sterile conditions have to be maintained in order to avoid contamination of the formulation. Hence, a protocol was standardized to obtain contaminant free alginate beads. Alginate beads consisting of nitrogen fixer, phosphorus solubilizer and potash solubilizer were prepared. Sodium alginate solution (3%) were treated at different temperatures such as 80 ^{o}C for 10 minutes, 85 ^{o}C for 10 minutes and 95 ^{o}C for 15 minutes. The optimum temperature and time required for obtaining sterile alginate beads was by maintaining the sodium alginate solution at 95^{o}C for 15 min. Ching *et al*. (2017) reported that alginate gels subjected to thermal treatment (boiling at 100 ^{o}C and 121 ^{o}C) resulted in changes in textural attributes of alginate beads which is in agreement with the present study where the sodium alginate solution at 121^{o}C resulted in dry and distorted beads. However, the alginate beads obtained from sodium alginate solution at 95 ^{o}C resulted in uniform and spherical beads.

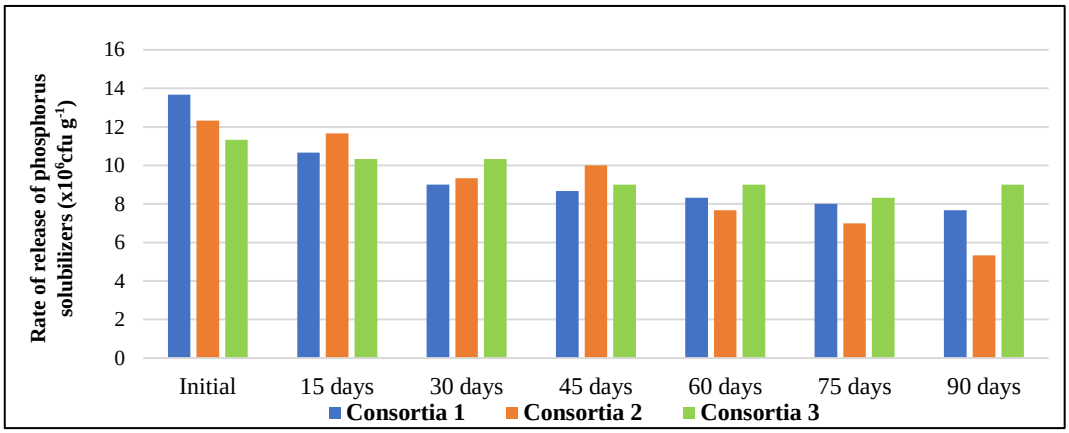
Previous studies by Roopa and Bhattacharya (2010) also found that heated gels were generally softer and less brittle but nevertheless maintained a gel structural integrity. Oates and Ledward (1990) stated that at temperature above the gel transition temperature (180 ^{o}C), thermal decomposition of the ionic alginate gel was recorded which is in agreement with the present study where the sterilization of sodium alginate solution at 121 ^{o}C resulted in distorted beads and shape less beads. Higher number of beads were taken to obtain one gram of beads in the case

of sterilized sodium alginate solution (Table 18). For the 5% concentration of sterilized solution, 103 number of beads constituted one gram of beads while 24 beads constituted one gram of beads in solution maintained at 95 ^{o}C. For 5% concentration of sterilized sodium alginate solution, the weight of an individual bead corresponds to 4.3 mg while for solution maintained at 95^{o}C the weight is 34.8 mg.

The alginate based consortiummaintained a viable population even after 90 days of storage (Table 19, 20 and 21). Higher population of nitrogen fixers were maintained in alginate beads. The population of bacterial isolates decreased gradually (Fig. 1). In earlier studies, it is reported that the population of the inoculated bacteria declines progressively over time, preventing the accumulation of a bacterial pool in the rhizosphere sufficient to promote beneficial effects (Bashan, 1998 and Sivakumar *et al*., 2014) which is in agreement with the present study where the initial population of bacteria in the alginate beads were significantly higher when compared with the population of microorganisms at 90th day. The initial population of nitrogen fixers in consortia 1 (41.67 x 10^{6} cfu g^{-1}), consortia 2 (39.33 x 10^{6} cfu g^{-1}) and consortia 3 (40.67x 10^{6} cfu g^{-1}) were statistically on par with each other. The population declined in consortia 1 (21.0 x 10^{6} cfu g^{-1}), consortia 2 (23.67 x 10^{6} cfu g^{-1}) and in consortia 3 (23.0 x 10^{6} cfu g^{-1}) at 90th day respectively.

A similar study was conducted by Archana and Brahmaprakash (2014), who immobilized the plant growth promoting bacterial consortium consisting of *Azotobacter chrococcum*, *Pseudomonas fluorescence* and *Acinetobacter* sp. in alginate beads. They reported that surviving population was maximum in alginate formulation after 240 days of storage. The results were comparable with Zago *et al*. (2017), who reported that *Azospirillum brasiliense* encapsulated in alginate beads remained viable which proved that encapsulation did not prevent cell growth. At 90th day, the population of bacteria in alginate beads were 12% lower when compared with the first day which is in agreement with the present study where the population of nitrogen fixers, phosphorus and potassium solubilizers gradually declined at 90th day.





