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Effect of biofilmed biofertilizer on plant growth and nutrient uptake of *Hevea brasiliensis* nursery plants at field condition

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Abstract

Beneficial microbes through their direct and indirect mechanisms support plant growth in a sustainable manner. This study was conducted to understand the effect of biofilmed biofertilizer (BFBF) on dry matter production and nutrient uptake of Hevea brasiliensis nursery plants. The BFBF was formulated by phosphorus solubilizing bacteria Bacillus spp. and commonly found fungi Aspergillus spp. associated with H. brasiliensis rhizosphere. Rubber Research Institute of Sri Lanka (RRISL) recommended inorganic fertilizers were applied at the rates of 0%, 50% and 100% of the recommended level with and without the application of BFBF at a rate of 100 ml per rubber nursery plant monthly. Plant dry matter production, nutrient contents of plant parts and their nutrient uptake were determined before and after bud grafting stages. Results showed that many observed growth parameters; diameter, height, shoot dry matter, root dry matter and total dry matter were significantly higher in BFBF applied treatments compared to its respective non BFBF treatments. There was no consistent trend on the effect of BFBF on leaf nutrient contents at both growth stages. There were no significant differences with 100%F, 50%F+BFBF and 100%F+BFBF treatments in relation to total N, P and K uptake at before bud grafting stage and total N and K uptake at after bud grafting stage. At after bud grafting stage, significantly higher total P uptake was observed in T5 (50%F+BFBF) and T6 (100%F+BFBF) treatments (81 mg P/plant and 87 mg P/plant respectively) than in T3 (100%F) (64 mg P/plant) treatment. This study suggest that reduced amount of inorganic fertilizer with BFBF gave no significant difference or significantly higher values in relation to plant growth and total nutrient uptake of rubber nursery plants compared to recommended fertilizer application.

Key words: biofilmed biofertilizer, *Hevea brasiliensis*, nursery plants, nutrient uptake, plant growth

Introduction

In general, soils in rubber growing areas of Sri Lanka are deficient in plant nutrients and consequently, considered as less productive (Dissanayake *et al.*, 1999). The same agricultural cropping system adopted with the same management practices throughout the last several decades have resulted in decreasing soil fertility (Samarappuli, 1995 and Samarappuli and Yogaratnam, 1996). Therefore, fertility improvement has been identified as an accepted practice (Samarappuli and Yogaratnam, 1997) and requires a number of nutrients for normal, healthy growth and development of rubber. At present, mineral fertilizers are used to compensate the shortages of nutrients and to manage the soil fertility (Bockman *et al*., 1990; Thennakoon, 1990). There is a potential loss of nitrogen, phosphorus, potassium and magnesium that could be occur through leaching and runoff while nitrogen could get lost through volatilization and denitrification. Hence, loss of applied fertilizers from the soils leads to marked economic losses and negative environmental impacts (Bockman *et al*., 1990). Therefore, minimizing leaching of nutrients and volatilization of nitrogen losses associated with chemical fertilizers are required for prevention of unbalanced nutrition promoted through indiscriminative fertilization practices (Ayoub, 1999).

Among the sources available for fertilizing, the use of biofertilizers in agriculture is considered to be an eco-friendly practice which is more cost effective than chemical fertilizers. They are highly advantageous in enrichment of soil fertility and fulfilling the plant nutrient availability. The conventional practice of plant inoculation with monocultures or mixed cultures of effective microbes as biofertilizers may not furnish the highest microbial effect, which may be achieved by biofilm formation (Bandara *et al*., 2006). Biofilm is a community of microbes and considerable attention has been focused recently on BFBF and their potential to increase the nutrient availability in soils (Jayasinghearachchi and Seneviratne, 2004a; Seneviratne and Jayasinghearachchi, 2005) and enhance plant growth (Bandara *et al*., 2006; Seneviratne *et al*., 2009). The bradyrhizobial-fungal biofilm with nitrogenase activity has showed N_2 fixing symbiosis with soybean, but the bradyrhizobial strain alone did not N_2 fixing symbiosis (Jasinghearachchi and Seneviratne, 2004b). Moreover, fungal-rhizobial biofilms could be used more effectively in biosolubilizing of poorly soluble Eppawala Rock Phosphate (ERP) (Seneviratne and Jasinghe-arachchi, 2005, Hettiarachchi *et al*., 2015). The application of BFBF developed from effective microbes have shown to stimulate restoring degraded tropical agricultural lands with improved ecosystem functioning and sustainability (Seneviratne *et al*., 2011; Seneviratne, 2012). Several studies conducted so far with the BFBF under laboratory, nursery and field conditions for soybean, mung bean, wheat, rice, anthurium and tea in Sri Lanka have shown positive results in relation to soil fertility and crop growth (Seneviratne and Jayasinghearachchi, 2005; Seneviratne *et al*., 2009 and Seneviratne *et al*., 2011; Seneviratne and Kulasooriya, 2013).

