Chapter 3
Comparative Study of Different Soil Amendments and Microbes for Integrated Nutrient Management and Growth Promotion of *Jatropha curcas*

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3.1 Introduction

Integrated nutrient management practice that can improve organic matter status and nitrogen availability of soil is important to attain sustainable production. Sustainable agriculture is the management and utilization of the agricultural ecosystem in a way that maintains its biological diversity, productivity, regeneration capacity, vitality and ability to function, so that it can fulfill- today and in the future - a significant ecological, economic and social function at the local, national and global levels and that does not harm other ecosystems (Spiertz 2010). Soil amendments have important role in improving the soil fertility and in prevention of nutrient loses. Microbial communities in the soil or rhizosphere contribute to plant growth by recycling nutrients and making them available (Lynch 1990), increasing root health through competition with root pathogens (Weller et al. 2002) or enhancing nutrient uptake (Smith and Read 1997).

The world's land surface occupies about 13.2×10^9 ha, no more than 7×10^9 ha are potentially arable, and only 1.5×10^9 ha are currently cultivated. Of the cultivated area, about 0.34×10^9 ha (23%) are saline and another 0.56×10^9 ha (37%) are sodic. Actually, the problem of soil alkalinization due to $NaHCO_3$ and Na_2CO_3, may be more severe than the problem of soil salinization caused by the neutral salts, such as NaCl and Na_2SO_4. These soils present a highly inhospitable environment to the plants (Shi and Sheng 2005). Soil pH is one of the most important chemical properties that influence nutrient solubility and, hence, the nutrients availability to plants. Calcium, potassium, magnesium and sodium are alkaline elements, which are lost with increasing acidity whereas phosphorous is more available in acidic soil conditions. Availability of nitrogen (N), as well as P, is lower at lower pH and improves in a quadratic fashion with increasing pH until around 7.0. The increase in N availability is associated mainly with improved activity of N turnover bacteria. The availability of P is associated with neutralizing of A1, Mn, and Fe compounds, which fix this element at lower soil pH (Fragiea and Stone 2006). The

majority of soil microbes thrive in neutral pH (6-7) due to the high availability of most nutrients in this pH range, but there are examples of microbes (especially fungi) that can tolerate pH of 1 to 13.

Development of plant growth promoting consortium (PGPC) could be a feasible strategy for increased activity and better viability of plant growth promoting rhizobacteria (PGPR). When these strains are made into an inoculum consortium, each of the constituent strains of the consortium not only out competes with the others for rhizospheric establishments, but complement functionally for plant growth promotion (Shenoy and Kalagudi 2003). Direct interactions occurring between members of different microbial types often result in the promotion of key processes benefiting plant growth and health. It is obvious that all interactions taking place in the rhizosphere are, at least indirectly, plant-mediated. However plant acts as a 'supporting actor' in the rhizosphere. Three types of interactions have been selected for discussion here because of their relevance to the development of sustainable agro-ecosystems. These are: (i) the co-operation between PGPRs for improving N_2-fixation, phosphate solubilization, IAA production, Siderophore production and other plant growth promoting attributes (ii) microbial antagonism for the biocontrol of plant pathogens and (iii) interactions between rhizosphere microbes and AM fungi to establish a functional mycorhizosphere.

Van Veen et al. (1997) critically reviewed the reasons for poor performance of agricultural bioinocula in natural environments and in the rhizosphere of host plants and suggested that instead of using a single strain, for a single trait, use of multiple microbial consortia for multiple benefits, can also thrive together in unique ecological niches in ideal proportions. On the other hand it has been found that these bacteria would also interact synergistically by providing nutrients, removing some inhibitory products, or stimulating each other through physical or biochemical mechanisms.

Macro-elements such as nitrogen, phosphorus and potassium as well as micro- elements such as iron and manganese are essential nutrients for plant growth and development. However, the widespread and heavy application of agrochemicals for crop production is

known to cause negative impacts on arable soils (Kozdrooj et al. 2004, Tate 1995).Soil microorganisms are important in agriculture as many of them are capable of promoting the circulation of plant nutrients and reducing the need for chemical fertilizers. For instance, plant growth promoting rhizobacteria (PGPR) may be used as biofertilizer to increase N and P uptake by plants and thereby, promote growth and production of crops (Cakmakci et al. 2005). The mechanisms of plant growth stimulation by PGPR are mobilization of nutrients, stimulation of root growth by production of phytohormones and antagonism against soilborne plant pathogens (Hoflich et al. 1994).

Other beneficial microbial partners of the plant are the ubiquitous symbiotic mycorrhizal fungi. Mycorrhizal symbioses are found in almost all ecosystems and can enhance plant growth through a number of processes which include improvement of plant establishment, increased nutrient uptake (particularly P and essential micronutrients such as Zn and Cu, but also N and, depending on soil pH, may enhance the uptake of K, Ca and Mg (Clark and Zeto 2000), protection against biotic and abiotic stresses and improved soil structure (Buscot 2005; Smith and Read 2008). The establishment of mycorrhizal fungi in roots changes key aspects of plant physiology, including mineral nutrient composition in tissues, plant hormonal balance and patterns of carbon allocation. Tawaraya et al. (2006) showed that exudates from fungal hyphae solubilized more P than root exudates alone, suggesting that the mycorrhiza contribute to increased P uptake through solubilization.

Vermicompost (VC) is a sustainable source of macro- and micro-nutrients, that can affect plant growth and manage soil-borne plant pathogens by modifying the physicochemical and microbiological characteristics of the plant growth beneficially. Vermicompost has a special place because of the presence of readily available plant nutrients, growth enhancing substances, and number of beneficial microorganisms like Nitrogen fixing, Phosphate solubilizing and cellulose decomposing organisms (Sultan 1997). Vermicompost has been found to have beneficial effects when used as a total or partial substitute for mineral fertilizer in peat-based artificial greenhouse potting media and as soil amendments in field studies. Likewise, some studies show that vermicomposting

leachates or vermicompost water-extracts, used as substrate amendments or foliar sprays, also promote the growth of tomato plants, sorghum, and strawberries (Lazcano and Dominguez 2011). Positive effects of vermicompost include stimulated seed germination in several plant species such as green gram, tomato, petunia and pine trees. Vermicompost also has a positive effect on vegetative growth, stimulating shoot and root development (Edwards et al. 2004). The effects include alterations in seedling morphology such as increased leaf area and root branching (Lazcano et al. 2009). Vermicompost has also been shown to stimulate plant flowering, increasing the number and biomass of the flowers produced (Atiyeh et al. 2002; Arancon et al. 2008), as well as increasing fruit yield (Atiyeh, et al. 2000; Arancon et al. 2004a, 2004b; Singh et al. 2008). In addition to increasing plant growth and productivity, vermicompost may also increase the nutritional quality of some vegetable crops (Lazcano et al. 2011).

Humic substances (HS) are natural organic polyelectrolytes present in the soil humus and stabilized soil organic matter (Chen et al. 2004b). These molecules have ecological importance, as intervene in the regulation of a large number of chemicals and biological processes that occur in natural ecosystems (Chen et al. 2004b). However, although these functional actions of humic substances directly influenced by their chemical configuration in solution, the relationships between humic substances structure and biological activity are not clear (Garcia-Mina 2007). Thus, the same humic system can present different functional properties depending on its molecular configuration in solution (molecular aggregration, molecular conformation) (Garcia-Mina 2007). Among the different functional actions of humic substance, their ability to plant growth has been well established in diverse plant species and growth conditions (Chen and Aviad, 1990; Chen et al. 2004b). Some author propose that humic substance promote plant growth by improving the soil bioavailability of certain nutrients, principally iron and zinc (Chen et al. 2004 a;b) and other suggest that humic substances can also directly affect plant metabolism (Nardi et al. 2002). Humic acid contains growth promoting substances and indirectly helps in promoting growth and yield of crops by decreasing IAA oxidase activity and promoting metabolic activities consequently accelerates growth and yield of crops. Humic substances have the abilities to both activate the root PM-ATPase activity

and increase nitrate uptake rates in roots (Quaggiotti et al. 2004). On the other hand, shows that nitrate can act as a type of pseudo-hormonal signal, promoting shoot growth under different conditions (Garnica et al. 2009).

Soil enzymes are important in catalysing several important reactions necessary for the life processes of micro-organisms in soils and the stabilisation of soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling (Dick et al. 1994). Phosphatase enzymes are believed to play critical roles in P cycles (Speir and Ross 1978) as they are correlated to P stress and plant growth. When there is a signal indicating P deficiency in the soil, acid and alkaline phosphatase secretion from plant roots is increased to enhance the solubilisation and remobilization of phosphate, and helping the plant to cope with P-stressed conditions.

Maintenance of soil quality is an integral part of agricultural sustainability. Soil organic matter influences a wide range of physical, chemical and biological properties of soil and is considered by some authors as the most important indicator of soil quality (Bolinder et al. 1999). Early changes in total soil organic matter may be small and detectable only by monitoring the active fractions of soil organic matter (SOM) such as the labile carbon fractions and microbial biomass carbon. Microbial activity in the rhizosphere is a major factor that determines the availability of nutrients to plants and has a significant influence on plant health and productivity. Soil enzyme activities and microbial biomass have been shown to be sensitive indicators of changes produced by management practices, crops, fertilizers or environmental conditions (Rolda´n et al. 2005). Soil structure is crucial to the success of sustainable agriculture. On all but the coarsest soils aggregation is essential to maintain soil porosity; it facilitates water infiltration, provides adequate habitat space for soil organisms and an adequate oxygen supply to roots and soil organisms, and helps avoid soil erosion (Dı´az-Zorita et al. 2002). We hypothesize that introduced micro-organisms can have a significant effect on soil properties and quality as they can interact with natural micro-organisms in the rhizosphere (Caravaca et al. 2002).

Jatropha curcas L or Physic nut is a drought resistant large shrub or small tree, belonging to genus Euphorbiaceae, producing oil containing seeds. Different parts of this plant could be used for various purposes, such as energy source, therapeutic uses, fertilizer and animal feed (Gao et al. 2008). Enhanced initial seedling vigor can help in better establishment of the plants especially under adverse conditions (Desai et al. 2007). *Jatropha* produce 30-35% of seed oil on weight basis. *Jatropha* is a multipurpose species with many attributes and considerable potential. Nearly 40% of the land area in India is wasteland. Importance is given on the plantation of *Jatropha* species on sodic lands, for the protection of the environment and fulfilling future energy requirements. Although this plant can grow on wastelands but its growth is limited. Inoculation of beneficial microbes to these lands may improve plant growth by enhancing plant resistance to adverse environmental stresses, e.g. water and nutrient deficiency and heavy metal contamination (Shen 1997).

A balanced application of both organic, inorganic and biofertilizers appear to be an ideal proposition to meet nutrient requirements of crops rather than single application. In view of this, the present investigation was undertaken to assess the comparison among different combination of organic, inorganic and biofertilizer sources of nutrients on the nutrient uptake and residual soil fertility. We hypothesize that inoculation with Mycorrhizal fungus and PGPR alone or in combination, can confer alkaline stress tolerance to vegetative growth parameters of *Jatropha*, mineral nutrient uptake and the acid and alkaline phosphatase activity related to assimilation of P.

3.2 Materials and Methods

3.2.1 Microorganisms

Enterobacter clocae (MSA), *Pseudomonas pseudoalcaligenes* (MSC) and *Bacillus cereus* (MSD) were isolated and characterized according to their plant growth promoting attributes as discussed in chapter 2 and were used in the present study. These three isolates were compatible with each other and selected for the study. A mycorrhizal fungus, *Glomus intraradices* was also used in the present study.

3.2.2 Combination of isolates showing compatibility

- *Enterobacter clocae* (MSA) + *Pseudomonas pseudoalcaligenes* (MSC)
- *Enterobacter clocae* (MSA) + *Bacillus cereus* (MSD)
- *Pseudomonas pseudoalcaligenes* (MSC) + *Bacillus cereus* (MSD)
- *Enterobacter clocae* (MSA) + *Pseudomonas pseudoalcaligenes* (MSC) + *Bacillus cereus* (MSD)

3.2.3 Interaction of microbes under cultural conditions (Growth profile study)

To observe the interaction of different microbes under liquid culture conditions, 100 ml broth in tryptic soybean broth (TSB), Hi media Laboratories, Mumbai, India was used to inoculate the organisms at 1% inoculum level. Growth curve of four isolates was determined by viable cell count method. Growth profile these four isolates in different combinations was determined by inoculating early exponential phase culture in 50 ml of TSB broth. The combination prepared during this study was MSA+MSC, MSA+MSD, MSC+MSD and MSA+MSC+MSD. In mixed culture, equal volume of early exponential phase each culture was mixed aseptically. Samples were withdrawn after every 4 hour. Mean growth rate constant (K) was calculated using the formula: $K = 3.322 (\log Zt - \log Z0)/Dt$; where Z0 and Zt are the initial and final cell populations, while Dt is difference in culture time (Pandey and Maheshwari 2007).

