AVAILABILITY OF NITORGEN AND PHOSPHORUS IN THE SOIL AND GROWTH OF NATURAL RUBBER PLANTS UNDER INTEGRATED NUTRIENT MANAGEMENT SYSTEM

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Received: 03 August 2020 Accepted: 14 August 2020

Joseph, M., Joseph, K., Hareeshbabu, G. and Elias, R.S. (2020). Availability of nitrogen and phosphorus in the soil and growth of natural rubber plants under integrated nutrient management system. *Rubber Science*, 33(2): 164-176.

A field experiment to study the effect of integration of chemical fertilizers with bio inoculants on soil fertility and growth of natural rubber plants was conducted at two locations in the traditional rubber growing region of South India experiencing humid tropical climate. After two to three rounds of application, build-up of phosphobacteria was significant in both the locations in both recordings. *Azotobacter* and *Pseudomonas* build up was noticed in a few recordings. Root infection of AMF was more than 90 per cent in both control and treated plants indicating natural infection in rubber roots. The inorganic nitrogen (N) in the soil, especially in the ammoniacal N form was improved by bio inoculant treatments either alone or in combination with chemical fertilizers. The beneficial effect of phosphorus (P) solubilizing microbes on P transformation was indicated through the significant difference in the P fractions, especially on Fe-P fraction. The positive effect was more pronounced in one location where the organic carbon status of the soil was very high. The influence of fertilizer P was directly reflected on the Ca-P fraction. Growth of rubber plants recorded at periodical interval did not show significant difference between the treatments indicating lack of response of rubber plants to applied nutrients. Better understanding of the nutrient dynamics, having continuous uptake with roots spreading to the deeper layers of the soil and release and availability from the slowly available fractions and organic forms is required to explain the soil test crop responses in a perennial crop like natural rubber.

Key words: Arbuscular mycorrhizal fungus, Bio inoculants, *Hevea brasiliensis*, Inorganic nitrogen, Nitrogen fixers, Natural rubber, Phosphorus fractions, Phosphorus solubilizers

INTRODUCTION

Soil fertility is controlled by the soil physical, chemical and biological properties. The highly weathered acid soils of South India subjected to continuous cultivation of natural rubber (*Hevea brasiliensis*) have medium to high organic matter status (NBSS and LUP, 1999; Karthikakuttyamma *et al.*, 2000). Establishment and maintenance of leguminous cover crops in the early years (Watson, 1989; Kothandaraman *et al.*, 1989; Philip *et al.*, 2005) and litter addition through annual leaf fall (Varghese *et al.*, 2001) contributed for the high levels of organic

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matter in the rubber growing soils. Large amount of organic residues and root exudates are incorporated to the rhizosphere from the cover crop in addition to the proven advantages of soil moisture conservation, nitrogen fixation, prevention of weed growth *etc.* The cultural operations with nearly zero tillage favour stabilization of organic matter at a high level (Krishnakumar and Potty, 1992). A detailed review on the rubber growing soils of India is available (Joseph, 2016). Indications of P build up in older plantations were reported by NBSS and LUP (1999). However, imbalance in nutrient availability and declining soil fertility was reported in recent years in the replanting fields from the traditional (Joseph *et al.*, 1995; Karthikakuttyamma, 1997; Karthikakuttyamma *et al.*, 1998; Ulaganathan *et al.*, 2010; 2012) and non-traditional (Mandal *et al.*, 2010; 2012; 2013) regions of cultivation.

In the immature phase of rubber, proper manuring is essential for good, uniform growth and early tappability (Karthikakuttyamma *et al.*, 2000). In poor soils, girth and girth increment of rubber show positive response to N and P fertilizer application and proper manuring is essential for good growth and early tappability. Good management in the immature phase of rubber plantation improves the soil fertility status and reduces the fertilizer requirement in the mature phase especially in the initial years of tapping (Punnoose *et al.*, 1976). Fertilizer requirement of rubber grown in association with legume ground cover was low in comparison with the plants grown in association with natural cover (Mathew *et al.*, 1989; Punnoose *et al.*, 1994).

