

Research Article

Synergistic Effect of Biofertilizers and Inorganic Fertilizers on Soil Microbial Activities in Rhizospheric Soil of *Eucalyptus*

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Integrated application of biofertilizers and inorganic fertilizers has potential to improve physical, chemical and biological characteristics of soil. The present investigation was carried out to evaluate the impact of biofertilizers and inorganic fertilizers on microbial activities in rhizospheric soil of *Eucalyptus* clone 413. Significantly higher total bacterial population and PGPR population was observed in treatment having consortium biofertilizer along with inorganic fertilizers, however highest bacterial population was found at 180 DAP and PGPR population at 60 DAP. Actinomycetes population was found to be higher in treatments having inorganic fertilizers alone. Significantly higher population of fungi, diazotrophs and PSB was found in treatments having PSB + N-75% P-100%, *Azotobacter* + N-100% P-100% and PSB + N-100% P-75% at 120 DAP, 60 DAP and 180 DAP respectively. Among rhizospheric soil enzyme activities, alkaline phosphatase activity was observed to be significantly higher in PSB + N-100% P-75% treatment at 180 DAP while dehydrogenase activity was recorded higher in treatment with consortium biofertilizer + N-100% P-75% at 180 DAP.

Urease activity also showed a variable pattern during different time intervals. The results indicated that the combined application of biofertilizers and inorganic fertilizers resulted in improved soil microflora which consequently boosts the soil health.

Keywords: Consortium, Enzyme, *Eucalyptus*, Microflora, Soil

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Introduction

Evergreens are trees that have foliage year around and are best known for enduring cold weather and dry seasons. *Eucalyptus camaldulenesis* is an evergreen tree belonging to family Myrtaceae which can grow upto 30 m by 20 m at a fast rate. It is capable of growing in sandy, loamy and clay soil. In spite of having medicinal use, it is used in paper and timber industries as well [1].

Chemical fertilization, especially NPK promotes the vegetative growth of tree seedlings. Over utilization of chemical fertilizers can bring about destruction, for example, leaching, contamination of water assets, devastation of microbes, crop susceptibility to disease, fermentation or alkalization of the soil or decrease in soil fertility — subsequently making unsalvageable harm to the general framework of soil. Supplements are effortlessly lost from soils through leaching or gas release and can result in lessened fertilizers effectiveness [2]

Biofertilizers are microbial inoculants used for application to either seeds or soil for expanding soil health with the target of expanding the quantity of such microorganisms and to quicken certain microbial procedures. Such microbiological procedures can change inaccessible types of supplements into accessible ones that can be effortlessly acclimatized by plants [3]. The process of organic matter degradation and nutrients availability to plant is organized by soil microbes. For sustainable agriculture, microbes play a significant role in managing all the dynamics in soil. Biofertilizer may contribute to plant development; compost dosages may decrease and extra supplements may be acquired from the soil [2]. So keeping all the points in concern, the study was proposed to evaluate the combined effect of biofertilizers and inorganic fertilizers on microbial dynamics of rhizospheric soil of *Eucalyptus*.

Materials and Methods***Experimental Design and Treatments***

The pot experiment was conducted during summer and rainy season of 2016 in experimental area of Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana, India. The experiment was laid in triplicate using clone 413. A total of 16 different fertilizer combinations were made. The experiment was conducted by following package and practices of PAU, using different nutrient levels of recommended doses of inorganic

fertilizers (nitrogen and phosphorus) in combination with biofertilizers (*Azotobacter*, PSB and consortium biofertilizers). Two levels of nitrogen and phosphorus fertilizers viz 75% and 100% of recommended dose of N and P were applied to soil in polybags in combination with each biofertilizers. The complete recommended dose (100%) of P and N fertilizers revealed 60 kg P₂O₅/ acre and 50 kg urea/acre respectively. Phosphorus fertilizer (75% and 100%) and biofertilizers were applied to poly bags at the time of plantation whereas nitrogen fertilizer was introduced in the treatments at 120 DAP.

