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Biofilm biofertilizer application in paddy cultivation: pioneering studies in Sri Lanka

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Abstract

The National Institute of Fundamental Studies (NIFS) developed Biofilm Biofertilizer (BFBF) *in-vitro* and it was tested for efficacy in paddy cultivation, progressing from controlled greenhouse experiments to large-scale field trials, conducted in collaboration with the Department of Agriculture (DoA) and other stakeholders. The outcomes of this research program have proven a substantial reduction of Chemical Fertilizer (CF) usage up to ca. 50% when combined with the BFBF applied at the rate of only 2.5 L/ha. With this fertilizer combination, the BFBF demonstrated the real and significant ($p<0.05$) crop yield improvement effects, boosting the yields on average ca. 25% compared to DoA's CF practices in the long-term extensive research. The widespread adoption of the BFBF across ca. 0.11 Mha of paddy cultivation in Sri Lanka revealed the practicality of its use. In conclusion, the BFBF integrated an eco-friendly fertilizer technology into commercial-scale paddy cultivation. As such, the BFBF

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stands out as a sustainable and transformative solution, providing a blueprint for future agricultural innovations with global implications. This research program serves as a testament to the vital role played by fundamental research in driving a meaningful change for the benefit of agriculture

Keywords: Chemical fertilizer, Biofilm biofertilizer, Organic agriculture, Rice production, Sustainable agriculture

Introduction

Rice (*Oryza sativa* L.) is the staple food for more than half of the global population, with at least 54 kg of annual per capita consumption (FAO, 2024). During the past 30 years, the area under global paddy cultivation has increased up to ca. 155 Mha at a rate of 0.39% per annum and it will gain ca. 10 million ha of rice land by 2050 (Samal et al., 2022; Van Nguyen & Ferrero, 2006). In Sri Lanka, more than 50% of the rural community is engaged in agriculture or agriculture-related livelihood, rice being the staple crop (Marambe et al., 2017; Weerahewa et al., 2010). Currently, ca. 2.7 Mt of paddy is produced by cultivating both *Yala* and *Maha* seasons annually, satisfying around 95% of the domestic requirement (Agriculture and Environmental Statistics, 2022).

Rice production depends on various factors such as climate, availability of water and nutrients, and socio-economic and political situations (Chidi et al., 2015; Obirih-Opareh, 2009; Stuecker et al., 2018). In the mid-1960s, the green revolution introduced user-friendly Chemical Fertilizers (CF), and hence the utilization of bulky organic inputs has declined dramatically (John & Babu, 2021). Initially, the application of CF with improved seeds helped to achieve higher productivity, however, as time passed, farmers tended to use excessive amounts of CF, believing that they would give higher yields. Consequently, soil quality declined causing decreased crop productivity (Aulakh et al., 2022; Horrigan et al., 2002; Nkoa, 2014; Sun et al., 2015) and adverse environmental impacts (Kahandage et al., 2023), indicating the need for urgent remedial measures. As alternatives, organic and mineral fertilizers made of compost, rock phosphate, and fish residue manure have been used. However, since they have relatively low Nitrogen (N), Phosphorus (P), and Potassium (K) concentrations, bulky quantities of them are required to produce a significant yield (Bhunia et al., 2021; Roba, 2018; Seufert et al., 2012; Thomas et al., 2019).

As an eco-friendly substitute, the conceptual use of developed Biofilms as Biofilm Biofertilizer (BFBF) was first introduced in 2003 from fundamental research conducted at the NIFS in Sri Lanka (Rana et al., 2020; Seneviratne, 2003; Seneviratne & Jayasinghearachchi, 2003), highlighting the importance of biofilm application in agriculture more than two decades ago. The BFBFs are being popularized among farmers in Sri Lanka due to its ability to reinstate degraded agroecosystems (Premarathna et al., 2021). The BFBF is a novel biotechnological product that differs from conventional Biofertilizers (BFs), as the former operates in a holistic ecosystem approach (Meepegamage et al., 2021; Premarathna et al., 2022).