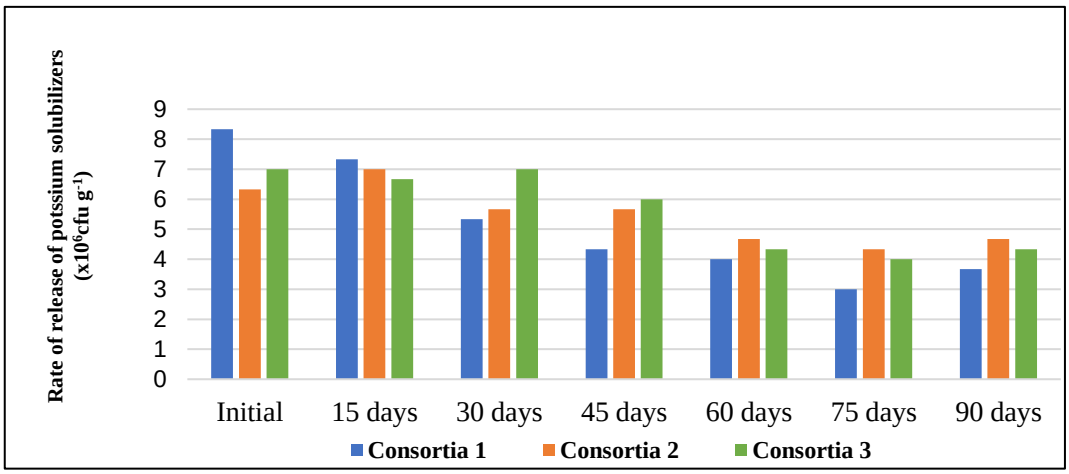


Fig. 1. Rate of release of bacteria from alginate beads

Consortia 1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

Consortia 2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

Consortia 3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

Alginate gel network supported the bacterial densities in stable numbers even after 5 months of storage (Galiana *et al*., 1994). Similar observations on the survival of bacteria even after 14 years in alginate beads were made (Bashan and Gonzalez, 1999 and Cassidy *et al*., 1997). The porous alginate gel matrix protects the cells against mechanical stress, facilitates the survival for prolonged storage period as well helps in cell release from the bead. Young *et al*. (2006) reported high viability of the encapsulated bacteria *Bacillus subtilis* in alginate beads enriched with humic acid with minimum cell loss upon storage for 5 months. Steady and constant cell release from the bead was observed for 1 week at different pH. In the present study, the initial population of phosphorus solubilizers in consortia 1 (13.67 x 10^6 cfu g^{-1}), consortia 2 (12.33 x 10^6 cfu g^{-1}) and consortia 3 (11.33 x 10^6 cfu g^{-1}) were statistically on par with each other. The population decreased gradually and at 90^{th} day, the population of consortia 1 (7.67 x 10^6 cfu g^{-1}) was on par with the population in consortia 3 (9.00 x 10^6 cfu g^{-1}). Lower population of bacteria was recorded in consortia 2 (5.33 x 10^6 cfu g^{-1}) at 90^{th} day.

Bashan and Gonzalez (1999) conducted a study with two plant growth promoting bacteria, *Azospirillum brasilense* and *Pseudomonas fluorescens*, immobilized in two types of alginate bead inoculant (with and without skim-milk supplement) and later dried and stored at ambient temperature for 14 years. The population in each type of bead had decreased, yet significant number survived (10^5 ± 10^6 cfu g^{-1} beads). The number of *A. brasilense* decreased to 10% of the original population after storage in beads for 14 years. In the present study, the initial population of potassium solubilizers in consortia 1 (8.33 x 10^6 cfu g^{-1}) was higher which was statistically on par with consortia 3 (7.00 x 10^6 cfu g^{-1}). The population of bacteria in consortia 1 (3.67 x 10^6 cfu g^{-1}), consortia 2 (4.67 x 10^6 cfu g^{-1}) and in consortia 3 (4.30 x 10^6 cfu g^{-1}) decreased at 90^{th} day. The low population in the present studies might be due to the absence of additional supplements in the alginate beads solution.

After *in vitro* screening of beneficial organisms and compatibility studies, the efficiency of alginate beads formulation was evaluated under both sterile and unsterile soil. Tomato was used as the test crop with the KAU variety "Anagha". Tomato is a heavy feeder and exhaustive crop which requires large quantities of inorganic and organic nutrient inputs for its growth and development. Anagha is a high yielding tomato variety with inbuilt resistance to bacterial wilt, released by Kerala Agricultural University. In the present study, the initial three treatments included the three most efficient and compatible isolates consisting of nitrogen fixer, phosphorus solubilizer and potassium solubilizer which were encapsulated in alginate beads, fourth treatment comprised of the combination of T_1, T_2 and T_3 respectively. Similarly, the treatments T_6, T_7 and T_8 consisted of talc based consortiumof efficient and compatible isolates of nitrogen fixer, phosphorus solubilizer and potash solubilizers. Treatments consisting of Package of Practices Recommendations (KAU) 2016 and Organic Package of Practices Recommendations (2017) were used for comparison with the microbial inoculants along with control. In the present study, inoculation was done at the time of transplantation at the rate of one gram per plant.

In the *in-planta* experiment, biometric characters and yield parameters of tomato grown under sterile and unsterile soil were recorded at the harvesting stage. In sterile soil, higher plant height was recorded in T_1 (alginate based consortium1) and T_2 (alginate based consortium2) with 129.17 cm and 126.07 cm respectively. It was followed by T_3 (alginate based consortium3) and T_4 (T_1 + T_2+ T_3) with 119.37cm and 119.63 cm which were statistically on par with each other (Table 32). Bashan *et al*. (2002) reported that *Azospirillum brasilense* encapsulated in the alginate microbeads in both wet and dry formulations, significantly increased plant height in wheat which is in agreement with the present study. In sterile soil, the number of days taken for flowering ranged from 52 to 67 days (Table 33). Minimum number of days for first blooming was recorded in T_1 (alginate based consortium1) and T_3 (alginate based consortium3) with 53.00 days and 55.67 days respectively. Earlier reports indicated that PGPR can induce early blooming in tomato (Brown *et*

al., 1968; Raj *et al*., 2005). In sterile soil, the population of nitrogen fixers were higher for all the treatments with bacterial consortia. The population of P-solubilizers were higher in alginate based consortia-1 while the population of K-solubilizers were higher in treatments with combined application of alginate based consortium (Fig. 2, Fig. 3, Fig. 4). Under unsterile soil conditions, the population of nitrogen fixers were found to be higher in treatments with both alginate and talc based consortia. Similarly, the population of potassium solubilizers were higher in treatment with combined application of alginate based consortia. Treatments with alginate based consortium showed a higher population of phosphorus solubilizers compared with talc based consortium under unsterile soil. However, the population of N fixers, P and K solubilizers decreased with time in all the treatments (Fig.5, Fig. 6 and Fig. 7).

Chauhan *et al*. (2014) reported that the inoculation of tomato seedlings with *Bacillus subtilis* resulted in maximum per cent increase in shoot length (24.93%), shoot dry weight (74.76%) and root length (107.4%) when compared with the control which is in agreement with the present study where a high dry weight was recorded in T_1 (44.60 g plant^{-1}). Walpola and Yoon (2013) reported that the plant height, root length, and dry weight of shoot and root was higher in tomato plants inoculated with *Pantoea agglomerans* or *Burkholderia anthina* or coinoculated with both strains compared to non-inoculated tomato plants. It is in agreement with the present study, where alginate based consortiumconsisting of *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus* resulted in greater plant height (129.17 cm) and dry weight (44.60 g). Galiana *et al*. (1994) reported that the height of *Acacia mangium* inoculated with alginate beads containing selected *Bradyrhizobium* strains showed a statistically significant increase of 9 to 26 % compared with uninoculated trees which is in agreement with the present study where higher plant height (129.17 cm) was recorded in T_1.