Treatments			
T1	N (75%) and P (75%)	T9	T1 + PSB biofertilizer
T2	N (75%) and P (100%)	T10	T2 + PSB biofertilizer
T3	N (100%) and P (75%)	T11	T3 + PSB biofertilizer
T4	N (100%) and P (100%)	T12	T4 + PSB biofertilizer
T5	T1 + <i>Azotobacter</i> biofertilizer	T13	T1 + Consortium biofertilizer
T6	T2 + <i>Azotobacter</i> biofertilizer	T14	T2 + Consortium biofertilizer
T7	T3 + <i>Azotobacter</i> biofertilizer	T15	T3 + Consortium biofertilizer
T8	T4 + <i>Azotobacter</i> biofertilizer	T16	T4 + Consortium biofertilizer

Soil Sampling and Analysis

Soil samples were collected with auger from rhizospheric soil (0-15 cm) of *Eucalyptus* grown at the experimental area at different time intervals- 0, 60, 120 and 180 DAP (Days after Plantation). Enumeration of bacteria, fungi, actinomycetes, diazotrophs, PGPR (Plant Growth Promoting Rhizobacteria) and PSB (Phosphorous solubilizing bacteria) was done on Nutrient Agar medium, Glucose Yeast Extract medium, Kenknight's medium, Jensen's medium, King's B medium and NBRIP medium respectively, using serial dilution spread plate technique. Activities of soil enzymes as: alkaline phosphatase by the method of Tabatabai and Bremner 1969, urease by the method of Douglas and Bremner 1971 and dehydrogenase by the method of Tabatabai 1982 was studied. The soil samples were dried, crushed and sieved to perform the enzyme activities assay.

A two-factor analysis of variance (ANOVA) was performed to determine the effect of different fertilizer treatments, stages of plant growth and their interaction on soil microbial population and enzyme activities. Correlation among different microbial population and enzyme activities was executed. For statistical analysis of data, CPCS1 and SPSS 16.0 software were used. The level of significance referred in the results is $P < 0.05$.

Results and Discussion

Microbial Population

The results obtained indicated that microbial population and soil enzyme activities were significantly affected by biofertilizer inoculation. The initial microbial population of soil was determined at zero day of the experiment i.e. before planting. Microbial population enumerated at zero day of the experiment included bacterial population (168×10^7 CFU/g of soil), fungal population (3×10^3 CFU/g of soil), actinomycetes (84×10^4 CFU/g of soil), diazotrophs (12×10^4 CFU/g of soil), PSB (7×10^4 CFU/g of soil) and PGPR population (113×10^4 CFU/g of soil).

Bacteria

The total bacterial count was found maximum (190×10^8 CFU/ g of soil) at 180 DAP in treatment with consortium biofertilizer + inorganic fertilizers, which was statistically higher than uninoculations and all other fertilization treatments as shown in **Table 1**. The increase in bacterial population at 60 DAP with biofertilization may be due to supplementation of soil with beneficial bacteria through biofertilizer inoculation which supported bacterial growth due to their role in phytohormone production, detoxification of soils contaminated with heavy metals and high salt levels, extracellular polysaccharide synthesis and other processes[4]. The increase in bacterial population from 120 DAP to 180 DAP may be due to establishment of bacterial population with increased rhizodeposits and availability of nutrients [5].

Fungi

The maximum count of fungal population (22×10^4 CFU/ g of soil) was observed in soil samples treated with PSB + N-75% P-100% at 120 DAP. The fungal population was found to be highest at 120 DAP due to higher litterfall during this period which resulted in increase of soil moisture content and might have contributed to the increase in the soil

organic matter and organic carbon contents. A decrease was observed at 180 DAP [6]. Fungal count was found to be lower in treatments having *Azotobacter* as compared to the control because of its antagonistic relationship with some fungi [7]. However, inorganic fertilizers seemed to have regressive effect on growth of fungal strains which can be related to production of toxic metabolites from mineral nitrogen [8].

Table 1 Microbial populations in different treatments at different time intervals in rhizospheric soil of *Eucalyptus*

