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Regular Article

Analysis of interaction between Arbuscular Mycorrhizal fungi and their Helper bacteria by MILPA model

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Many recent researches carried out the research in mycorrhizal symbiosis to improve crop growth in the agriculture but they differ in their approaches like crops and the agricultural perspectives. The study focused on the interaction of Arbuscular mycorrhizal (AM) fungi and helper bacteria, influence maize growth through milpa as a model system to analyze soil enzyme activity of acid, alkaline phosphatase and signaling molecule of phospholipids fatty acid (PLFA) profile. Bioinoculants plays an important role in plant growth like nutrient mobilization, biocontrol and prevent the plants from stress. The maize crops were treated with bio inoculants such as *Azospirillum, Azotobacter, Rhizobium, Pseudomonas* and AM fungi. Significant diverse effects were observed with bioinoculants in the crops compared to control crop. The number spores formed was proportional to the rate of colonization.AM fungi association in plant roots helps the plants in nutrient uptake especially phosphorus (P) from soil materials. The enzyme activities were also found to influence the growth of the plant and phospholipids fatty acid (PLFA) profile influences the interaction between bacterium and AMF. Plant and phospholipids fatty acid analysis is a sensitive and accurate method in determining microbial community structures, because it depends on living cell contents of microorganisms under *in situ* conditions. It confirmed with gas chromatographic analysis of PLFA to determination of the structure and total biomass of microbial community in treated soil samples.

Keywords: Biofertilizers, Crop improvement, Plant-soil interactions.

Currently agricultural practices are neither economically nor environ-mentally sustainable and India's yields for many agricultural supplies are low. Poorly maintained irrigation systems, soil fertility and almost universal lack of good extension services are among the factors responsible. The root-soil is an active environment where roots, soil and microorganisms were interact each other. Many bacteria are known to be able to stimulate plant growth through direct or indirect interactions with plant roots. The association between soil fungi and plant roots is called mycorrhiza. The establishment of mycorrhiza implies profound morphological and physio-logical changes in the root, which operates in an included manner with the fungus, thus promoting increasing in the adaptability and survival of symbionts (Costa, 2002). The symbiotic association

between mycorrhizal fungi and the roots of plants are extensively present in the natural environment. Many type of fungus that forms these associations, but important for agriculture was arbuscular mycorrhizal fungi (AMF). The first report that root fungi may be beneficial to plants was observed on Indian pipe. Arbuscular mycorrhizal fungi belong to the fungal phylum Glomeromycota. Frank 1885 named the symbiosis between fungi and root as "fungus root". An arbuscular mycorrhiza is a type of mycorrhiza in which the fungus penetrates the cortical cells of the roots of a vascular plant and among the mycorrhizal associations; the AM association is the most common one (Schubler *et al.,* 2001). The numerous and different interactions with its physical, chemical and biological components, adapted by the common environmental conditions to determine the soil complexity. The genetic diversity and functional activities of the extensive microbial populations have a critical impact on soil structure, because microorganisms are dynamic forces for essential metabolic processes involving specific enzyme activities. Processes affecting the soil structures that are mediated by root physical force or penetration, rhizode position, root decomposition, root entanglement of soil particles. Soil biota are significant components in the natural soil sub-ecosystem because not only for nutrient availability in the soil, but also combine soil particles into the stable aggregates, which improve soil structure and reduce erosion (Shetty *et al*., 1994). Arbuscular mycorrhizal symbiosis affects the community and diversity of the organisms present in the soil. This can be directly observed by the release of exudates, or indirectly by a change in the plant species. The extent of arbuscular mycorrhizal colonization affects the bacterial population in the rhizosphere. Differ in their abilities to compete for carbon as compound root exudates. A change in the amount or composition of root exudates and fungal exudates due to the existing Arbuscular mycorrhizal colonization. There are three major important components of the mycorrhizal root system the root itself, the intra radical mycelium (the fungi within the root) and the extra radical mycelium (the fungi within the soil). Colonization of roots by AM fungi can occur through spores. The spores are formed on the extra radical hyphae, but some species also may form spores inside the roots. Several AM fungal has excellent root colonizers (Lugtenberg and Dekkers, 1999; Barea*et al.,* 2002) and in the physical interactions between bacteria and plant roots (Bianciotto and Bonfante, 2002). Bianciotto and colleagues reported that some *Rhizobium* and *Pseudomonas* species attached to germinated AM fungal spores and hyphae under sterile conditions, and that the degree of attachment varied with the bacterial strains. Duponnois and Garbaye (1991) proposed first the term Mycorrhization Helper Bacteria (MHB), referring only to bacteria that promoted the establishment of the root-fungus symbiosis. This concept was reinforced and clarified latter by Garbaye (1994). The two major important functional categories about the MHB bacterial action: the primary, Mycorrhization Helper Bacteria, those that stimulate the process of mycorrhiza and the next, Mycorrhiza Helper Bacteria, that interact positively with the functioning of the already-established symbiosis (Frey-Klett 2005). A milpais a traditional intercropping system of maize, bean, squash etc. it is not only rich in inter- and infra-crop species diversity, but also in landraces of maize, which are building blocks for the future improvements of important staple crop. Based on rotation the maize fields and fallows, to replenish organic matter and nutrients. The cultivation is coupled land-use and land-tenure system sustained on poor soils by regulating the number, timing of

milpa fields and the effects of population growth on land resources through generally non-divisible inheritance rights (Plaza, 2000). The *milpa* system is a poly-cropping system characterized by species and variety richness as well as genetic diversity, particularly in maize landraces (Roseland 2002; Bellon and Berthaud 2004; Van Dusen and Taylor 2005). Many rhizosphere colonizing bacteria, including *Azotobacter*, *Azospirillum*, *Bacillus*, *Clostridium* and *Pseudomonas*, typically produce substances that stimulate the plant growth or inhibit root pathogen. Different microbial inoculants used *Azospirillum*, *Pseudomonas and Trichoderma* were colonization on maize roots, the effects processes such as bacterial populations and enzyme activities in rhizosphere soil (Vasquez, 2000). There is much scope for experimental analyses of the mechanism of bacteria and AM fungal interactions with plant roots and the investigations of their functions will be one of the biggest challenges for the future mycorrhizal research.

MATERIALS AND METHODS

Milpa arrangement: Talc formulated inoculum prepared from (AM) fungi *Azospirillum, Azotobacter, Pseudomonas, Rhizobium* and *Arbuscular Mycorrizhal* collected from biofertilizer production unit, Mannarpuram and Meenakshi Agroservice, Tiruchirappalli, Tamil Nadu. For the maize seed implantation the red soil, sandy soil, fertile soil and organic manures were mixed thoroughly in the ratio of 2:2:2:1 and filled in the nursery bag sit uphold for up to one month for the growth of roots and plantlets. The grown fit maize plants were uprooted from the bags without damaging the root system. Following the, roots were segregated into equal four parts and inserted into the separate new bags, inoculated with 5gms of AM fungi. Apart from that, talc formulated prepared inoculum were introduced independently into every bag before sowing the seeds of most optimized host like chilly, onion, green gram and groundnut. According to the milpa model system, the maize plant was positioned centrally adjacent to the four bags along with the experimental treatments as T1 – Control without inoculum, T2 – *Azospirillum* with AMF, T3 – *Azotobacter* with AMF, T4 – *Rhizobium* with AMF, T5 – *Pseudomonas* with AMF, T6 - *Azospirillum, Azotobacter Rhizobium, Pseudomonas* altogether with AMF (***Figure 1***).

