Research Article

Influence of Biofertilizers and Inorganic Fertilizers on Soil Microbial Population and Enzyme Activities in Rhizosphere of Poplar

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Abstract

Sustainable agriculture involves optimizing the benefits from biological and inorganic inputs through their interactive application for the maintenance of soil health and productivity. The present study was aimed to investigate the synergistic effect of inorganic fertilizers (75% and 100% of recommended dose of nitrogen and phosphorus fertilizer) and biofertilizers (*Azotobacter*, PSB Consortium) on microbial population and enzyme activities in the rhizospheric soil of Poplar under nursery conditions. The results indicated significantly higher bacterial, fungal and PGPR population under conjoint application of consortium biofertilizer with recommended dose of fertilizers. Soil actinomycetes population improved where only inorganic fertilizers were applied as uninoculated treatments. Statistically higher diazotroph and PSB population was observed when *Azotobacter* and PSB biofertilizer respectively; were used as inoculum with inorganic fertilizers. Among the rhizospheric soil enzyme activities, statistically higher dehydrogenase and urease activity were recorded under Consortium biofertilizer + 100 % of NP fertilizer at each time interval. However, the highest activity of phosphatase enzyme varied among the treatments with consortium inoculation. The correlation analysis revealed significant positive interaction between microbial population (except actinomycetes) and the enzyme activities at different time intervals. It is concluded that integrated use of inorganic fertilizers and microbial inoculants was markedly effective in improving rhizospheric microbial dynamics of Poplar than sole application of inorganic fertilizers. The improved soil fertility might contribute in nutrition and rapid growth of Poplar, providing quality nursery stock which could establish better in fields and satisfy the demands of wood.

Keywords: Biofertilizer, Consortium, Enzyme activities, Microbial population, Poplar

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Introduction

Tree plantations are known to improve soil properties through the addition of organic matter via litter fall and translocation of nutrients to surface soil through their deep and extensive root systems. Now a day, growing trees in combination with agricultural crops for augmenting biomass production has also become popular among farmers [1]. Poplars (*Populus deltoides*) are among the commercial plantations of forestry as they provide leaves as fodder for livestock, timber and potentially fibre for making paper. Poplar based agroforestry systems are economically viable and more profitable due to their rapid growth, multipurpose soft wood, less competition with associated crops and stress tolerant nature [2].

Growth of trees is determined by quality of planting stock, climate and management practices. Chemical nitrogen and phosphorus fertilizers are common artificial inputs to supplement soil nutrients but they do little to improve soil texture, stimulate soil biota and benefit long term soil fertility. Moreover, the inappropriate dosage of chemical fertilizers can degrade the natural nutrients of soil by inhibiting the growth of beneficial microbes [3]. Biofertilizers based on renewable energy sources is a sustainable and cost effective supplement to chemical fertilizers. Biofertilization involves the introduction of specific living microorganisms which add, conserve and mobilize plant nutrients in the soil [4]. These biological agents can release deposited nutrients from inorganic fertilizers as well as degrade their toxic chemical residues. This forms the basis of more reliable and an interactive method of nutrient supply based on combination of chemical and biological fertilizers to maintain soil fertility and plant nutrient supply [5]. The efficiency of any fertilization system can be determined by its influence on overall rhizospheric interactions in a plant- soil system. It is useful to target the changes in soil microbial population and their activities to analyse the effect of different sources and amount of fertilizers on soil properties during experimental trials [6]. This study was conducted by keeping all these points in concern to evaluate the effect of combined application of biofertilizers and different doses of inorganic fertilizers on microbial dynamics in rhizosphere of Poplar (clone PL-5).

Materials and Methods

Experimental Design and Treatments

The present investigation was carried out at the nursery site of Department of Forestry and Natural Resources, PAU, Ludhiana, India during 2016 to study the effect of biofertilizers with inorganic fertilizers on microbial population and enzyme activities in the rhizospheric soil of Poplar. Initially Poplar cuttings (clone PL-5) of same age and length (5 inches) were drawn from one year old plantation from the nursery. These were planted in polybags using nursery soil and later shifted to pots after 120 DAP (Days After plantation). The experiment was laid down with sixteen treatments (given below) and three replications in completely randomized design. Two levels of nitrogen and phosphorus fertilizer (75% and 100%) were arranged in four combinations and each of them was applied alone and in conjunction with different biofertilizers (*Azotobacter*, PSB and Consortium) to the soil in polybags. The complete recommended dose (100%) of P and N fertilizers revealed 60 kg P_2O_5/ acre and 50 kg urea/ acre respectively. Phosphorus fertilization was done at the time of plantation whereas doses of nitrogen fertilizer were incorporated after 120 DAP. Five grams of each biofertilizer having 10^8cells/g was introduced to respective polybag soil at the time of plantation. Both inorganic and biofertilizers were applied in a ring around the cutting at depth of 10 cm which were mixed in soil and covered.