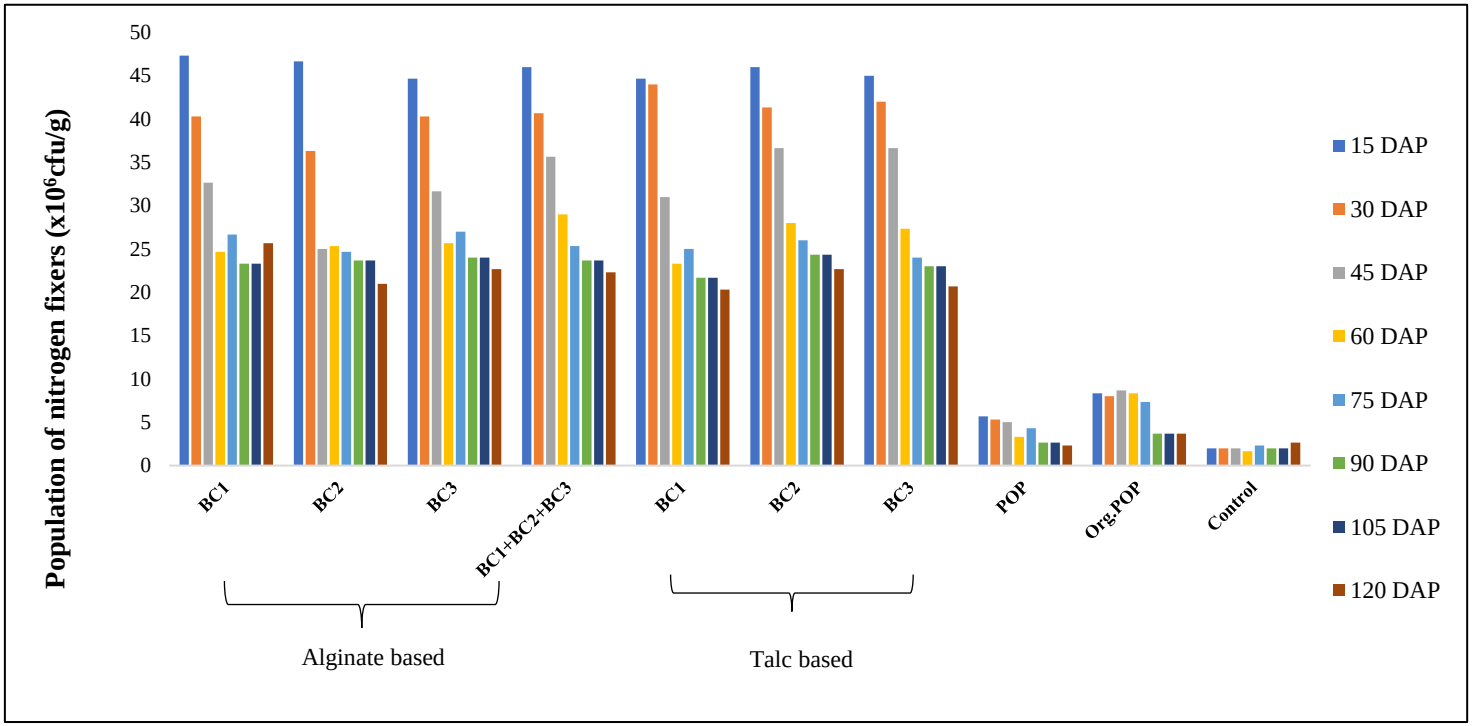


Fig. 2. Population of nitrogen fixers in sterile potting mixture at fortnightly intervals

BC1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

BC2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

BC3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

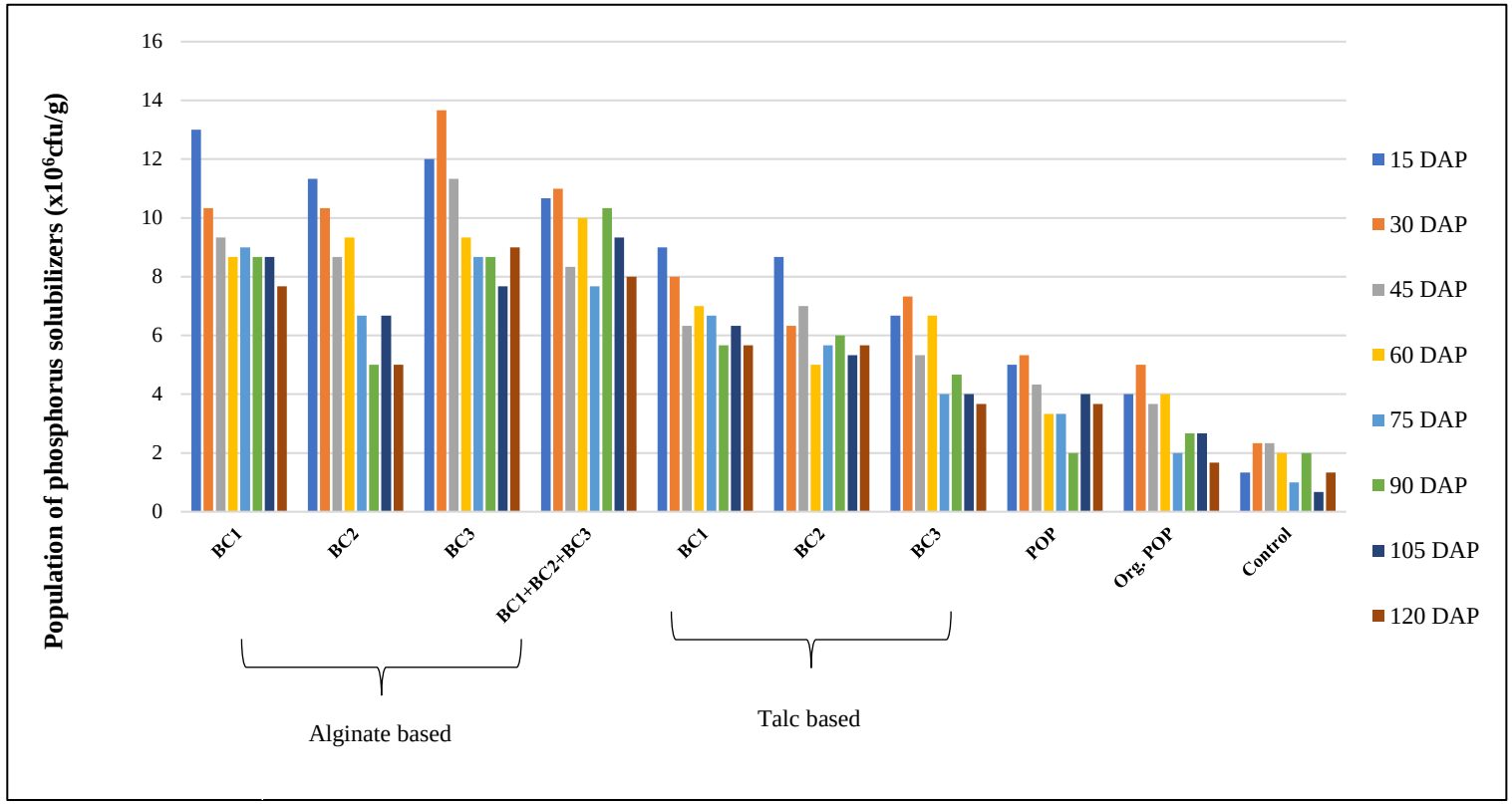


Fig. 3. Population of phosphorus solubilizers in sterile potting mixture at fortnightly intervals