Isolation of AM fungi

For the soil analysis and for the AM fungal isolation the soil collected from various bioinoculated rhizosphere soil sat the MILPA system experimental field site of Microbiology department in Bharathidasan University, Tiruchira-ppalli, Tamil Nadu. Subsequently roots from the plants were washed and fixed using FAA (Philips & Hayman, 1970) and be observed under a dissection microscope (40X) for intact AM fungal spores. After assessment, the roots cut into 1 cm bits, cleared in 10% KOH for 15min. (Koske and Gemma, 1989), acidified the roots with 2.5N HCl for 5 min. and stained with tryphan blue in lactophenol are immersed for overnight. The stained roots were examined with a compound microscope for AM fungal structures and for root colonization percentage through magnified inter-section method (McGonigle *et al.,* 1990).

Enumeration and identification of AM fungal spores

AM fungal spores in the rhizosphere soil samples were estimated by a wet-sieving and decanting technique of Gerdemann & Nicolson (1963). Spores were recognized based on spore morphology and sub-cellular characters and compared with original description (Schenck and Perez, 1990). Spore morphology was also compared with the culture database established by INVAM (http://invam.caf.wvu.edu). According to the dilution plate method, the colonies were counted for bacterial populations to estimate the microorganisms in soils.

Determination of acid, alkaline phosphatase and extraction of phospholipid fatty acid methyl esters:

Stocks and working standards prepared using1 gm of p- nitro phenol in 1000 ml distilled water and latter 1 ml of the stock solution was diluted to 100 ml with distilled water. The 1 gm of soil sample and 4 ml of Modified Universal Buffer (MUB) with1 ml of buffered substrate used as Test control. 4 ml of MUB and 1 ml of buffered substrate as substrate control and 1 gm of soil sample with 1ml of MUB as a Sample control. All the tubes were incubated at 37°C for 1 hour. After incubation, 1 ml of 0.5M $CaCl_2$ was added. Then 4 ml of 0.5M NaOH was added and mixed well. All the test tubes were then filtered using Whatman No. 1 filter paper and the solutions were read at 420 nm (Tabatabai and Bremner, 1969). The extraction of phospholipid fatty acid **(**PLFA) was carried out using Sherlock microbial identification system (MIS; Microbial ID Inc. MIDI, 1992).

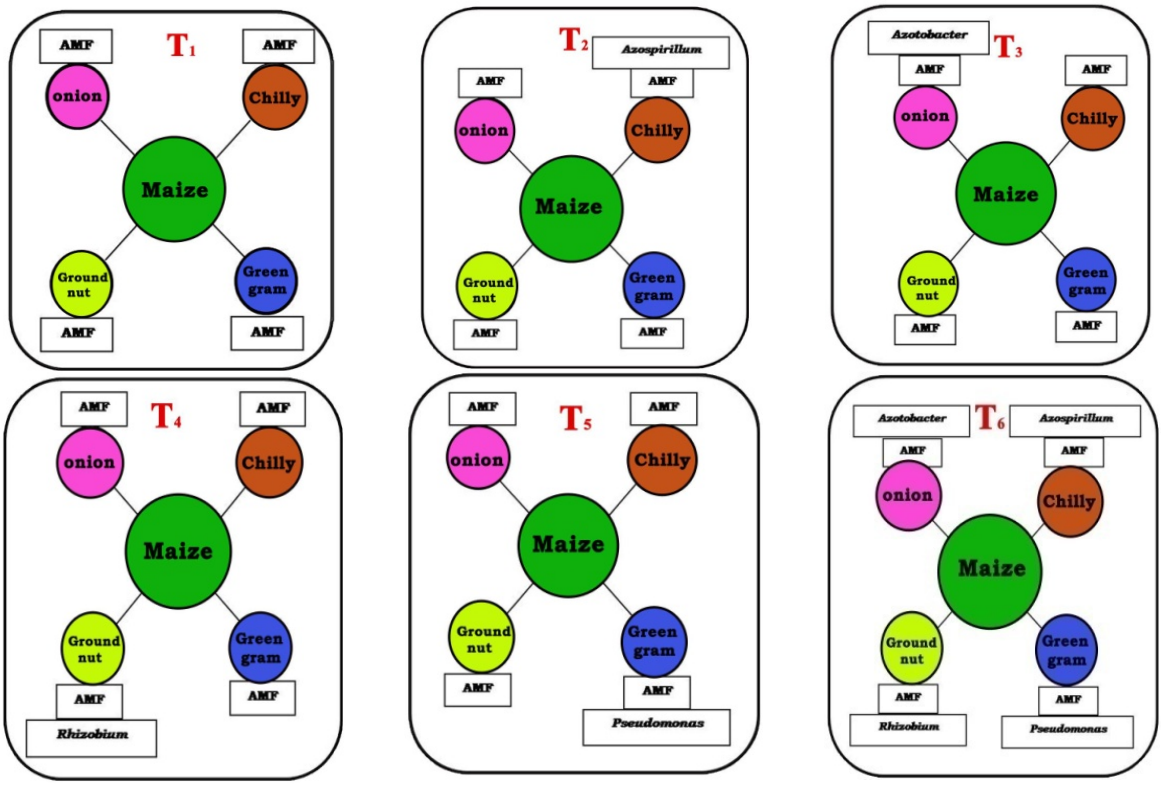


Figure 1: Milpa model, T1-Control, T2-*Azospirillum* with AMF, T3-*Azotobacter* with AMF, T4-*Rhizobium* with AMF, T5-*Pseudomonas* with AMF, T6-*Azospirillum, Azotobacter, Rhizobium, Pseudomonas* with AMF

Results

Plant morphological growth can be either increased or decreased according to the environmental conditions from which they grow. By utilizing the nutrients from soil, the growth level of plant will be high and obviously, reduces when they starve under insufficient nutrient contents. Plant morphological characters such as plant shoot height, root length, shoot fresh weight, root fresh weight, root and shoot dry weight was measured and the number of leaves present was counted. The shoot and root length was found to be greater in T_4 whereas other treatments also showed considerable growth regarding the control. The fresh weight and

dry weight of the shoot and root was also seemed to be higher in T_4 and $T_5$with minimal variations between them. Finally, the leaf number was greater in T_5 and represented in the **Table 1 & 2**.