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 + *Azotobacter* biofertilizer	T13	T1 + Consortium biofertilizer
T6	T2 + *Azotobacter* biofertilizer	T14	T2 + Consortium biofertilizer
T7	T3 + *Azotobacter* biofertilizer	T15	T3 + Consortium biofertilizer
T8	T4 + *Azotobacter* biofertilizer	T16	T4 + Consortium biofertilizer

Soil Sampling and Analysis

Soil samples were collected from each replication of the treatments at 60, 120 and 180 DAP with the help of auger without harming the cutting. Sampled soil was air dried and enumerated for bacteria, fungi, actinomycetes, diazotrophs, PSBs and PGPRs on Nutrient agar, Glucose yeast extract medium, Kenknight's medium, Jensen's medium, NBRIP and Kings B medium respectively, using serial dilution spread plate technique. Soil enzyme activities as: dehydrogenase activity [7], alkaline phosphatase activity [8] and urease activity [9] were estimated. The data was analysed to compare sixteen treatment means by following completely randomized design technique and correlation between microbial population and enzyme activities was analysed using SPSS [10].

Results and Discussions

Microbial Population

Soil microbial population was enumerated before plantation as: bacteria (168 x 10^7 CFU/g of soil), fungi (3 x 10^3 CFU/g of soil), actinomycetes (84 x 10^4 CFU/g of soil), diazotroph (12 x 10^4 CFU/g of soil), PSB (7 x 10^3 CFU/g of soil) and PGPR (113 x 10^4 CFU/g of soil) to evaluate the effect of different treatments. The results indicated that fertilization had improved microbial population and it was significantly higher when inorganic fertilizers were applied in combination with biofertilizer.

Bacteria

Significantly higher bacterial population was recorded in the rhizosphere of inoculated treatments than uninoculated treatments at each time interval (**Table 1**). This could be attributed to the establishment of inoculated microbial species and their activities like phyto-hormone production, heavy metal detoxification and secretion of polysaccharides which might have favoured growth of indigenous bacterial population [11]. Highest bacterial count (195 x 10^8 CFU/g of soil) was recorded in treatment T16 ($N_{100}P_{100}$ + Consortium biofertilizer) at 180 DAP. The synergistic effect of coinoculated species on soil bacterial population in addition to the indirect effect of N fertilizer on improved soil organic returns could be explanatory for this [12].

Table 1 Microbial population in different treatments at different time intervals in rhizospheric soil of Poplar