Thus far, a large number of research studies have been conducted to evaluate the potential of the BFBF in agriculture and plantations and it has been found that the BFBF is more effective than conventional BFs, particularly for non-legumes such as paddy (Buddhika et al., 2016; Jayasinghearachchi & Seneviratne, 2004; Premarathna et al., 2021; Rathnathilaka et al., 2023; Seneviratne, 2021; Seneviratne et al., 2010, 2017), tea (De Silva et al., 2014), rubber (Hettiarachchi et al., 2014), wheat (Domínguez-González et al., 2022; Swarnalakshmi et al., 2013), maize (Korniichuk & Zayarnyuk, 2018; Mariana et al., 2017) and other field crops (Hassani et al., 2018; Herath et al., 2015; Jayasinghearachchi & Seneviratne, 2004; Ricci et al., 2019; Seneviratne & Kulasooriya, 2013; Singhalage et al., 2018, 2021; Sudadi et al., 2018; Triveni et al., 2012; Velmourougane et al., 2017). All these studies have proven that the BFBF can be profitably used in various crops. Even though pioneering studies on the use of BFBF and their large-scale applications in paddy cultivation, in particular have been conducted in Sri Lanka, the studies have not been sufficiently reported yet in full publications. Therefore, this paper aims to present the results of those studies in a chronological order to report the potential of the BFBF in paddy cultivation of the country.

Materials and methods

The NIFS employed a research project for the investigation of the effect of BFBF on paddy cultivation, which encompassed a multifaceted approach since its inception in 2016. From 2016 to 2018, the project initially conducted greenhouse studies. Then, field experiments were started in agricultural research stations and farms in the Department of Agriculture (DoA) and Mahaweli Authority of Sri Lanka (MASL), respectively. The project started large-scale trials in farmers' fields in collaboration with the Irrigation Management Division (IMD) of the Ministry of Irrigation, and DoA in 2018 and 2019, respectively.

Greenhouse studies

During *Yala*-2016, a pot experiment was conducted in the greenhouse without temperature control, and under natural light conditions at the NIFS. In this experiment, soil was collected from a paddy field at Kimbissa, Dambulla (7°56'24.94''N 80°43'47.19''E). Initial soil properties were; pH 6.8, Soil Organic Carbon (SOC) 1.7%, Soil Total Nitrogen (STN) 0.073%, and Soil Total Phosphorus (STP) 8.4 mg/kg. Five treatments *viz*, (a) 100% CF [425 kg CF/ha (2001 DoA recommendation) (Table 1)], (b) 50% CF (212.5 kg CF/ha), (c) BFBF (2.5 L/ha of BFBF applied two times at 2^{th} and 6^{th} week after transplanting) {BFBF is a patented commercial product [Sri Lanka patent no. 15958 (2013), and hence the exact composition cannot be revealed due to Intellectual Property Rights reasons]}, (d) 50% CF + BFBF (212.5 kg CF/ha with 2.5 L/ha of BFBF), and (e) the control (no amendments) were applied. The treatments and the control were triplicated, and the pots (12 cm in diameter and 15 cm in height) were arranged in a completely randomized design (CRD). Rice variety Bathalagoda (Bg)-360 was used. Five kilograms of paddy soil and equal amounts of water were added to each pot. Grain yield was measured at maturity (90 days after planting). The numbers of tillers (NT) and panicles (NP) per plant were counted and taken as the plant parameters. Fungal and bacterial colony-forming units (CFU) were taken as microbial parameters by following both streak plate and pour plate methods (Sanders, 2012), mentioned in the microbial analyses section under laboratory analysis of this paper.

Table 1. Fertilizer rates for 3.5-month-old paddy varieties in the dry zone of Sri Lanka as recommended by the Department of Agriculture, Sri Lanka in 2001 and 2013.

