Availability and leaching of nutrients after biofilm biofertilizer applications into a Red Yellow Podsolic soil

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Abstract

Nutrient leaching in RYP soils lowers the fertilizer use efficiency in plantation agriculture of Sri Lanka. The study was conducted to determine nutrient leaching and their availability in a rubber (Hevea brasiliensis *Mull Arg.*) *growing RYP soil supplied with biofilm biofertilizers (BFBF). Microorganisms isolated from rubber roots were formulated as BFBF under in-vitro conditions, and applied with or without 50% and 100% recommended chemical fertilizers into rubber seedlings growing in RYP soils packed in PVC columns. Combine use of inorganic fertilizer with BFBF reduced leaching loss of soil organic carbon throughout the experimental period. The application of BFBF had a marginal influence on leaching losses of K but it significantly reduced Mg losses. The leaching loss of N was not affected in the first two months, but from three months onward a significant reduction was observed with combine use of full recommended inorganic fertilizer with BFBF (100%F+BFBF) compare to inorganic fertilizer alone (100%F). Available nutrients, organic carbon and cations exchange capacity (CEC) in these two treatments were also comparable. Reduced leaching losses of some nutrients in the combine use of inorganic fertilizer with BFBF treatments (50%F+BFBF &100%F+BFBF) were not reflected in the nutrient status of the top soil layer (0-5 cm) but high plant dry matter accumulation was observed in BFBF treated plants. There is a possibility to reduce leaching loss of some nutrients and induce dry matter accumulation of rubber plants by BFBF application into RYP (Hapludults) soils.*

Key words: biofilm biofertilizer, dry matter accumulation, growth, *Hevea brasiliensis*, leaching, plant nutrients, red yellow podzolic soil

Introduction

Many individual rubber plantations have undergone around four planting cycles since their first establishment. The continuous cultivation of monocultural cropping system lower fertility and productivity of soils due to deteriorated of physical, chemical and biological properties as a result of soil erosion and nutrient removal by and crop as well as leaching loss. At present mineral fertilizers is the major factor in the maintenance of soil fertility (Bockman *et al*., 1990; Thennakoon, 1990). However, with the increased usage of chemical fertilizers alone, particularly in an unbalanced manner, problems such as diminishing soil productivity and multiple nutrient deficiencies has appeared. With only half of the applied fertilizers getting into the crop (Bockman *et al*., 1990), there is a potential for marked economic losses and for negative environmental impacts. Minimizing leaching and volatilization losses of both mineral and organic fertilizers and prevention of unbalanced indiscriminative fertilization are important strategies for modern agriculture (Ayoub, 1999).

Through the use of biofertilizers, healthy plant can be grown while enhancing the sustainability of the soil. Biofertilizers have found to be highly advantageous in enrichment of soil fertility and fulfilling the plant nutrient requirements by supplying the nutrients through microorganisms and their byproducts. Moreover, biofertilizers do not contain any chemical which are harmful to the living soil. Biofertilizers are eco-friendly organic agro-input and more cost effective than chemical fertilizers. As a recent development in biofertilizer research, biofertilizers have been produced from fungal-bacterial biofilms developed under *in-vitro* conditions (Seneviratne *et al*., 2008a), which are now known as BFBFs (Seneviratne *et al*., 2008b). The BFBFs have showed increased biological nitrogen fixation (BNF), mineral nutrient release in the soil, organic acids and plant growth hormone production *etc*., compared to mono or mixed cultures of the microbes without biofilm formation (Seneviratne *et al*., 2008a).

The aim of the study was to examine the effect of BFBFs on nutrient availability of soil and their effect on leaching loss of nutrients.

Materials and Methods

The production of young budding plants is very much advantageous for successful establishment of rubber. For this purpose rubber seedlings were raised in polybags dimension with 15x30cm for a period of about 4-5 months before transplanted to the field. The experiment was conducted in RYP (Hapludults) soil columns which were prepared using 30cm long PVC pipes with 11cm diameter. Soil was selected from the upper 15cm of *Agalawatta* soil series. These columns were prepared by packing 4 kg of air-dried, 2-mm sieved soil that were thoroughly mixed with 50 g of compost and 50 g of higher grade Eppawala rock phosphate (HERP), into lengths of the column which was sealed at the bottom end. The average bulk density of the packed soil in the column was1.2 gcm^{-3} and assuming particle density was 2.65 gcm^{-3} porosity was calculated as 52%. The pore volume of the column is the total volume of the voids in the column. This can be computed as follows.