Treatment	Bacteria ($x10^8$ CFU g^{-1} soil)			Fungi ($x10^4$ CFU g^{-1} soil)			Actinomycetes ($x10^5$ CFU g^{-1} soil)		
DAP	60	120	180	60	120	180	60	120	180
T1	87±3.5	58±1.7	98±3.6	11±2	14±2	12±2	35±5.2	51±2.6	32±3.5
T2	107±2.6	94±3.5	112±1.7	13±3.6	18±2.6	15±3	28±4	43±1.7	26±4.4
T3	91±1.5	61±2	115±3	10±1.7	16±4.6	14±1.7	33±3.5	49±5.6	24±2.0
T4	110±1.2	97±3.6	121±6.1	15±3	19±1	17±2.6	27±3	45±4.6	23±2.6
T5	143±2.5	132±4.6	152±2	09±5.3	13±4.6	14±3	15±2	17±2.6	13±1.7
T6	155±3.6	145±4.2	159±5.9	07±3.5	09±1.7	11±2	12±2.6	12±3.5	11±2
T7	145±3.1	139±3.6	147±2.3	11±1	14±2	17±3.6	13±1.5	15±3.6	10±4.4
T8	157±2.6	148±4	151±5.3	10±2.6	11±3.5	15±2	09±5.3	13±2	06±3.6
T9	134±1.7	124±2.6	136±3.8	13±1.7	17±2.6	14±1.7	17±2	24±3	15±2
T10	125±3.6	115±4.4	128±2.6	17±2	22±3	18±2	14±2.6	20±4	12±2.6
T11	136±2.1	122±3.5	144±1.5	15±3.5	20±1.7	16±3.6	18±2	26±3.5	11±1.7
T12	127±3.1	117±4	139±3.1	18±3.6	23±2.6	21±1.7	12±1.7	19±2.6	09±3
T13	167±1.5	153±3	172±3.6	16±4.4	18±1	16±3.5	13±3.6	14±3.6	11±2
T14	189±2.6	178±3.1	180±2.6	20±4	27±3.5	23±2	10±4.4	12±3	08±3.5
T15	168±1	155±1.7	187±3	17±1.7	20±3.6	19±2.6	11±2	16±1.7	09±2
T16	191±2	183±3.1	195±3.1	22±2	29±1.7	25±1.7	09±1	11±2.6	05±1.7
CD@5%	Treatments:2.97			Treatments:1.98			Treatments:2.41		
	Days:1.28			Days:0.85			Days:1.04		
	Interaction:5.14			Interaction:3.43			Interaction:4.17		
Treatment	**Diazotroph ($x10^5$ CFU g^{-1} soil)**			**PSB ($x10^4$ CFU g^{-1} soil)**			**PGPR ($x10^5$ CFU g^{-1} soil)**		
DAP	60	120	180	60	120	180	60	120	180
T1	18±1.7	16±1.7	14±1.7	12±2	15±1.7	17±2.6	74±1	68±4	74±1
T2	24±2.5	20±3	17±1.5	08±3	11±4.4	14±3	91±3.5	87±1.7	91±3.5
T3	20±3.6	18±2.3	12±1	13±3.6	17±2	21±2	89±3.1	72±2.1	89±3.1
T4	27±1	23±2.1	15±1.7	10±2.6	13±2.6	18±1.7	104±1.7	91±3.6	104±1.7
T5	65±2	58±1	43±2	16±1.7	22±1.7	25±3.6	146±3	125±2.3	146±3
T6	73±2.6	66±2.5	47±2.9	12±2	18±1	23±4.4	152±1.7	140±3.5	152±1.7
T7	68±1	62±3.5	32±1.7	17±3.6	24±3	27±2.6	158±2.6	127±3.5	158±2.6
T8	77±1.7	71±2	36±3	14±1	19±2	20±1.7	162±3	143±3	162±3
T9	23±3.1	19±1.7	16±1	23±4	31±3.6	37±1	131±3.2	128±1	131±3.2
T10	31±2.5	26±2.5	22±1.7	18±1.7	25±2.6	34±3.5	129±1.7	121±2.5	129±1.7
T11	25±1.7	21±2.9	14±3.2	25±1	33±2.3	42±2	142±4.2	132±2	142±4.2
T12	34±1	28±4.2	19±2.5	21±2.6	27±4.4	39±4.6	135±2.1	123±1	135±2.1
T13	53±2.1	46±1	39±1	20±4.6	28±1.7	33±1.7	162±4.2	149±3	162±4.2
T14	69±3	57±1.7	41±2.9	16±3.6	23±2.5	28±1	181±1	153±1	181±1
T15	55±4.2	49±3.6	32±1.7	22±2	30±3	37±2	178±2.1	150±3.8	178±2.1
T16	72±2.6	68±1	35±1	19±3.5	21±1.7	35±1.7	186±2.9	151±4	186±2.9
CD@5%	Treatments:1.54			Treatments:1.54			Treatments:1.48		
	Days:0.67			Days:0.67			Days:0.64		
	Interaction:2.68			Interaction:2.68			Interaction:2.57		

*All values represent mean of three replications

Fungi

Inoculation with PSB or Consortium biofertilizer significantly increased fungal population over uninoculated treatments (Table 1). Treatment T16 ($N_{100}P_{100}$ + Consortium biofertilizer) was found with maximum fungal count (29 x 10^4 CFU/ g of soil) at 120 DAP (Table 1). This may be the result of increased nutrient availability in the rhizosphere due to mineral fertilizers and activities of inoculated species which might have improved growth of fungal hyphae [13]. Lowest fungal population under *Azotobacter* inoculation could be attributed to the fact that *Azotobacter* species are known to secrete an antibiotic structurally similar to fungicidal anisomycin [14]. In contrary to this, the

stimulatory effects of N fertilizer on fungal growth whereas inhibitory on nitrogen fixers [15] could have improved fungal population under *Azotobacter* inoculation at 180 DAP

Actinomycetes

Application of N and P fertilizer as uninoculated treatments was found favourable for the growth of actinomycetes than microbial inoculants (Table 1). Soil sample from treatment T1 ($N_{75}P_{75}$) was recorded with highest actinomycetes population among the treatments at each time interval. This may be due to the fact that actinomycetes are efficient decomposers of nutrient poor carbon compounds and can increase in the number under lower mineral nitrogen application rates [16]. Maximum actinomycetes population (51 x 10^5 CFU/ g of soil) was recorded in uninoculated control (T1) whereas lowest actinomycetes population (5 x 10^5 CFU/ g of soil) was found in treatment (T16) with consortium inoculation.