3.2.4 Compatibility test

To study the antagonistic properties of single characterized isolates MSA, MSC and MSD a single bacterial strain was streaked as a straight line in the center of tryptic soya agar plate. Cultures to be tested were streaked perpendicularly across the initial culture and incubated at 28°C for 48 to 96 hours. Lack of microbial growth (zone of inhibition) at the intersections was indicative of the antagonism of the cultures (Raja et al. 2006) but the cultures growing in the close proximity were compatible to each other.

3.2.5 Characterization of consortia for their Plant growth promoting potentials

3.2.5.1 Phosphate solubilization for co-inoculated strains

The co-inoculated strains were also tested in the liquid Pikovskaya's medium for quantitative phosphate solubilization and off change in the pH (Gaur 1990). To check the amount of phosphate solubilized in the liquid medium by microbial consortia, 1 ml of supernatant and add 9 ml of double distilled water was added. Then 10 ml of Chloromolybdic acid was added. Contents in flasks were diluted up to 40 ml by adding double distilled water. Chlorostannous acid (Five drops) reagent was added and mixed well till blue colour develops. Final volume was adjusted up to 50 ml with double distilled water as quickly as possible and OD at 600 nm (Shimadzu UV-1800, Japan) was taken. The total amount of phosphate solubilized and present in 1 ml of supernatant was calculated from the standard curve.

3.2.5.2 Phytase Production for co-inoculated strains

Quantative phytase production was carried by inoculating 24 hrs old consortium in medium containing pea flour as a substrate and incubate at 45°C. Centrifuged at 10,000 rpm for 10 min and assayed for phytase activity. Culture filtrate was assayed for phytase activity by incubating 150 µl with 600 µl of substrate solution for 30 min at 39°C (Shimizu 1992). Substrate solution contains 0.2 % w/v sodium phytase in 0.1 M sodium acetate buffer pH 5.0. The reaction was stopped by adding 750 µl of 5% trichloroacetic acid solution and liberated phosphate was determined by a modification of the method of Fiske and Subbarow (1925).

3.2.5.3 Indole acetic acid production by developed consortia

The production of indole-3-acetic acid (IAA) was determined by following Bric et al. (1991). Each consortium was grown in glycerol-peptone broth with tryptophan (500 mg ml^{-1}) and incubated at 28°C for 3 days. A 5 ml culture was taken from each tube and centrifuged at 10,000 rpm for 15 min. One millilitre of the supernatant fluid was taken to a clean dry tube to which 100 ml of 10 mM orthophosphoric acid and 2 ml of reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) were added. After one hour of incubation at dark, the absorbance of the pink colour was measured spectrophotometrically at 530 nm

(Shimadzu UV-1800, Japan). The IAA concentration in the microbial consortial broth was determined by using a standard curve of pure IAA (Bano and Musarrat 2003).

3.2.5.4 Siderophore production by developed consortia

The siderophore production was determined by performing the chrome azurol S (CAS) assay (Schwyn and Neiland 1987). All the glassware was cleaned with 6 N HCl. The medium was deferrated by extracting with 3% 8-hydroxyquinoline in chloroform. The medium was then autoclaved to remove any residual chloroform. Multispecies consortia were raised in DF minimal medium at 30°C to a density of 10^8 CFU/ml. Cells in late log phase were removed by centrifugation at 3000 rpm, and the filtrate was tested for siderophore in CAS agar medium. A simultaneous change in the growth pattern of the isolates was also carried out. The quantitative estimation was performed according to the method of Chambers et al. (1996). A catecholate type of siderophore was checked by Arnow's method (1937), and for hydroxymate-type siderophores the Gibson and Magrath method (1969) was used.

3.2.5.5 Ammonia production by developed consortia

Each consortium was tested for the production of ammonia in peptone water. Overnight broth cultures (100 µl inoculum with approximately 3 x 10^8 c.f.u. ml^{-1}) were inoculated in 10 ml peptone water and incubated at 30°C for 48–72 h. Nessler's reagent (0.5 ml) was added to each tube. Development of brown to yellow color was recorded as a positive test for ammonia production (Cappucino and Sherman 1992).

3.2.5.6 HCN (Hydrocyanic acid production) production by developed consortia

Production of hydrocyanic acid (HCN) was determined by the modified method of Millar and Higgins (1970). Consortial strains (24 h old) were grown combined and then streaked on TSM supplemented with 4.4 g glycine l^{-1} with simultaneous addition of a filter paper soaked in 0.5% picric acid in 1% Na_2CO_3 in the upper lid of Petri plate. The plates were sealed with parafilm. The control plates were not inoculated. After incubation at 28 ± 1°C changes in colour from yellow to light brown, moderate (brown) or strong (reddish) brown were examined.

3.2.6 Seed bacterization

Jatropha seeds (*Jatropha curcas* SDAU J1 Chhatrapati) collected from Regional research station S.D. Agriculture University, Sardarkrushinagar, Gujarat, were soaked in 0.02% sodium hypochlorite for 2 min. and washed five times with sterilized distilled water. Seeds were coated with 1% carboxymethylcellulose as adhesive. Then seeds were treated with bacterial strain for 30 min. Each consortium was inoculated in 150 ml flask containing 60 ml medium and incubated at 28 ± 1°C for three days. An optical density of 0.5 recorded at λ 535 nm was achieved by dilution to maintain uniform cell density (10^8-10^9 CFU/ml) (Gholami et al. 2009).

Pots were filled with soil and watered as per protocols. Twenty grams of *Glomus intraradices* (19 spores /g soil) were added into the planting hole. Vermicompost was added 50 g per kg of soil.

3.2.7 Seed germination testing during nursery condition

Daily record of seed that had emerged out of the surface of soil was kept. Recording of germination was continuing for 21 to 28 days. At the end of 28 days all the seeds that had not germinated are taken out and ungerminated seeds were counted and they were cut open to find whether they are still viable or not. Under germination parameter: germination percent, germination energy, germination capacity, and seedling vigor were calculated (Abdul-Baki and Anderson 1973).

3.2.8 Biometric observations of *Jatropha* in greenhouse conditions under different treatments

Pot experiments were conducted to test the influence of impact of integrated nutrient management on growth and nutrient uptake by *Jatropha curcas* L. Soil used during the experiment was detailed in Table 1. Pot study was carried out in triplicates of each treatment on *Jatropha*. Study was carried out in a soil developed by using different treatments like:

T1 - Consortia of bacterial culture MSA, MSC and MSD

T2 - Consortia + Vermicompost

T3 - Consortia + Mycorrhiza

T4 - Consortia + Humic Acid

T5 - Mycorrhiza

T6 - Vermicompost

T7 - Humic Acid

T8 – Consortia + Vermicompost + Mycorrhiza + Humic acid

Control - without any treatment

Jatropha plant was harvested after 30, 60 and 90 days of seed sowing. For each observation, three plants were randomly selected from each treatment and the mean of two plants was used as one replication. Number of leaves, number of branches, number of roots, root length, shoot length and fresh weight of root and shoot of each plant were recorded. Dry weights of shoot and root were recorded after drying in an oven for 1 day at 70°C. Chlorophyll content of each treatment was also recorded after 90 days (Tank and Saraf 2008).

3.2.9 Soil sampling and physico-chemical properties

Soil samples were taken immediately after harvest and after clearing the litter layer. The soils samples were taken from the inner two-thirds of each bed, bulked to obtain a composite sample, cleared of any organic debris and transferred for storage in sealed plastic bags. Once in the laboratory, the soils were sieved (<2 mm), and stored at 4°C for not more than one week before analyses. Soils pH, salinity and electrical conductivity (EC) was measured in 1:2 soil: water suspension (Rhoades 1982). Soil organic C (SOC) was determined by the Walkley–Black method (Anderson and Ingram 1996). Available N by alkaline permanganate method (Subbiah and Asija 1956), available P by Olsen's method (Olsen et al. 1954), exchangeable K and sodium was determined by flame photometry by using ammonium acetate (Schollenberger and Simon 1945) and exchangeable Ca and Mg by using ammonium acetate extracts (Hanway and Heidel 1952).

3.2.10 Plant Analysis

Two months after planting, *Jatropha* plants were harvested and stem diameters were measured with calipers. The roots were washed free soil and fresh and dry weights of leaves and roots were recorded. Plant tissues were ground before chemical analysis. Nitrogen was analyzed using the standard Kjeldahl method. The analysis of the rest of the elements was carried out by digesting the samples in a di-acid digestion mixture (HNO_3: $HClO_4$, 9:4). Phosphorus content was estimated as described by Taussky and Shorr, (1953). Potassium and sodium content was determined by flame photometry. Micronutrient cations (Zn, Fe, and Cu) concentrations were measured by atomic absorption spectrophotometer (Bhargava and Raghupathi 1993).

3.2.11 Soil enzyme activity

Alkaline and Acid phosphatase activity was determined using p-nitrophenyl phosphate disodium (PNPP, 25 mM) as substrate. Take 0.2 ml of toluene than add four millilitres of 0.1 M Trishydroxymethyl aminomethane (THAM) buffer (pH 6.5 for acid phosphatase and pH 11.0 for alkaline phosphatase and 1 mL of substrate were added to 1 g of soil and incubated at 37 ^{0}C for 1 hr. After incubation add 1mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were added, and filter the soil suspension. Yellow colour intensity was measured at 420 nm (Tabatabai and Bremner 1969). Controls were made in the same way, although the substrate was added after incubation.

3.2.12 Statistical analysis

Statistical analysis of all tests was carried out using SPSS 15.0 design. Data was analyzed with ANOVA at $P<0.05$ level. All tests were conducted in triplicates.

3.3 Results and Discussion

3.3.1 Growth Profile study

Enterobacter cloacae (MSA), *Pseudomonas pseudoalcaligenes* (MSC) and *Bacillus cereus* (MSD) were grown in monoculture and mixed species consortium. All isolates were fast growing. *K* value of *Enterobacter cloacae* (MSA), *Pseudomonas pseudoalcaligenes* (MSC) and *Bacillus cereus* (MSD) was 0.79 ± 0.02, 0.87 ± 0.04 and

0.71 ± 0.01 h^{-1} respectively, in single-species cultures. When grown as multi-species mixed-culture, *K* value of *Enterobacter cloacae* (MSA) and *Pseudomonas pseudoalcaligenes* (MSC), *Enterobacter cloacae* (MSA) and *Bacillus cereus* (MSD), *Pseudomonas pseudoalcaligenes* (MSC) and *Bacillus cereus* (MSD) and *Enterobacter cloacae* (MSA), *Pseudomonas pseudoalcaligenes* (MSC) and *Bacillus cereus* (MSD) was 1.24 ± 0.06, 1.66 ± 0.08, 1.91 ± 0.09 and 1.52 ± 0.05 h^{-1} respectively (Fig. 3.1). This shows that growth profile was increased in mixed culture consortium as compared to monoculture. Data represent commensalisms between the isolates.

3.3.2 Phosphate solubilization

Maximum solubilization of phosphate was observed on the seventh day of incubation. Maximium solubilization was achieved by *Enterobacter cloacae* (MSA) + *Pseudomonas pseudoalcaligenes* (MSC) + *Bacillus cereus* (MSD). The level of soluble P gradually increased up to 7th day, with a maximum value of 73 µg/ml (Fig. 3.2). In our study we found that in co-inoculated culture, maximum P was solubilized relative to single species culture. In previous study single isolate MSA (35 µg/ml) showed maximum phosphate solubilization after 7 days of incubation. In the multispecies consortium phosphate solubilization was two times higher than single species results suggest that the process of P-solubilization, which is governed by a complex group of mechanisms, is substantially affected by a mixed culture.