Pore volume = A ($95cm^2$) x n (.52) x L (30.5cm)

Where A is cross sectional area of the column, n is the porosity and L is the length of the column. The average pore volume of the packed soil column was $1500cm^3$, before adding fertilizer treatments. Prior to-leaching one germinated seed was planted in each column and tap water was added to bring the moisture content of the soil to 60% of its water holding capacity (Klute, 1986). The water content was checked weekly, by weighing columns and then the original water content was restored. During each leaching event, 1740 ml of distilled water was applied and the amount of water used per column per leaching event was equivalent to 120% of the total pore volume of the soil column. One month after the experiment, fertilizer treatments were initiated. The RRISL recommended NPKMg mixture was applied at zero, 50% and 100% of the currently recommended level with or without the application of developed BFBF. This resulted in 6 treatment combinations (Table 1). The RRISL recommended NPKMg fertilizer was applied at zero level was considered as control treatment. Throughout the experimental period, NPKMg fertilizers were applied at monthly intervals while freshly prepared BFBF was applied at biweekly intervals. These columns were place on a rack and kept at room temperature. Treatments were arranged in a completely randomized block design with ten replicates. There were five leaching events with one month interval in between and the first event was done before adding fertilizer treatments. After each leaching, the leachates were collected until leachates droplets stopped. The effect of different treatment was tested during the course of the experimental period of 4 months.

Table 1. *Treatment combinations of the experiment*

Treatment	**Combination**
Zero	Not any fertilizer
50%F	50% recommended inorganic fertilizer
100%F	100% recommended inorganic fertilizer
BFBF	Biofilm biofertilizer only
50%F + BFBF	50% recommended inorganic fertilizer + BFBF
100%F + BFBF	100% recommended inorganic fertilizer + BFBF

Soil analysis

Soil samples were collected at the end of the experiment was analyzed for nutrient contents. The pH (1:2.5 water) and organic carbon (Walkley, 1947) were determined as basic soil properties. Nutritional status of the soil was evaluated by determining total N by Se/H_2SO_4 digestion, available phosphorus by NH_4F/HCI extraction, and exchangeable K, Ca and Mg by ammonium acetate extraction. Nitrogen and phosphorus contents in the extractions were determined colorimetrically using a Skalar auto analyzer whereas K, Ca and Mg in the extractions were determined using a GBC 9000 atomic absorption spectrophotometer. These methods have been described in details by Yapa (1983) and Jayasundara (1984).

Leachate analysis

Periodically, the volume of leachate from each column was measured, and a sample retained was used for the measurements of nitrate, total nitrogen, calcium, potassium, magnesium and organic carbon concentrations. Nitrate concentration was determined by the sodium salicylate method (Yang *et al.*, 1998) and other elements were analyzed same as the procedures followed for soil nutrient analysis.

Statistical analysis

Statistical analysis of the experimental data was done by analysis of variance followed by a mean separation procedure, Duncan's Multiple Range test (DMRT), at a probability level of 0.05.

Results and Discussion

All of the combinations of BFBFs were found to enhance soil organic carbon status compared to their non BFBF application (Table 2) and BFBF only treatment gave significantly higher value for soil organic carbon over no fertilizer application control (zero). Similar observation has been made by Seneviratne *et al*., (2009) where organic carbon content in tea growing soils has increased significantly with the use of BFBF. This increase in OC content in soil after BFBF application could be attributed to increased storage of root exudate C by the fungal components of the BFBF forming biofilms on the root surface and in the rhizosphere.