Diazotroph

Highest diazotroph population was recorded at 60 day interval (Table 1) which could be attributed to the phosphorus fertilization (at plantation) that doubles the rate of nitrogen fixers by satisfying their energy requirements for nitrogen fixation [17]. However, the significant decrease in population upto 180 DAP might be the result of slow exhaustion of phosphorus reserves with time and addition of N fertilizer to soil (after 120 DAP) which has been reported to have suppressive effect on *nif*H gene of diazotrophs [15]. Maximum diazotroph population (77 x 10^5 CFU/ g of soil) was recorded under *Azotobacter* inoculation (T8) which could be supported by the fact that soils usually respond interactively to the inoculated species whose population was initially suboptimal in it [11].

PSB

The present study resulted in significantly higher PSB population in inoculated treatments than uninoculated treatments (Table 1). This might be due to the increased nutrient availability in the rhizosphere of inoculated treatments favourable for the growth of heterotrophs including PSBs [18]. Irrespective of the doses of N fertilizer, higher PSB population in treatments having lower dose (75%) of P fertilizer pointed towards the inhibitory effects of available inorganic phosphate on the growth of PSBs. Among the inoculated treatments, highest PSB population (42 x 10^4 CFU/ g of soil) was recorded under inoculation of PSB biofertilizer (T11) which could be the result of successful establishment of inoculum in rhizosphere. Raut and Patale [19] had also reported a significant increase in inoculated and indigenous microbial species in the presence of one or more essential nutrients.

PGPR

The population of plant growth promoting microbes was significantly affected by different fertilizer combinations (Table 1). This is due to the fact that bacteria, as the most sensitive group of soil microbes respond earlier and sensitively to agricultural practices. Inoculated treatments were recorded with significantly higher PGPR population than uninoculated control T4 ($N_{100}P_{100}$). This could be supported by the fact that biofertilizers along with inorganic fertilizers improve nutrient availability to plants which in turn produce more root exudates and favours growth of native PGPR population [20, 21].The maximum PGPR count (186 x 10^5 CFU/ g of soil) in T16 (Consortium biofertilizer + $N_{100}P_{100}$) might be the synergistic effects of coinoculation on plant nutrition and native microbes as compared to individual inoculations. Similar results were recorded in previous study conducted by Korir et al [22].

Soil Enzyme Activities

Soil enzyme activities can provide an early picture of improved soil health during fertilization practices. The present study resulted in significantly higher soil dehydrogenase, alkaline phosphatase and urease activity in rhizosphere of inoculated treatments as compared to uninoculated treatments (**Figure 1**).

Dehydrogenase

Maximum dehydrogenase activity (14.35 µg TPF formed/ min/ g of soil) was recorded in the treatment T16 (Consortium biofertilizer + $N_{100}P_{100}$) in which highest bacterial population was observed (Figure 1a). This could be due to the fact that dehydrogenase activity reflects the total oxidative activity of viable soil microflora and is directly related to the change in microbial biomass and activities as a result of inoculation. As a consequence of greater

biological activity of soil due to consortium inoculation and the presence of complete dose of inorganic fertilizers favored highest dehydrogenase activity in T16. These results were supported by Aseri et al [23] and Adak et al [24].