All the mixed cultures were found to lower the pH of the growth medium. Decrease in pH indicates the production of acids, which is considered to be responsible for P solubilization (Rashid et al. 2004). The pH of the medium showed a decrease from 7.2 to a maximum of 3.95 after 21 days in case of MSA+MSC+MSD (Table 3.1). However, from the observations it is clear that no correlation could be established between the degree of P-solubilization and final pH of the medium. Many of the PSMs lower the pH of the medium either by H^+ extrusion (Illmer and Schinner 1995) or by secretion of organic acids such as acetic, lactic, malic, succinic, tartaric, gluconic, 2-ketogluconic, oxalic and citric acids.

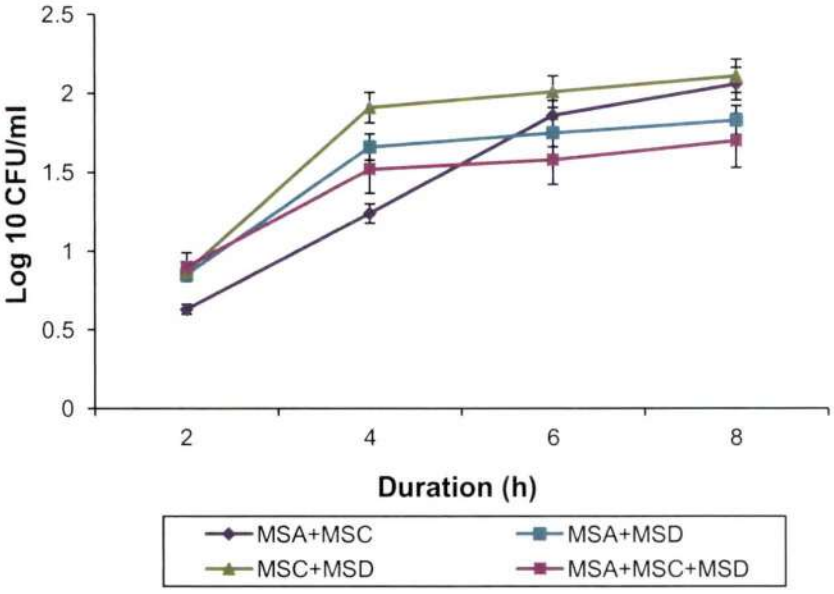


Figure 3.1 Growth profile study of the developed consortia

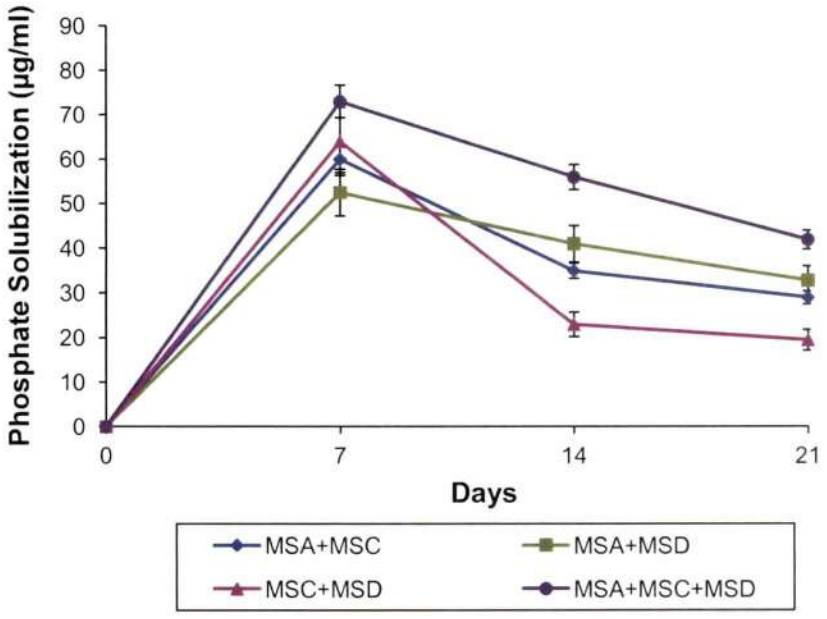


Figure 3.2 Phosphate solubilization by the co-inoculated strains

3.3.3 Phytase Production

Phytase activity was found to be maximum in microbial consortia *Enterobacter cloacae* (MSA) + *Pseudomonas pseudoalcaligenes* (MSC) + *Bacillus cereus* (MSD) i.e. 1.55 unit/ml after 72 h of incubation. Other microbial consortia MSA +MSC, MSA + MSD and MSC + MSD showed considerable amount of phytase activity 1.21 Unit/ml, 1.34 Unit/ml and 1.28 Unit/ml after 72 h of incubation thenafter activity found to be decreased in all the multispecies consortia (Fig. 3.3). Gulati et al. (2007) reported that *Bacillus laevolacticus* is a good source of thermostable alkaline phytases insensitive to the presence of inorganic phosphate in the medium that produces high levels of phytase as compared to other reported wild type strains of *Bacillus* sp. Yadaf and Tarafdar (2007) showed increase in phytase activity with crop age in pearl millet. A significant higher phytase activity was observed after 4 weeks onwards due to inoculation of *Emericella rugulosa*. The phytase activity was increased by 46% after inoculation of *Emericella rugulosa*.

3.3.4 IAA Production

Maximum IAA production was observed in *Enterobacter cloacae* (MSA) + *Pseudomonas pseudoalcaligenes* (MSC) + *Bacillus cereus* (MSD) i.e. 84 µg/ml after 96 hrs of incubation (Fig. 3.4). However, in single inoculation MSC shows maximum IAA production i.e. 45 µg/ml after 96 h. The pattern of production of indole acetic acid by plant growth promoting consortia showed to be increased after 96 hrs and thenafter kept decreasing subsequently. Consortium produced IAA 2.0 times higher compared to maximum amount of IAA produced individually by these strains. In mixed cuture IAA production almost increased by 50% with respect to monospecies cultures. Chatterjee et al. (2011) reported that *Bacillus firmus* KUCr1 isolate produced significantly more IAA than *Cellulosimicrobium cellulans* KUCr3. It produced 14 mg mL^{-1} IAA, whereas KUCr3 produced almost 5 mg mL^{-1} IAA after 5 days of incubation. IAA production was greater (19 mg mL^{-1}) when KUCr1 and KUCr3 were co-cultured and the production is comparable with the co-culture of three isolates, but it was found less in other combinations. Increased phytohormone production when grown in mixed culture has been reported with *Azospirillum* under *in vitro* condition (Janzen et al. 1992).

Isolate	Change in pH			
	0 day	7th day	14th day	21st day
MSA+MSC	7.2	5.25	5.20	4.30
MSA+MSD	7.2	5.67	5.57	4.58
MSC+MSD	7.2	5.13	5.20	5.06
MSA+MSC+MSD	7.2	5.01	5.08	3.95

Table 3.1 Change in pH due to solubilization of tricalcium phosphate up to 21st day after inoculation

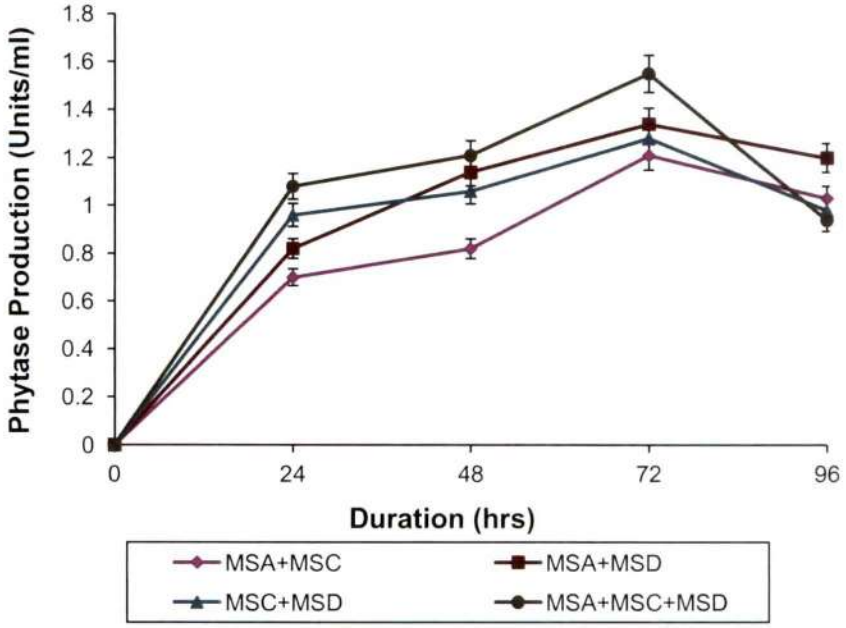


Figure 3.3 Phytase activity by the co-inoculated isolates

3.3.5 Siderophore Production

Significant amount of siderophore production was observed in the *Enterobacter cloacae* (MSA) + *Pseudomonas pseudoalcaligenes* (MSC) + *Bacillus cereus* (MSD) i.e. 63 µg/ml after 24 hrs of incubation (Fig. 3.5). Maximum amount of siderophore production was observed in MSA (23 µg/ml) after 24 hrs but in combination it was found to increase to almost double quantity. MSC produced only 16 µg/ml after 24 hrs was increase by co-inoculation with the MSA and MSD. Production of siderophore results in siderophore mediated competition among the bacteria which further results into exclusion of siderophore non producer pathogens from the rhizosphere due to lack of iron depletion for sclerotia germination and hyphal growth. This was supported by Manwar (2004) observed the ability of *Pseudomonas aeruginosa* to produce the pyoverdine type of siderophores that has good antifungal activity against plant deleterious fungi namely *Aspergillus niger, A. flavus, A. oryzae, F. oxysporum and Sclerotium rolfsi.* Dileep Kumar et al. (2001) reported that although all isolates showed inhibition of phytopathogens, strains RBT 13 showed biocontrol potential even in presence of iron while other isolates lost their biocontrol efficiency. This shows that although siderophore acts as biocontrol agent there can be other mechanisms of biocontrol by PGPR, like HCN, phenazines, chitinase, cellulose, β-1,3 glucanase etc.

3.3.6 Ammonia Production

Maximum amount of ammonia production was observed in microbial consortia MSA + MSC + MSD i.e. 112 µg/ml, 119 µg/ml, 102 µg/ml and 98 µg/ml after 10^{th}, 11^{th}, 12^{th} and 13^{th} of incubation respectively (Fig. 3.6). Other microbial consortia showed considerable amount of ammonia production in MSA +MSC, MSA + MSD and MSC + MSD is 98 µg/ml, 76 µg/ml and 82 µg/ml respectively on 10^{th} day of incubation, then after there is continuous decrease in ammonia production. Joseph et al. (2007) reported ammonia production in 95% of isolates of *Bacillus* followed by *Pseudomonas* (94.2%), *Rhizobium* (74.2%) and *Azotobacter* (45%).