Positive effect of plant growth promoting rhizobacteria (PGPR) on the growth of rubber seedlings was reported (Joseph *et al.*, 2015; Syamala *et al.*, 2015). Occurrence of mycorrhizal spores in rubber growing soils and colonization in roots of mature rubber plants from 16 locations were studied and reported that the spore population ranged from 214 to 428 in 50 g of soil and the arbuscular mycorrhizal fungus (AMF) infection in rubber roots ranged from 67 to 92 per cent (Joseph, 1997; Joseph *et al.*, 2002; Jessy *et al.*, 2007).

Our hypothesis was that integration of chemical fertilizers with N fixers, P solubilizers and PGPR will improve the availability and uptake of nutrients and thus improve the growth of plants, with the added advantage of reducing the use of chemical fertilizers and sustaining soil health.

MATERIALS AND METHODS

Field experiment with young natural rubber plants, clone RRII 414 was conducted at two locations. Location I was Central Experiment Station of the Rubber Research Institute of India at Chethackal (9.41°N 76.82°E) in Ranni, Central Kerala and Location II was Puthukkad estate (10.21N 76.26E), Thrissur, Kerala. The experiment was conducted for five years from 2008 to 2012 at both the locations and continued for four more years up to 2016 in Location I. The experiment was laid out in randomised block design with three replications and the treatments were graded levels of N and P in combination with bio inoculants, bio inoculants alone, chemical fertilizer alone (as per the general recommendations for natural rubber) and also a no fertilizer control. The general recommendation for young rubber up to four years is 40:40:16:6 kg NPKMg per hectare applied in two equal split doses during April-May and September-October season. From fifth year onwards the general recommendation is 30:30:30 kg NPK per hectare per year applied in two equal split doses (Karthikakuttyamma *et al.*, 2000). Nitrogen and P treatments (graded levels) along with common dose of K and Mg as per the general recommendation was applied in the form of urea, rock phosphate, muriate of potash and magnesium sulphate, respectively, once during September in the first year of planting and during May and September from second year onwards. The consortia of bio inoculants (BI) applied were one isolate of N fixing bacteria (*Azotobacter* sp.), two strains of P solubilizing bacteria (*Bacillus* spp.) and two strains of PGPR (*Pseudomonas* spp.) isolated from the rubber growing soils of Kerala and multiplied at RRII and arbuscular mycorrhizal fungus (AMF) from The Energy and Resources Institute, New Delhi. Twenty gram each of lignite based preparation of *Azotobacter*, Phosphobacteria and PGPR having 10^8 cells g^{-1} and five gram of AMF having 200 propagules g^{-1} were applied at the time of planting during the first year and twice during the subsequent years with a gap of 21 days between the chemical fertilizer application and bio inoculant application. Luxuriant growth of leguminous cover crop was maintained in the inter row areas of rubber in every treatment.

With the continuous application of bio inoculants for four years and recording the build-up of inoculated microbes, experiment in one location was continued with chemical fertilizer treatment alone to study the effect of chemical modifications in the soil on the availability of nutrients. Growth of the plants in terms of girth at 150 cm height from the bud union from second year onwards was recorded at periodical interval.

Before initiating the experiment, soil sample from both the locations were collected for assessing the soil fertility and microbial population. Soil samples from the individual plots from 0-30 cm depth were collected during 2012 from both the locations and during 2015 from location one where the experiment was continued with chemical fertilizer treatment alone and analysed for N and P availability. Rhizosphere soil samples were collected in the second year during May 2009 (before the application of BI during the pre-monsoon season) and during September 2009 (before the application of BI during the post-monsoon season). Soil samples were processed and analysed for organic carbon (OC) by Walkley and Black wet digestion method (Walkley and Black, 1934). Available P was extracted by Bray II extractant (Bray and Kurtz, 1945) and the P in the extract was estimated colorimetrically by chlorostannous blue colour method (Jackson, 1958). pH, available K, Ca and Mg were estimated as per the standard procedure outlined in Jackson (1958). Available N was estimated by alkaline permanganate method (Subbiah and Asija, 1956), ammoniacal and nitrate N from KCl extract as per Bremner (1965), and P fractions (Chang and Jackson, 1958). Leaf samples from individual plots were collected following the standard procedure (Karthikakuttyamma *et al.*, 2000) and concentration of N and P in the leaves were estimated (Piper, 1942). From the rhizosphere soil samples, the total count of the different groups of inoculated bacteria were estimated by serial dilution plate method of Timonin (1940) using apatite agar medium for Phosphobacteria, King's B agar for *Pseudomonas* spp. and Jensen's agar for *Azotobacter* sp. Mycorrhizal infection in the root was estimated as per Phillips and Hayman (1970). The data were subjected to statistical analysis as outlined in Panse and Sukhatme (1961).