BC1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

BC2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

BC3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

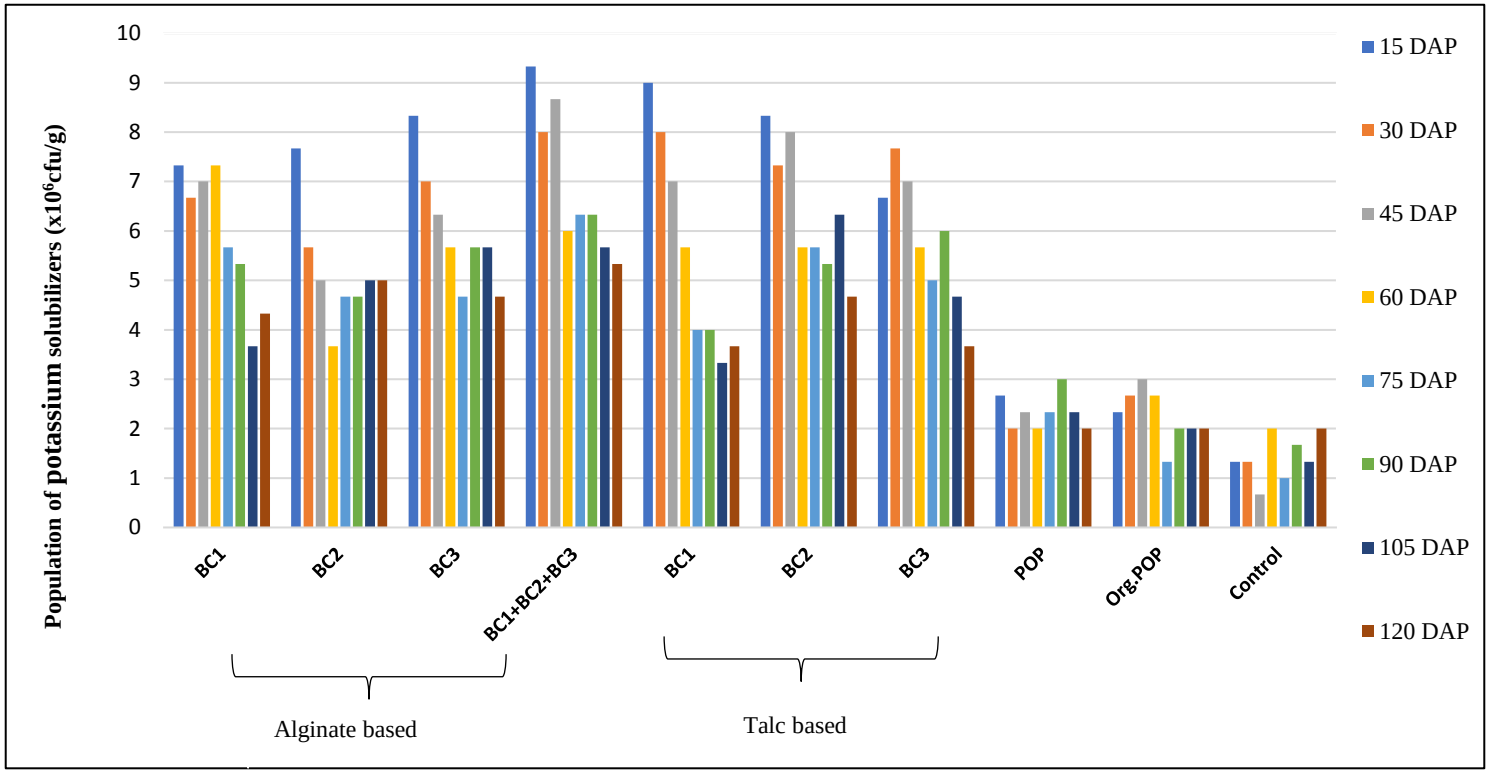


Fig. 4. Population of potassium solubilizers in sterile potting mixture at fortnightly intervals