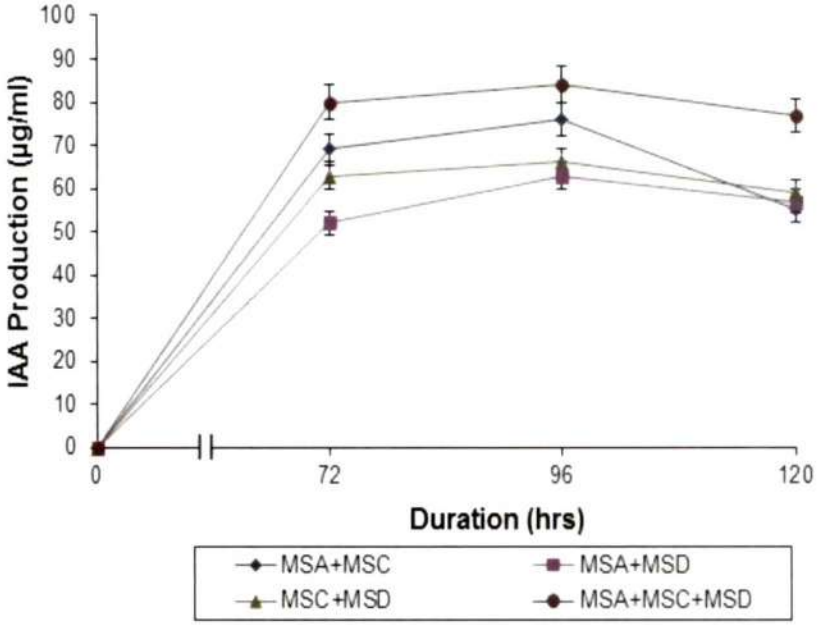


Figure 3.4 Indole acetic acid production by the co-inoculated isolates

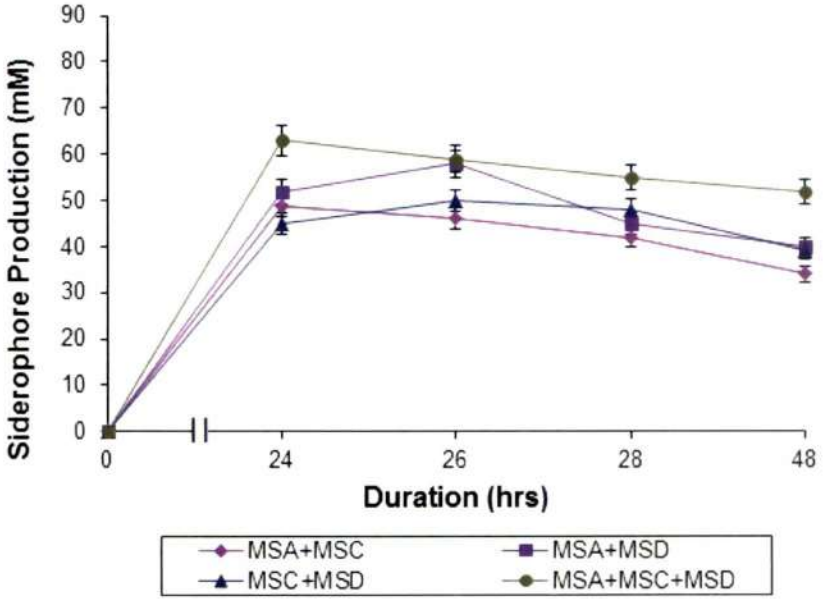


Figure 3.5 Siderophore production by the co-inoculated isolates

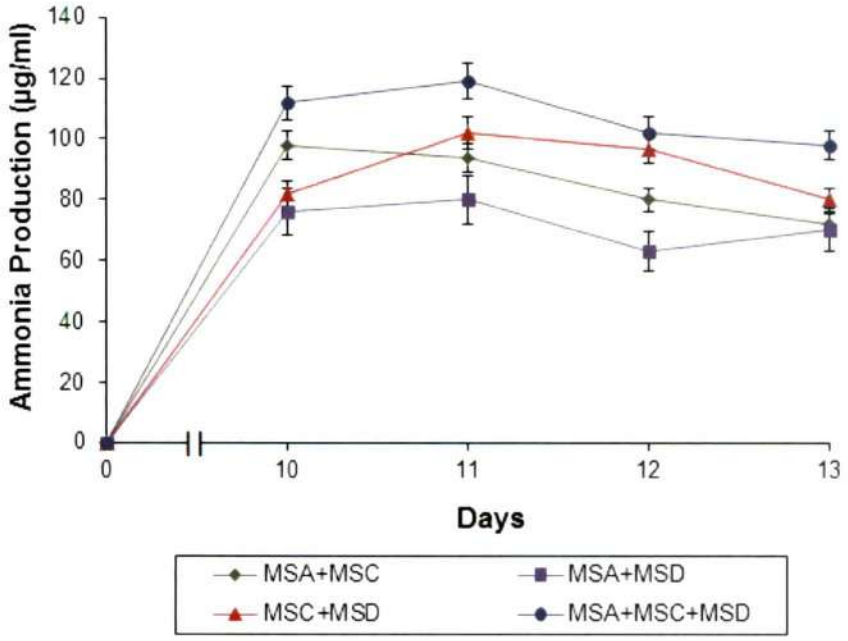


Figure 3.6 Ammonia production by the co-inoculated isolates

Isolate	24 h	48 h	72 h
MSA+MSC	nd	+	+
MSA+MSD	nd	+	+
MSC+MSD	nd	+	+
MSA+MSC+MSD	nd	+	++

Table 3.2 HCN productions by the co-inoculated PGPR (+ low; ++ medium; +++ good; nd not detected)

3.3.7 HCN Production

Maximum HCN production was observed in MSA + MSC + MSD followed by in MSA +MSC, MSA + MSD and MSC + MSD moderate HCN production was reported (Table 3.2). Presence or absence and intensity of HCN production can play a significant role in antagonistic potential of bacteria against phytopathogens. Similar results were also reported by Chandra et al. (2007) reported production of HCN by the PGPR which was inhibitory to the growth of *S. sclerotium*. Kumar et al. (2007) also reported in vitro antagonism by HCN producing PGPR against charcoal rot of chickpea by *M. phaseolina*. Production of HCN along with siderophore production has been reported as the major cause of biocontrol activity for protection of Black pepper and ginger (Diby 2004).

3.3.8 Seed germination testing during nursery conditions

Seed germination is a process where the radicle and plumule of the seed emerge out from the coat when favourable environment is acquainted. Germination parameters was observed to know the extent of completeness of germination, rapidity of germination and peak of germination which reflects the quality of seed lot and seedling produced by different treatments. Daily recording of the seeds that had emerged out of the surface of the soil was carried out up to 28 days. Maximum germination percentage (76.66%) was observed in treatment T1 (consortia) with germination capacity (83.33%) followed by treatment T3 (consortia + mycorrhiza) (73.33%) with the germination capacity (80%). The lowest germination (63.33%) was recorded in control as compared to other treatments. Treatments help in the increased germination from control between the ratios of 5.55% to 27.77%. Similarly the highest germination energy (31.67) was in treatment T1 followed by (29.77) in treatment T3. Minimum germination energy was recorded (25.63) in control in comparison to other treatments (Table 3.3).

The germination capacity of one seed, based on a binary answer (germinated/non germinated), is one qualitative attribute of the germination process, generally converted in a quantitative attribute, commonly percentage. According to Labouriau (1983), germinability of one seed sample is the percentage of seeds in which the germination process reaches the end, in the experimental conditions, by means of the intraseminal

growth that results in the protrusion (or emergence) of one live embryo. In general, it is presented as percentage, accompanied by some dispersion measurement, but it is possible to use proportion rather than percentage, and the proportion values for one or several samples can be submitted to statistical tests.

Vigor index reflects the health of the seedlings produced and so it takes into account the germination percent and radicle length. In the present study the highest seedling vigor index (1149.13) was reported in treatment T1 and minimum (958.18) was in control. The effect of treatment T1 (consortia) showed 19.93% increase in the seedling vigor index against the control (Table 3.3). The developed microbial consortia with the PGPR characteristics like production of IAA and ACC deaminase helped in breaking seed dormancy with mechanical scarification and environmental impacts increased germination. Nainar et al. (1999) has shown that among the seed pre-treatments including mechanical scarification, hot water treatment (with or without removing the testa) and sulfuric acid treatment (with or without breaking the testa), mechanical scarification gave the highest germination percentage (60 %) in *Terminalia chebula*. However according to our results the effect of the multispecies bacterial consortia on *Jatropha curcas* plant was excellent with respect to untreated control and individual traits of PGPR.

3.3.9 Biometric observations of *Jatropha* under greenhouse conditions

The effects of co-inoculation on plants depend on the particular combinations used. The efficiency of PGPR in growth promotion and resistance induction is well documented in various crops (Ramamoorthy et al. 2001). In the present study multispecies consortia of PGPR and mycorrhizal fungus inoculation supported improved growth and nutrient uptake. Inoculation with multispecies consortia increases the number of roots, root length, shoot length, fresh weight of root and shoot, dry weight of root and shoot and chlorophyll content of *Jatropha curcas* as compared to untreated control and other treatments. All the treatments showed increase in number of leaves as compared to untreated plants whereas maximum increase in number of leaves was observed in plants with treatment T1 (100%, 42.85% and 58.33%) after 30, 60 and 90 days of seed sowing.

Treatments	No. of seed shown	No. of seed germinated	Percentage Germination	Germination capacity %	Germination energy	Seedling vigour index
Control	30	18	60	63.33	25.63	958.18
T1	30	23	76.66	83.33	31.67	1149.13
T2	30	21	70	76.66	28.65	1034.6
T3	30	22	73.33	80	29.77	1089.68
T4	30	20	66.66	73.33	28.20	979.32
T5	30	21	70	76.66	25.85	1022
T6	30	19	63.33	70	27.25	980.18
T7	30	19	63.33	70	27.42	956.12
T8	30	22	73.33	80	29.18	1102.2
Total	270	185	68.51	70.74	28.18	-

Table 3.3 Germination parameter study shown by different treatments in comparison with the control. These parameters were calculated after the germination count up to 28th day after the seeds sown in the pot

However, there is no such significant increase in number of branches was observed in plants with all the treatments in comparison to untreated control. Plants treated with treatment T3 also showed increase in number of leaves (50%) after 90 days as compared to all the treatments and untreated control (Table 3.5).

A substantial increase in root length may be responsible for increased nutrient uptake by plants. Enhancement of root length was maximum in treatment T1 (72.81%, 55.63% and 56.09%) after 30, 60 and 90 days as compared to untreated control. Plants treated with treatment T6 (Mycorrhizal fungi) also showed maximum increase in root length (35.92%, 38.73% and 31.09%) after 30, 60 and 90 days of seed sowing compare to untreated control. Increase in number of roots was observed in plants treated with T1 (68%, 73.33% and 53.84%) after 30, 60 and 90 days as compared to untreated control and other treatments. Treatment with T1 also showed increase in shoot length by 52.80%, 43.22% and 48.43% respectively (30, 60 and 90 DAS) compare to untreated control (Table). Increase in shoot length was observed in plants treated with T5 (48.80%, 35.48% and 31.77%) as compared to untreated control after 30, 60 and 90 days. Maximum increase in fresh weight of shoot was observed in plants treated with treatment T6 (Mycorrhiza) (158.37%, 108.15% and 107.61%) after 30, 60 and 90 days, followed by plants with treatment T1, T2 and T5. Increase in fresh weight of root was also observed to be maximum in treatment T1 (80%, 42% and 42.10%) after 30, 60 and 90 days of seed sowing followed by treatment T5 (Mycorrhiza) and T2 (Consortia and vermicompost) as compared to control (Table 3.4, 3.5 and 3.6).

Maximum chlorophyll content (Chl a and Chl b) (Fig. 3.7) was found in Treatment T1 (1.465 mg/g and 0.763 mg/g) at 30 days, (1.635 mg/g and 0.908 mg/g) at 60 days and (1.752 mg/g and 1.021 mg/g) at 90 days, followed by treatment T5 (1.365 mg/g and 0.763 mg/g) at 30 days, (1.552 mg/g and 0.85 mg/g) at 60 days and (1.726 mg/g and 0.993 mg/g) at 90 days compared to untreated control and other treatments(Fig. 3.7).

The study of various vegetative parameters, that is, number of lateral roots, root length, shoot length, fresh weight of root and shoot, dry weight of root and shoot and chlorophyll

content showed that the best growth promotion was observed in plants treated with T1 (Consortia). Treatment with mycorrhizal fungi (T5) was the next most effective treatment in terms of growth promotion. T2, T3 and T6 showed negligible increases in root length and shoot length compared to untreated control and other treatments.

PGPR in conjugation with effective *Rhizobium* strains have been reported to affect growth and nitrogen fixation in pigeonpea by inducing the occupancy of introduced Rhizobium in the nodules of legume (Tilak et al. 2006). Kumar and Chandra (2008) reported combined inoculation treatment of *Rhizobium* sp. +PSB+PGPR produced the highest and significantly more number and dry weight of nodules and dry weight of lentil plant. The next effective growth promoter was mycorrhizal fungus which also showed increase in vegetative growth of *Jatropha*. Treatment with vermicompost showed increase in number of leaves and number of branches compared to untreated control. Similarly, Kannan et al. (2006) reported that application of vermicompost registered the higher plant height and number of branches per plant of tomato and it was significantly superior over supplementation of urea and FYM. Co-inoculation of microbial consortia and mycorrhizal fungus and microbial consortia and vermicompost showed negligible increase in plant growth as compared to uninoculated control.