RESULTS AND DISCUSSIUON

Fertility status of the soil

Soils of the experiment locations were highly weathered red ferruginous soils belonging to the great group Kandihumults as per the USDA system of soil classification (NBSS & LUP,1999). The initial fertility status of the surface soil for the two locations is provided in Table 1. The soil was strongly acidic in both the locations. The OC status was medium in Location II (1.16 %) and very high in Location I (2.35 %) as per the rating followed in rubber. Organic carbon status of 1.0 to 1.5 per cent is rated as medium and above 1.5 per cent is high and below 1.0 per cent is low (Karthikakuttyamma *et al.*, 2000). The beneficial effect of leguminous cover crops in building up the organic matter and addition of nutrients was reported earlier (Kothandaraman *et al.*, 1989; Philip *et al.*, 2005). The available P (Bray II P) status was low (< 10 mg kg^{-1}) and the available K status was medium (50-125 mg kg^{-1}) in both the locations as per the fertility rating (Karthikakuttyamma *et al.*, 2000). The available Ca status ranged from low (<100 mg kg^{-1}) in Location II to high (>150 mg kg^{-1}) in Location I. The available Mg status was high (>25 mg kg^{-1}) in both locations as per the rating followed (Karthikakuttyamma *et al.*, 2000).

Table 1. **Initial fertility status of the experimental field**

Experiment	pH	OC (%)	Available nutrients (mg kg^{-1})			
			P	K	Ca	Mg
Location I	4.8	2.35	4.4	101	236	52
Location II	4.6	1.16	6.4	65	80	31

Population of introduced microbes

The microbial population (population of BI in the initial soil samples from the experiment fields) is provided in Table 2. *Azotobacter* and *Pseudomonas* population was high in location I compared to location II. Population of introduced microbes *viz.* Phosphobacteria, *Pseudomonas* and *Azotobacter* in the soil were recorded during the second year during pre-monsoon and post-monsoon period before incorporation of the treatments. Population of *Azotobacter* (Table 3) recorded statistical significance during the second recording in location I and during the first recording in location II. Positive effect of *Azotobacter* isolated from rubber growing soils on the growth of rubber seedlings was reported by Joseph *et al.* (1997). Build-up of Phosphobacteria was significant in both the locations in both recordings (Table 4). The highest recording was observed in BI alone treatment. Very high population of Phosphobacteria was recorded in location one during the first recording during May.

Root infection of AMF was recorded during the second year after three rounds of incorporation and the values ranged from 89.3 to 96.0 per cent in location I and from 94.7 to 100 per cent in location II (Table 5). Treatment effect was not statistically significant. AMF infection of roots was more than 90 per cent in both control and treated

Table 2. **Initial soil analysis for microbial population (CFUg^{-1} of soil)**

Experiment	*Azotobacter sp.* x10^4	Phosphobacteria x10^5	*Pseudomonas sp.* x10^5
Location I	23.0	3.6	15.8
Location II	16.0	8.0	9.8

Table 3. **Effect of treatments on the population of *Azotobacter* (CFUg^{-1} soil)**

Treatments	Location I		Location II	
	May-09	Sept-09	May -09	Sept-09
	x10^2	x10^3	x10^2	x10^3
Control	10.0 (2.69)	17.0 (4.12)	0 (1.22)	15.3 (3.90)
100 % N & P	10.0 (3.08)	24.3 (4.85)	0 (1.22)	10.7 (3.22)
25 % N & P + BI	16.7 (4.22)	21.7 (4.64)	16.7 (4.22)	15.7 (3.83)
50 % N & P + BI	23.3 (4.96)	26.3 (5.09)	13.3 (3.80)	19.7 (4.42)
75 % N & P + BI	23.3 (4.96)	23.0 (4.78)	10.0 (3.39)	18.0 (4.19)
100 % N & P +BI	23.3 (4.96)	25.0 (4.99)	3.3 (1.95)	14.7 (3.73)
BI alone	30.0 (5.56)	29.0 (5.37)	20.0 (4.54)	33.7 (5.76)
SE	0.75	0.23	0.41	0.48
CD (P=0.05)	NS	0.72	1.28	NS