Table 2. *Nutrient contents of the top 0-5cm soil layer measured at the end of the experiment*

Treatments	pH	OC%	Kppm	CEC (cmol+/kg)
Zero	5.32^{c}	0.702^{b}	11.8^{d}	4.07^{a}
50%F	5.50^{b}	0.716^{b}	118.04^{b}	4.54^{a}
100%F	5.42^{bc}	0.664^{b}	123.11^{b}	4.56^{a}
BFBF	5.73^{a}	0.842^{a}	39.88^{c}	5.06^{a}
50%F+BFBF	5.53^{b}	0.728^{b}	124.27^{b}	4.73^{a}
100%F+BFBF	5.43^{bc}	0.70^{b}	168.89^{a}	5.46^{a}

Means with same letters in a column are not significantly different at $p< 0.05$

Dissolved organic carbon (DOC) fluxes play a critical role in terrestrial ecosystems. It may be a main energy source for microorganisms (Tranvic, 1992). Over longer time scales, DOC fluxes through soil may be responsible for soil organic matter (SOM) formation via the sorption of DOC onto mineral surfaces in soils (Mcdowell and Wood, 1984; Mcdowell and Likens, 1988). Dissolved organic carbon may be produced by microbial processes, physical deposition in the surface layer, microbial decomposition and sorption processes affect DOC fluxes in the deeper part of the soil profile (Neff *et al*., 2000). Dissolved organic carbon recovered in the leachates following the six leaching events indicated that the lowest DOC was in the 0%F treatment probably due to poor root growth and resulting lower root exudates. The application of BFBF treatments (50%F+BFBF&100%F+BFBF) has resulted lower organic carbon concentration when compared to those in without BFBF treatments (50%F & 100%F), except two or three incidences, throughout the incubation period. Compared to 50% recommended fertilizer treatment, its respective BFBF treatment (50%F+BFBF), showed a significant decrease in leach down of organic carbon from three months onwards. Compared to 100% recommended fertilizer (100%F), its respective BFBF treatment (100%F+BFBF) showed a significant decrease in leach down of organic carbon at first two months but thereafter such a reduction was not observed thereafter (Fig. 1). Carbon export from these soils have not been completely identified in this study. Processes that occur below this sample depth, for example, root exudates and uptake, sorption and desorption, growth of microbial biomass and microbial decomposition, further influence water chemistry and element export through the ecosystem (Hedin *et al*., 1998; Fang *et al*., 2008). Rubber generally grows well in acid soils in Sri Lanka. However, extreme pH conditions are not favourable for good performance of rubber trees and stunted growth has been observed. The effect of soil pH on plant growth is partly through its effects on root function and on soil properties (Samarappuli, 2001). Increasing soil pH from extremely acidic levels upto some extent is important for sustaining soil fertility and such an improvement was appeared significantly ($p>0.05$) in this study with BFBF only (BFBF) compared to their non-application (zero) (Table 2).

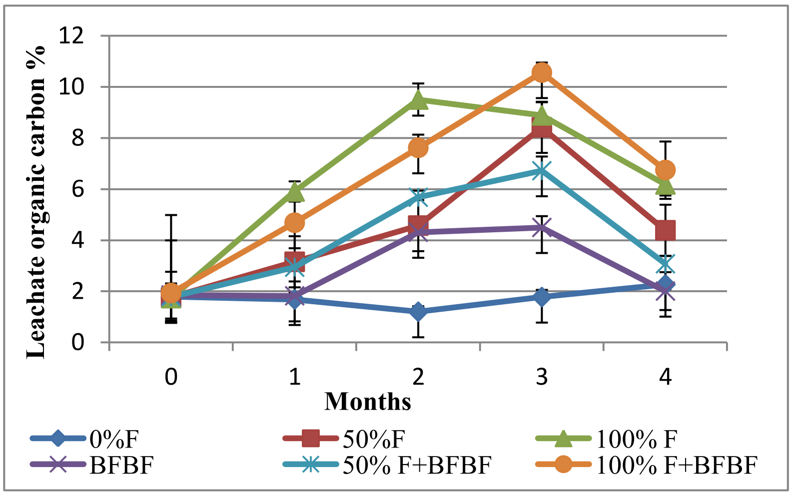

Fig. 1. Organic carbon recovered in the leachates during four months experimental period