Table 1. Morphological studies of maize plant shoot performance under nursery conditions in Milpa model: Shoot and root length was increase in T_4 and T_5 with least variations between them. Finally, the leaf number was greater in T_5 when compare with all treatments.

S. No	Treatments	Shoot length (cm/plant)		Shoot fresh weight (gm/plant)		Shoot dry weight (gm/plant)	Leaf number / plant
		Initial	Final	Initial	Final		
1.	T_1	47	51	4.76	7.58	2.848	7
2.	T_2	43	52	5.12	7.62	1.668	4
3.	T_3	48	59	4.98	7.54	1.609	4
4.	T_4	56	68	6.34	9.12	2.885	6
5.	T_5	51	64	6.16	8.82	3.526	8
6.	T_6	49	54	5.96	8.75	2.467	5

Table 2. Morphological studies of maize plant shoot performance under nursery conditions in Milpa model. Under nursery treatments of the plants, T_4 and T_5 showed minimum increase in root length and root fresh weight but T_6 greater than all the treatments.

S. No	Treatments	Root length (cm/plant)		Root fresh weight (gm/plant)		Root dry weight (gm/plant)
		Initial	Final	Initial	Final	
1.	T_1	23	41	1.678	1.976	1.442
2.	T_2	25	43	1.428	1.690	0.442
3.	T_3	25	44	1.276	1.606	0.905
4.	T_4	26	44	1.729	2.003	1.543
5.	T_5	27	48	1.927	2.117	1.405
6.	T_6	21	41	1.660	1.998	0.975

According to the observed results, T_4 (*Rhizobium* + AMF) and T_5 (*Pseudomonas* + AMF) had shown better growth performance by showing induced root and shoot length and weight when compared to control. Simultaneously population dynamics of the AM fungi were also identified under nursery trials; the control plants associated with AMF was about 88% of colonization along with 24 vesicles and 16 arbuscules number of structures and was shown in a **Figure 2 & 3a, b.**

The highest spore count of 97/15 gm rhizosphere soil was also observed. On contrast with other treatments, the control plants provided much support for the colonization of AMF. T_5 (Maize) application showed increased colonization level of about 94% but the vesicle 21, arbuscules 14 and spore count was 92/15 gm, which was lesser than control. Similarly, T_5 (Maize) application increased the colonization level 93% with 20 vesicles, 12 arbuscules and the spore count was 87 which is represented in **Figure 4 a& b.**

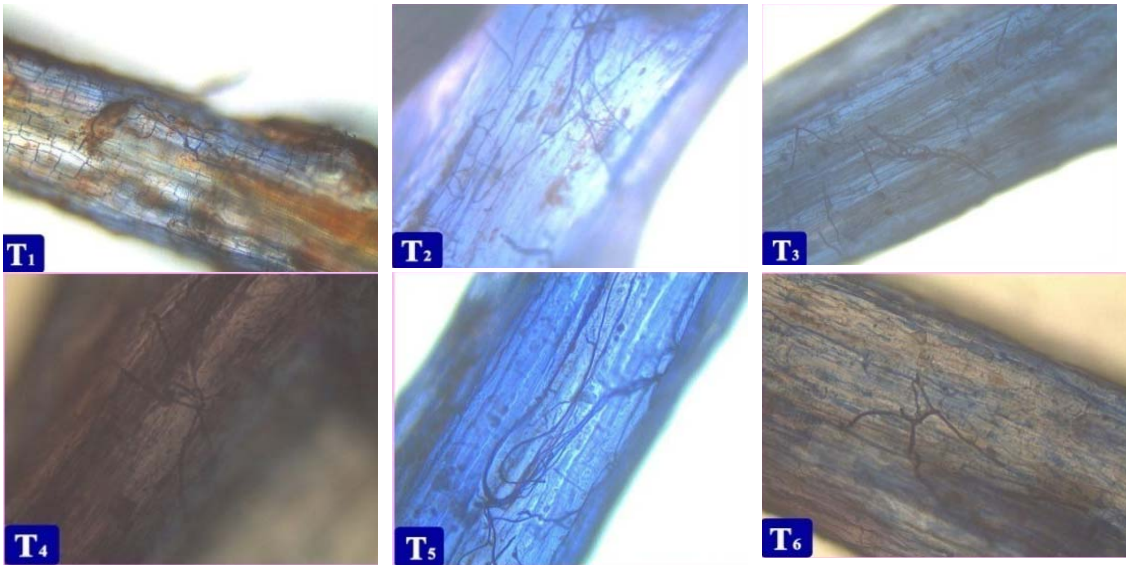


Figure 2. AMF colonization in maize and in nursery trials: T_4 (*Rhizobium*+ AMF) and T_5 (*Pseudomonas* + AMF) had shown better growth performance by showing induced root and shoot length and weight when compared to control; **T1(Control), T2 (*Azospirillum* with AMF), T3 (*Azotobacter* with AMF), T4 (*Rhizobium* with AMF), T5 (*Pseudomonas* with AMF), T6 (*Azospirillum, Azotobacter, Rhizobium, Pseudomonas* with AMF)**

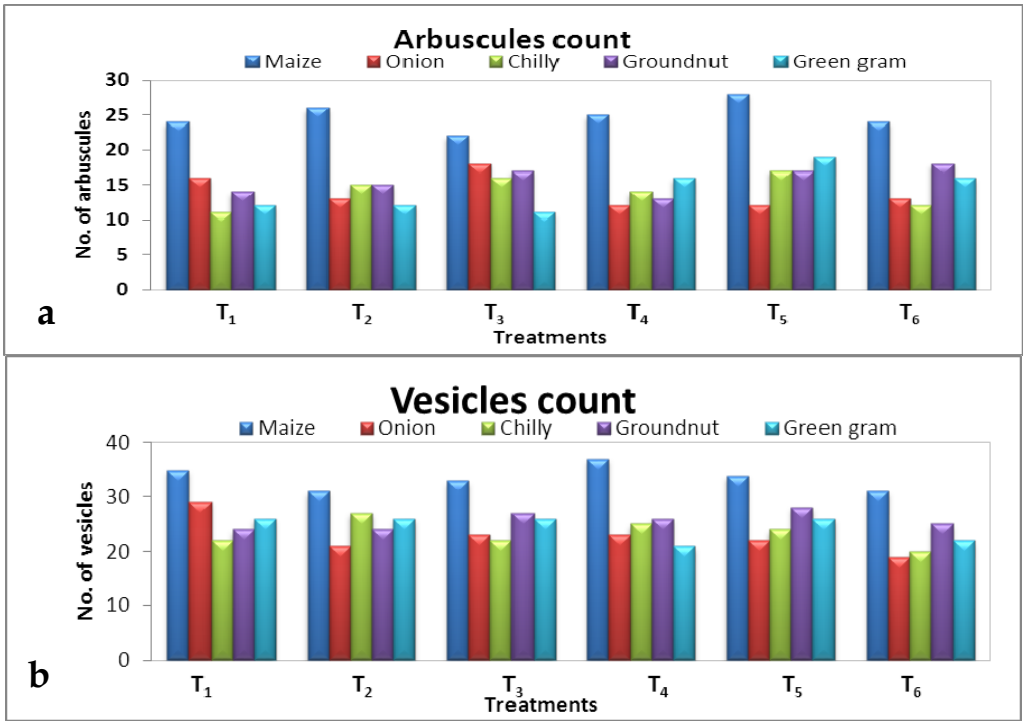


Figure 3 a & b. Arbuscules and vesicles colonized in roots grown under nursery in milpa model: 3 a:T_4 (Maize)showed increased colonization level in arbuscules vesicle but**3 b:** T_5(Maize) showed increase colonization in vesicles.