Figures in parenthesis are the square root transformed values

plants indicating natural infection in rubber roots. Occurrence of mycorrhizal spores in rubber growing soils and colonization in roots of mature rubber plants from 16 locations were studied and reported that the spore population ranged from 214 to 428 in 50 g of soil. The common genera of AM fungi included *Glomus* spp., *Gigaspora* spp., *Acaulospora* spp. *and Sclerocystis* spp. The frequency of occurrence was in the order *Glomus* spp. >*Acaulospora* spp. > *Sclerocystis* spp. Only a few spores of *Gigaspora* spp. were recorded. The AMF infection in rubber roots ranged from 67 to 92 per cent (Joseph, 1997; Joseph *et al.*, 2002; Jessy *et al.*, 2007).

Build-up of phosphobacteria in the soil contributed for enhanced solubility of the P either from the organic form or from the fixed forms. AMF colonization in the rubber

Table 4. **Effect of treatments on the population of *Phosphobacteria* (CFUg^{-1} soil)**

Treatments	Location I		Location II	
	May-09	Sept-09	May-09	Sept-09
	x10^3	x10^4	x10^3	x10^4
Control	16.7 (3.9)	20.3 (4.5)	3.3 (1.8)	9.3 (3.0)
100 % N & P	10.0 (2.5)	18.7 (4.3)	9.0 (3.0)	12.3 (3.5)
25 % N & P + BI	100.0 (9.9)	38.0 (6.2)	16.7(4.0)	37.0 (6.1)
50 % N & P + BI	73.3 (8.5)	27.0 (5.2)	13.3 (3.6)	30.7 (5.5)
75 % N & P + BI	96.7 (9.8)	25.0 (5.0)	30.0 (5.4)	33.0 (5.7)
100 % N & P +BI	86.7 (9.1)	38.7 (6.2)	40.0 (6.3)	34.7 (5.9)
BI alone	146.7 (12.1)	39.0 (6.2)	33.3 (5.6)	39.0 (6.2)
SE	1.1	0.2	0.57	0.2
CD(P=0.05)	3.3	0.7	1.8	0.7

Figures in parenthesis are the square root transformed values

Table 5. **Effect of treatments on the root infection of AMF (%)**

Treatments	Location I	Location II
Control	96.0 (9.79)	97.3 (9.86)
100 % N & P	93.3 (9.65)	100.0 (10.00)
25% N & P + BI	89.3 (9.45)	91.3 (9.54)
50% N & P + BI	96.0 (9.80)	97.3 (9.86)
75% N & P + BI	92.0 (9.58)	96.0 (9.79)
100 % N & P + BI	92.0 (9.58)	97.3 (9.86)
BI alone	96.0 (9.79)	94.7 (9.73)
SE	0.17	0.15
CD (P=0.05)	NS	NS

Figures in parenthesis are square root transformed values

roots is promoting the mobilization of P from the soil and uptake is enhanced and could possibly be one of the reason for the maintenance of optimum P concentration in the plant even when the soil is tested low in available P as observed in the present study as well as in earlier studies (Joseph *et al.*, 2018). Increased solubility coupled with enhanced mobilization through the AMF colonized in the roots might have contributed for enhanced uptake of P by the plants as reported in many tree crops. In the rubber growing soils of South India, 64 to 86 per cent of the total P was reported to be in the organic form (Prasannakumari *et al.*, 2008) and solubilisation of these organic forms through bio agents contributes increased availability for plant uptake.