DAP	Treatments	Bacteria × 10 ⁸ CFU/g soil	Fungi × 10 ⁴ CFU/g soil	Actinomycetes × 10 ⁵ CFU/g soil	Diazotrophs × 10 ⁵ CFU/g soil	PSB × 10 ⁴ CFU/g soil	PGPR × 10 ⁵ CFU/g soil
60	T1	85±3.60	10±4.35	33±1.73	38±4.32	13±4.35	52±1
	T2	95±1	15±1.73	25±1	40±2	9±2.64	54±7
	T3	98±2	12±2	36±1	45±4.58	12±2.64	55±5.29
	T4	100±1	16±1.73	28±1.73	48±1.73	10±6.24	58±4.58
	T5	142±2.64	12±2.64	16±1.732	78±2.64	15±3.60	72±1.73
	T6	150±2	9±3	10±6.24	84±3.60	16±1.73	85±5.56
	T7	164±1.73	15±1	13±1.732	84±5.29	18±5.29	76±2
	T8	168±3.60	13±2.64	12±2.64	89±7.39	19±1.73	82±4.35
	T9	130±1.73	12±2.64	19±3.60	42±2	24±4.35	56±2
	T10	115±2.64	16±1	15±2.64	58±2.64	17±3.60	58±3.60
	T11	134±1.73	14±1.73	20±4.22	45±2.64	22±9.53	55±4.58
	T12	120±2	19±2.64	18±7.87	65±3.60	18±5.29	62±3
	T13	162±2	14±2.64	11±8.71	74±3.60	20±1.73	77±3.60
	T14	172±2	16±2.64	8±1.16	78±4.58	19±3.60	84±5.29
	T15	180±6.24	15±1	12±1	84±6.08	23±1.73	72±5.29
	T16	184±1.73	18±4.58	9±2.64	86±3	16±4.58	86±1
120	T1	60±2	14±1.73	42±2	32±2.64	16±3.60	45±1
	T2	74±2.64	18±1	45±1	35±2.64	15±2.64	48±1.73
	T3	94±2.64	18±2.64	48±3	38±2.64	19±1	50±4.35
	T4	95±1	19±1	39±1	40±2.64	12±1.73	52±2
	T5	125±2.64	10±1.73	18±5.29	65±2.64	20±6.24	67±1.73
	T6	134±2	8±2.64	12±1.73	77±1.73	21±5.56	71±3.60
	T7	129±1	9±3.60	16±1	68±2.64	22±5.29	65±2.64
	T8	139±3.60	7±3.60	15±1.73	75±3.60	24±2	74±3.46
	T9	112±2	18±4.58	25±4.35	40±4.58	35±2.64	58±1.53
	T10	95±1	22±3.46	20±2	52±2.64	27±2.64	62±1.73
	T11	120±1.73	19±3.60	24±1.38	42±2	33±1	57±1.73
	T12	98±3	20±3.46	22±2.68	60±5.56	25±2.64	64±5.29
	T13	153±1.73	17±1	14±1.73	67±1	26±1.73	68±6.08
	T14	164±1.73	17±3.60	12±2.64	69±1	24±1.73	71±1.73
	T15	158±4.35	18±2.64	15±7	72±5.29	29±1.73	63±4.58
	T16	178±2	20±2	11±4.58	78±1.73	28±3.60	72±2
180	T1	98±3	12±2	25±3.60	20±2	25±4.58	54±1
	T2	100±2.64	16±1.73	27±1.73	22±2	20±2.64	55±2
	T3	108±3.60	15±1	23±1.73	23±4.35	28±3.60	57±2.64
	T4	112±2	18±3.60	22±2.64	25±1	24±5.29	58±3.60
	T5	138±3.60	13±1	11±1	38±4.58	30±5.29	65±1.03
	T6	144±1.73	14±1.73	8±1.73	34±1.73	28±4.35	62±5.29
	T7	135±1	15±1	14±2.64	30±2	34±5.29	58±7.9
	T8	140±2	17±3.60	13±1.73	35±1.73	32±2.64	62±3.46
	T9	129±4.58	14±3.60	15±3.60	30±2	35±4.35	64±1.73
	T10	132±1	15±1	13±1.74	28±2	34±5.29	68±1
	T11	148±2.64	17±2	11±1.92	24±1.73	48±6.08	71±1
	T12	138±5.29	20±5.29	12±6.08	25±1.73	40±5.29	75±3.60
	T13	174±5.29	14±2.64	14±2.64	50±7.21	38±5.29	78±1.93
	T14	178±2.64	16±4.58	11±4.58	52±2	35±2.64	77±5.29
	T15	186±2.64	17±3.60	10±4.35	48±4.58	44±2.64	81±1
	T16	190±1.73	19±3.60	9±3.60	45±3.60	42±2.64	84±2
CD@5%							
Treatments		2.58	2.62	7.12	3.26	3.89	4.23
Days		1.11	1.13	3.08	1.41	1.68	1.83
Interactions		4.46	4.54	NS	5.65	NS	7.34

*All values represent mean of three replications

Actinomycetes

Highest population of actinomycetes (48×10^5 CFU/ g of soil) was observed in treatment having inorganic fertilizers alone at 120 DAP. The adversary effect of microbial inoculants on actinomycetes growth may be attributed to secretion of antibiotics by inoculated PGPR strain, such as 2, 4-diacetylphloroglucinol which exhibits antifungal properties. The decrease in actinomycetes population at 60 DAP and 180 DAP may be due to progressive increase in PGPR population. Significantly higher actinomycetes population in treatment without any fertilization can be supported by the fact that they are efficient decomposers of nutrient poor carbon sources and exhibit high activity in nitrogen limited soils [9, 10].