Therefore, this study was conducted with the aim of understanding the effect of BFBF on plant growth and nutrient uptake of *Hevea* seedling nursery plants.

Materials and Methods

The top soil belonged to Red Yellow Podzolic (RYP) great soil group and classified as Hapludults according to the USDA soil taxonomy (Mapa *et al*., 1999) was used without stones, pebbles and roots particles. This top soil was the recommended medium for filling poly bags (Tillekeratne and Nugawela, 2001) with a size of 6” wide, 15” long (lay flat dimension) and contain about 2 kg of soil. Prior to filling the poly bags the soil was air dried and crushed gently to pass through 2 mm sieve. Fifty grams of Higher Grade Eppawala Rock Phosphate (HERP) and 50 g of compost per bag were mixed with soil prior to filling according to the Rubber Research Institute Sri Lanka (RRISL) recommendation for rubber nursery plants. Bags in a single row were arranged close to each other and kept 5 cm distance between two rows. Two weeks old one germinated seed per bag was placed and Rubber Research Institute recommended management practices were conducted throughout the experimental period (Tillekeratne and Nugawela, 2001). Germinated seeds were grown in poly bags to raise young buddings were bud-grafted by the green budding technique at the age of 3-4 months. Considering that bud-grafting activity two periods were identified as before the bud-grafting and after the bud-grafting. Two weeks after planting of seedlings, fertilizer application was started and it was continued throughout the before and after bud-grafting stages until two weeks before planting at field conditions following the recommendation of fertilizer application for rubber nursery plants by RRISL (Tillekeratne and Nugawela, 2001). Recommended N, P, K and Mg fertilizers were used at 0% (0%F), 50% (50%F) and 100% (100%F) of the currently recommended level with and without the application of developed BFBF. There were 6 treatment combinations. The required quantities of N, P, K and Mg fertilizers were dissolved in water and 100 ml of the solution per bag was applied at monthly intervals. Isolated phosphorus solubilizing bacteria *Bacillus* spp. and fungi *Aspegillus* spp. from rubber root rhizosphere had an ability to form complex, multicellular biofilm community called BFBF. It was evaluated under *in-vitro* conditions and proved its ability of phosphorus solubilizing, N_2 fixing and secreting plant growth promoting hormones. The BFBF medium was examined under the microscope using a hemocytometer and cell density was adjusted to 10^8 – 10^9 cells ml^{-1}. The freshly prepared BFBF was applied as a liquid at equal rate for inorganic fertilizers. Each treatment had 5 replicates and each replicate consisted of 10 poly baged plants. Each replicate was maintained in different steps of the land and there was a variation in sunlight at the site. Hence, treatments were arranged in a randomized complete block design.

When the seedling plants reached their grafting stage, 6 mm in diameter above the soil level (Tillekeratne and Nugawela, 2001) was grafted with the clone RRISL 203. One month after, successfully grafted seedling plants were pollarded and facilitated for sprouting the desired bud patch. Therefore, both stages (before and after bud grafting) important for the final outcome of the preparation of quality planting material for rubber plantation. End of four months of before and after bud grafting stages, randomly selected five plants from each treatment were collected for data collection.

Growth assessments

Plant height was measured as the total height above the soil level and diameter was measured at 1 cm above the base of the plant and bud union at before bud grafting stage and after bud grafting stage respectively throughout the experimental period at one month interval.

Plant dry matter

At the end of four months at before and after bud grafting stages each plant was separated into leaves, stems, roots and their dry weights only were recorded by drying the components at 60ºC for constant weight. At the time of separating plants into their parts special care was taken to collect almost entire root system. Soil and dust particles adhered to the root system were removed carefully.

Plant analysis

Dried samples were ground in a Willy mill. 0.2g of finely ground plant materials were weighed into a pyrex tube and 5 ml Se/H_2SO_4mixture and 1 ml of '100 vol' H_2O_2were added to each. The tubes were then heated to 350-400°C for around two hours in a digestion unit. When the heated samples became colorless, the solution was kept to cool for another 2 hours. Ten ml of distilled water were added to the digest and the digest was thoroughly washed with distilled water and transferred to 50 ml volumetric flasks and made up to volume. This solution was used for the determination of nutrient contents (RRIM 1971b).