BC1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

BC2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

BC3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

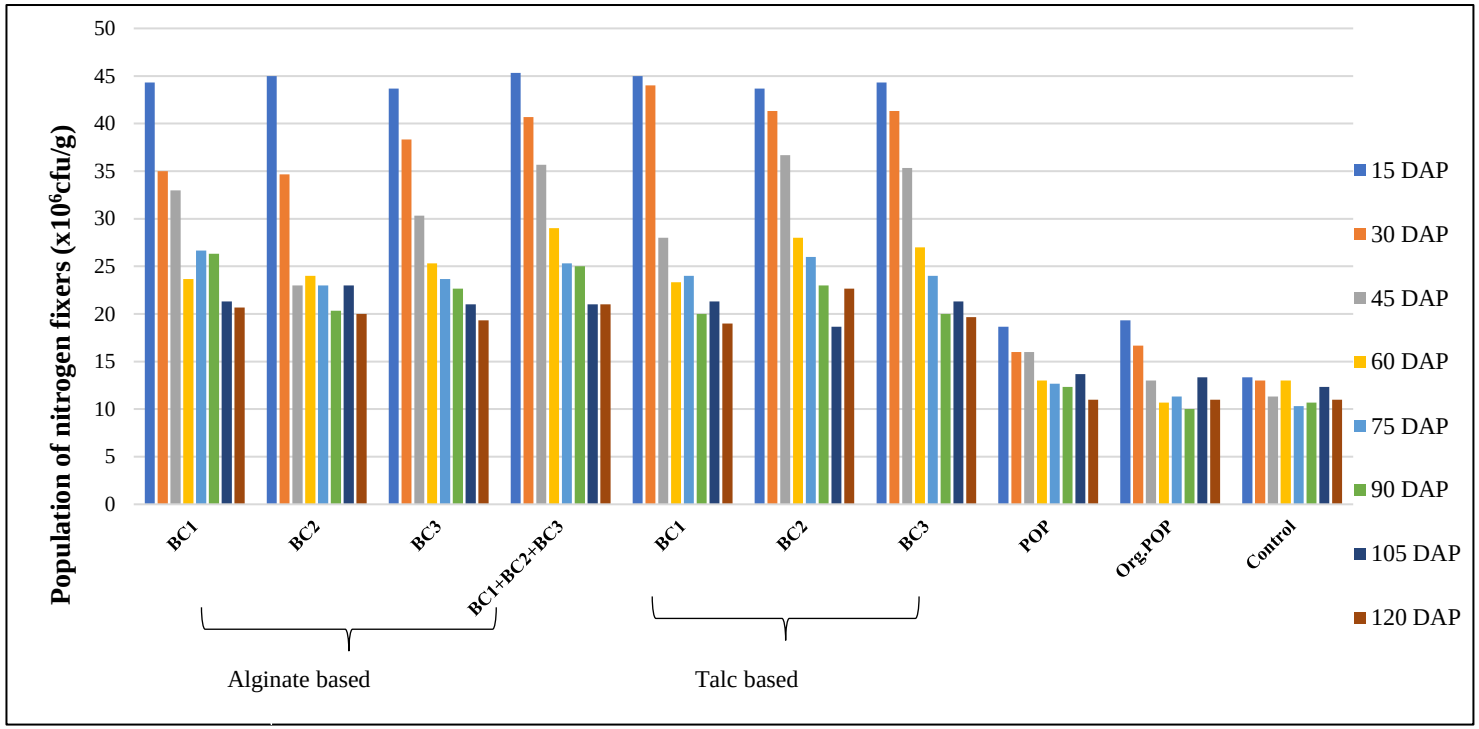


Fig. 5. Population of nitrogen fixers in unsterile potting mixture at fortnightly intervals

BC1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

BC2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

BC3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

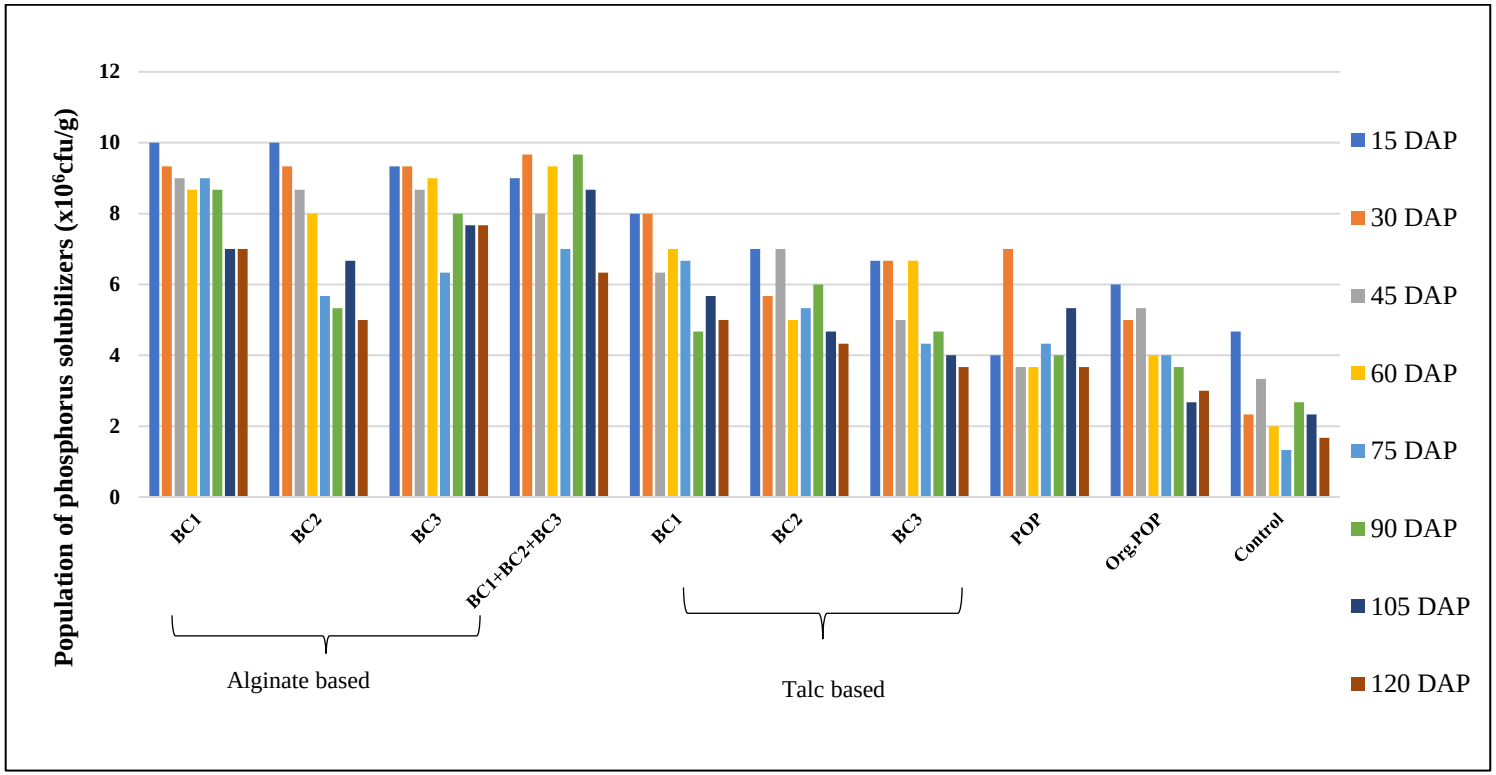


Fig. 6. Population of phosphorus solubilizers in unsterile potting mixture at fortnightly intervals