In treated soil, the density of microbial population in rhizosphere soil was enumerated initially and finally under nursery conditions. The initial population count for *Azotobacter sp.* was 19 × 10^{-4} CFU/g; *Azospirillum sp.* 11 × 10^{-4} CFU/g; *Rhizobium sp.* 21 × 10^{-4} CFU/g and *Pseudomonas sp.* had 32 × 10^{-4} CFU/g.

The final population count was *Azotobacter sp.* was 24 × 10^{-4} CFU/g; *Azospirillum sp.* 17 × 10^{-4} CFU/g; *Rhizobium sp.* 32 × 10^{-4} CFU/g and *Pseudomonas sp.* had 49 × 10^{-4} CFU/g and was tabulated in Table 3. Between the four types of biofertilizers, *Rhizobium* and *Pseudomonas* possess increased population density than the other two types of biofertilizers.

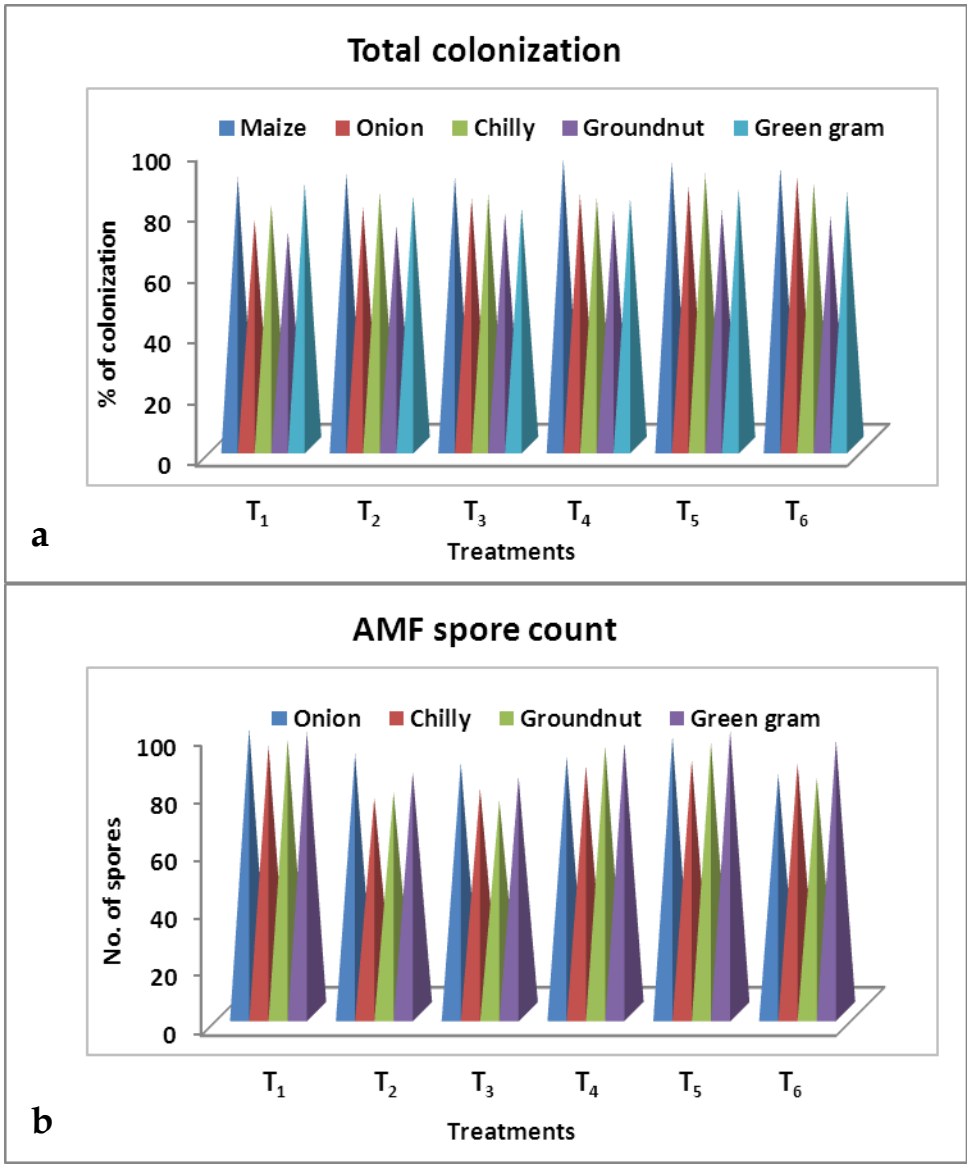


Figure 4 a & b: Percentage of AMF colonization and spore population grown under nursery conditions in milpa model. T_5 (Maize) application increased in the total colonization level and spore count in arbuscular and vesicles.

Table 3. Microbial populations under nursery conditions in milpa model: According to the microbial load in 10^{-4}dilution between four microbes, *Rhizobium* species in showed increased in the microbial growth but the *Pseudomonas* showed high microbial load than other all biofertilizer.

S. No	Microbes	Microbial load at 10^{-4} dilution (cfu/g)	
		Initial load	Final load
1.	*Azotobacter* sp.	19	24
2.	*Azospirillum* sp.	11	17
3.	*Rhizobium* sp.	21	32
4.	*Pseudomonas* sp.	32	49

Azotobacter and *Azospirillum* also showed increased growth but not as much like that of *Rhizobium* and *Pseudomonas*. Among the 24 microbial inoculated soil samples, enzyme activity was analyzed chiefly the acid and alkaline phosphatase activity was between 8 to 16 µg/g and 7 to 15 µg/g respectively. Regarding acid phosphatase, the highest activity was found in T_3 (chilly) rhizosphere soil so as to is 15 µg/g and the lower activity of 8.026 µg/g was seen in T_6 (groundnut) rhizosphere soil. Likewise in alkaline phosphatase, the higher activity was observed in T_5 (green gram) rhizosphere soil to is 14.973 µg/g and lower activity of 7.223 µg/g was found in T_4 (groundnut) and shown in **Figure 5 a & b.**