Potassium is one of the major nutrient required and taken up in large amounts by *Hevea* besides nitrogen and phosphorus. According to the results of the study, BFBFs have improved the soil exchangeable K content which was observed by the application of BFBF compared to their non-BFBF (Table 2). Meena *et al*. (2013) observed that potassium solubilizing microrganisms are rhizospheric and they solubilize insoluble K to soluble forms promoting plant growth and yield. Santaella *et al*., (2008) reported that *Rhizobium* forms biofilm on non-legume plant root and produces exopolysaccharides (EPS) which is not essential for biofilm formation, but contributes to the colonization of specific zones that increases nutrient availability. The importance of magnesium for the rubber nutrition has been emphasized by several workers (Shorrocks, 1965; RRISL, 1995; Lowe, 1962). Leachate Mg concentrations at every month are given in Figure 2. This data showed significant reduction in leachate Mg with BFBF application. This could be observed with BFBF and 100% recommended fertilizer treatment (100%F+BFBF) compared to 100% recommended fertilizer treatment (100%F). Also BFBF with 50% recommended fertilizer (50%F+BFBF) gave lower leachate Mg than their inorganic fertilizer alone (50%F) treatment particularly towards the latter part of the experiment. Therefore, magnesium leach down was considerably decreased when the soil was colonized by BFBF. Considering the soil data at the end of the experiment did not give higher values of exchangeable soil magnesium with BFBF treatments but it has given much higher plant dry weight than the recommended fertilizer alone treatments.

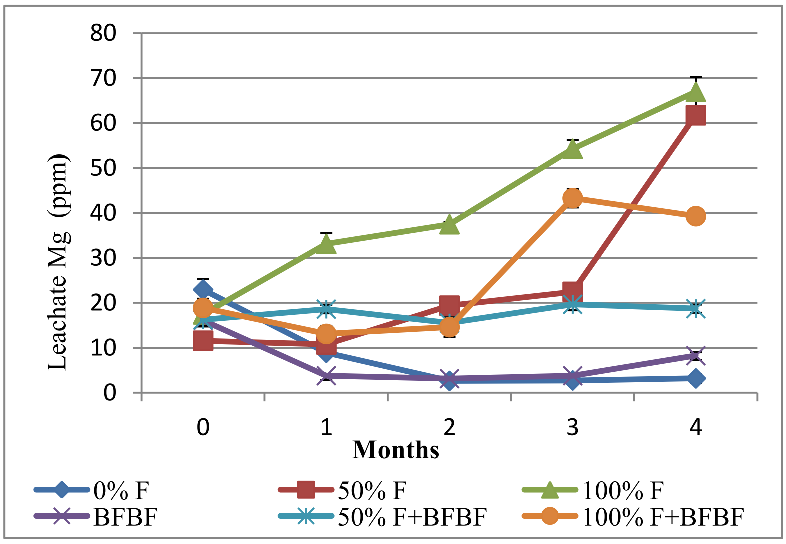

Fig. 2. Available Mg recovered in the leachates during four months experimental period

Leaching of applied fertilizer nitrogen result in reduced uptake efficiency of applied N by the target crop and is an agricultural and environmental problem. The portion of applied N that was not taken up by the crop is either adsorbed by soil components, incorporated into organic matter, volatilized, denitrified or leached below the effective root zone of crops (Wang and Alva, 1996; Vernimmn, 2007; Fox *et al.*, 2001; Kessel *et al.*, 2009). Loss of the applied nitrogenous fertilizers can be reduced through fertilizer management practices (Rao, 1987; Fiez, *et al.*, 1995). The results of nitrate levels in leachate after treatment with fertilizer application and their respective BFBF treatment are presented in Table 3. There was no significant difference observed between the half recommended fertilizer application (50%F) and its respective BFBF application (50%F+BFBF) throughout the experimental period. But leachate nitrate was significantly reduced by the application of BFBF with recommended fertilizer application (100%F+BFBF), from month 3 onwards.

Dissolved organic nitrogen (DON), losses from forested ecosystems have been found to exceed NO_3^- leaching losses (Qualls *et al.*, 2000; Perakis and Hedin, 2002). Neff *et al.* (2003) recorded that "over centuries, DON leaching may represent a significant leak of N as plant and microbes cannot prevent DON losses, even in times of high N demand". Nitrogen is considered to have leaked out of the system when the biological system cannot fully prevent the loss of N and therefore leakage cannot be avoided. The BFBFs have showed increased BNF, mineral nutrient release in the soil, organic acid and plant growth hormone production *etc*., (Seneviratne *et al.,* 2008a). Beneficial biofilms attached to the plant roots of some crops may help cycle nutrient as well as biocontrol of pest and diseases and, consequently, improve the productivity of the crops (Seneviratne *et al*., 2008b). Seneviratne *et al*., (2011) reported that the effect of developed microbial biofilms with N_2 fixers on degraded soil in tea cultivation, with reducing recommended chemical fertilizer use by 50% significantly increased soil microbial biomass and BNF, and decreased soil NO_3^- and pest infestation. Observations made on plant dry matter accumulation in the present study support the observations made in previous studies for other crops (Fig. 3).