BC1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

BC2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

BC3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

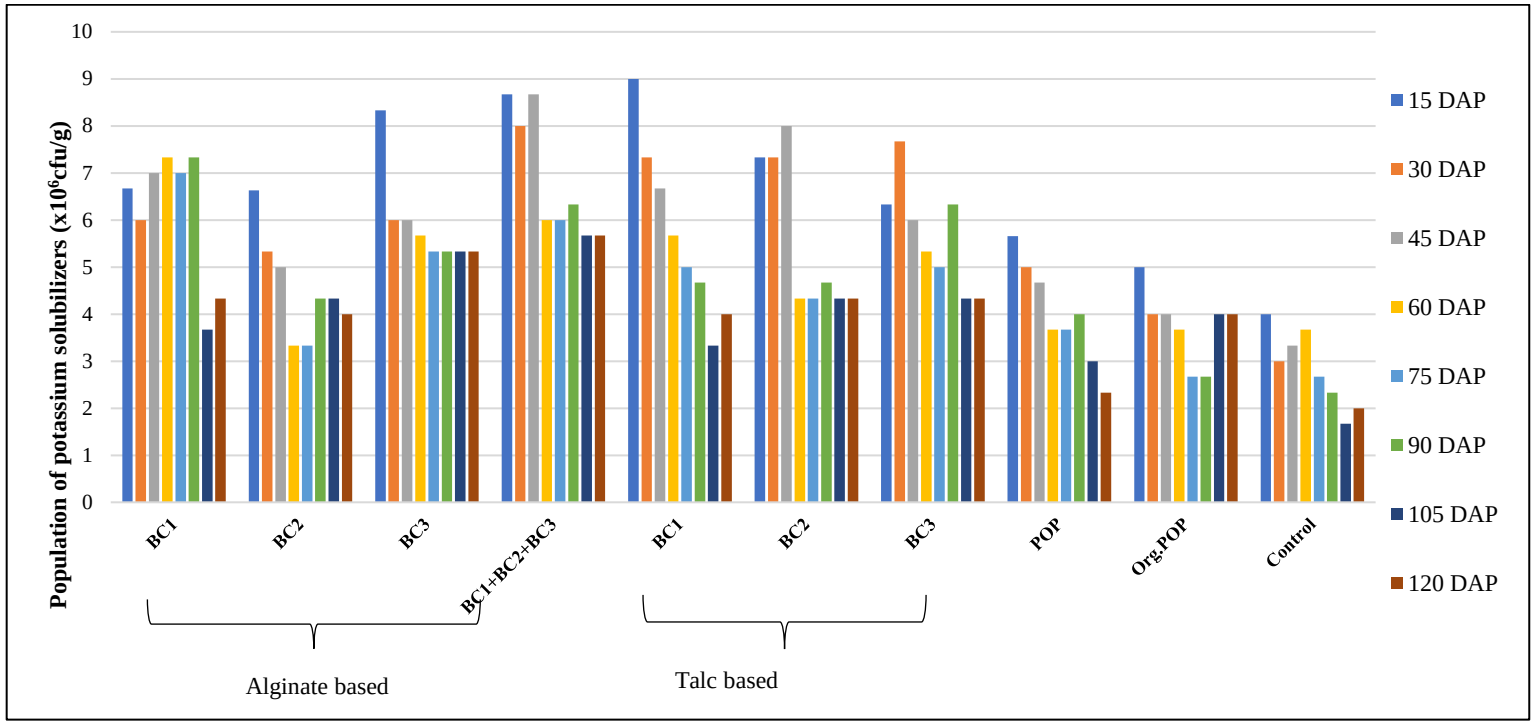


Fig. 7. Population of potassium solubilizers in unsterile potting mixture at fortnightly intervals

BC1: *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*

BC2: *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus*

BC3: *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp.

1In the present study, T_1 (alginate based consortium1), T_3 (alginate based consortium3), T_5 (talc based consortium1) and T_8 (Package of Practices Recommendations, KAU, 2016) recorded a higher yield of 897.0g, 781.33g, 743.33 g and 734.33 g respectively for the tomato grown under sterile potting mixture. (Table 35). Andrade *et al*. (2014) showed that the strawberry crop responded positively to inoculation with the consortia of *Azospirillum brasilense* (Ab-V5) + *Burkholderia cepacia* (CCMA 0056) + *Enterobacter cloacae* (CCMA 1285) compared to the uninoculated control. This indicated that a consortium consisting of nitrogen fixer, phosphorus solubilizer and potassium solubilizer could effectively increase the growth and development of plants which is in agreement with the present study.

Hernandez-Montiel *et al*. (2017) reported the use of plant growth promoting rhizobacteria (PGPR) as biofertilizers by means of microcapsules could be an alternative in agricultural management and sustainable production of tomato. Immobilization of *Pseudomonas putida* rhizobacteria by alginate microcapsules confers protection and gradual release, improving adhesion, permanency, and colonization of cells on the roots, promoting a better effect as PGPR and productivity in tomato plants which is in agreement with the present study where a higher yield was recorded in T_1 (897.0 g plant^{-1}). Miao *et al*. (2015) reported that *Burkholderia* sp. enhanced the tomato yield and significantly promoted activities of soil urease, phosphatase, sucrase, and catalase. All these results demonstrated *Burkholderia* sp. as a valuable PGPR and a candidate of biofertilizer which is in agreement with the present study where *Burkholderia cepacia* was a promising phosphorus solubilizer. Members of *Burkholderia* were known for their bio-control ability, bioremediation and plant growth promotion (Kang *et al.* 2010).

Singh *et al*. (2013) reported that inoculation with IAA overproducing strains of *Burkholderia cepacia* can change the level of IAA at the site of rice root interiors, and possibly in different other parts of plants which helps in root proliferation, and as a result of that, nutrient harnessing may be enhanced to a greater extent for the growth of host plants. Kloepper *et al*. (1989) reported that *Bacillus* sp. has been found to be effective in increasing the yield of wheat up to 43% and also for other crops which is in agreement with the present study, where consortia consisting of *Bacillus subtilis* gave a higher yield (606.0 g plant^{-1}).

Ozaktan *et al*. (2016) stated that the highest rate in the germination of tomato seedlings was observed in seeds treated with *Pantoea agglomerans* followed by *Ochrobactrum pseudintermedium*, *Pseudomonas putida* and *Pseudomonas fluorescens*. The result suggest that use of rhizosphere associated microorganisms as biofertilizers derived from indigenous plant growth promoting rhizobacteria (PGPR) can be employed for the organic production of tomato in near future. In sterile soil, the population of nitrogen fixers were higher for all the treatments receiving bacterial consortia. The population of P-solubilizers were higher in T_1 (alginate based consortium-1) while the population of K-solubilizers were higher in treatments with combined application of alginate based consortia. Under unsterile soil conditions, the population of nitrogen fixers were found to be higher in treatments with both alginate and talc based consortium. Similarly, the population of potassium solubilizers were higher in treatment with combined application of alginate based consortium. Treatments with alginate based consortium showed a higher population of phosphorus solubilizers compared with talc based consortium under unsterile soil. However, the population of N fixers, P and K solubilizers decreased with time in all the treatments.