Year	Time of fertilizer application (weeks after planting)	Fertilizer type and amount (kg/ha)			
		Urea	TSP	MOP	Total
2001	Basal	12.5	87.5	37.5	137.5
	2nd	75.0	-	-	75
	4th	125.0	-	-	125
	6th	50.0	-	37.5	87.5
	8th	-	-	-	0
	Total	262.5	87.5	75.0	425
2013	Basal	-	55.0	-	55
	2nd	50.0	-	-	50
	4th	75.0	-	25.0	100
	6th	65.0	-	35.0	100
	8th	35.0	-	-	35
	Total	225.0	55.0	60.0	340

TSP: Triple super phosphate, MOP: Muriate of potash. Source: Department of Agriculture (2013)

During *Maha*-2016/2017, another pot experiment was conducted using soils collected from Dehiaththakandiya in Mahaweli system C (7°40'29.70''N 81°2'45.86''E) and Rice Research and Development Institute (RRDI), Bathalagoda (N 7° 31' 52.82 E 80° 26' 5.95). Six different treatments *viz*., (a) 100% CF [(340 kg CF/ha) 2013 DoA recommendation (Table 1)], (b) 80% CF

(272 kg CF/ha), (c) 80% CF + BFBF (272 kg CF/ha with 2.5 L/ha of BFBF), (d) 65% CF (221 kg CF/ha), (e) 65% CF + BFBF (221 kg CF/ha with 2.5 L/ha of BFBF), and (f) the control (no amendments) were applied. Since the rates of CF have been reduced in the DoA recommendation in 2013 in comparison to the recommendation in 2001, the 50% CF level had to be increased up to 65% CF to match the nutrient requirement of the rice plant when the CF was coupled with the BFBF. In addition to the 65% CF, an 80% CF level was included to maintain the continuity of the range of CF levels in the study (Table 1). The experiment was arranged in the CRD with four replicates in each treatment. Plant growth parameters and SOC, STN, STP, and soil potassium (SP) were measured in both the tillering and 50% flowering stages by following the methodology mentioned in the section under laboratory analysis.

Field experiments in agriculture research stations and farms

During the *Yala*-2017, field experiments were carried out at the DoA research stations at Ambalantota (N 6°7'35.58 E 81°1'51.0852), and Mahailluppallama (N8°6'42.8472 E80°28'3.2736), and also in Aralaganvila (N7°47'23.184785 E81°9'36.063168) MASL farm. Initial soil properties in the three sites were not significantly different and ranged as follows; pH 6.9 - 7.5, SOC 1.1 - 2.3%, STN 0.07 - 0.26%, and STP 9.8 - 11.9 mg/kg. Six different treatments *viz*. (a) 100% CF [340 kg CF/ha (2013 DoA recommendation) (Table 1)], (b) 80% CF (272 kg CF/ha), (c) 80% CF + BFBF (272 kg CF/ha with 2.5 L/ha of BFBF), (d) 65% CF (221 kg CF/ha), (e) 65% CF + BFBF (272 kg CF/ha with 2.5 L/ha of BFBF), and (f) the control (no amendments) were applied. The BFBF was applied using a spray tank by mixing 2.5 L of BFBF with ca. 400 L of water for one hectare. And, the same treatments tested in the last season were used to evaluate the effect of the BFBF under field conditions. The plots of 3 m × 4 m were arranged in a randomized complete block design (RCBD) with three replicates for each treatment. Direct sowing of 120 g of pre-germinated paddy seeds of 90 days-old variety Bg-300 was done in each plot. Irrigation water was managed separately for each plot, and only weedicides were applied one week before or after the BFBF application. Generally, pesticides were not required due to the bio-controlling effect of the BFBF (Buddhika et al., 2013). Soil physicochemical, plant growth, and microbial parameters were measured as explained in the methodology mentioned in the section under laboratory analysis. Grain yield was evaluated by harvesting the whole plot after removing the borders. The study was continued in *Maha* 2017/2018 at the RRDI, Bathalagoda, and MASL Thoda farm, Dehiattakanadiya (N 7° 31' 10.002 E 81° 2' 59.2728). Initial soil properties in the two sites were not significantly different and ranged as follows; pH 5.4 - 6.5, SOC 1.1 - 2.3%, STN 0.06 - 0.53%, and STP 0.3 - 1.1 mg/kg. The rice variety used was Bg-360.