Population of *Pseudomonas* (Table 6) recorded statistical significance during the first recording in both the locations. Among the 17 fluorescent rhizobacteria (*Pseudomonas*) collection from different rubber growing areas of Kerala seven selected isolates having phosphate solubilization and antagonistic activity were also found to produce IAA and gibberellins (Joseph *et al.*, 2003). Positive effect of these PGPR on growth of rubber seedlings was reported (Joseph *et al.*, 2015; Syamala *et al.*, 2015).

Availability of nitrogen and phosphorus in the soil

Effect of treatments on N status in the soil in the first location during 2012 after four

Table 6. **Effect of treatments on the population of *Pseudomonas* (CFUg^{-1} soil)**

Treatments	Location I		Location II	
	May-09	Sept-09	May-09	Sept-09
	x10^{3}	x10^{4}	x10^{3}	x10^{4}
Control	30.0 (5.3)	34.7 (5.9)	13.3 (3.6)	21.0 (4.6)
100 % N & P	24.0 (4.4)	45.3 (6.7)	15.0 (3.7)	25.3 (4.1)
25 % N & P + BI	40.0 (6.3)	31.7 (5.5)	56.7 (7.5)	39.7 (6.1)
50 % N & P + BI	39.7 (6.2)	40.3 (6.2)	36.7 (6.0)	37.7 (6.0)
75 % N & P + BI	63.3 (8.0)	55.0 (7.4)	26.7 (5.1)	29.7 (5.4)
100 % N & P + BI	63.3 (8.0)	32.3 (5.7)	26.7 (5.1)	41.3 (6.3)
BI alone	70.0 (8.4)	47.7 (6.9)	76.7 (8.6)	55.3 (7.4)
SE	0.84	0.47	0.62	0.63
CD(P=0.05)	2.62	NS	1.91	NS

Figures in parenthesis are the square root transformed values

Table 7. **Effect of treatments on the nitrogen status in soil –Location I**

Treatments	OC (%)	Available N (kg ha^{-1})	NH_4-N (mg kg^{-1})	NO_3-N (mg kg^{-1})
T1-Control	2.28	319	8.23	0.68
T2-Standard FR	2.69	396	20.93	14.47
T3-25% N & P+BI	2.77	372	13.67	1.70
T4-50% N & P +BI	2.83	388	17.33	5.33
T5-75% N & P +BI	2.45	370	15.10	5.83
T6-100% N & P+BI	2.36	378	13.10	7.40
T7- BI alone	2.27	317	12.40	2.57
SE	0.19	21.45	3.43	1.34
CD (P=0.05)	NS	NS	NS	4.14

years of continuous treatment incorporation is presented in Table 7. Soil is having very high OC status and the treatment effect is not significant on OC, total N, and available N or on ammoniacal N. However, nitrate N recorded significant difference between treatments. The highest value (8.47 mg kg^{-1}) was recorded by 100 per cent N and P alone treatment which was on par with 100 per cent N and P in combination with BI (7.40 mg kg^{-1}) treatment. Effect of treatments on the N status in the soil in the second location is presented in Table 8. Treatment effect was not significant on OC and available N status. However, total N, ammoniacal N and nitrate N recorded statistical significance between treatments. The highest ammoniacal N was recorded in 100 per cent N and P + BI and 75 per cent N and P + BI treatments followed by N and P alone treatment and these treatments were on par with N and P alone or combination of N and P at 50 and 75 per cent with BI. Positive effect of BI on ammoniacal N was reflected on the high value recorded in BI alone treatment compared to control.

Table 8. **Effect of treatments on the nitrogen status in soil- Location II**

Treatments	OC (%)	Total N (%)	Available N (kg ha^{-1})	NH_4-N (mg kg^{-1})	NO_3-N (mg kg^{-1})
Control	1.33	0.140	327	2.07	2.77
100 % N & P	1.21	0.176	366	12.80	6.53
25%N & P + BI	1.34	0.175	334	6.23	2.23
50% N&P+ BI	1.09	0.183	337	11.00	5.30
75% N&P+BI	1.36	0.187	357	14.83	4.43
100 % N&P +BI	1.18	0.177	368	15.47	2.90
BI alone	1.42	0.151	328	7.70	3.17
SE	0.165	0.008	19.3	1.52	1.07
CD (P=0.05)	NS	0.024	NS	4.70	3.31