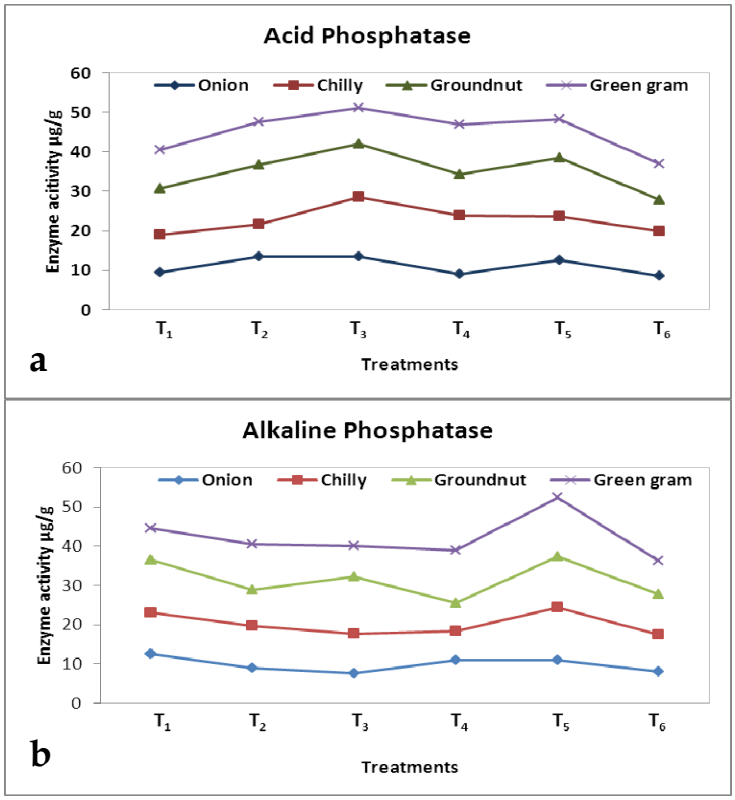
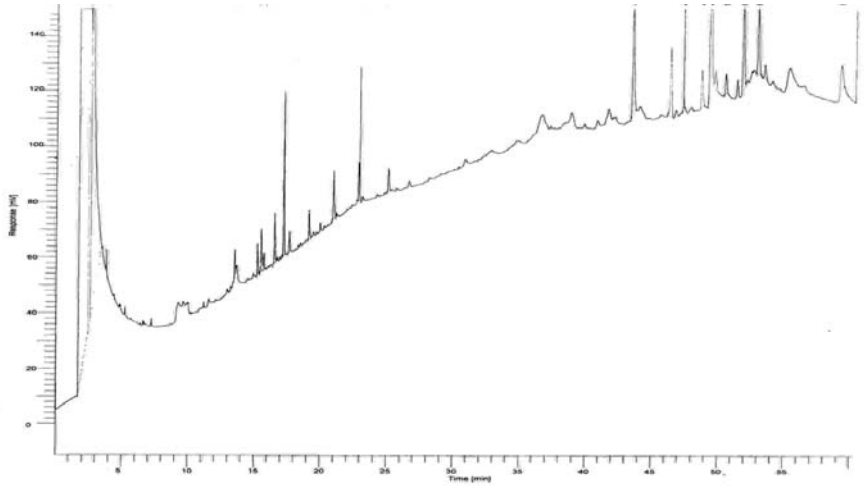
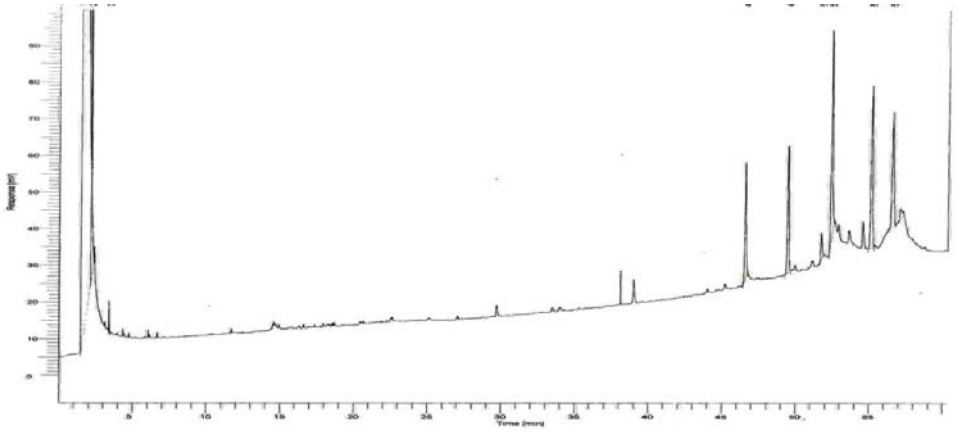


Figure 5a & b: Enzyme activity of acid & alkaline phosphatase under nursery conditions in milpa model. T_3 (chilly) rhizosphere soil showed high enzyme activity in acid phosphatase and T_5 (green gram) rhizosphere soil alkaline phosphatase enzyme activity.