Nitrogen (N) and phosphorus (P) contents were determined using a SKALAR San^{++} Auto analyzer and potassium (K) using a GBC 203 Atomic Absorption Spectrophotometer. Nutrient uptake was calculated as the nutrient content of plant part; leaf, stem and root multiply by their total dry weight of each plant part.

Statistical analysis

Statistical analysis of the experimental data *viz*; plant height, plant diameter, total plant dry matter, nutrient contents; N, P and K of leaf, stem and root, N, P and K nutrient uptake of shoot, root and total plant were done by Analysis of variance (ANOVA) is for the RCBD. Subsequently, mean separation was done employing Duncan's Multiple Range Test (DMRT).

Results

Growth parameters were measured at before and after bud grafting stages are given in Table 1 and 2 respectively.

Plant growth parameters; diameter, height and shoot dry weight gave significantly higher values with 100%F (T3), 50%F+BFBF (T5) and 100%F+BFBF (T6) treatments compared to other treatments; 0%F (T1), 50%F (T2) and BFBF (T4) treatments at before bud grafting stage. Same pattern could be observed with diameter, shoot dry weight, root dry weight and total dry weight at after bud grafting stage. There is no significant difference in many growth parameters with 100% F (T3), 50%F+BFBF (T5) and 100%F+BFBF (T6) treatments. Other growth parameters were higher in BFBF included treatments; 50%F+BFBF (T5) and 100%F+BFBF (T6) compared to 100%F (T3) treatment.

Table 1. *Plant growth parameters were measured at before bud grafting stage of nursery plants as affected by different combinations of fertilizer applications*

Treatment	Diameter (mm)	Height (cm)	Shoot dry matter (g/plant)	Root dry matter (g/plant)	Total dry matter (g/plant)
0%F	6.79^{c}	69.4^{c}	12.7^{c}	6.6^{c}	19.3^{c}
50%F	7.8^{b}	68.4^{c}	21.4^{b}	8.6^{b}	30^{b}
100%F	8.5^{a}	78.0^{ab}	25.3^{a}	9.6^{b}	34.9^{ab}
BFBF	7.3^{bc}	69.75^{c}	19.3^{b}	8.7^{b}	28^{b}
50%F+BFBF	9.05^{a}	82.16^{a}	25.9^{a}	11.1^{a}	37^{a}
100%F+BFBF	8.7^{a}	85.08^{a}	28.6^{a}	12.6^{a}	41.2^{a}

(Means with same letters along the column are not significantly different at 5% probability level)

Table 2. *Plant growth parameters were measured at after bud grafting stage of nursery plants as affected by different combinations of fertilizer applications*

Treatment	Diameter (mm)	Height (cm)	Shoot dry matter (g/plant)	Root dry matter (g/plant)	Total dry matter (g/plant)
0%F	6.3^{c}	69.7^{c}	15.9^{c}	7.0^{c}	22.9^{d}
50%F	7.0^{b}	71.1^{c}	18.4^{b}	8.7^{b}	27.1^{c}
100%F	8.1^{a}	78.6^{b}	22.6^{a}	9.9^{a}	32.5^{b}
BFBF	7.5^{b}	68.7^{c}	17.75^{bc}	7.6^{c}	25.3^{c}
50%F+BFBF	8.5^{a}	78.9^{b}	29.3^{a}	10.3^{a}	39.6^{a}
100%F+BFBF	8.6^{a}	85.7^{a}	29.6^{a}	10.2^{a}	39.7^{a}

(Means with same letters along the column are not significantly different at 5% probability level)

Nutrient concentrations were measured in the leaves collected at before and after bud grafting stages are given in Table 3. In both stages P concentrations of the leaf samples were not significantly affected by the treatments. 0% fertilizer (T1) treatment and BFBF treatment (T4) gave significantly lower values of leaf N concentrations compared to other treatments; 50%F (T2), 100%F (T3), 50%F+BFBF (T5) and 100%F+BFBF (T6) at both growth stages. At before bud grafting stage 100%F (T3) treatment increased leaf K level significantly compared to other treatments. Furthermore, significantly lower value of leaf K contents could be observed with 0%F (T1) treatment compared to other treatments.

At after bud grafting stage, BFBF applied treatments; BFBF (T4) and 50%F+BFBF (T5) gave significantly higher values of leaf K contents compared to their respective non BFBF treatments; 0%F (T1) and 50%F (T2).