Compared to initial soil status, application of alginate based bacterial consortia-1 resulted in a significant increase in available nutrient content among treatments with bacterial consortia under sterile soil conditions. Yield and yield attributes were higher in the alginate based bacterial consortia-1(897.00 g/plant) under sterile condition (Table 35). However, yield was higher in treatment based on POP in the case of unsterile conditions (845.00 g/plant) (Table 36).

The efficient isolates selected for consortia preparation were *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus* ($N_1P_1K_1$), *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus* ($N_2P_2K_2$) and *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp. ($N_3P_3K_3$).

Based on the parameters standardised, beads formed at 3% concentration of sodium alginate solution and calcium chloride, a moisture per cent of 15 % and an ideal temperature of 95 ^oC for 15 min. were found to be the optimum condition for contaminant free alginate beads.The diameter of alginate beads ranged between 2.0 -2.7 mm. From the pot culture study, it is clear that under sterile condition alginate based consortium1 (*Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*) proved to be a promising consortium which enhanced the crop growth and yield of tomato under sterile conditions. However, under unsterile conditions among alginate and talc based consortium, alginate based consortium gave a higher yield. The present studies indicated that the alginate based consortium could be a potential formulation which is less bulky, free from contamination, biodegradable and non-toxic. Encapsulation enables slow and controlled release of cells and thus, maintains a uniform bacterial population. However, further studies are required to study its shelf life and its performance under field condition.

Summary

6. SUMMARY

A study was conducted on "Alginate based consortial formulation of native microbial fertilizers" in Department of Agricultural Microbiology, College of Horticulture, Vellanikkara during 2017-2019. The main objectives of the study were screening of bacterial isolates for growth promotion traits, to study the compatibility among the selected bacterial isolates, standardisation of alginate based formulation, encapsulation of bacterial consortia in alginate and evaluation of alginate based bacterial consortia for growth enhancement under pot culture studies. The important findings of the study are summarized below:

- Five isolates each of nitrogen fixers (*Cellulosimicrobium* sp., *Paenibacillus* sp. *Microbacterium testaceum*, *Nguyenibacter vanlangensis*, *Microbacterium arborescence*), phosphorus solubilizers (*Pseudomonas putida*, *Bacillus subtilis* strain (KASB5), *Burkholderia vietnamiensis, Bacillus subtilis* strain (H4) and *Burkholderia cepacia*) and potassium solubilizers (*Acinetobacter calcoaceticus*, *Pseudochrobactrum* sp., *Burkholderia* sp., *Stenotrophomonas maltophila* and *Brevibacterium* sp.) were screened for nitrogen fixation, phosphorus and potassium solubilization and indole acetic acid production under *in vitro*.
- Amount of nitrogen fixed (22.63 mg of N g^{-1} sucrose utilized) and indole acetic acid produced (6.0 µg ml^{-1}) were highest in the case of *Microbacterium arborescence.* Similarly, *Burkholderia cepacia* recorded the highest amount of phosphorus solubilization (64.83 µg ml^{-1}) and indole acetic acid production (8.67 µg ml^{-1}). Among the potassium solubilizers, *Acinetobacter calcoaceticus* solubilized the highest amount of potassium (41.63 µg ml^{-1}).
- *Microbacterium arborescence*, *Microbacterium testaceum* and *Nguyenibacter vanlangensis* were selected as the three most promising nitrogen fixers based on *in vitro* screening tests. Similarly, the phosphorus

solubilizers selected were *Burkholderia cepacia*, *Bacillus subtilis* (KASB5) and *Bacillus subtilis* (H4). The potassium solubilizers selected for further studies were *Acinetobacter calcoaceticus*, *Burkholderia* sp. and *Brevibacterium* sp.

- Compatibility studies conducted among the efficient bacterial isolates using showed no inhibition at the intersection of two bacterial isolates and it was further confirmed by dual culture method which showed compatibility among all the isolates.

- The efficient isolates selected for consortia preparation were *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus* ($N_1P_1K_1$), *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus* ($N_2P_2K_2$) and *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp. ($N_3P_3K_3$).

- In order to develop sterile and uniform sized alginate beads, a protocol was standardized for the preparation of alginate beads. The optimum concentration of sodium alginate solution and temperature required for alginate beads formation were 3 per cent and 95 ^oC for 15 minutes respectively. The diameter of alginate beads ranged between 2.0 – 2.7 mm.

- The rate of release of nitrogen fixers from alginate based consortia-1 during the initial 24 hours was high (41.67 x 10^6 cfu g^{-1} beads) which reached to a population of 21 x 10^6 cfu g^{-1} of beads at 90^{th} day. Population of nitrogen fixers, phosphorus solubilizers and potassium solubilizers showed a decreasing trend up to the 90^{th} day when compared to initial population of bacteria released from the alginate beads.

- Under unsterile soil conditions, the population of nitrogen fixers were found to be higher in treatments with both alginate and talc based consortia. Similarly, the population of potassium solubilizers were higher in treatment with combined application of alginate based consortia.

- Treatments with alginate based consortia showed a higher population of phosphorus solubilizers compared with talc based consortia under unsterile soil. However, the population of N fixers, P and K solubilizers decreased with time in all the treatments.

- Compared to initial soil status, application of alginate based bacterial consortia-1 resulted in a significant increase in available nutrient content among treatments with bacterial consortia under sterile soil conditions. From the pot culture study, it is clear that under sterile condition alginate based consortia 1 (*Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus*) proved to be a promising consortia which enhanced the crop growth and yield of tomato under sterile conditions

- Yield and yield attributes were higher in the alginate based bacterial consortium-1 (897.0 g/plant) under sterile condition. However, yield was higher in treatment based on POP in the case of unsterile conditions (845.00 g/plant). Under unsterile conditions among alginate and talc based consortium, alginate based consortium gave a higher yield. The present studies indicated that the alginate based consortium could be a potential formulation which is less bulky and free from contamination. Encapsulation enables slow and controlled release of cells and thus, maintains a uniform bacterial population. However, further studies are required to study its shelf life and its performance under field condition.

