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Research Article

INFLUENCE OF ORGANIC MANURES (BIOFERTILIZERS) ON SOIL MICROBIAL POPULATION IN THE RHIZOSPHERE OF MULBERRY (*MORUS INDICA* L.)

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Abstract

The effect of different kinds of organic manures on soil microbial population and mulberry production was assessed. A field experiment was carried out at Periyar EVR College, Tamil Nadu, India in basic soil to study the influence of organic manures on soil bacterial population and mulberry production. The 4 groups of mulberry plants of MR2 variety were biofertilized with FYM, Azospirillum, Phosphobacteria and Vermicompost respectively. The biofertilizers lodged bacteria on the rhizosphere of mulberry plants. When the root microorganism are analyzed Farm yard manure biofertilized mulberry plant root tips had *Gluconacobacter diazotrophicus*, *Bacillus pumilus*, *Pseudomonas putida*, *Bacillus coagulans*, *Bacillus sonorensis*, *Azotobacter chroococcum*; Azospirillum biofertilized mulberry plants root tips had *Bacillus coagulans*, *Azotobacter chroococcum*, *Azotobacter vinelandii*, *Bacillus subtilis* and *Azospirillum brasilense*. Phosphobacteria biofertilized mulberry plant root tips had *Pseudomonas putida*, *Bacillus stearothermophilus*, *Brevibacillus borslelensis* and *Streptomyces thermonitrificans* and vermicompost biofertilized mulberry plant root tips had lodged bacterias like *Bacillus megaterium*, *Bacillus subtilis*, *Gluconacobacter diazotrophicus*, *Pseudomonas putida*, *Azotobacter chroococcum*, *Azotobacter vinelandii*, *Bacillus stearothermophilus*, *Brevibacillus borslelensis* and *Bacillus sonorensis*. Microbiology work reveals luxuriant growth of bacteria in all the biofertilizer treated rhizosphere in the order FYM < Azospirillum < Phosphobacteria < Vermicompost. Increased availability of NPK and other micronutrients were noticed in T4 treated plants compared to other treatments.

Key words: *Azospirillum*; Phosphobacteria; Vermicompost; Biofertilizers; Mulberry.

Introduction

Organic agriculture is a production system which avoids largely excludes the use of synthetically compounded fertilizers, pesticide, fungicides, growth regulators and livestock feed activities (Lampkin, 1990). The organic matter of a particular material may be of the beneficial effects of organic matter on improvement of soil properties and productivity (Dahama, 1999). The organic production system aims at largely to utilize the available biomass of the farm to enrich soil fertility, to supply balanced nutrients to the plants and also to reduce the cost production as well as the environmental pollution (Ramakrishna *et al.*, 2002). Organic amendments not only support sustained crop production, but also improve the soil microbial activity (Dagar and Gautam, 2002). The mulberry leaf yield and quality depends on the soil type, plant variety, availability of plant nutrients and agro-ecological conditions, which reflects on the quality of silk production. Mulberry leaf productivity is highly dependent on plant nutrients like NPK and known to respond well to the addition of organic manures. The chemical fertilizers are becoming costlier day by day due to escalating costs and scarce availability of commodities. The highly intensive mulberry cropping

system causes depletion of nutrients in soil and excess usage of inorganic fertilizers and pesticides caused deleterious effect on soil health.

In this context, supply of major nutrients to mulberry through organic manures and biofertilizers is highly imperative. Organic manures are bulky in nature which supplement the crop with small amounts of major nutrients like NPK and other minor nutrients required by the crop and encourages the proliferation of soil microflora (Lakshmi, V *et al.*, 1977). Introduction of crop benefiting microbial inoculants in the soil play a significant role in the mobilization of various nutrients needed by the crop (Kumar and John, 1990) reported that, application of microbial inoculants in conjunction with organic manures has significantly increased the productivity of mulberry leaf.

With this background the present investigation was carried out to know the effect of major nutrients supplied through organic sources on soil microflora and fertility status of mulberry garden under irrigated condition.

Materials and Methods

A field experiment was carried out during 2012 -2013 in an established irrigated MR2 Mulberry garden planted at a spacing of 50cm × 50cm. The experiment was laid out with 4 treatment of 3 replications each. After the establishment period of mulberry plantation, biofertilizers were applied at 20 Kg/ha/yr.