Diazotroph

Maximum diazotrophic population (89×10^5 CFU/ g of soil) was observed at 60 DAP in treatment having *Azotobacter* + N-100% P-100%. The reason behind high population at 60 DAP may be attributed to the fact that nitrogen fertilizer was not added. Moreover, the fertilization supplements the soil with high amount of organic matter leading to increased carbon and energy sources for rapid diazotroph growth. The increasing mineral nitrogen rates significantly decreased *Azotobacter* population at 180 DAP [11].

Phosphate solubilizing bacteria

The results indicated increase in PSB population with biofertilizer inoculation (Table 1). Maximum PSB population (48×10^4 CFU/ g of soil) was observed in treatment with PSB + N-100%-P-75% at 180 DAP. The PSB population in treatments with biofertilizer inoculation was significantly higher than treatments without biofertilizer inoculation. This may be attributed to the supplementation of PSB to the soil by the addition of PSB and consortium biofertilizer. Treatments containing inorganic sources of phosphorus had significantly lower PSB population because of the inhibitory effect of inorganic phosphate applied via chemical fertilizer on the growth of PSB. Maximum population was observed at 180 DAP owing to the establishment of microflora in the rhizosphere.

Plant Growth Promoting Rhizobacteria

Significantly higher PGPR population (86×10^5 CFU/ g of soil) was observed in treatments with biofertilizer inoculation in comparison with treatments without biofertilizer inoculation (Table 1). This may be due to various activities associated with microbial inoculants such as regulation of soil nitrogen regime, phytohormone production, detoxification of soils contaminated with heavy metals and high salt levels, extracellular polysaccharide synthesis and other processes [12, 13, 14]. Higher PGPR population in treatments having combination of biofertilizers and inorganic may be due to the fact that bacteria are the most delicate variety of microorganisms which can proceed quickly to the ecological changes than other varieties of microorganisms.

Enzyme Activities

Soil enzymes are indirect indication of the microbial activities of the soil. It is considered to be a major contributor of overall soil microbial activity. In the present research, the effect of incorporation of biofertilizers and inorganic fertilizers on the soil biological activity in terms of, dehydrogenase activity, alkaline phosphatase activity and urease activity was studied in *Eucalyptus* rhizospheric soil at different time intervals under nursery conditions.

Dehydrogenase

The combined use of biofertilizers and inorganic manures noticeably affected soil dehydrogenase activity. The highest dehydrogenase activity ($8.83 \mu\text{g TPF formed/min/g}$ of soil) was observed in treatment having consortium + N-100%- P-75% at 180 DAP. The results indicated that dehydrogenase activity was higher in treatments having high bacterial population (**Figure 1a**), the reason being that dehydrogenase activity is only present in viable cells and it is considered to reflect the total range of oxidative activity of soil microflora [15]. The treatments having integrated application of consortium biofertilizer and inorganic fertilizers were observed with higher dehydrogenase activity as compared to soil with inorganic fertilizers alone. This may be due to stimulation of bacterial population by incorporation of biofertilizers.