Table 9. **Effect of treatments on the phosphorus status in soil – Location I**

Treatments	Bray II P (mg kg^{-1})	Phosphorus fractions (mg kg^{-1})			
		Saloid P	Al-P	Fe-P	Ca-P
Control	4.3	T	1.00	2.50	6.17
100 % N & P	10.2	T	1.67	4.50	28.33
25 % N & P + BI	16.1	T	2.50	4.83	10.67
50 % N & P + BI	18.7	T	2.00	5.17	11.17
75 % N & P + BI	30.0	T	2.33	5.17	20.00
100 % N & P + BI	29.7	T	2.00	5.17	26.50
BI alone	6.5	T	1.33	3.50	8.00
SE	4.0	-	0.41	0.65	1.94
CD (P=0.05)	11.5	-	1.26	2.00	5.98

T-Traces

Effect of treatments on the soil P status in the first location is presented in Table 9. Available P (Bray II-P) values ranged from 4.3 to 89.7 mg kg^{-1}. The lowest value was recorded by the control treatment and the highest was recorded by 100 per cent N and P in combination with bio inoculants. The 100 per cent N and P in combination with BI recorded very high availability of P and was significantly superior to all other treatments. Similarly, 75 per cent N and P in combination with BI also recorded high availability of P. The 75 or 100 per cent N and P in combination with BI recorded very high availability of P in comparison with the N and P alone treatment indicating the beneficial effect of BI on P solubilisation in soil which was favoured by the high OC status of the soil. Saloid P was traces in all the treatments. Aluminium P, Fe-P and Ca-P fractions recorded statistical significance between treatments and the effect was more pronounced on Ca-P fraction. The highest Ca-P was recorded by 100 per cent N and P

Table 10. **Effect of treatments on the phosphorus status in soil - Location II**

Treatments	Bray II P (mg kg^{-1})	Phosphorus fractions (mg kg^{-1})			
		Saloid P	Al-P	Fe-P	Ca-P
Control	6.2	T	1.33	2.17	3.00
100 % N & P	17.2	T	1.17	4.17	5.33
25% N & P + BI	7.7	T	1.33	3.20	6.50
50% N & P + BI	7.7	T	1.33	3.17	5.50
75% N & P + BI	11.7	T	1.83	3.83	8.83
100 % N & P + BI	16.0	T	1.17	4.83	10.67
BI alone	7.7	T	1.33	3.00	4.67
SE	1.5	-	0.27	0.53	1.47
CD (P=0.05)	4.8	-	NS	1.64	4.60

T-Traces

Table 11. **Effect of treatments on the availability of N and P in the soil and on the concentration of N and P in leaf during 2015 sampling**

Treatments	OC (%)	Av.N ($kg\ ha^{-1}$)	BrayIIP ($mg\ kg^{-1}$)	Concentration in leaf (%) N	P
Control	2.40	299	8.83	3.31	0.23
100 % N & P	2.33	330	8.67	3.34	0.24
25% N & P + BI	2.12	310	7.00	3.44	0.23
50% N & P + BI	2.20	350	6.67	3.39	0.21
75% N & P + BI	2.44	365	9.00	3.59	0.26
100 % N & P + BI	2.42	354	9.50	3.53	0.25
BI alone	2.08	308	9.17	3.21	0.23
SE	0.13	15.5	1.34	0.14	0.02
CD (P=0.05)	NS	NS	NS	NS	NS

alone or in combination with BI and was on par indicating the direct positive effect of fertilizer P on the Ca-P fraction. Among the reduced levels, 75 per cent N and P in combination with BI also recorded statistically significant value compared to control and BI alone treatment.