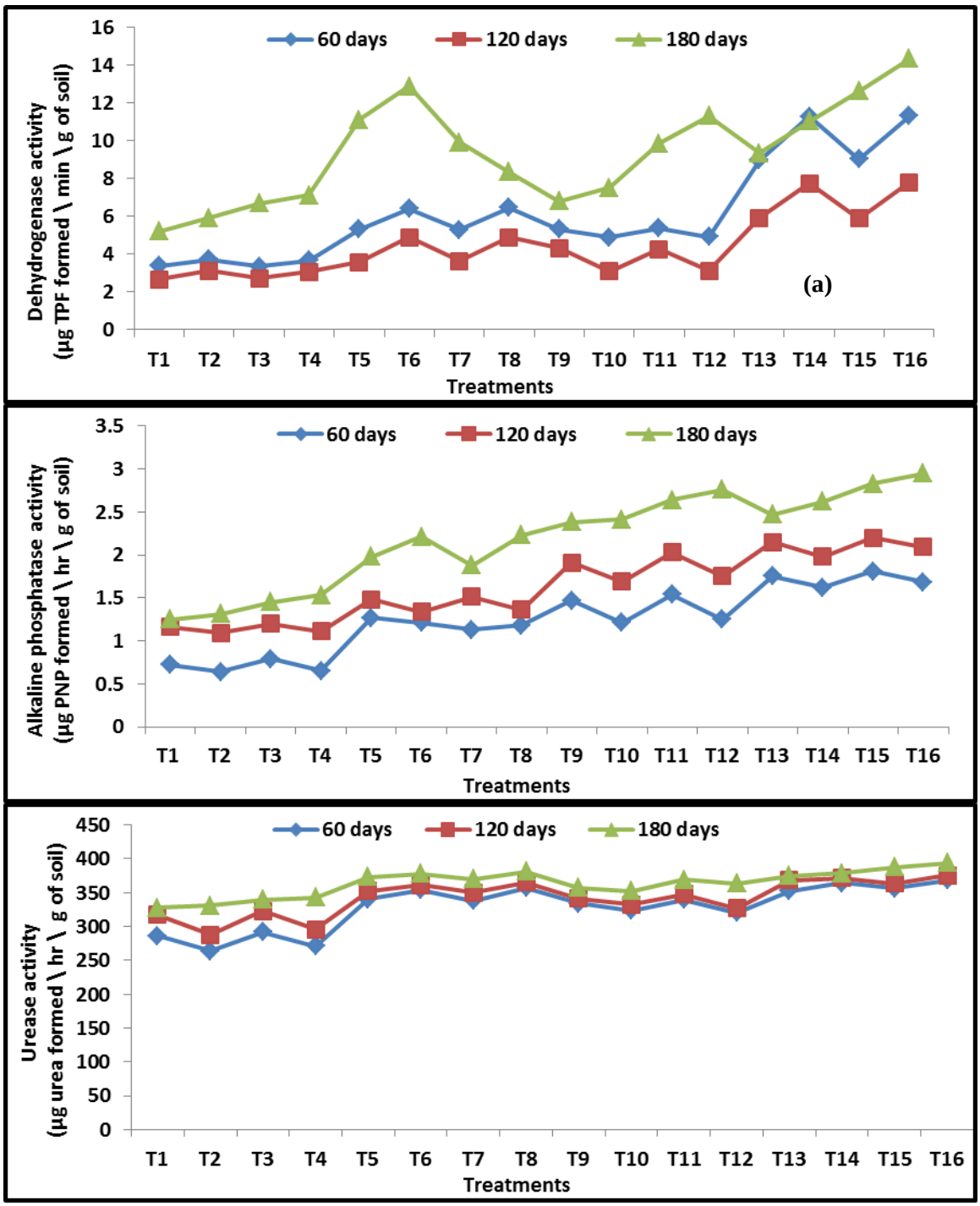

Figure 1 (a-c) Comparison of enzyme activities in different treatments at different time intervals

Alkaline phosphatase

Alkaline phosphatase activity was observed higher in treatments with lower dose of inorganic P fertilizer (Figure 1b). This could be attributed to the fact that soil phosphatase activity is inversely proportional to the amount of mineral phosphorus present in soil. Application of N fertilizer improved alkaline phosphatase activity at 180 DAP which indicated stronger control of N fertilization on phosphatase activity as N is an essential part of phosphatase enzyme [25]. Inoculated treatments were recorded with significantly higher alkaline phosphatase activity than uninoculated treatments. This could be explained by the fact that phosphatase activity is highly correlated with microbial biomass C

of soil [26]. Maximum phosphatase activity (2.95 µg PNP formed/ hr/ g of soil) in T16 (Consortium + $N_{100}P_{100}$) may be the result of improved microbial growth and synthesis of phosphatase enzyme under combined inoculation.

Urease

Highest urease activity at 180 DAP might be due to the incorporation of N fertilizer to soil acting as substrate for enzyme [27]. Inoculation of biofertilizer with inorganic fertilizers significantly improved soil urease activity (Figure 1c) which may be attributed to the improved rhizodepositions and thus microbial activities under inoculation [28]. Maximum urease activity (394.3 µg urea formed /hr /g of soil) under Consortium inoculation with $N_{100}P_{100}$ (T16) could possibly be the result of higher nutrient availability and microbial population in the rhizosphere. *Azotobacter* inoculation was found more effective at 75% dose of N fertilizer in increasing soil urease activity. This may be due to the inhibitory effects of N compounds (ammonium and nitrates) produced as result of urease activity on nitrogen fixing ability of *Azotobacter* population [29]. Despite of inhibition, higher urease activity with *Azotobacter* than PSB inoculation indicated its urea adaptation and potential in reducing the use of chemical fertilizers [30].