Table 3. *Effect of different combinations of fertilizer applications on leaf nutrient contents of nursery plants at before and after bud grafting stages*

Treatment	Leaf nutrient contents (%) at before bud grafting stage			Leaf nutrient contents (%) at after bud grafting stage		
	N	P	K	N	P	K
0%F	1.83^{c}	0.23^{a}	0.597^{c}	1.77^{c}	0.23^{a}	0.63^{b}
50%F	2.27^{ab}	0.215^{a}	0.720^{b}	2.42^{ab}	0.22^{a}	0.69^{b}
100%F	2.59^{a}	0.233^{a}	0.807^{a}	2.64^{a}	0.24^{a}	0.8^{a}
BFBF	1.78^{c}	0.245^{a}	0.740^{b}	1.76^{c}	0.25^{a}	0.8^{a}
50%F+BFBF	2.05^{b}	0.233^{a}	0.700^{b}	2.08^{b}	0.25^{a}	0.76^{a}
100%F+BFBF	2.2^{ab}	0.24^{a}	0.765^{b}	2.56^{ab}	0.26^{a}	0.76^{a}

(Means with same letters along the column are not significantly different at 5% probability level)

Table 4. *Effect of different combinations of fertilizer applications on total nutrient uptake of nursery plants at before and after bud grafting stages*

Treatment	Total nutrient uptake (mg/plant) at before bud grafting stage			Total nutrient uptake (mg/plant) at after bud grafting stage		
	N	P	K	N	P	K
0%F	116^{c}	19^{c}	123^{c}	205^{c}	38^{c}	176^{c}
50%F	319^{b}	41^{ab}	262^{b}	283^{b}	50^{c}	270^{b}
100%F	377^{a}	46^{ab}	334^{ab}	421^{a}	64^{b}	342^{ab}
BFBF	159^{c}	37^{b}	193^{c}	173^{c}	48^{c}	209^{c}
50%F+BFBF	340^{ab}	53^{a}	410^{a}	403^{a}	81^{a}	407^{a}
100%F+BFBF	347^{ab}	52^{a}	390^{a}	450^{a}	87^{a}	398^{a}

(Means with same letters along the column are not significantly different at 5% probability level)

Total nutrient uptakes of the plant at both growth stages were calculated by using the data of nutrient concentrations in different plant parts and their dry weights are given in Table 4.

Calculation of nutrient uptake

Nitrogen uptake of shoot = leaf dry wt. x leaf nitrogen content + shoot dry wt. x shoot nitrogen content

Phosphorus uptake of shoot = leaf dry wt. x leaf phosphorus content + shoot dry wt. x shoot phosphorus content

In both stages, total N uptake gave significantly higher value with 50%F (T2), 100%F (T3), 50%F+BFBF (T5) and 100%F+BFBF (T6) treatments compared to 0%F (T1) and BFBF (T4) treatments. Same pattern could be observed with total K uptake at both growth stages. Moreover, there were no significant differences with 100%F (T3), 50%F+BFBF (T5) and 100%F+BFBF (T6) treatments in relation to total N and K uptake at both stages. At after bud grafting stage significantly higher total P uptake with 50%F+BFBF (T5) and 100%F+BFBF (T6) treatments (81 mg P/plant) and 87 mg P/plant) than those in 100%F (T3) (64 mg P/plant) treatment.

Discussion

Fertilizer application according to RRISL recommendations is an accepted practice for rubber in their different stages of rubber nursery, immature rubber and mature rubber to achieve their maximum growth and yield. This was further confirmed by an observation of significantly higher growth parameters of nursery plants with recommended fertilizer application treatment (T3) compared to no fertilizer application treatment (T1) in both growth stages on this study.

Due to low value of cation exchange capacity (CEC) in rubber growing soils added fertilizers can be frequently lost through leaching as well. Phosphorus availability is very low in rubber growing soils and found that P fixation is very high in respect to their high availability of Fe and Al contents in the soil (Dharmakeerthi *et al*., 2010; Silva *et al*., 1977).

Biofilm showed high nitrogenase activity (Jayasinhearachchci and Seneviratne, 2004b; Seneviratne and Jayasinghearachchi, 2005) and biosolubilization of rock phosphate enhanced availability of nitrogen and phosphorus in the soil (Jayasinghearachchi and Seneviratne, 2006a; Seneviratne and Indrasena, 2006). Bandara *et al*. (2006) observed that the conventional practice of plant inoculation with monocultures or mixed cultures of microbes may not facilitate the highest microbial effect in biological N_2 fixation, mineral nutrient release organic acids and plant growth hormone production etc. and may only be better achieved by biofilm formation. Hettiarachchi *et al*., 2012 observed that BFBF treated soils showed significantly higher microbial biomass content (MBC) which reflected the buildup of soil microbial communities. Soil microorganisms are of critical importance in nutrient cycling processes and also source and sink of plant nutrients.