Effect of treatments on the P status of soil in the second location (Table 10) recorded statistical significance for available P, Fe-P and Ca-P fractions. The highest available P of 17.2 $mg\ kg^{-1}$ was recorded by the 100 per cent N and P alone followed by 100 per cent N and P in combination with BI treatment and these two treatments were on par. In the second location, the OC status was low (initial value of 1.16 %) compared to the first location (initial value of 2.35%) and the effect of BI was significant only in 100 per cent N and P in combination with BI treatment. Positive effect of BI was more pronounced in soils having very high OC status. Though statistical significance on Fe-P was recorded, the treatment effect is not that pronounced. Similar observation was recorded in the first location also. Cacium -P values ranged from 3.0 to 10.7 $mg\ kg^{-1}$ and were considerably lower than the values recorded in the first

Table 12. **Effect of treatments on the growth (girth at 150 cm height) of plants (cm)**

Treatments	Location I 2009	Location I 2012	Location I 2016	Location II 2009	Location II 2012
Control	7.9	33.2	55.4	8.5	35.5
100 % N & P	9.0	33.5	57.9	8.5	36.2
25% N & P + BI	10.0	35.0	59.7	8.6	37.7
50% N & P+ BI	9.8	34.6	57.5	8.8	37.0
75% N & P+BI	9.6	37.9	57.4	8.9	36.6
100 % N & P +BI	9.7	34.8	57.9	9.1	38.4
BI alone	9.6	35.6	58.4	8.7	38.2
SE	0.5	1.2	1.2	0.4	1.0
CD (P=0.05)	NS	NS	NS	NS	NS

location. Compared to 100 per cent N and P alone treatment (5.3 mg kg^{-1}), statistically significant higher value was recorded only by the combination of 100 per cent N and P plus BI treatment (10.7 mg kg^{-1}) indicating the positive beneficial effect of phosphobacteria on Ca-P values which might be through the reduced fixation of released P or increased solubilisation of P from rock phosphate. Effect of treatments on the availability of nutrients in the soil and N and P concentration in leaves sampled after eight years did not indicate statistical significance (Table 11). No difference was recorded between control and 100 per cent N and P application indicating the nutritional sufficiency of the soil.

Growth of natural rubber plants

Growth of rubber plants did not show significant difference between the treatments in both locations indicating lack of response to N and P fertilizers (Table 12). Response to added fertilizer depends on the soil fertility status and there is possibility of reducing the N and P fertilizer doses in good soil. Studies conducted in the seedling nursery indicated that growth of plants with 50 per cent reduced level of N and P fertilizer applied in combination with bio inoculants was on par with the standard recommended dose of chemical fertilizers alone or in combination with bio inoculants (Joseph *et al.*, 2015). Similarly, in young natural rubber plants grown in Tripura, North East India where the soil OC status was only 0.92 per cent, positive response to fertilizer application was recorded on the availability of nutrients and growth of plants and the possibility of reducing the chemical fertilizer by integration with BI was reported (Mandal and Joseph, 2018). Enhanced nutrient uptake, especially P uptake through AMF may be one of the reasons for the lack of response to P fertilizer treatments. Lack of response to fertilizer P in rubber, especially in mature rubber was reported by many workers (Jessy, 2004; Jessy *et al.*, 2004). Continuous uptake of nutrients from the deeper layers of the soil and release of nutrients from the organic pool may be the reason for the lack of response to applied fertilizers. This is more applicable in the case of N and P as the dynamics of these two nutrients is more dependent on the organic pool of the soil. The rubber growing soils in general are rich in organic matter status compared to the same soil being cultivated with coconut or tapioca or other crops in the same agroclimatic zone. Large amount of organic residues are added to the system both in the young phase either through the leguminous cover crops or intercrops and in the mature phase through litter addition and recycling through annual leaf fall. Nutritional self-sustainability of the mature rubber system and dependence of the response to fertilizers on the fertility of the soil was reported earlier by George and Joseph (2011).

CONCLUSION

Growth of rubber plants was not influenced by treatments in both the locations. Build-up of Phosphobacteria was significant in both the locations in both recordings. Regarding *Azotobacter* and *Pseudomonas*, build-up was noticed in few recordings. Natural infection of rubber roots with AMF was indicated contributing towards enhanced nutrient uptake, especially P uptake. Influence of bio inoculants on the availability of P was more pronounced and significant and that too in the first location with very high OC status. The OC rich soil contributed towards the maintenance of sufficiency level of N and

P in the soil in the control plots and external application of chemical fertilizers alone or in combination with bio inoculants could not bring in additional advantage on the growth of plants. Results from the study clearly indicated that the response to fertilizers depends on the fertility status of the soil.

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