Collaborative trials with the irrigation management division and the Department of Agriculture conducted in farmers' fields

During *Yala*-2018 and *Maha*-2018/2019, the efficacy of BFBF was tested under farmers' field conditions by comparing two practices *viz*. (a) farmers' CF alone practice, which was equal to the CF rate recommended by the DoA in 2001 [Chemical Fertilizer Practice (CFP), 425 kg CF/ha (Table 1)], and (b) BFBF practice [BFP, (2.5 L/ha BFBF with 225 kg CF/ha)] selected based on the results of the previous studies explained above. In 2018, a novel method of the application of BFBF was introduced to paddy fields in which the BFBF liquid was soaked into a small amount of dry fine sand or biochar and then mixed with the CF and broadcasted to the field. Thereby, the spraying cost was removed in the BFBF practice. The trials were conducted in collaboration with the IMD in Ampara (N 7° 18' 7.1424 E 81° 40' 30.4428), Kurunegala (N 7° 44' 50.2044 E 80° 7' 54.156), and Polonnaruwa (N 7° 56' 25.3356 E 81° 1' 7.8276). The average annual temperature and average annual precipitation of the locations ranged from 23 - 34 °C and 950 - 1070 mm, respectively. Two consecutive uniform paddy fields (ca. 0.4 ha each) were selected for the two practices. Short-duration (3.5-month-old) paddy varieties, Bg-94-1, Bg-352, Bg-366, Bg-357, Bg-300, Bg-360, and Ambalantota (At)-302 were used. The study was continued only in Ampara during *Yala*-2019 season.

After the successful completion of the collaborative field trials with the IMD, the NIFS collaborated with the DoA for the field trials from *Maha*-2019/2020 season onwards. These trials were conducted in 14 farmers' fields in 14 Agrarian divisions in Anuradhapura (N 8° 18'43.2 E 80° 24'15.4), Polonnaruwa (8°08'28.4"N 80°58'47.8" E), Kurunegala (N 7° 29' 10.3524 E 80° 21' 31.5972), Kegalle (N 7° 16' 51.6396 E 80° 20' 38.1696), and Ampara districts, according to the protocols given by the DoA. Two practices *viz*. (a) CFP [340 kg CF/ha

(2013 DoA recommendation) (Table 1)] and (b) BFP (2.5 L BFBF with 225 kg CF/ha) together with a reference treatment *viz*. (c) 65% CFP (225 kg CF/ha) alone, were applied. The reference treatment was used to evaluate the effect of BFBF on top of the CF. Consecutive uniform paddy fields (up to ca. 0.4 ha each) were selected for each practice. After Maha-2019/2020 season, the DoA decided to continue the trials only in Polonnaruwa and Ampara districts during *Yala*-2020 season. After verifying the effect of BFBF using the reference treatment, the DoA decided to compare only the BFP and CFP in the next trials in 46 farmers' fields of 37 Agrarian divisions in Ampara (N 7° 40' 15.4092 E 81° 2' 47.0184), Polonnaruwa (8°08'28.4"N 80°58'47.8" E), Kurunegala (N 7° 29'10.3524 E 80° 21' 31.5972), Hambantota (N 6° 8' 34.764 E 81° 7' 16.3344), Monaragala (N 6° 53' 26.1168 E 81° 20' 44.7), Trincomalee (N 8° 35' 14.3196 E 81° 12' 54.5328) and Batticaloa (N 7° 43' 29.6256 E 81° 41' 48.1956) from *Maha*2020/2021 season onwards.

During *Maha*-2020/2021 and *Yala*-2021, in response to the fertilizer distribution policy changes implemented by the National Fertilizer Secretariat (NFS) of Sri Lanka, which involved further reduction of the fertilizer quantities supplied to farmers, we were prompted to study the response of the BFBF practice under the reduced rate of CF. This led us to change the rates of fertilizer application of the two practices *viz*. (a) CFP (the NFS rate, 283 kg CF/ha) and (b) BFP (a 30% reduction from the NFS rate, i.e. 198 kg CF/ha + 2.5 L/ha BFBF) under the reduced fertilizer rates (Table 2). These trials were conducted across 23 farmers' fields, spanning 19 Agrarian divisions in Ampara, Polonnaruwa, Monaragala, Trincomalee, and Batticaloa. During the *Yala*-2022 and *Maha*-2022/2023 seasons, the trials were conducted in three agriculture schools of the DoA upon their request. The two practices *viz*. (a) CFP [340 kg CF/ha (2013 DoA recommendation) (Table 1)] and (b) BFP (2.5 L BFBF with 225 kg CF/ha) were compared in the agricultural schools situated in Vavuniya (N 8° 46' 29.4744 E 80° 29' 9.6792), Pelwehera (N 7° 53' 46.0068 E 80° 39' 56.4624), and Agrunakolapalassa (N 6° 9' 0.8028 E 80° 53' 56.9004). Altogether, 232 farmers' field trials in 13 districts were conducted from 2018 to 2023. Seasons, locations, number of trials, treatments, and the fertilizerapplication rates of the respective field trials explained above are summarized in Table 2.