T1: MR2 variety of mulberry biofertilized with FYM

T2: MR2 variety of mulberry biofertilized with Azospirillum

T3: MR2 variety of mulberry biofertilized with Phosphobacteria

T4: MR2 variety of mulberry biofertilized with vermicompost.

Each treatment consisted of 125 plants. The biofertilizers were used by mixing with powdered FYM and applied near the rhizosphere of mulberry by making 8 to 10 inch deep furrows. Soil nutrient status of different biofertilizers treated mulberry plants were estimated. The soil P^H, Organic Carbon (OC), Electric conductivity (EC), available Phosphorus (P), Potassium (K), available Zinc (Zn), available Copper (Cu), available Iron (Fe), available Manganese (Mn), were recorded as per the standard analytical methods. The microbial inoculants in the rhizosphere of all the four treatments were found out by pour plate method and streak culture method (Fig. 1).

Results and Discussion

Soil microbes in the rhizosphere of mulberry

Biochemical tests for microbial identification revealed that the FYM biofertilized rhizosphere had *Gluconactobacter diazotrophicus*, *Bacillus pumilus*, *Bacillus coagulans* and *Azotobacter chroococcum*. *Azospirillum* biofertilized rhizosphere had *Bacillus coagulans*, *Azotobacter chroococcum*, *Azotobacter vinelandii*, *Bacillus subtilis* and *Azospirillum brasilense*. Phosphobacteria biofertilized mulberry rhizosphere had *Bacillus stearothermophilus*, *Bravibacillus borstelensis*, *Pseudomonas putida* and *Streptomyces thermonitrificans*. Vermicompost biofertilized rhizosphere had *Gluconactobacter diazotrophicus*, *Pseudomonas putida*, *Azotobacter chroococcum*, *Bacillus stearothermophilus*, *Azotobacter vinelandii*, *Bacillus megaterium*, *Brevibacillus borstelensis* and *Bacillus subtilis* (Table 1.).

Yadav and Nagendra Kumar (1989) also opined better N fixing ability of *Azospirillum* contributing to nitrogen economy and soil fertility. Goswami (1997) had also reported that 20 to 400 kg/ha of N can be added to the soil by N fixing bio-inoculants. Similar results were also obtained earlier by Rashmi *et al.* (2007) and Murali *et al.* (2006) who have asserted increased availability of NPK when biofertilizer were integrated with different types of

organics. According to Alemu and Bayu (2005) four years application of FYM at the rate of 10 and 15 kg/ha had increased total N of the soil, organic carbon, available P, K and Mg when compared to the plants lacking FYM application in the 0 – 20 cm soil depth. Similarly Sori *et al.* (2008) found that the mulberry gardens treated with bio-inoculants at 20 kg / ha of *Azotobacter* + 25 kg/ha of *A. awamori* + 20 kg/ha of *T. harzianum* + 75% recommended nitrogen and phosphorus each through chemical fertilizer with full recommended dose of FYM and K had recorded maximum available NPK.

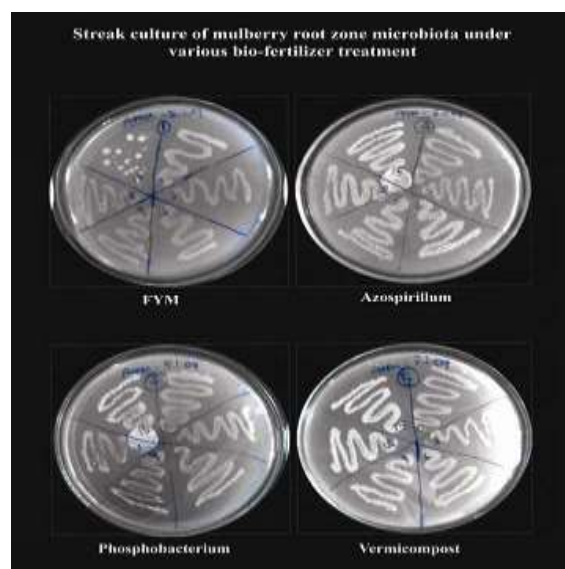


Fig. 1: Streak cultures of mulberry rhizosphere revealed luxuriant growth of bacteria in vermicompost biofertilized soil.