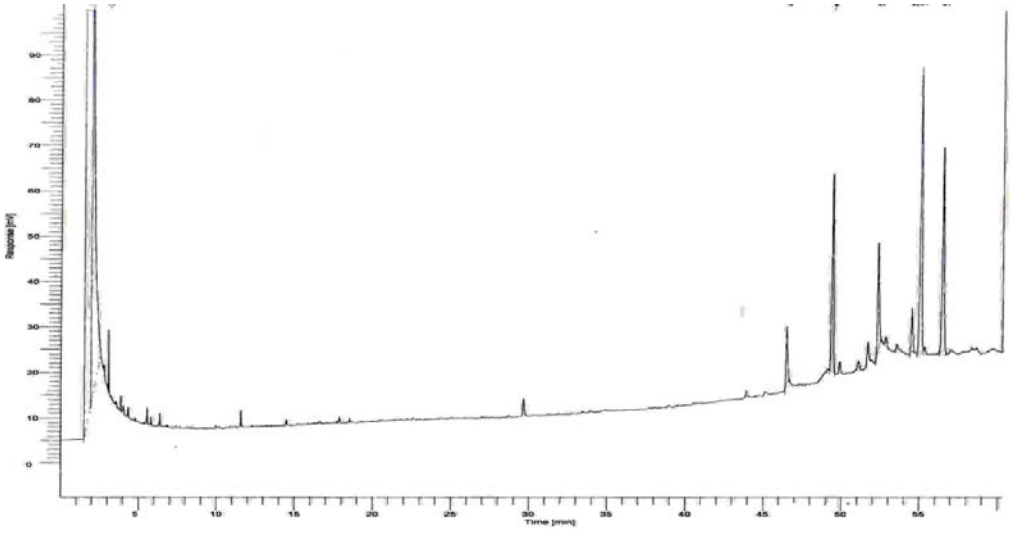
The phospholipids fatty acid extraction was through by using silica column and analyzed by gas chromatography. In this, the highest peak was found at 1 hour. The peak value was 59.18, followed by 52.87, 51.73, 50.40, 49.25, 48.60, 47.23, 46.26, and 43.45. Increased retention time results in increased peak values and vice versa. The lowest peak was observed in 5 min as 1.72, 2.46, and 3.79while the intermediate peak was observed between 15-30 minutes of sample injections. The peaks values react to intermediate were 13.49, 15.21, 15.69, 16.49, 17.17, 17.60, 19.08, 20.93, 22.80, and 25.03. The fatty acids there in T_1be Butyric acid- 17.60, Capric acid- 0.18, Undecanoic acid- 0.17, Lauric acid- 0.53, Tridecanoic acid- 0.19, Myristic acid- 0.21, cis-10-pentadecanoic acid- 0.11, Behenic acid- 0.93, Arachidonic acid- 0.43, Tricosanoic acid- 1.19, Eicosadienoic acid- 0.26, Lignoceric acid- 1.90, Eicosa- pentaenoic acid- 0.12, Nervonic acid - 0.70 and Docosahexanenoic acid- 0.51. In T_2, the uppermost value was observed in 53 minutes as 52.36, followed by 55.11 at 54 minutes. The lowermost peak value was observed as 1.5, 2.15 in 5 minutes between 5-30 minutes, the peaks were merged together and no fatty acids were found at that time butyric acid- 4.01, Arachidonic acid- 0.84, Lignoceric acid- 0.94, Nervonic acid- 0.19, Docosa-hexanenoic acid- 1.61. In T_3, the highest peak value was observed as 54.47 in 54 minutes followed by 49.38 and 56.46 at 48 and 52 minutes. The solvent peak was experiential as 1.53, 1.97. The fatty acids found between 5-45 minutes are butyric acid- 11.80, Arachidonic acid- 0.39, Lignoceric acid- 1.34, Docosa= hexanenoic acid- 0.80 accurately. In T_4, between 40-60 minutes the maximum peak value was revealed inside 43.45, 47.23, 49.25, 51.73, and 52.87. At 5-30 minutes, the peaks were merged together and showed subsequent fatty acids such as butyric acid- 7.76, Arachidonic acid- 0.44, Lignoceric acid- 1.26, Docosa-hexanenoic acid- 0.86. The total retention time for this entire treatment was 60.33minutes. In T_5, the peak value between 45-60 minutes to be very high where as the lowest peak found at 12, 30 & 49 minutes and the value was observed as 3.23. The fatty acids present in this treatment were butyric acid- 8.20, Arachidonic acid- 0.33, Lignoceric acid- 0.96, Docosahexanenoic acid- 0.68. The common fatty acids found in all the five treatments *(T1, T2, T3, T4, T5)* were butyric acid, Arachidonic acid, Lignoceric acid and Docosahexanenoic acid in ***Figure 6.***



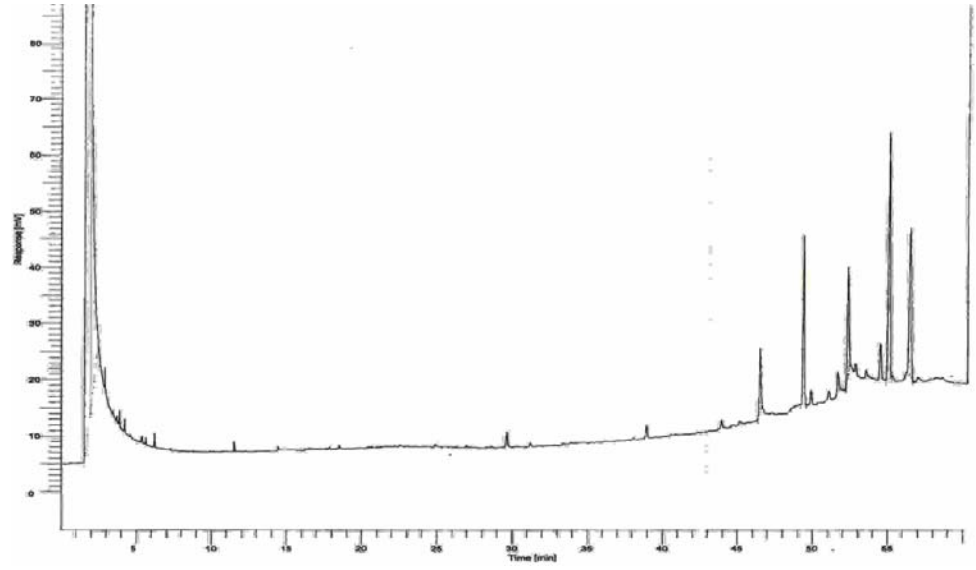
T1



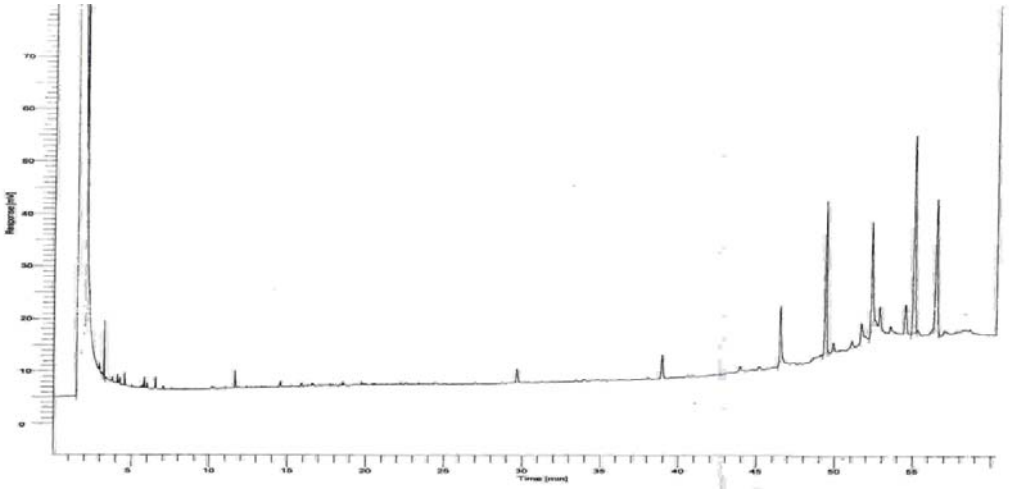
T2



T3



T4



T5

Figure 6. Gas chromatogram peak showed common fatty acids peaks in all the treatments. T1(Control), T2(*Azospirillum* with AMF), T3(*Azotobacter* with AMF), T4(*Rhizobium* with AMF), T5(*Pseudomonas* with AMF).