Table 2. Summary of the field trials conducted in collaboration with the Irrigation Management Division of the Ministry of Irrigation, and the Department of Agriculture since *Yala*-2018.

Season	Locations	Number of trials	Treatments	CF rates (kg/ha) and BFBF(L/ha)				
				Urea	TSP	MOP	BFBF	Total
Yala-2018 and *Maha*-2018/2019	Ampara Kurunegala Polonnaruwa	61	BFP CFP	150 284	40 76	35 66	2.5 -	225 425
Yala-2019 to Yala-2020	Ampara Anuradhapura Batticaloa Hambantota Kegalle Kurunegala Monaragala Polonnaruwa Trincomalee	102	BFP CFP 65% CFP	150 225 150	40 55 40	35 60 35	2.5 - -	225 340 225
Maha-2020/2021 and *Yala*-2021	Ampara Polonnaruwa Monaragala Trincomalee Batticaloa	59	BFP CFP	146 198	12 25	40 60	2.5 -	198 283
	Pelwehera	10	BFP	150	40	35	2.5	225

Yala-2022 and *Maha*-2022/2023	Vavuniya Angunakolape-lessa		CFP	225	55	60	-	340
Total number of trials		232						

RRDI: Rice Research and Development Institute, Bathalagoda, CF: Chemical fertilizer, CFP: Chemical fertilizer practice, BFBF: Biofilm Biofertilizer, BFP: BFBF practice, TSP: Triple superphosphate, MOP: Muriate of potash

Sample collection

In the pot and field experiments, three (from replicated pots) and five plants (hills) from random positions, respectively were carefully uprooted at the 50% flowering stage with root-zone soil (ca. 0.2 m and 0.3 m depth in pot and field, respectively) without damaging the root system. Root-zone soil was sampled because it is the main sphere in which the root system explores resources that are important for plant growth, and also it is the main soil region of the water cycle. The collected samples were immediately brought to the laboratory of the NIFS. Then, the soil was carefully removed without damage to the root system of each plant. The soil sample was mixed thoroughly and divided into two subsamples; one subsample was used for analyzing moisture, pH, and microbial parameters and the second subsample was air-dried and stored for further analyses.

Laboratory analyses

Plant analyses

After soil was removed from the root system, it was washed carefully without damaging the roots. Roots and shoots were separated and oven-dried at 65 °C until a constant weight was reached, and then root dry weight (RDW) and shoot dry weight (SDW) were recorded using a top loading balance. Plant growth parameters such as NT, shoot length (SL), root length (RL), root volume (RV), and chlorophyll content (CC) (SPAD) were measured.

Soil parameters

Soil moisture was determined by oven-drying fresh soil at 105 °C until a constant weight is reached. Soil pH was measured using a glass electrode by mixing soil: water 1:2.5 (w/v) ratio. A portion of the stored soil was passed through a 0.5 mm sieve after crushing. By using those soils, SOC was determined by the Walkley-Black colorimetric method (McIntyre & Baker, 1978). STN and STP were measured using the distillation and titration method (Bremner & Mulvaney, 1982) and the colorimetric method, respectively. And, exchangeable SP was estimated using a modified Morgan-extraction method (Anderson & Ingram, 1990).