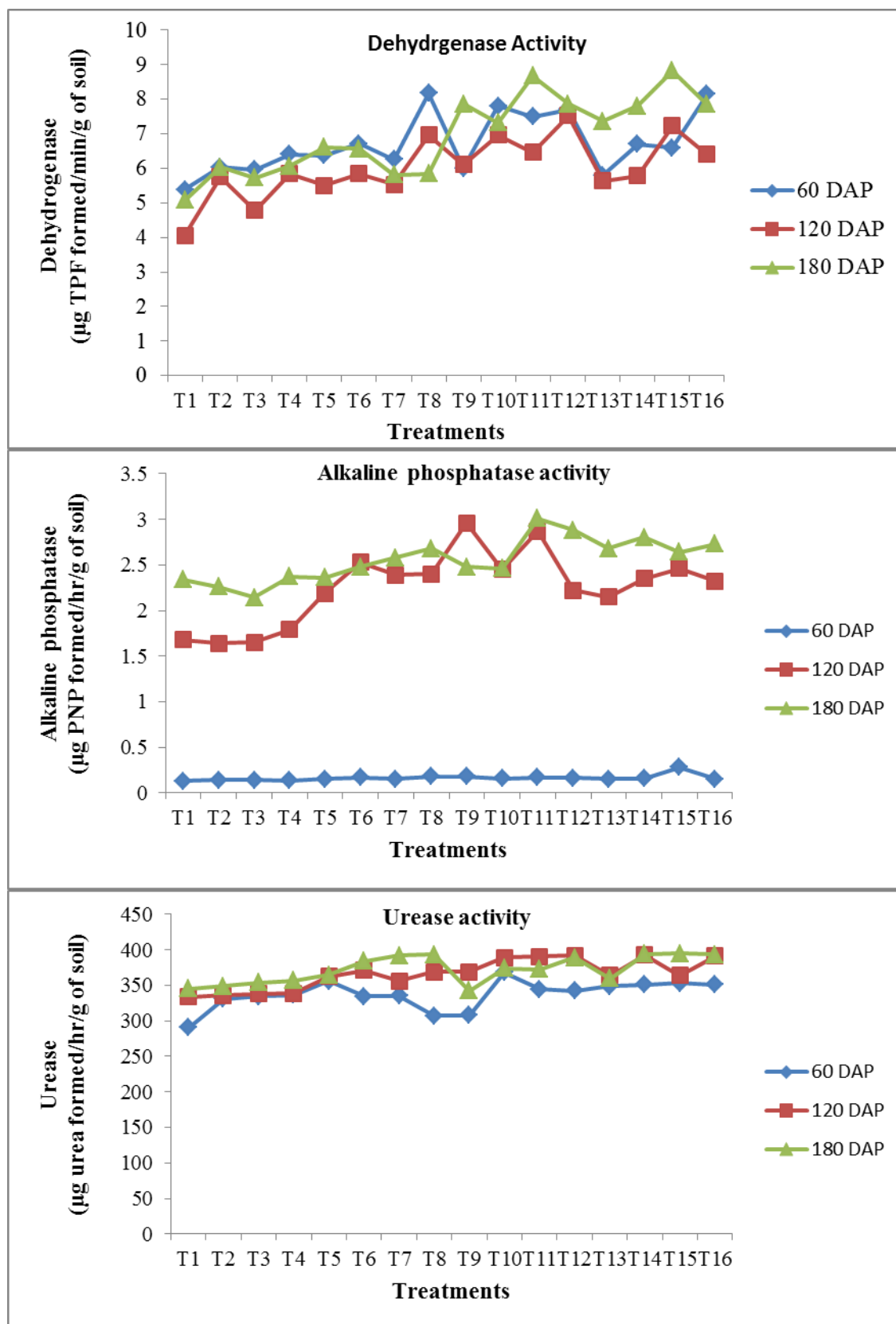


Figure 1 (a-c) Enzyme activities in different treatments at different time intervals

Alkaline phosphatase

The results indicated that alkaline phosphatase activity was significantly higher in treatments having biofertilizers inoculations as compared to uninoculated treatments (Figure 1b). Maximum activity of alkaline phosphatase (3.01 μg PNP formed/hr/g of soil) was observed in treatment with PSB + N-100%-P-75% biofertilizer at 180 DAP. Alkaline

phosphatase activity was found to be positively correlated to PSB population. The treatments having PSB biofertilizer and inorganic fertilizers showed higher alkaline phosphatase activity as compared to treatments with inorganic fertilizers alone. This might be due to the reason that microbes continuously produce and secrete the enzyme necessary for degradation of their substrate. [16]. There was increase in enzyme activities with corresponding increase in microbial population.