Results showed that 60% of the observed plant growth parameters were significantly higher in BFBF treatments compared to its respective non BFBF treatments at both growth stages. No significant differences between 100%F (T3) treatment and 50%F+BFBF (T5) treatment for more growth parameters and rest of them were significantly higher with 50%F+BFBF treatment compared to 100%F treatment (Table 1&2).

Several studies conducted so far with biofertilizers and BFBF have shown positive results. Hettiarachchi *et al*., 2012 observed that the combined use of 50% recommended fertilizer with BFBF recorded the highest values for most growth parameters in rubber nursery plants under greenhouse conditions. Seneviratne *et al*., (2008a) observed that biofilm attached to the plant root of some crops help in cycling of nutrients and biocontrol of pests and diseases, resulting in enhancing of plant growth accompanied with improved agricultural productivity. Improved growth parameters with reduced rates of chemical fertilizer and BFBF were also recorded with tea plantations in Sri Lanka (Seneviratne *et al.*, 2009 and 2011) and China (Hvistentendahl, 2010). Khan, 2018 observed the performances of rice plant were better when 25% less inorganic N was applied with *Trichoderma* and combined application of *Trichoderma* and *Azospirillum.* Nafady *et al*., 2018 observed that the total dry mass of *Vicia faba* was increased significantly by the application of arbuscular mycorrhizal fungi and biofertilizer compared with non-inoculated plants. Mahanta *et al*., 2014 observed that the inoculation of phosphorus solubilizing bacteria (BSB) and vesicular arbusculatar (VAM) could substitute 50% P of soybean – wheat cropping system with better root property and higher grain yield.

According to the enhancement of growth parameters, improvement of leaf nutrient status could not be observed under present study. Further leaf nutrient contents and total nutrient uptake showed that 25% of the observed nutrient levels were significantly higher in BFBF treatments compared to its respective non BFBF treatments (Table 3 & 4). Rest of the observation gave no significant differences between BFBF treatments compared to its respective non BFBF treatments except one occasion. Out of 12 combinations of 100%F (T3) and 50%F+BFBF (T5) for different leaf nutrients and their total nutrient uptake showed no significant differences except three occasions. Dawwam *et al*., 2013 reported that inoculated potato plants showed significant differences in vegetative growth parameters as well as photosynthetic pigments and N, P and K concentrations compared with control. Similar to that Silva *et al*., 2016 observed high rate application of bio protector and biofertilizer gave increased chemical characteristics and nutrient uptake of melon plant compared to soluble fertilizer applied in the recommended rate.

Moreover, some other studies have reported similar to above mentioned observations of their studies by the application of microbial inoculants as biofertilizers (Mukhtar *et al*., 2017; Dutta *et al.*, 2017; Andrade *et al*., 2013; Gupta *et al*., 2012; Tejada *et al*., 2016). Biofertilizer or BFBF is a term used for the products including living or dormant micro-organisms (Rai, 2006). They are an alternative to mineral fertilizers for increasing soil productivity and plant growth in sustainable agriculture. Recently, there is an increasing interest in this type of environmental friendly, sustainable agricultural practices to alleviate deterioration of nature and environmental pollution (Gauda *et al.*, 2018). They may support the plant growth by several mechanisms such as decomposing organic materials and release inorganic nutrients, increasing the availability of nutrients in the soil by solubilization, chelation, oxidation and reduction processes, increasing root surface area by inducing root growth promotion, enhancing other beneficial symbiosis associated with plant and by combination of mode of actions *etc.* (Vessely, 2003; Anderson *et al.*, 1993; Whiting *et al.*, 2001; Jing *et al*., 2007).

Conclusion

RRISL recommended fertile top soil is not available in most of the rubber nurseries in Sri Lanka. Soil Analysis of the poly bag filling indicated mean values for the: pH 4.6; CEC 2.3 (c mol (+) kg^{-1}; total nitrogen 0.097%; available phosphorus 10 – 12 ppm; potassium 112 ppm and magnesium 223 ppm. Due to low values of CEC in the soils added fertilizers can be frequently lost through leaching as well. Therefore, fertility management is an important aspect for the production of quality planting material of rubber.

This study showed that the enhancement of many growth parameters (around 60%) could be observed with BFBF treatments compared to its respective non BFBF treatments. Further, 50% F + BFBF (T5) and 100%F (T3) gave comparable growth parameters for more assessments and rest of them were significantly higher with 50%F+BFBF (T5) treatment compared to 100%F (T3) treatment. Moreover, their total nutrient uptake showed no significant differences frequently.

It can therefore be concluded that there is a possibility of using BFBF to improve growth and nutrient uptake of rubber seedling plants with modified levels of chemical fertilizers under field condition.

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