DISCUSSION

Plants growth be directly propotinal to the soil nutirents.Hence by estimating the shoot height, root length, weight and number of leaves provides a better measurment for plant growth. By supplying the bioferitlizers to the soil the plant growth will elevate when compared to the control.The necessary elements (generally N) are required for initial growth for plants to synthesizea new cells and organic compounds (Troeh and Thompson, 1993). The deficiency of nitorgen(N) during seed germination will affects the plant growth and results in poor yield. The availability of Phosphorous (P) in soil is a complex condition, determined by the major factors like soil type, acidity, soil temperature, soil water content and Ca concentration. therefore the biofertilizers improve plant growth by providing large amount of N and P contents required for fixing the nitrogen and to transfer the phosphorus from soil. AMF colonization with plant roots results in better nutrient uptake especially phosphorus. Simirarily the MHB might be exploited to improve the colonization of roots by mycorrihal fungi . It been shown a beneficial to plants in taking up nutrients such as P, Cu, Zn and Fe; increasing plant drought tolerance and providing benefits to the plant against pathogens. T_4 and T_5 showsutmost colonization while other treatments affect to some extent. The rate of colonization results in better plant growth and nutrient uptake (Ryan and Angus, 2003). Phosphate solubilizing bacteria releases soluble phosphate that are taken up by mycorrhizal roots and was transferred to the plants (Kapoor *et al.,* 1989). The number of spores formed was proportional to the rate of colonization. Enhanced uptake of P was considered to be a beneficial effect of AM in plant-microbe interaction. The high diversity of fungal partners allows optimal foraging and mobilisation of various N and P forms from organic soil layers (Buscot *et al.,* 2000). The adding up of biofertilizers increased colonization and spores may be due to the symbiotic relationship with AM fungi which provide nutrients for their growth. Soil microbial population may be affected mainly by environmental changes like watering, temperature, pH and addition of chemical substances, etc. unnaturally introducing an alien microorganism into the soil was a

challenge because of competition exerted by the tenant microflora along with the specific physico-chemical properties of the soil resolved the equilibrium of microbial community (Garbaye, 1994). Soil microorganisms may produce compounds that increase root cell permeability as well asthe root exudation (Barea *et al.,* 2002). Dissimilar populations of bacteriarecognized themselves under the influence of different AM plant-fungus amalgamations. (Andrade *et al.,* 1997). Higher concentrations of chemicals in the soil may be harmful to microbes because they may enter into the cell and disturbs its metabolism and affects the microbial population. Initial and final microbial load varies in a population according to the environmental condition. Biofertilizers such as *Azospirillum sp., Azotobacter sp., Rhizobium sp.,* helps the soil to fix the nitrogen and *Pseudomonas sp.* solubilizes the native soil phosphate as well as phosphorus from rock phosphate for the enhancement of plant growth.*Rhizobium* strains assist to colonize the rhizosphere of non-legume hosts with the intention to establish positive interactions with mycorrhizal fungi (Galleguillos *et al.,* 2000). The N_2-fixing *Azospirillum* bacteria are recognized to benefit plant development and yield under appropriate conditions (Okon, 1994; Bashan, 1999). The treatments inoculated with both AM fungi and bacteria significantly increased plant biomass and N & P accumulation in plant tissues compared with their controls (Artursson *et al.,* 2006). Soil enzyme acivity of the T_3 (*Azotobacter*+ AMF) was found to contain high amount of acid phosphatase activity, followed by T_4 (*Rhizobium* + AMF) and T_5 (*Pseudomonas* + AMF). Lower activity was found in T_2 (*Azospirillum* + AMF) and T_6 (*Azospirillum* + *Azotobacter* + *Rhizobium* + *Pseudomonas* + AMF). Higher alkaline phosphatase activity was found in T_5 (*Pseudomonas* + AMF) and T_3 (*Azotobacter* + AMF). T_4 (*Rhizobium* + AMF) was seemed to contain lower activity. The higher phosphatase activity may be due to the presence of essential phosphorus contents and lower activity may be due to deficiency of the phosphate content in the soil. According to the availability of phosphorus in soil the microbes play a role to absorb and transport it to the plants with the secretion of these enzymes. Phosphatases were found to hydrolyzes esters and anhydrides of phosphoric acid (Balota *et al.,* 2004). When there is P deficiency in the soil, acid phosphatase secretion from plant roots is increased to enhance the solubilisation with remobilisation of phosphate, thus influencing the ability of the plant to cope with P-stressed conditions (Nakas *et al.,* 1987; Chrost, 1991; Muchhal *et al.,* 1996; Li *et al.,* 1997; Daram *et al.,* 1999; Hayes *et al.,* 1999; Kai *et al.,* 2002; Karthikeyan *et al.,* 2002; Mudge *et al.,* 2002; Versaw & Harrison, 2002;). Under phosphorus starvation plant cells produce a major acid phosphatase with a pronounced preference for phosphoenol pyruvate through glycolysis. (MacDonald and Lewis, (1978) has cytochemically demonstrated the presence of acid phosphatase in *Glomus mosseae*. The level of alkaline phosphatase depends on the quantity of microbial biomass containing the materials (Dick 1984). The different treatments used for PLFA analysis, the high fatty acids content was attained in T_3 followed by T_4 and T_5 while compared to other treatments. The common fatty acids present in the samples were butyric acid, capric acid, undecanoic acid, lauric acid, tridecanoic acid, myristic acid, cis-10-pentadecanoic acid, behenic acid, arachidonic acid, trichosanoic acid, lignoceric acid, cis 11, 14 eicosadienoic acid, eicosapentaenoic acid, nervonic acid, docosahexanenoic acid andsome unknown fatty acids were formed which may be high carbon polyunsaturated fatty acids in all treatments due to PLFA it acts as a signal between AMF and MHB. In addition to characterization of the microbial

community structure, PLFA analysis may be used for quantification of the total microbial association (Silvie*et al.,* 2003). As PLFAs, the quantitative measurable biomolecules in soil microorganisms are rapidly twisted and indicate the current living community, both qualitatively and quantitatively, have a tendency to elevate the indicative value to assess and monitor the microbial community structure, physiological and stress state. The determination of PLFA profiles provides a broad diversity extent of microbial community at the phenotypic level and discriminates flanked by communities of different origin. As the analyzed dataof quantitative and qualitative diestribution of fatty acids with treatments inclusive to know the associations between the microorganisms and rhizosphere microial community. Based on the results attained from the present study, it clear showed that the necessity of fertilizer application is to improve the plant growth and soil nutrient status but the improper usage of agrochemicals had lead to disruption of ecology. The lower concentration of agrochemicals aided the augmentation of soil nutrient status, crop improvement and microbial communities. Although the relevance of agrochemicals at recommended dosage, but there are, long-term application of agrochemicals had led to environmental problems. In turn, the application of biofertilizers (*Azotobacter* sp.) acted friendly to the soil nutrients, plant growth metabolism and rhizosphere microbial communities. The revise put forwarded about the use of microbial bioinoculants for improvement of agricultural productivity and sustainability.

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REFERENCES

Andrade, G., Mihara, K.L. Linderman, R.G., Bethlenfalvay, G.J. 1997. Bacteria from rhizosphere and hyposhere soils of different arbuscular mycorrhizal fungi. plant and soil 192:71-79.