Table 2 Correlation between microbial population and enzyme activities at (a) 60 DAP (b) 120 DAP (c) 180 DAP

	Bac	Fungi	Actino	Diazo	PSB	PGPR	Deh	Phos	Urease
(a) 60 DAP									
Bac	1	.118	-.699**	.748**	.568**	.757**	.179	.456**	.261
Fungi		1	-.110	.124	.096	.016	.321*	.036	.194
Actino			1	-.665**	-.552**	-.647**	-.279	-.227	-.230
Diazo				1	.266	.894**	.297*	.313*	.149
PSB					1	.194	.150	.366*	.395**
PGPR						1	.182	.187	.202
Deh							1	.194	.401**
Phos								1	.201
Urease									1
(b) 120 DAP									
Bac	1	-.132	-.725**	.818**	.478**	.735**	.281	.447**	.245
Fungi		1	.274	-.343*	.200	-.267	.1b00	-.056	.052
Actino			1	-.767**	-.472**	-.851**	-.508**	-.540**	-.300*
Diazo				1	.223	.866**	.288*	.310*	.229
PSB					1	.349*	.524**	.725**	.214
PGPR						1	.458**	.463**	.180
Deh							1	.448**	.161
Phos								1	.020
Urease									1
(c) 180 DAP									
Bac	1	.206	-.638**	.866**	.666**	.858**	.647**	.618**	.673**
Fungi		1	-.096	-.042	.254	.284	.180	.340*	.317*
Actino			1	-.533**	-.476**	-.498**	-.513**	-.426**	-.581**
Diazo				1	.350*	.712**	.401**	.358*	.491**
PSB					1	.710**	.738**	.606**	.426**
PGPR						1	.676**	.618**	.530**
Deh							1	.520**	.344*
Phos								1	.597**
Urease									1

. Correlation is significant at the 0.01 level (2-tailed).* **. Correlation is significant at 0.05 level (2-tailed)*Bac: Bacterial population; Actino: Actinomycetes population; Diazo: Diazotrophic population; PSB: Phosphate Solublising Bacteria; PGPR: Plant Growth Promoting Rhizobacteria; Phos: Alkaline Phosphatase activity; Deh:Dehydrogenase

Urease

The effect of biofertilizers and inorganic fertilizers on enzyme activity indicated that the urease activity showed variable pattern during the different time intervals as shown in Figure 1c. Urease activity is dependent on soil organic matter content as well as nitrogen levels. Among the entire time interval, significantly higher urease activity was observed at 180 DAP since urea was added [16]. Urease enzyme activities are highly related to soil organic matter content and microbial activity of the soils. The urease activity was observed to be positively correlated with bacterial population and available nitrogen in soil. This might be due to the reason that urease enzyme is responsible for the hydrolysis of urea fertilizer thus its activity increases with increase in the level of N-fertilizer in the form of urea.

Correlation between microbial population and enzyme activities

At 60 DAP, bacterial population was highly correlated to phosphatase ($r=0.456$), urease ($r=0.261$) and dehydrogenase ($r=0.179$) activities (**Table 2a**). Fungal population was positively correlated with phosphatase ($r=0.036$), dehydrogenase ($r=0.321$) and urease activity ($r=0.194$). Actinomycetes population was negatively correlated with alkaline phosphatase ($r=0.227$), urease ($r=0.230$) and dehydrogenase ($r=0.279$) activities.

At 120 DAP, correlation between microbial population and enzyme activities shows that bacteria, PSB and PGPR populations were strongly correlated with all the three enzyme activities viz., alkaline phosphatase, urease and dehydrogenase (Table 2b). Fungal population was negatively correlated with phosphatase ($r=0.056$). However, actinomycetes population showed negative correlation with enzyme activities [17].

At 180 DAP, bacterial population was highly correlated to phosphatase ($r=0.618$), urease ($r=0.673$) and dehydrogenase ($r=0.647$) activities (Table 2c). Fungal population was positively correlated with phosphatase ($r=0.340$) and dehydrogenase ($r=0.180$) activities. Actinomycetes population was negatively correlated with alkaline phosphatase ($r=-0.426$), urease ($r=-0.581$) and dehydrogenase ($r=-0.513$) activities [18].

Conclusion

It was concluded that microbial inoculants had huge effect on dynamics of rhizospheric soil. The application efficiency of chemical fertilizer with biofertilizers showed significant increase in soil microbial activities over the uninoculated treatment. In addition to that, it was obvious that NP fertilizer was more efficient when mixed with biofertilizers due to its characters and enrichment of nutrients in comparison with biofertilizers or NP fertilizer alone. This depicts the synergistic effect of biofertilizers and inorganic fertilizers. Therefore, the combined application of biofertilizers and inorganic fertilizers helps in improving the soil health leading to increased soil fertility.

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