3.3.10 Soil Physico-chemical properties

Soil analysis after 30, 60 and 90 days of plant growth revealed that there is no significant difference in soil pH in any of the treatment. Electrical conductivity found to be decreased as the Na^{-} content from the soil decreased. Maximum decrease in electrical conductivity was recorded in plants treated with T5 followed by T1 after 90 days of soil analysis. Soil organic carbon content was found to be considerably greater in treatment T2 (34.61%, 40% and 41.18%) compared to control after 30, 60 and 90 days and 92% compared to soil before plantation was carried out i.e. before 90 days of plantation (Table 3.9). Nitrogen content in the soil was observed maximum in treatment T1 and T4. Nitrogen content in the soil was found to be increased by 42.18 kg/hec to 52.95 kg/hec, 56.92 kg/hec and 60.54 kg/hec in treatment T1 respectively after 30, 60 and 90 days of growth of *Jatropha*, i.e. showed increase in nitrogen content (43.53%) as compared to

untreated soil before *Jatropha* plantation. Treatment with T1 (consortia) also showed an increase in nitrogen content (21.27%, 24.09% and 26.78%) after 30, 60, and 90 days as compared to control and other treatments. There are positive and significant relations between the soil organic C and total N. Plants treated with T1, T2, T3, T5 and T6 showed maximum increases in both total organic carbon and total N as compared to untreated control and other treatments. Similarly, Li et al. (2007) reported that the dynamics of N in mineral soil is closely linked to C, because most N exists in organic compounds and heterotrophic microbial biomass, which utilize organic C for energy as a result in soil if organic carbon increases than total N increases.

Phosphorus content in the soil was found to be increased in plant treated with T1 (Consortia) by solubilizing the phosphate. Increase in the phosphate content was found from 16.2 kg/hec to 29.8 kg/hec, 25.8 kg/hec and 34.9 kg/hec after 30, 60 and 90 days of growth (Table 3.7, 3.8 and 3.9). In comparison to untreated control and other treatments plants treated with treatment T1 showed increase in phosphorus content (46.59%, 55.21% and 49.15%) after 30, 60 and 90 days of plantation. Plants treated with T2, T3 and T5 also showed increase in phosphorus content as compared to untreated control. Uptake of potassium from the soil was found to maximum in treatment T2 from 8.8 ppm to 8 ppm, 6.2 ppm and 5.6 ppm after 30, 60 and 90 days compared to soil before any treatment. Similarly, decrease in sodium content was observed in soil treated with treatment T6 and T1 after 30, 60 and 90 days from 39.5 ppm to 11.4 ppm and 12.4 ppm, 10.2 ppm and 11.2 ppm and 9.8 ppm and 10.4 ppm respectively. Other micronutrients like calcium and magnesium were also observed to be decrease in soil treated with treatment T6, T5 and T1.

Kumar and Chandra (2008) reported that combined inoculation of PGPR, PSB and Rhizobium facilitate P supply to plant by solubilizing insoluble P and resulted in better P uptake. It also results in to better N_2 fixation and provides nitrogen to plants and improves number of nodules and plant dry weight. Decrease in soil potassium was observed resulting into uptake of potassium by the plant. Maximum decrease in soil potassium was recorded in plants inoculated with treatment T2 and T4. Similarly, other

micronutrients like calcium and magnesium found to increases in plants treated with treatment T1 and T4. Naidu et al. (2009) reported the uptake of major nutrients N (78.46 kg ha^{-1}), P (16.69 kg ha^{-1}), K (75.20 kg ha^{-1}) and micronutrients Viz., Zn (119.69 g ha^{-1}), Fe (367.18 g ha^{-1}), Cu (48.91 g ha^{-1}), Mn (103.71g ha^{-1}) were noticed with the application of 50 per cent RDN + 50 per cent N through FYM + BF + Panchagavya.

3.3.11 Plant Analysis

The reactivation of microbial activity in the rhizosphere can increase plant nutrient availability, as the soil microbial community mediates the processes of organic matter turnover and nutrient cycling. In the present study, the uptake of nitrogen, phosphorus, potassium and other micronutrients were significantly influenced by the effect of application of microbial consortia and mycorrhizal fungus source of nutrient on *Jatropha*. Uptake of nutrients can be attributed to plant growth promoting consortia and mycorrhizal fungus which mobilize and solubilize nutrients in the soil. The uptake of nitrogen, phosphorus, potassium and micronutrients were significantly influenced by the effect of application of organic and inorganic source of nutrient on *Jatropha*. The highest N uptake was recorded in treatment T1 (0.057%, 0.060% and 0.075%) after 30, 60 and 90 days of plant growth and it was on par with untreated control and with other treatments. Treatment with T2 (consortia + vermicompost) also showed significant uptake of nitrogen. Sahni et al. (2008) reported that the combined effect of vermicompost and seed bacterization is more likely to synchronize nutrient release from the vermicompost and soil with plant nutrient demand. The uptake of macronutrients (N and P) and micronutrients (Zn, Mn, and Fe) was increased in the combined application of vermicompost and seed bacterization, resulting in improvement in plant vigour, which normally restricts the ability of pathogens to proliferate and to invade plants and thus they protected the plants from collar rot. Plants treated with treatment T1 also showed highest uptake of phosphate (0.2 ppm, 0.24 ppm and 0.45 ppm) after 30, 60 and 90 days and it was on par with other treatments. The highest K uptake (2.4 ppm) was recorded in treatment T2 after 30 days compared to other treatments and untreated control. However, highest K uptake was observed in treatment T1 (2.8 ppm and 5.2 ppm) after 60 and 90 days.

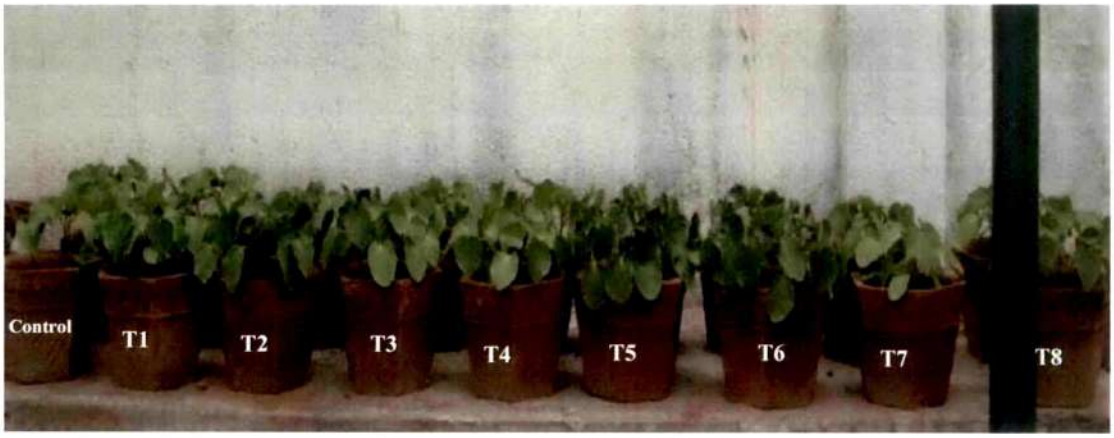
Maximum sodium content was observed in treatment T5 (0.34 ppm, 0.6 ppm and 0.8 ppm) after 30, 60 and 90 days as compared to untreated control. Micronutrient analysis showed highest uptake of iron (10.98 ppm, 11.08 ppm and 11.12 ppm) in plants treated with treatment T8 after 30, 60 and 90 of plant analysis. Similarly, highest uptake of zinc and copper (5.00 ppm, 5.09 ppm and 5.23 ppm) and (0.75 ppm, 0.89 ppm and 0.93 ppm) was observed in plants treated with treatment T3 after 30, 60 and 90 days. Han and Lee (2005) reported an increased uptake of P and K when soil was fertilized with rock P and K and coinoculated with P solubilizing bacteria *B. megaterium* and K solubilizing bacteria *B. mucilaginosus*.

3.3.12 Soil enzyme activity

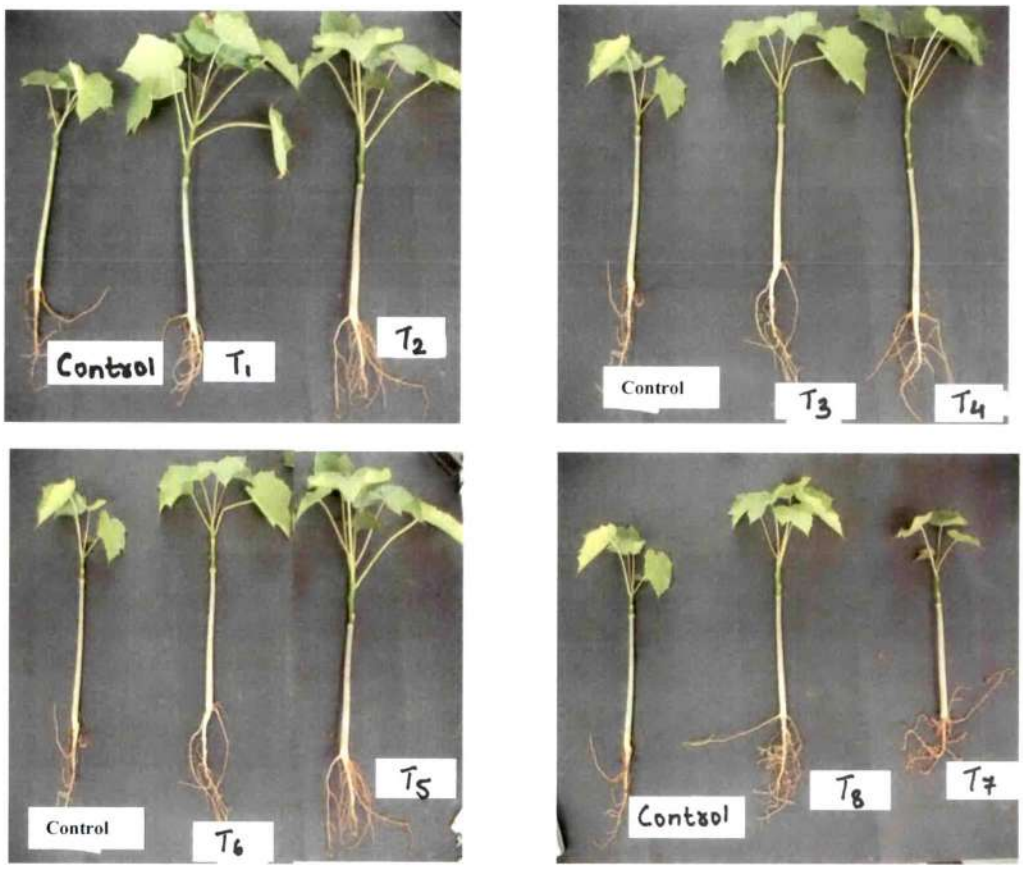
Phosphatases are produced when the available P content reaches critical levels for plant and microorganism growth. Acid and alkaline phosphatase has been studied extensively because of their optimum activities under acid and alkaline conditions and their importance in soil organic P mineralization and plant nutrition. Microorganisms would be the most productive phosphatase sources in soil, due to their high metabolic activity and short lifespan, with several generations a year, allowing the production of high amounts of enzymes (Balota et al. 2010).

The activity of acid phosphatase (ACP) and alkaline phosphatase (ALP) enzymes significantly increased from 30 to 90 DAS (Fig 3.8 and 3.9). The application of vermicompost + consortia, and mycorrhiza + consortia, showed increase in acid and alkaline phosphatase activity as compared to control. Maximum acid phosphatase activity was observed after 90 days of plantation in plants treated with multispecies consortia (160 µg p-nitrophenol kg^{-1} soil) compared to control and other treatment. Increase in acid phosphatase activity observed in all the treatments between the ratios of 2.96% to 18.51% as compared to control after 90 DAS. Treatment T7 (humic acid) does not showed significant increase in acid phosphatase over control after 30, 60 and 90 days of plantation. Chakraborty et al. (2010) reported that application of the *Serratia marcescens* TRS-1 bacterium, soil P content decreased, root and leaf phosphate increased, and soil acid and alkaline phosphatase activities were enhanced.

Treatment with T5 and T1 also showed increase in alkaline phosphatase activity (350 µg p-nitrophenol kg^{-1} soil, 382 µg p-nitrophenol kg^{-1} soil and 420 µg p-nitrophenol kg^{-1} soil) and (346 µg p-nitrophenol kg^{-1} soil, 377 µg p-nitrophenol kg^{-1} soil and 405 µg p-nitrophenol kg^{-1} soil) in comparison to other treatments and untreated control. Application of microbial consortia also showed increase in acid and alkaline phosphatase activity after 30 and 60 DAS. Plant growth promoting consortia showed maximum production of acid and alkaline phosphatase as compared to other treatments and untreated controls. Soil microbial biomass C and enzyme activity were significantly correlated with total N, available P, and available K concentration (Zhang et al. 2004). This revealed that soil biological communities played crucial role in soil fertility formation and nutrient cycling and they could not only provide plant-available nutrients, but also accumulate soil organic carbon.