Artursson,V., Finaly, R.D., Jansson, J.K. 2006. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Envn Microbiol 8:1-10

Balota,E.L., ColozziFilho, A., Andrade, D.S., Dick,R.P. 2004. Long –term tillage and crop rotation effects on microbial biomass and C and N mineralization in a Brazilian Oxisol. Soil Till .Res.77:137-145.

Barea, J. M., Azcon-Aguilar, C. and Azcon, R. 2002. Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie van leeuwenhoek 81:343-351.

Bashan .Y. 1999. Interactions of *Azospirillum* spp. in soils-a review. BiolFertil Soils 29:246-256

Bellon, M.R. and J. Berthaud. 2004. Transgenic maize and the evolution of landrace diversity in Mexico. The importance of farmers. *Plant Physiology* 134: 883-888.

Bianciotto, V., and Bonfante, P. 2002. Arbuscular mycorrhizal fungi: a specialised niche for rhizospheric and endocellular bacteria. *Antonie Van Leeuwenhoek* **81:** 365–371.

Buscot, F., Munch, J.C., Charcosset, J.Y., Gardes, M., Nehls,U., Hampp,R. 2000 .recent advances in exploring physiology and biodiversity of ectomycorrhizas highlight the functioning of these symbioses in ecosystems. FEMS Microbiol Rev 24:601-614

Chrost, R.J. 1991. Microbial enzymes in aquatic environments. Springer-verlag, NewYork, USA.

Costa, M.D.; Pereira, O.L.; Kasuya, M.C.M.; Borges, A.C. 2002. Ectomicorrizas: A Face Oculta das Florestas. *Biotecno.Ci. Desenvol.* 29, 38-46.

Daram, P., Brunner, S., Rausch, C., Steiner, C., Amrhein, N., Bucher, M. 1999. Pht2;1 encodes a low affinity phosphate transporter from Arabidopsis. Plant cell 11:2153-2166.

Dick, W.A. 1984. Influence of long term tillage and crop rotation combinations on soil enzyme acitivites. Soil Sci. Soc. Am. J., 48: 569-574.

Duponnois, R.; Garbaye, J. 1991. Effect of dual inoculation of Douglas fir with the ectomycorrhizal fungus *Laccarialaccata* and mycorrhization helper bacteria (MHB) in two bare root forest nurseries. *Plant Soil* 138: 169-176.

Frey-Klett, P.; Chavatte, M.; Clausse, M.L.; Courrier, S.; Le Roux, C.; Raaijmakers, J.; Martinotti, M.G.; Pierrat, J.C.; Garbaye, J. 2005. Ectomycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent pseudomonads. *New Phytol.*165, 317–328.

Galleguillos, C., Aguirre, C., Barea, J.M., Azcon, R. 2000. Growth promoting effect of two sinorhizoiummeliloti strains (a wild type and its genetically modified derviate) on a non –legume plant species in specific interaction with two arbuscular mycorrhizal fungi. Plant sci 159:57-63

Garbaye, J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. *New Phytol.*128:197-210.

Hayes, J.E., Richardson, A.E., Simpson, R.J. 1999. Phytase and acid phosphatase acitivites in roots of temoerate pasture grasses and legumes. Austr. J. Plant Physiology. 26:801-809.

Kai,M., Takazumi,K., Adachi, H., Wasaki,J., Shinano,T., Osaki,M. 2002. Cloning and characterization of four phosphate transporter cDNAs in tobacco. Plant Sci 163:837-846.

Kapoor, K.K., Mishra, M.M., Kukreja, K 1989. Phosphate solubilization by soil microorganisms : A review. Ind J. Microbiol. 29:119-127.

Karthikeyan, A.S., Varadarajan, D.K., Mukatira, U.T., D'Urzo, M.P., Damaz, B., Raghothama, K.G. 2002. Regulated expression of Arabidopsis phosphate Transporters. Plant Physiol. 130:221-233.

Li, M., Shinamo,T., Tadano,T. 1997. Distribution of exudates of lupin roots in the rhizosphere under phosphorous deficient conditions. Soil Sci. Plant Nutr. 43:237-245.

Lugtenberg, B.J., and Dekkers, L.C. 1999. What makes *Pseudomonas* bacteria rhizosphere competent? *Environ* Microbiol**1:** 9–13.

MacDonald, R.M., Lewis, M.1978.The occurrence of some acid phosphatises and dehydrogeneases in the vesicular–arbuscular mycorrhizal fungus *Glomus mosseae*, NewPhytol .80:135-141.

Muchhal, U.S., Pardo, J.M., Raghothama, K.G. 1996. Phosphate transporters from the higher plant *Arabidopsis*. Proc. Natl. Acad. Sci USA. 93:10519-10523.

Mudge, S.R., Rae,A.L., Diatloff,E., Smith,F.W. 2002. Expression analysis suggests novel roles for members of Pht 1 family of phosphate transporters in Arabidopsis. Plant J.31:341-353.

Nakas,J.P., W.D.Gould, D.A.Klein. 1987. Origin and expression of phosphatise acitivity in a semi-arid grassland soil. Soil Biol. Biochem.19:13-18f

Okon,Y. 1994. *Azospirillum*/plant associations. CRC press, Boca Raton, FL.

Plaza, C.R., 2000. Gender roles, inheritance patterns, and female access to land in an ejidal community in Veracruz, Mexico. In: Zoomers, A., van der Haar, G. (Eds.), Current Land Policy in Latin America: Regulating Land Tenure under Neo-

liberalism. Royal Tropical Institute, Amsterdam, pp. 161–173.

Roseland, C.R. 2002. LMOS and the environment: Proceedings of an International Conference Paris: Organization of Economic Cooperation and Development.

Ryan, M. H. and Angus, J. F. 2003. Arbuscular mycorrhizae in wheat and field pea crops on a low P soil: increased Zn-uptake but no increase in P-uptake or yield. *Plant Soil*. **250**:225–239.

Schubler, A., Schwarzott, D. & Walker, C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research 105*, 1413-1421.

Shetty, K. G., Hetrick, B. A. D., Figge, D. A. H. and Schwab, A. P. 1994. Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil. *Environ. Pollut.***86**: 181– 188.

SilvieSalomonova, JarmilaLamacova, MartinRulik, JakubRolcik, LubomirCap, PetrBednar and Petrz Bartak. 2003. Determination of phospholipid fatty acid in sediments. Chemica 42:39-49

Troeh, F. R. and Thompson, L. M. 1993. Soils and Soil Fertility, Oxford University Press Inc., New York. 3-15, 193-253.

Van Dusen, M.E. and J.E. Taylor. 2005. Missing markets and crop diversity: Evidence from Mexico. *Environment and Development Economics* 10(4): 513-531.

Vazquez, M.M., C'Esar,S., Azc'on, R and Barea, J.M. 2000.Interaction between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma)* and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Appl. soil Ecol.15:261-272

Versaw, W.K., Harrison, M.J. 2002. A chloroplast phosphate transporter, PHT2;1 influences allocation of phosphate within the plant and phosphate –starvation responses. Plant cell 14:1751-1766.