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Appendices

APPENDIX I

MEDIA USED AND COMPOSITION

a) Jensen's agar

Sucrose	: 20.00 g
Dipotassium phosphate	: 1.00 g
Magnesium suphate	: 0.50 g
Sodium chloride	: 0.50 g
Ferrous sulphate	: 0.10 g
Sodium molybdate	: 0.005 g
Calcium carbonate	: 2.00 g
Agar	: 20.00 g
Distilled water	: 1000 ml

b) Nutrient agar

Beef extract	: 3.00 g
Peptone	: 5.00 g
Sodium chloride	: 5.00 g
Agar	: 29.00 g
Distilled water	: 1000 ml
pH	: 6.8-7.2

c) Pikovskaya's agar

Glucose	: 10.00 g
Tri calcium phosphate	: 5.00 g
Ammonium sulphate	: 0.50 g
Sodium chloride	: 0.20 g
Magnesium sulphate	: 0.10 g
Potassium chloride	: 0.20 g

Yeast extract : 0.50 g
Magnesium sulphate : 0.002 g
Ferrous sulphate : 0.002 g
Distilled water : 1000 ml
pH : 7.00

d) Aleksandrov's agar

Glucose : 5.00 g
Magnesium sulphate : 0.50 g
Ferric chloride : 0.005 g
Calcium carbonate : 0.10 g
Calcium phosphate : 2.00 g
Potassium aluminosilicate : 2.00 g
Distilled water : 1000 ml
pH : 7.2

APPENDIX II

REAGENTS USED

a) Ammonium molybdate reagent

12 g of Ammonium molybdate is dissolved in 250 ml of distilled water. 0.291 g of antimony potassium tartarate is dissolved in 100 ml of distilled water. Both these solutions are added to 1000 ml of approx. 5 N H_2SO_4. This solution is mixed thoroughly and made up to 2L with distilled water.

b) Boric acid indicator mixture

0.2 % Bromocresol green + 0.2 % Methyl Red in alcohol in 5:1 ratio

c) Salkowski reagent

2 % of 0.5 M $FeCl_3$ in 35 % Perchloric acid

ALGINATE BASED CONSORTIAL FORMULATION OF NATIVE MICROBIAL FERTILIZERS

by

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ABSTRACT OF THE THESIS

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Abstract

At present, the available biofertilizers are bulky and have short shelf life due to contamination problem. Hence, a suitable formulation needs to be developed which is less bulky and has increased shelf life. Alginate is one of the most commonly used polymers for microbial encapsulation. It is commercially extracted from seaweeds like *Macrocystis pyrifera, Ascophyllum nodosum, Laminaria etc*. The present study was undertaken in the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara to develop an alginate based formulation of nitrogen, phosphorus, and potash biofertilizers consortia and evaluate for growth enhancement using tomato as the test crop. Five isolates each of nitrogen fixers, phosphorus and potassium solubilizers from Wayanad district were obtained from the repository maintained in the Department of Agricultural Microbiology, COH, Vellanikkara. The bacterial isolates were screened for nitrogen fixation, phosphorus and potash solubilization along with indole acetic acid production. Amount of nitrogen fixed (22.63 mg of N g^{-1} sucrose utilized) and indole acetic acid production (6 μg ml^{-1}) were highest for *Microbacterium arborescence.* Similarly, *Burkholderia cepacia* recorded the highest amount of phosphorus solubilization (64.83 μg ml^{-1}) and indole acetic acid production (8.67 μg ml^{-1}). Among the potassium solubilizers, *Acinetobacter calcoaceticus* solubilized the highest amount of potassium (41.63 μg ml^{-1}) under *in vitro* conditions.

Microbacterium arborescence, Microbacterium testaceum and *Nguyenibacter vanlangensis* were selected as the three most promising nitrogen fixers. Similarly, the phosphorus solubilizers selected were *Burkholderia cepacia, Bacillus subtilis* (KASB5) and *Bacillus subtilis* (H4). The potassium solubilizers selected were *Acinetobacter calcoaceticus, Burkholderia* sp. and *Brevibacterium* sp. Compatibility studies were conducted among the selected bacterial isolates using cross streak method which showed no inhibition at the intersection of two bacterial isolates. The compatible isolate was further confirmed by dual culture method. The efficient isolates selected for consortia preparation were *Microbacterium arborescence* + *Burkholderia cepacia* + *Acinetobacter calcoaceticus* ($N_1P_1K_1$), *Microbacterium arborescence* + *Bacillus subtilis* (KASB5) + *Acinetobacter calcoaceticus* ($N_2P_2K_2$) and *Microbacterium testaceum* + *Burkholderia cepacia* + *Burkholderia* sp. ($N_3P_3K_3$).

In order to prepare a sterile and uniform sized alginate beads, a protocol was standardized for temperature, time and concentration of sodium alginate solution with calcium chloride solution. The optimum concentration of sodium alginate solution and temperature required for alginate beads preparation was 3% and 95 ^{o}C for 15 minutes. The diameter of alginate beads ranged between 2 mm – 2.7 mm. The rate of release of nitrogen fixers from alginate based consortia-1 during the initial 24 hours was high (41.67 x 10^6 cfu $^{-1}$ g of beads) which reached to a population of 21 x 10^6 cfu $^{-1}$ g of beads at 90th day. Population of nitrogen fixers, phosphorus solubilizers and potassium solubilizers decreased towards the 90th day when compared with the initial count of bacteria released from the alginate beads.

A pot culture experiment using tomato as a test crop was conducted under sterile and unsterile potting mixture separately to evaluate Alginate based consortia (T_1, T_2 and T_3), combination (T_1 + T_2 + T_3), Talc based consortia (T_5, T_6 and T_7) and POP (T_8), Organic POP (T_9) and Absolute control (T_{10}). In sterile soil, the population of nitrogen fixers were higher for all the treatments receiving bacterial consortia. The population of P-solubilizers were higher in alginate based consortium-1 (13.0 x 10^6 cfu g^{-1}) while the population of K-solubilizers were higher in treatments with combined application of alginate based consortia. Under unsterile soil, the population of nitrogen fixers were found to be higher in treatments with alginate and talc based consortia. The population of potassium solubilizers was higher in treatment with combined application of alginate based consortia. Treatments with alginate based consortium showed a higher population of phosphorus solubilizers compared with talc based consortia in unsterile soil. However, the population of N fixers, P and K solubilizers decreased with time in all treatments. Compared to initial soil status, application of alginate based bacterial consortium-1 resulted in a significant increase in available nutrient content among treatments receiving bacterial consortia in sterile soil conditions. Yield and yield attributes were higher in the alginate based bacterial consortia-1(897.0 g/plant) under sterile condition. However, yield was higher in treatment based on POP under unsterile soil conditions (845.0 g/plant). Among alginate based treatments, alginate based consortium-1 recorded a higher yield (707.33g/plant) under unsterile condition also. The present studies indicated that the alginate based consortium could be a potential microbial inoculant formulation which is less bulky, free from contamination, biodegradable and non-toxic. Encapsulation enables slow and controlled release of cells and thus, maintains a uniform bacterial population. However, further studies are required to study its shelf life, its performance under field condition before commercialization and develop a suitable protocol for large scale production.