Picture 3.1 Effect of different treatments on the growth of *Jatropha curcas* after 30 DAS



Picture 3.2 Comparative study of the vegetative structure of *Jatropha* with different treatments

Treatment	No. of leaves	No. of Branches	Root Length (cm)	No. of roots	Fresh weight of root (gm)	Dry weight of root (gm)	Shoot Length (cm)	Fresh weight of shoot (gm)	Dry weight of shoot (gm)
Control	4 ± 0.2^{ns}	2 ± 0.01^{ns}	10.3 ± 1.8^{ns}	25 ± 3.6^{ns}	0.1 ± 0.09^{ns}	0.06 ± 0.008^{ns}	12.5 ± 1.4^{ns}	4.54 ± 0.2^{ns}	$1.12 \pm 1.8^{*}$
T1	8 ± 1.3^{ns}	4 ± 0.05^{ns}	17.8 ± 2.1^{ns}	42 ± 5.2^{ns}	$0.9 \pm 0.2^{*}$	0.49 ± 0.02^{ns}	19.1 ± 2.3^{ns}	7.17 ± 0.9^{ns}	$3.22 \pm 1.6^{**}$
T2	6 ± 0.3^{ns}	3 ± 0.02^{ns}	$13.5 \pm 1.4^{*}$	35 ± 6.8^{ns}	0.76 ± 0.1^{ns}	0.32 ± 0.05^{ns}	16.2 ± 2.1^{ns}	8.87 ± 1.8^{ns}	4.12 ± 2.8^{ns}
T3	5 ± 0.9^{ns}	4 ± 0.02^{ns}	11.4 ± 1.2^{ns}	26 ± 4.7^{ns}	0.68 ± 0.9^{ns}	0.24 ± 0.1^{ns}	$18.2 \pm 3.4^{*}$	$7.70 \pm 1.1^{*}$	3.10 ± 1.4^{ns}
T4	5 ± 0.7^{ns}	3 ± 0.01^{ns}	10.8 ± 1.7^{ns}	27 ± 2.9^{ns}	0.62 ± 1.4^{ns}	$0.19 \pm 0.4^{*}$	15.4 ± 2.5^{ns}	6.91 ± 1.5^{ns}	3.72 ± 2.6^{ns}
T5	6 ± 0.7^{ns}	5 ± 0.08^{ns}	12.5 ± 1.4^{ns}	30 ± 9.5^{ns}	0.73 ± 1.2^{ns}	0.34 ± 0.06^{ns}	$18.6 \pm 3.8^{*}$	8.67 ± 2.1^{ns}	$4.42. \pm 3.4^{*}$
T6	7 ± 0.9^{ns}	5 ± 0.06^{ns}	$14 \pm 2.1^{*}$	32 ± 8.2^{ns}	$0.76 \pm 2.6^{*}$	0.41 ± 0.2^{ns}	14.9 ± 1.9^{ns}	$11.73 \pm 3.2^{**}$	5.09 ± 1.2^{ns}
T7	4 ± 0.4^{ns}	4 ± 0.09^{ns}	12 ± 1.5^{ns}	$39 \pm 2.4^{*}$	0.84 ± 0.3^{ns}	0.46 ± 0.8^{ns}	17.5 ± 2.8^{ns}	8.87 ± 2.4^{ns}	4.10 ± 1.9^{ns}
T8	5 ± 0.6^{ns}	6 ± 0.04^{ns}	15 ± 2.0^{ns}	35 ± 1.8^{ns}	$0.82 \pm 0.4^{*}$	0.39 ± 0.09^{ns}	16.8 ± 1.9^{ns}	6.77 ± 1.2^{ns}	3.52 ± 2.4^{ns}

Table 3.4 Effect of combined application of different treatments on vegetative growth of *Jatropha curcas* after 30days. Result shows means of three replicate ± SE (standard error), significance test by using ANOVA software at 30 DAS, * Significant at 5%; ** Significant at 1%; ns, non-significant as compared to control (ANOVA)

Treatment	No. of leaves	No. of Branches	Root Length (cm)	No. of roots	Fresh weight of root (gm)	Dry weight of root (gm)	Shoot Length (cm)	Fresh weight of shoot (gm)	Dry weight of shoot (gm)
Control	7 ± 0.8^{ns}	4 ± 0.05^{ns}	14.2 ± 2.6^{ns}	45 ± 2.7^{ns}	1.5 ± 1.2^{ns}	0.25 ± 0.06^{ns}	15.5 ± 3.3^{ns}	8.95 ± 2.6^{ns}	2.54 ± 0.9^{ns}
T1	10 ± 1.1^{ns}	5 ± 0.08^{ns}	22.1 ± 2.9^{ns}	78 ± 8.4^{ns}	2.7 ± 1.8^{ns}	0.45 ± 0.1 **	22.2 ± 2.1^{ns}	12.79 ± 4.8^{ns}	5.76 ± 0.2^{ns}
T2	8 ± 0.9^{ns}	4 ± 0.1^{ns}	16.9 ± 1.9^{ns}	60 ± 5.5^{ns}	1.89 ± 2.4^{ns}	0.31 ± 0.08^{ns}	19.5 ± 4.2*	11.80 ± 5.7^{ns}	5.34 ± 1.4^{ns}
T3	8 ± 1.0^{ns}	4 ± 0.09^{ns}	15.3± 1.6*	72 ± 7.6^{ns}	1.97 ± 3.1*	0.35 ± 0.02^{ns}	18.5 ± 1.6^{ns}	9.55 ± 3.5 *	3.63 ± 1.6**
T4	7 ± 1.2^{ns}	4 ± 0.2^{ns}	15.1 ± 1.2^{ns}	52 ± 9.1*	1.79 ± 0.9^{ns}	0.29 ± 0.09^{ns}	17 ± 5.1^{ns}	9.09 ± 2.9^{ns}	3.12 ± 0.8^{ns}
T5	10 ± 1.3*	5 ± 0.02^{ns}	17.9 ± 1.4^{ns}	55 ± 8.2^{ns}	2.54 ± 1.4^{ns}	0.49 ± 0.1*	21 ± 2.2^{ns}	19.80 ± 7.9^{ns}	7.56 ± 0.9^{ns}
T6	9 ± 1.4^{ns}	5 ± 0.06^{ns}	19.7 ± 1.9*	70 ± 4.7^{ns}	1.88 ± 0.6^{ns}	0.36 ± 0.08^{ns}	18.8 ± 3.5^{ns}	18.63 ± 7.5*	6.89 ± 1.2^{ns}
T7	7 ± 0.9^{ns}	4 ± 0.1^{ns}	14.9 ± 1.4^{ns}	75 ± 3.9^{ns}	1.89 ± 0.9^{ns}	0.40 ± 0.06*	17 ± 2.5^{ns}	9.12 ± 5.6^{ns}	3.53 ± 1.2^{ns}
T8	8 ± 1.2^{ns}	4 ± 0.3^{ns}	17 ± 1.5^{ns}	56 ± 4.2^{ns}	1.54 ± 3.1*	0.27 ± 0.02^{ns}	18 ± 1.4^{ns}	9.89 ± 6.2^{ns}	3.72 ± 0.6^{ns}

Table 3.5 Effect of combined application of different treatments on vegetative growth of *Jatropha curcas* after 60days Result shows means of three replicate ± SE (standard error), significance test by using ANOVA software at 60 DAS, * Significant at 5%; ** Significant at 1%; ns, non-significant as compared to control (ANOVA)

Treatment	No. of leaves	No. of Branches	Root Length (cm)	No. of roots	Fresh weight of root (gm)	Dry weight of root (gm)	Shoot Length (cm)	Fresh weight of shoot (gm)	Dry weight of shoot (gm)
Control	12 ± 1.8^{ns}	7 ± 1.8^{ns}	16.4 ± 3.2^{ns}	78 ± 5.6^{ns}	1.9 ± 0.9*	0.46 ± 0.04^{ns}	19.2 ± 2.8^{ns}	10.11 ± 1.9*	4.21 ± 1.2**
T1	19 ± 1.3^{ns}	12 ± 1.6*	25.6 ± 6.4^{ns}	120 ± 12.8^{ns}	2.7 ± 0.6^{ns}	0.63 ± 0.03*	28.5 ± 1.9*	14.95 ± 1.6^{ns}	6.95 ± 1.3^{ns}
T2	17 ± 2.3^{ns}	11± 2.3^{ns}	19.4 ± 4.1^{ns}	110 ± 17.6^{ns}	2.6 ± 0.8^{ns}	0.42 ± 0.04^{ns}	22.6 ± 1.6^{ns}	13.65 ± 1.9**	6.63 ± 1.9^{ns}
T3	18 ± 1.9^{ns}	9 ± 1.2^{ns}	18.2 ± 8.5^{ns}	98 ± 5.4^{ns}	2.42 ± 1.1*	0.57 ± 0.08**	24.1 ± 1.9^{ns}	10.72 ± 1.2*	4.86 ± 1.3*
T4	14 ± 1.2^{ns}	8 ± 1.8^{ns}	17.9 ± 5.6^{ns}	81 ± 5.1*	2.12 ± 0.4^{ns}	0.55 ± 0.06^{ns}	19.7 ± 2.2^{ns}	10.44 ± 1.4^{ns}	4.24 ± 1.2^{ns}
T5	14 ± 1.6^{ns}	10±1.6*	19.6 ± 2.8^{ns}	115 ± 10.2^{ns}	2.62 ± 0.9^{ns}	0.65 ± 0.03^{ns}	25.3 ± 3.6^{ns}	20.71 ± 2.8^{ns}	8.21 ± 1.8^{ns}
T6	15 ± 1.3^{ns}	10 ±1.9*	21.5 ± 3.9^{ns}	112 ± 10.3^{ns}	1.99 ± 0.3^{ns}	0.44 ± 0.1^{ns}	22.2 ± 2.6*	20.99 ± 2.6^{ns}	4.34 ± 1.3*
T7	13 ± 1.6^{ns}	8 ± 1.8^{ns}	17.2 ± 4.7^{ns}	85 ± 5.4^{ns}	2.58 ± 0.8^{ns}	0.68 ± 0.04^{ns}	20.2 ± 2.0*	10.85 ± 2.5^{ns}	4.30 ± 1.6^{ns}
T8	15 ± 1.4^{ns}	11 ± 2.1^{ns}	19.1 ± 5.2^{ns}	95 ± 5.4^{ns}	2.40 ± 1.2^{ns}	0.56 ± 0.06^{ns}	23.4 ± 2.5^{ns}	11.34 ± 3.1^{ns}	5.14 ± 1.8^{ns}

Table 3.6 Effect of combined application of different treatments on vegetative growth of *Jatropha curcas* after 90 days Result shows means of three replicate ± SE (standard error), significance test by using ANOVA software at 90 DAS, * Significant at 5%; ** Significant at 1%; ns, non-significant as compared to control (ANOVA)

(A)

Test	pH	Salinity	Electrical conductivity (ds ms^{-1})	TOC	Nitrogen (Kg ha^{-1})	Phosphorus(Kg ha^{-1})	Potassium (ppm)	Calcium (me/100gm soil)	Magnesium (me/100gm soil)	Sodium (ppm)
	8.5	0.18 %	0.30	0.25%	42.18	16.2	8.8	11.8	16.7	39.5

(B)