Table 3. *Nitrate (ppm) recovered in the leachates during four months*

Treatment	0 months	1 month	2 months	3 months	4 months
0%F	0.708^{a}	0.7374^{c}	0.9^{d}	0.8104^{d}	0.8482^{d}
50%F	0.654^{ab}	3.0802^{b}	3.0472^{c}	2.852^{c}	5.078^{c}
100%F	0.6292^{b}	7.5906^{a}	7.6654^{b}	9.1012^{a}	16.4622^{a}
BFBF	0.6866^{ab}	0.7298^{c}	0.6596^{d}	0.6984^{d}	0.7444^{d}
50%F+BFBF	0.6836^{ab}	4.6136^{b}	2.825^{c}	3.0808^{c}	4.3248^{c}
100%F+BFBF	0.5688^{c}	8.4806^{a}	9.4094^{a}	7.3868^{b}	12.0976^{b}

Means with same letters in a column are not significantly different at $p< 0.05$.

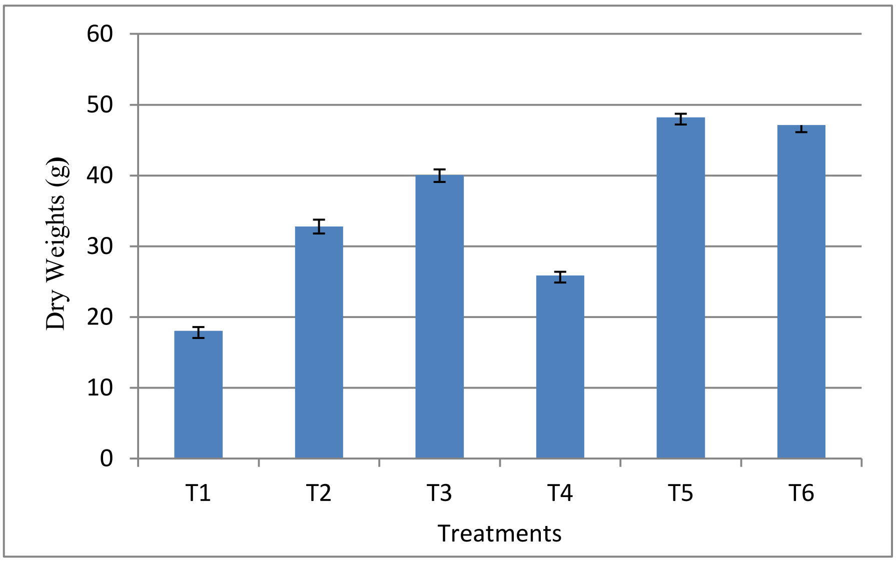

Fig. 3. Effect of different fertilizer applications on dry matter accumulation of rubber plants

Conclusions

In this study it was revealed that the leaching loss of dissolved organic matter, exchangeable Mg, K and nitrate nutrients can be reduced to a significant level by the combine use of chemical fertilizer with BFBF. However this reduction in leaching loss was not reflected in available nutrient contents in the top 0-5cm soil. A slight but significant increase in pH due to BFBF application was also observed. High plant dry matter production by the combine use of chemical fertilizer with BFBF together with reduced leaching losses of Mg, NO_3^- and OC suggests application of BFBF could be an important management practice to increase fertilizer use efficiency in rubber growing RYP (Hapludults) soils. Further studies are needed to investigate the processes that occur throughout the rooting zone affecting fertility parameters in order to realize maximum benefits that could be obtained by the combined used of chemical fertilizers and BFBFs in rubber growing soils, that there is a possibility of using BFBF to enhance plant growth with reduce leaching loss of nutrients upto their optimum levels.

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