Correlation between microbial population and enzyme activities

Correlation analysis revealed significant interaction between soil microbial population and enzyme activities at different time intervals (**Table 2**). At each time interval, significant positive correlation was found between microbial population (except actinomycetes population) and enzyme activities under study.

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	.416**	-.860**	.817**	.402**	.945**	.914**	.828**	.561**
Fungi		1	-.313*	.005	.448**	.356*	.555**	.450**	.168
Actino			1	-.747**	-.493**	-.930**	-.684**	-.757**	-.659**
Diazo				1	.022	.743**	.650**	.480**	.517**
PSB					1	.561**	.392**	.760**	.462**
PGPR						1	.812**	.881**	.611**
Deh							1	.827**	.485**
Phos								1	.605**
Urease									1
(b) 120 DAP									
Bac	1	.323*	-.905**	.798**	.430**	.954**	.868**	.688**	.663**
Fungi		1	-.207	-.068	.215	.221	.471**	.547**	.102
Actino			1	-.758**	-.579**	-.954**	-.665**	-.669**	-.665**
Diazo				1	.044	.732**	.642**	.245	.613**
PSB					1	.607**	.295*	.814**	.433**
PGPR						1	.776**	.743**	.681**
Deh							1	.685**	.613**
Phos								1	.537**
Urease									1
(c) 180 DAP									
Bac	1	.522**	-.810**	.712**	.502**	.952**	.864**	.768**	.941**
Fungi		1	-.469**	-.125	.400**	.515**	.473**	.556**	.492**
Actino			1	-.643**	-.631**	-.915**	-.766**	-.836**	-.900**
Diazo				1	-.002	.759**	.653**	.369**	.754**
PSB					1	.538**	.494**	.821**	.521**
PGPR						1	.830**	.801**	.969**
Deh							1	.721**	.875**
Phos								1	.793**
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 Solubilizing Bacteria; PGPR: Plant Growth Promoting Rhizobacteria; Phos: Alkaline Phosphatase activity; Deh: Dehydrogenase activity

At 60 DAP, bacterial population (r = 0.914, p<0.01) followed by PGPR population (r = 0.812, p<0.01) showed significant positive correlation with soil dehydrogenase activity (Table 2a). Whereas, soil phosphatase (r= 0.881, p<0.01) and urease (r= 0.611, p<0.01) activity had greater positive correlation with PGPR population than other microbial populations. PSB population showed significant correlation with soil phosphatase activity (r = 0.760, p<0.01) greater than soil urease (r= 0.462, p<0.01) and dehydrogenase activity (r = 0.392, p<0.01). However, actinomycetes population had significant negative correlation with soil dehydrogenase (r = -0.684, p<0.01), alkaline phosphatase (r = -0.757, p<0.01) and urease activity (r = -0.659, p<0.01).

At 120 DAP, the correlation between bacterial population and dehydrogenase activity (r= 0.868, p<0.01), PGPR population and urease activity (r= 0.681, p<0.01) whereas of PSB population and alkaline phosphatase activity (r= 0.814, p<0.01) was found significantly higher than others (Table 2b). However, the correlation analysis at 180 day interval revealed greater positive correlation of bacterial (r= 0.941, p<0.01), diazotroph (r= 0.754, p<0.01) and PGPR (r= 0.969, p<0.01) population with soil urease activity than dehydrogenase and phosphatase activity (Table 2c). Whereas, fungal (r=0.556, p<0.01) and PSB (r= 0.821, p<0.01) population showed greater association with alkaline phosphatase activity as compared to other enzyme activities. Negative correlation (p<0.01) was found between actinomycetes population and enzyme activities at 120 and 180 DAP.

Conclusion

It is concluded that synergistic application of biofertilizers and inorganic fertilizers was effective than individual application of inorganic fertilizers in improving the microbial dynamics of Poplar rhizosphere. At different time intervals, the positive correlation of microbial population with soil enzymes activities indicated enhanced metabolic activities of microbial communities. The elevated soil fertility could benefit plantations with better establishment and higher growth rates.

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