Parameters	Control	T1	T2	T3	T4	T5	T6	T7	T8
pH	8.4 ± 0.4*	8.5 ± 0.4*	8.1 ± 0.1*	8.2 ± 0.2*	8.5 ± 0.4*	8.4 ± 0.3*	8.2 ± 0.2*	8.4 ± 0.4*	8.4 ± 0.3*
Salinity (%)	0.15 ± 0.2*	0.14 ± 0.1^{ns}	0.15 ± 0.2*	0.13 ± 0.09^{ns}	0.14 ± 0.1*	0.12 ± 0.08^{ns}	0.13 ± 0.09*	0.15 ± 0.2*	0.14 ± 0.1*
EC (ds/ms)	0.22 ± 1.4^{ns}	0.20 ± 1.1^{ns}	0.22 ± 1.4^{ns}	0.18 ± 0.9*	0.21 ± 1.1^{ns}	0.17 ± 0.8^{ns}	0.18 ± 0.9*	0.22 ± 1.4^{ns}	0.20 ± 1.1^{ns}
Nitrogen (Kg/ha)	43.66 ± 5.9^{ns}	52.95 ± 5.6**	48.32 ± 4.2^{ns}	50.63 ± 4.6^{ns}	49.63 ± 5.4^{ns}	51.78 ± 5.2*	49.91 ± 5.9^{ns}	46.12 ± 4.4^{ns}	48.50 ± 4.8^{ns}
Total organic carbon (%)	0.26 ± 0.2^{ns}	0.28 ± 0.4^{ns}	0.35 ± 0.06*	0.30 ± 0.04^{ns}	0.29 ± 0.1^{ns}	0.32 ± 0.2*	0.32 ± 0.1^{ns}	0.29 ± 0.3^{ns}	0.28 ± 0.4^{ns}
Phosphorus (kg/ha)	17.6 ± 2.3^{ns}	25.8 ± 5.1^{ns}	19.4 ± 3.4*	20.5 ± 4.6*	17.9 ± 2.5^{ns}	22.6 ± 4.9^{ns}	22.3 ± 3.8^{ns}	18.6 ± 3.2^{ns}	19.2 ± 4.1**
Potassium (ppm)	8.3 ± 1.7**	8.1 ± 1.4*	7.8 ± 2.8^{ns}	8 ± 2.8^{ns}	8.2 ± 1.6^{ns}	7.4 ± 1.2^{ns}	7.9 ± 1.3^{ns}	7.2 ± 3.5^{ns}	7.0 ± 4.2^{ns}
Calcium (me/100gm soil)	11.6 ± 1.9^{ns}	10.7 ± 2.8^{ns}	11.5 ± 2.4**	11.2 ± 2.8^{ns}	11.3 ± 4.2*	10.9 ± 3.2^{ns}	10.2 ± 2.6*	10.8 ± 3.9^{ns}	10.8 ± 5.3^{ns}
Magnesium(me/ 100gm soil)	16.1 ± 4.2*	15.5 ± 4.3^{ns}	16.3 ± 2.8^{ns}	15.9 ± 1.98^{ns}	16.4 ± 3.2^{ns}	15.1 ± 4.2^{ns}	15.6 ± 4.6^{ns}	15.3 ± 5.8^{ns}	15.7 ± 7.2*
Sodium (ppm)	15 6 ± 4.8^{ns}	12.4 ± 2.8*	14.8 ± 3.2^{ns}	12.8 ± 2.8**	15.1 ± 6.2^{ns}	14.7 ± 3.6^{ns}	11.4 ± 3.2^{ns}	12.2 ± 3.5^{ns}	13.0 ± 5.8^{ns}

Table 3.7 (A) Soil physico-chemical properties before any treatments (B) Effect of combined application of different treatments on soil physico-chemical properties after 30 days

Result shows means of three replicate ± SE (standard error), significance test by using ANOVA software at 30 DAS, * Significant at 5%; ** Significant at 1%; ns, non-significant as compared to control (ANOVA)

Parameters	Control	T1	T2	T3	T4	T5	T6	T7	T8
pH	8.4 ± 0.4*	8.31 ± 0.3*	8.4 ± 0.4*	8.2 ± 0.2*	8.4 ± 0.4*	8.42 ± 0.3*	8.3 ± 0.2*	8.3 ± 0.2*	8.4 ± 0.3*
Salinity (%)	0.13 ± 0.08^{ns}	0.11 ± 0.08^{ns}	0.13 ± 0.09*	0.1 ± 0.03^{ns}	0.13 ± 0.06^{ns}	0.1 ± 0.07^{ns}	0.12 ± 0.09*	0.14 ± 0.08^{ns}	0.13 ± 0.08^{ns}
EC (ds/ms)	0.18 ± 0.9^{ns}	0.14 ± 0.7^{ns}	0.18 ± 0.03^{ns}	0.14 ± 0.03^{ns}	0.18 ± 1.0^{ns}	0.12 ± 0.04*	0.16 ± 0.06^{ns}	0.20 ± 1.2^{ns}	0.18 ± 0.06^{ns}
Nitrogen (Kg/ha)	45.87 ± 8.2^{ns}	56.92 ± 10.2*	52.27 ± 9.4^{ns}	54.75 ± 9.8*	51.24 ± 8.7^{ns}	53.29 ± 6.8^{ns}	54.45 ± 7.4^{ns}	50.21 ± 7.1^{ns}	51.14 ± 8.2^{ns}
Total organic carbon (%)	0.30 ± 0.02^{ns}	0.35 ± 0.2^{ns}	0.42 ± 0.04*	0.33 ± 0.03^{ns}	0.32 ± 0.04^{ns}	0.40 ± 0.02^{ns}	0.36 ± 0.07^{ns}	0.33 ± 0.06^{ns}	0.34 ± 0.02^{ns}
Phosphorus (kg/ha)	19.2 ± 5.4^{ns}	29.8 ± 4.2**	22.3 ± 3.2^{ns}	25.6 ± 3.6^{ns}	19.5 ± 3.5^{ns}	27.6 ± 3.4*	21.7 ± 2.8^{ns}	20.6 ± 2.9*	23.1 ± 3.4^{ns}
Potassium (ppm)	8.0 ± 2.8^{ns}	6.5 ± 1.2^{ns}	6.2 ± 1.8^{ns}	7.4 ± 2.8^{ns}	7.8 ± 1.8^{ns}	6.9 ± 1.8^{ns}	7.1 ± 1.4*	6.6 ± 2.5^{ns}	6.7 ± 3.3^{ns}
Calcium (me/100gm soil)	11.4 ± 1.6^{ns}	10.5 ± 2.6^{ns}	11.3 ± 1.8^{ns}	10.8 ± 3.2^{ns}	10.4 ± 3.1^{ns}	10.1 ± 2.9^{ns}	10.0 ± 2.8*	10.2 ± 2.9^{ns}	10.5 ± 3.3^{ns}
Magnesium(me/100gm soil)	15.7 ± 3.9^{ns}	15.0 ± 3.6^{ns}	15.7 ± 3.9*	15.0 ± 3.8^{ns}	15.8 ± 4.1*	14.8 ± 2.6^{ns}	14.6 ± 2.2^{ns}	15.1 ± 3.8^{ns}	14.9 ± 2.6^{ns}
Sodium (ppm)	14.8 ± 3.6^{ns}	11.2 ± 1.8*	13.5 ± 2.8^{ns}	11.4 ± 1.8*	14.3 ± 3.8^{ns}	13.5 ± 2.6^{ns}	10.2 ± 1.2^{ns}	11.2 ± 1.9*	12.4 ± 4.6^{ns}

Table 3.8 Effect of combined application of different treatments on soil physico-chemical properties after 60 days
Result shows means of three replicate ± SE (standard error), significance test by using ANOVA software at 60 DAS,
*** Significant at 5%; ** Significant at 1%; ns, non-significant as compared to control (ANOVA)**

Parameters	Control	T1	T2	T3	T4	T5	T6	T7	T8
pH	8.3 ± 0.3*	8.5 ± 0.5*	8.4 ± 0.4*	8.2 ± 0.2*	8.5 ± 0.4*	8.3 ± 0.3*	8.2 ± 0.2*	8.4 ± 0.4*	8.5 ± 0.4*
Salinity (%)	0.12 ± 0.09*	0.09 ± 0.1^{ns}	0.11 ± 0.08^{ns}	0.06 ± 0.03^{ns}	0.12 ± 0.06*	0.07 ± 0.04^{ns}	0.11 ± 0.08^{ns}	0.12 ± 0.09*	0.12 ± 0.09*
EC (ds/ms)	0.16 ± 0.06^{ns}	0.10 ± 0.02^{ns}	0.14 ± 0.04*	0.04 ± 0.01^{ns}	0.16 ± 0.08^{ns}	0.06 ± 0.01*	0.14 ± 0.04*	0.16 ± 0.08^{ns}	0.16 ± 0.1^{ns}
Nitrogen (Kg/ha)	47.75 ± 5.6^{ns}	60.54 ± 8.1^{ns}	57.86 ± 6.4^{ns}	59.79 ± 10.4*	54.66 ± 6.1^{ns}	58.09 ± 8.7^{ns}	57.28 ± 7.9^{ns}	55.42 ± 4.6^{ns}	55.68± 9.2^{ns}
Total organic carbon (%)	0.34 ± 0.06^{ns}	0.46 ± 0.8^{ns}	0.48 ± 0.4^{ns}	0.44 ± 0.3^{ns}	0.36 ± 0.08**	0.45 ± 0.6^{ns}	0.41 ± 0.09^{ns}	0.38 ± 0.06*	0.39 ± 0.08*
Phosphorus (kg/ha)	23 4 ± 8.1^{ns}	34.9 ± 6.5*	27.6 ± 8.2^{ns}	29.4 ± 4.8^{ns}	24.7 ± 7.5^{ns}	28.2 ± 8.1^{ns}	25.8 ± 4.5^{ns}	24.6 ± 7.6^{ns}	26.8 ± 6.6^{ns}
Potassium (ppm)	7.8 ± 1.4*	5.9 ± 1.2*	5.6 ± 2.4^{ns}	7.0 ± 2.9^{ns}	7.1 ± 2.4^{ns}	6.0 ± 2.4^{ns}	6.6 ± 2.2^{ns}	6.0 ± 2.5^{ns}	6.2 ± 2.6^{ns}
Calcium (me/100gm soil)	11.0 ± 1.0^{ns}	10 1 ± 2.8^{ns}	10.9 ± 1.9*	10.6 ± 4.2^{ns}	10.1 ± 3.4^{ns}	9.8 ± 3.1^{ns}	9.6 ± 3.2*	9.9 ± 3.2^{ns}	10.2 ± 3.6^{ns}
Magnesium(me/ 100gm soil)	15.2 ± 3.9^{ns}	14.6 ± 3.6^{ns}	15.1 ± 3.9^{ns}	14.8 ± 3.8*	15.0 ± 4.1^{ns}	14.2 ± 2.6^{ns}	13.9 ± 2.7^{ns}	14.8 ± 3.8*	14.7 ± 4.2^{ns}
Sodium (ppm)	12.6 ± 2.2^{ns}	10.4 ± 1.2^{ns}	11.6 ± 2.6^{ns}	10.8 ± 1.8*	12.1 ± 2.1^{ns}	12.0 ± 2.0^{ns}	9.8 ± 3.1^{ns}	10.5 ± 1.1^{ns}	11.4 ± 2.8^{ns}

Table 3.9 Effect of combined application of different treatments on soil physico-chemical properties after 90 days
Result shows means of three replicate ± SE (standard error), significance test by using ANOVA software at 90 DAS,
*** Significant at 5%; ** Significant at 1%; ns, non-significant as compared to control (ANOVA)**

Treatment	Nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)	Sodium (ppm)	Iron (ppm)	Zinc (ppm)	Copper (ppm)
Control	0.013 ± 0.01^{ns}	0.10 ± 0.06^{ns}	1.2 ± 1.5^{ns}	$0.26 \pm 0.09^{*}$	4.98 ± 2.1^{ns}	3.45 ± 1.8^{ns}	0.25 ± 0.09^{ns}
T1	$0.056 \pm 0.09^{*}$	0.2 ± 0.09^{ns}	2.2 ± 1.4^{ns}	0.33 ± 0.04^{ns}	8.091 ± 1.8^{ns}	$2.49 \pm 1.2^{*}$	0.62 ± 0.08^{ns}
T2	0.045 ± 0.04^{ns}	0.15 ± 0.04^{ns}	2.4 ± 1.1^{ns}	0.31 ± 0.02^{ns}	$5.80 \pm 3.2^{*}$	4.50 ± 2.2^{ns}	0.30 ± 0.02^{ns}
T3	0.033 ± 0.06^{ns}	$0.12 \pm 0.01^{*}$	1.8 ± 0.2^{ns}	0.32 ± 0.1^{ns}	$4.55 \pm 2.5^{*}$	5.00 ± 3.7^{ns}	0.75 ± 0.09^{ns}
T4	0.011 ± 0.02^{ns}	0.14 ± 0.07^{ns}	1.7 ± 1.2^{ns}	$0.29 \pm 0.04^{*}$	$5.10 \pm 1.9^{*}$	4.25 ± 1.9^{ns}	0.45 ± 1.2^{ns}
T5	$0.07 \pm 0.03^{*}$	0.14 ± 0.02^{ns}	1.4 ± 0.6^{ns}	0.34 ± 0.02^{ns}	6.02 ± 3.9^{ns}	$3.10 \pm 2.6^{*}$	0.28 ± 0.02^{ns}
T6	0.09 ± 0.02^{ns}	0.13 ± 0.04^{ns}	$1.9 \pm 0.9^{*}$	0.32 ± 1.4^{ns}	8.86 ± 1.4^{ns}	4.34 ± 1.8^{ns}	0.33 ± 0.06^{ns}
T7	0.041 ± 0.01^{ns}	$0.11 \pm 0.08^{*}$	2.5 ± 0.4^{ns}	0.28 ± 0.06^{ns}	9.8 ± 1.6^{ns}	4.01 ± 1.4^{ns}	0.31 ± 0.04^{ns}
T8	0.037 ± 0.06^{ns}	0.13 ± 0.02^{ns}	2.3 ± 1.9^{ns}	$0.25 \pm 0.08^{*}$	10.98 ± 3.6^{ns}	3.77 ± 1.8^{ns}	$0.40 \pm 1.1^{*}$