The application of bacterial inoculants as biofertilizers has been reported to result in improved plant growth and increased yield (Bashan and Holguin, 1998 and Vessay, 2003). Gupta *et al.*, (1995) reported that the inoculation with *Azospirillum* sp., *Azotobacter chroococcum* and *Pseudomonas fluorescense* significantly improved seedling emergence rate. *A. chroococcum* was most effective in increasing the total dry weight of root and shoot length.

Gluconacetobacter diazotrophicus is a variety of diazotrophic bacteria have been isolated from rhizosphere and roots of sugarcane plants. In 1998, Cavalcante and Dobereiner reported an acid-tolerant N-fixing bacterium *Acetobacter diazotrophicus*, associated with sugarcane which contributed abundant N to sugarcane crop with a capability to excrete almost half of the fixed N in a form that is potentially available in plants, *G. diazotrophicus* is a gram-negative, acid-tolerant, obligate aerobe and the cells are straight rods with rounded ends (0.7 – 0.9 µm by 1 – 2 µm). The cells can be seen under 48 microscope as single, pair or chain like structures without endospores. Recent studies on association of *G. diazotrophicus* with rice seedlings seem to be encouraging, where a substantial increase in growth of rice plants (ponni) was recorded.

Table 1: Dominant Bacterial species in the Rhizosphere of Biofertilized Mulberry plants

Organism	Bio-fertilizers			
	FYM	<i>Azospirillum</i>	Phosphobacteria	Vermicompost
<i>Gluconactobacter diazotrophicus</i>	+	-	-	++++
<i>Bacillus pumilus</i>	+	-	-	-
<i>Pseudomonas putida</i>	+	-	++++	+++
<i>Bacillus coagulans</i>	+	+	-	-
<i>Azotobacter chroococcum</i>	+	+++	-	+
<i>Bacillus stearothermophilus</i>	-	-	+	+
<i>Azotobacter vinelandii</i>	-	+	-	+
<i>Bacillus megaterium</i>	-	-	-	+++
<i>Brevibacillus borstelensis</i>	-	-	++++	+
<i>Bacillus sonorensis</i>	-	-	-	+
<i>Bacillus subtilis</i>	-	+	-	+++
<i>Azospirillum brasilense</i>	-	+	-	-
<i>Streptomyces thermonitrificans</i>	-	-	+++	-

+ Present; - Absent ; +++ Dominant ; +++++ Abundant

With the increasing cost of chemical fertilizers and concern about environmental pollution, the role of biological nitrogen fixation (BNF) in supplying plants with needed N, which can make agriculture more productive and sustainable without harming the environment has to be harnessed efficiently. As BNF is not restricted to legumes only, for sustainable agriculture it becomes necessary to increase the amount of biologically fixed N in non-legume crops also. Significant nitrogen input into the global nitrogen cycle, has been reported through the Azolla-Anabaena symbiosis (Plazinski, 1989), nitrogen fixation by free living cyanobacteria, *Azotobacter*, *Azospirillum* and *Pseudomonas* species (Krotzky *et al.*, 1987; Cavalcante *et al.*, 1998).

Studies indicate that rhizosphere, roots, stems and leaves of even healthy plants harbour diverse microbial communities that include N fixing bacteria (Boddey *et al.*, 1991) and part of the N accumulated by non-leguminous plants was proved to have been fixed by the root-zone bacteria (Loganathan *et al.*, 1999). A special term diazotrophic associative symbiosis, was suggested to describe the process of N fixation in the plant root zone (Vose and Ruschel, 1982).

Pseudomonas putida is a rod shaped, flagellated, gram negative bacterium that is found in most soil and water habitats where there is oxygen. The surface of the root, rhizosphere, allows the bacteria to thrive from the root nutrients. In turn, the *Pseudomonas putida* induces plant growth and protects the plants from pathogens because *P. putida* assist in promoting plant development, researchers use it in bioengineering research to develop biopesticides and to improve plant health (Espinosa-Urgel *et al.*, 2000).

Azotobacter vinelandii is a diazotroph that can fix nitrogen while grown aerobically. It is a free living N₂ fixer which is known to produce many phytochromes and vitamins in the soil.