Table 3.10 Effect of nutrient management on nutrient uptake of *Jatropha curcas* after 30 days

Result shows means of three replicate ± SE (standard error), significance test by using ANOVA software at 30 DAS, * Significant at 5%; ** Significant at 1%; ns, non-significant as compared to control (ANOVA)

Treatment	Nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)	Sodium (ppm)	Iron (ppm)	Zinc (ppm)	Copper (ppm)
Control	0.015 ± 0.009^{ns}	$0.15 \pm 0.04^{*}$	1.4 ± 0.9^{ns}	0.29 ± 0.04^{ns}	5.08 ± 1.3^{ns}	3.79 ± 1.3^{ns}	0.30 ± 0.06^{ns}
T1	0.060 ± 0.02^{ns}	0.24 ± 0.03^{ns}	$2.8 \pm 0.6^{*}$	0.35 ± 0.07^{ns}	$8.16 \pm 1.2^{*}$	2.51 ± 1.2^{ns}	0.66 ± 0.07^{ns}
T2	$0.058 \pm 0.007^{*}$	0.16 ± 0.04^{ns}	2.6 ± 0.7^{ns}	0.38 ± 0.07^{ns}	$5.82 \pm 1.6^{*}$	$4.54 \pm 1.3^{*}$	$0.33 \pm 0.04^{*}$
T3	0.049 ± 0.02^{ns}	0.15 ± 0.04^{ns}	2.4 ± 0.8^{ns}	$0.32 \pm 0.05^{*}$	$4.65 \pm 1.5^{**}$	$5.09 \pm 1.7^{**}$	$0.89 \pm 0.07^{**}$
T4	0.017 ± 0.01^{ns}	0.19 ± 0.02^{ns}	1.8 ± 0.2^{ns}	$0.35 \pm 0.06^{*}$	5.24 ± 1.4^{ns}	4.31 ± 1.6^{ns}	0.32 ± 0.09^{ns}
T5	0.054 ± 0.01^{ns}	$0.21 \pm 0.06^{*}$	2.0 ± 0.3^{ns}	0.6 ± 0.09^{ns}	$6.20 \pm 1.9^{**}$	$4.39 \pm 1.3^{**}$	$0.53 \pm 0.04^{**}$
T6	0.019 ± 0.01^{ns}	0.21 ± 0.06^{ns}	$2.0 \pm 0.3^{*}$	0.4 ± 0.08^{ns}	$8.92 \pm 1.5^{**}$	$3.13 \pm 1.4^{*}$	$0.47 \pm 0.07^{*}$
T7	0.048 ± 0.04^{ns}	$0.16 \pm 0.1^{*}$	2.7 ± 0.6^{ns}	0.29 ± 0.04^{ns}	9.94 ± 2.4^{ns}	4.36 ± 1.5^{ns}	0.33 ± 0.05^{ns}
T8	0.039 ± 0.02^{ns}	0.26 ± 0.02^{ns}	2.5 ± 0.9^{ns}	0.22 ± 0.02^{ns}	$11.08 \pm 4.8^{*}$	3.80 ± 1.1^{ns}	$0.43 \pm 0.08^{*}$

Table 3.11 Effect of nutrient management on nutrient uptake of *Jatropha curcas* after 60days

Result shows means of three replicate ± SE (standard error), significance test by using ANOVA software at 60 DAS, * Significant at 5%; ** Significant at 1%; ns, non-significant as compared to control (ANOVA)

Treatment	Nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)	Sodium (ppm)	Iron (ppm)	Zinc (ppm)	Copper (ppm)
Control	0.020 ± 0.8^{ns}	0.24 ± 0.2^{ns}	2.1 ± 0.6*	0.32 ± 0.4^{ns}	5.12 ± 2.4^{ns}	3.85 ± 1.8^{ns}	0.32 ± 0.9^{ns}
T1	0.075 ± 0.09^{ns}	0.45 ± 0.04*	5.2 ± 2.8^{ns}	0.48 ± 0.2^{ns}	8.21 ± 4.5*	2.93 ± 2.0^{ns}	0.69 ± 1.1^{ns}
T2	0.066 ± 0.4^{ns}	0.36 ± 0.08^{ns}	4.4 ± 1.4*	0.41 ± 1.4^{ns}	5.86 ± 3.2^{ns}	4.67 ± 1.8*	0.35 ± 0.1*
T3	0.052 ± 0.09^{ns}	0.30 ± 0.4^{ns}	3.1 ± 1.8^{ns}	0.38 ± 0.8^{ns}	4.75 ± 1.8^{ns}	5.23 ± 2.4*	0.93 ± 0.6**
T4	0.033 ± 0.04*	0.26 ± 0.09^{ns}	2.9 ± 0.8^{ns}	0.42 ± 0.02^{ns}	5.30 ± 3.6^{ns}	4.46 ± 2.1^{ns}	0.36 ± 1.8^{ns}
T5	0.068 ± 0.8^{ns}	0.38 ± 0.6^{ns}	3.8 ± 2.1^{ns}	0.8 ± 1.2^{ns}	6.39 ± 5.1*	3.17 ± 1.9*	0.62 ± 0.1**
T6	0.024 ± 0.02*	0.40 ± 0.08*	4.0 ± 1.4^{ns}	0.52 ± 0.9^{ns}	9.01 ± 7.3^{ns}	4.39 ± 3.2*	0.53 ± 0.09*
T7	0.056 ± 0.08^{ns}	0.29 ± 0.09^{ns}	3.5 ± 1.6*	0.36 ± 0.2^{ns}	9.96 ± 2.8^{ns}	4.24 ± 2.6^{ns}	0.38 ± 0.1^{ns}
T8	0.048 ± 0.2^{ns}	0.34 ± 0.04^{ns}	3.6 ± 1.4^{ns}	0.38 ± 0.2^{ns}	11.12 ± 7.6^{ns}	3.93 ± 1.8*	0.48 ± 0.8*

Table 3.12 Effect of nutrient management on nutrient uptake of *Jatropha curcas* after 90 days

Result shows means of three replicate ± SE (standard error), significance test by using ANOVA software at 90 DAS, * Significant at 5%; ** Significant at 1%; ns, non-significant as compared to control (ANOVA)

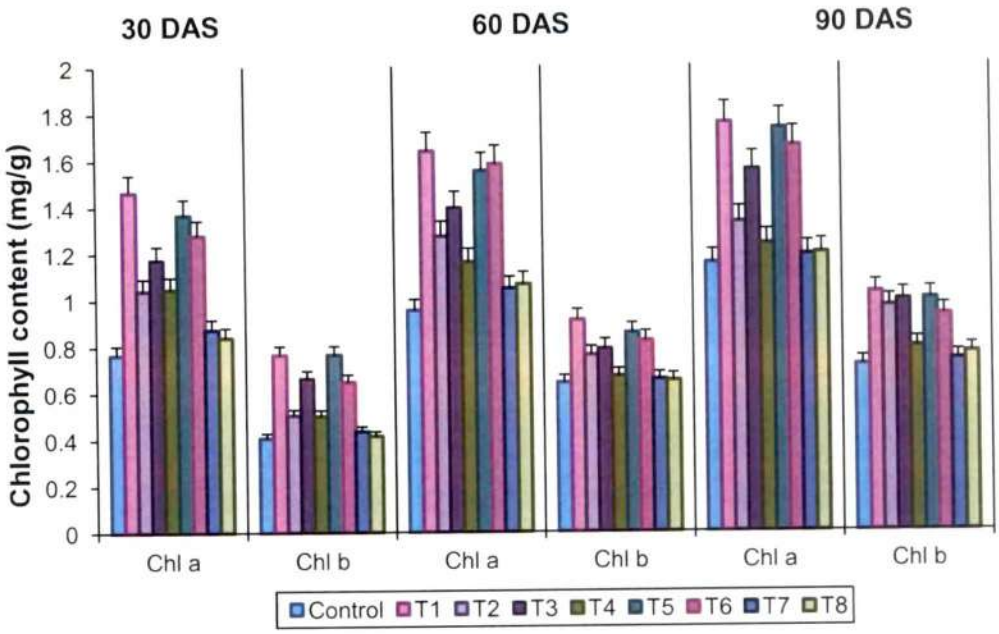


Figure 3.7 Effect of combined application of different treatments on chlorophyll content of *Jatropha curcas* after 30, 60 and 90 days of plantation

Conclusions

Microbial biomass plays a key role in soil nutrient cycling. Strong positive correlations have been found between the amount of nutrients held in the microbial biomass and amounts of mineralizable nutrients in the soil indicating that nutrient cycling in organic resources is tightly linked to the turnover of microbial biomass. Measurements of microbial biomass have been used to assess the effect of different farming systems on soil fertility and to characterize the status of soil restoration. The beneficial effects of combined application of chemical fertilizers with organic manures viz., farmyard manure vermicompost, biofertilizers and many more of such materials are universally known. Application of organic manures in general improves the availability of micronutrients like zinc, iron, manganese and copper. A balanced application of both organic, inorganics and biofertilizers appear to be an ideal proposition to meet nutrient requirements of dry land crops rather than single application. Present study showed that multispecies consortia of all the three isolates *Enterobacter cloacae* (MSA), *Pseudomonas pseudoalcaligenes* (MSC) and *Bacillus cereus* (MSD) have the ability to produce IAA, solubilize inorganic P, production of ammonia, exopolysaccharide, HCN and siderophore. Maximum germination percentage (76.66%) was observed in treatment T1 (consortia) with germination capacity (83.33%). Besides, result of coinoculated seeds showed increase in number of lateral roots, root length, shoot length, fresh weight of root and shoot, dry weight of root and shoot and chlorophyll content. Data were higher with respect to control, as well as other treatments. This supported the *in vitro* findings of PGP potentials in the multi-species consortium. Treatment with multispecies consortia showed increase in nitrogen, phosphorus and decrease in potassium, sodium content and other micronutrients in the soil. Soil treated with T1 (consortia) showed maximum uptake of nitrogen, phosphorus, content in the plant from soil. Treatment with T1 also showed increase in alkaline phosphatase activity (346 µg p-nitrophenol kg^{-1} soil, 377 µg p-nitrophenol kg^{-1} soil and 405 µg p-nitrophenol kg^{-1} soil) in comparison to other treatments and untreated control. Results of this study revealed that all the treatments showed increase in soil fertility as well as growth of *Jatropha* as compared to untreated control. Among all the treatments, T1 (consortia) was most effective in increasing nutrient status of plants and soil structural stability, leading to enhanced growth of

Jatropha plant. It could be concluded that multispecies consortia of PGPR technology should be employed with appropriate doses of fertilizers to get maximum benefit in terms of fertilizer savings and better plant growth. Treatment with T3 (microbial consortia + mycorrhizal fungi) and T5 (mycorrhizal fungi) was the next most effective treatment in increasing nutrient status of plants and soil structural stability, leading to enhanced growth of *Jatropha*. Considering the plant growth promoting abilities of these three isolates *Enterobacter cloacae* (MSA), *Pseudomonas pseudoalcaligenes* (MSC) and *Bacillus cereus* (MSD), a non-specific, multi-species PGPC for bioinoculant preparation is possible.