Azotobacter chroococcum, was discovered and described in 1901 by the Dutch Microbiologist and Botanist, Martinus Beijerinck. They are found in neutral and alkaline soils (Gandora *et al.*, 1998). *Azotobacter* synthesize some biologically active substances, including some phytohormones such as auxins (Ahmad *et al.*, 2005). They also facilitate the mobility of heavy metals in the soil and thus enhance bioremediation of soil from heavy metals, such as cadmium, mercury and lead (Chen *et al.*, 1995).

Table 2: Data on soil nutrient status of different biofertilizer treated mulberry plots

Name of the parameter	Biofertilizer used				F Test
	FYM	Azospirillum	Phosphobacteria	Vermicompost	
pH	7.06	7.59	7.16	6.89	**
Electrical Conductivity (dsm-1)	0.26	0.36	0.22	0.16	*
Organic carbon (%)	0.22	0.42	0.45	0.56	*
Organic matter (%)	0.44	0.90	0.84	1.03	*
Available nitrogen (kg/ac)	87.6	103.9	106.2	118.5	**
Available phosphorus (kg/ac)	3.56	4.26	6.39	4.78	**
Available potassium (kg/ac)	105	125	146	178	***
Available zinc (ppm)	0.96	1.20	1.26	1.36	**
Available copper (ppm)	0.56	0.85	0.89	1.09	**
Available iron (ppm)	4.58	5.20	5.63	6.25	**
Available manganese (ppm)	2.63	3.20	3.26	3.45	*
Cation exchange capacity (C.mole proton+ / kg)	22.6	28.1	28.9	29.6	*
Exchangeable bases (C. mole proton +/kg)					
Calcium	9.6	12.8	13.0	13.6	**
Magnesium	6.9	8.9	9.2	9.6	**
Sodium	1.26	1.58	1.67	1.68	*
Potassium	0.21	0.28	0.29	0.32	*

F value marked with *, ** and *** are significant at 0.05, 0.01 and 0.001 levels respectively.

Similar results were also obtained earlier by Rashmi *et al.*, (2007) and Murali *et al.*, (2006) who have established the increased availability of NPK when biofertilizers were integrated with different types of organics.

Soil nutrient status of different biofertilizer treated mulberry plots

The results of the present investigation revealed that the pH of FYM soil was 7.06 and pH of biofertilized mulberry plots ranged between 6.89–7.59. This may be due to interaction of biofertilizers in the rhizosphere of soil. Significant variations in mineral nutrients were found. They were found to be highest in vermicompost biofertilized soil, higher in phosphobacteria biofertilized soil, high in *azospirillum* biofertilized soils and low in FYM biofertilized soils. Available nitrogen ranged 87.6 – 118.5 kg/ac, available phosphorus 3.56 – 6.39 kg/ac and potassium 105 – 178 kg/ac. Available manganese ranged 2.63 – 3.45 ppm, available iron ranged 4.58 – 6.25 ppm (Table 2), available zinc ranged 0.96 – 1.36 ppm, available copper ranged 0.56 – 1.09 ppm (Table 2). The data revealed that the microbial

inoculants in the biofertilizers influenced the available nutrients status in the rhizosphere of soil. Similar results were reported by Sharma *et al.*, (2002).

According to Alemu and Bayu (2005), four years application of FYM at the rates of 10 and 15 t ha⁻¹ increased the soil total N, organic carbon, available P, K and Mg compared to the plot lacking FYM application in the 0 – 20 cm soil depth. Similarly, according to Sori *et al.*, (2008) the mulberry gardens treated with bio-inoculants at 20 kg/ha of *Azotobacter* + 25 kg/ha of *A. awamori* + 20 kg/ha of *T. harzianum* + 75 percent recommended nitrogen and phosphorus each through chemical fertilizer with full recommended dose of FYM and K has recorded maximum available NPK. The increased availability of NPK in soil may be attributed to better nitrogen fixing capacity of *Azotobacter*, solubilisation and mobilization by *A. awamori*

and *T. harzianum* in the presence of FYM and Inorganic Fertilizer (IF).

Conclusion

From the above findings it is concluded that application of different biofertilizers plays a significant role in enhancing the soil fertility in terms of macro-nutrients, secondary nutrients and microbial population. The microbial population helped in enriching the soil with major nutrients like N and P which are mainly essential for luxuriant growth of plants.

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