Microbial analyses

The quantification of Endophytic Diazotrophs (ED) present in plant leaves was accomplished by employing specific culture media. The ED was cultured at a 10^{-6} dilution in a Combined Carbon Medium (CCM, (Rennie, 1981). Endophytic non-diazotrophs (END) was cultured in a modified CCM medium, supplemented with NH_4NO_3. Before the extraction of endophytes, leaf surfaces were thoroughly sterilized using a 70% ethanol solution, thereby ensuring the purity of the surfaces of the sample. Colony counts were documented 48 hours post-inoculation to evaluate their respective populations. Soil-based microorganisms, encompassing soil fungi (SF), total bacteria (SB), diazotrophic bacteria (DB), and plant-associated microorganisms, endophytic fungi (EF), endophytic bacteria (EB) were enumerated using classical serial dilution and the spread plate technique. The media employed for these procedures included Potato Dextrose Agar (PDA), Czapek-Dox agar, N-free CCM, and Nutrient Agar (NA), with a dilution ratio of 10^{-3}. This rigorous methodology facilitated the accurate quantification of these microbial populations, thereby contributing to the robustness of the study.

Grain yield parameters

In each practice or treatment, the yield was measured using a similar method. In the pot experiments, the grain yield was measured by only measuring the hundred-grain weight (HGW) or the thousand-grain weight (TGW) by using a top loading balance. In the small field plots, the yield was recorded by performing three to five (depending on the plot size) 1×1 m^2 crop cuts in each plot. For the large field plots, the field was divided into four equal sub-sections and numbered them in a random order. Using a random matrix table, the harvesting plot was selected. To avoid the border effect, 1 m of distance from the field bunds was removed. If there was any physical damage due to some reason, the next random number was selected from the matrix. In evaluating grain yield, the collected crop-cuts were threshed and cleaned separately, grains were dried to 14% moisture and weighed, and crop yield per hectare was calculated.

Statistical analysis

Confirmation of the normal distribution of data was done using the normality test. Means were compared using a one-way analysis of variance (ANOVA), followed by Tukey's HSD test. Probability < 0.05 was used as the threshold for significance. Relationships between parameters were constructed using regression analysis. All data were analyzed using the Minitab 17 version.

Results and Discussion

Greenhouse studies

Microbial parameters

The results showed a significant increase in root EB in the 50% CF + BFBF treatment over the 100% CF treatment ($p<0.05$, Fig. 1).

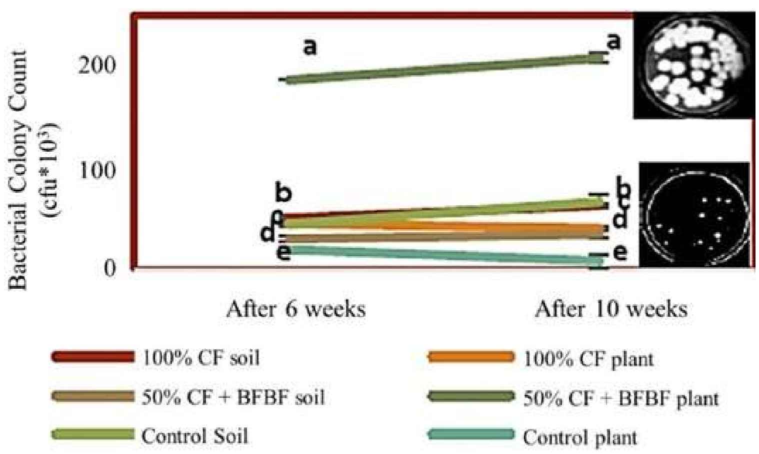

Figure 1. Plant root (endophytic) bacterial and soil bacterial colony counts (CFUper g fresh root or soil) at 6 and 10 weeks in 100% CF (425 kg CF/ha), 50% CF, 50% CF + BFBF, and BFBF only and the control in the pot experiment in 2016. Inset agar plates

Further, the EB count in the root tissues was higher than that of the soil, possibly indicating the activation of dormant bacterial spores even endophytically (Jayasinhearachchi & Seneviratne, 2006; Li et al., 2023). This suggests that fertilization strategies have influenced the population dynamics of microbes in the root. The BFBF application with a reduced rate of CF has led to endophytic biofilm formation, as reflected by larger colonies (Figure 1). This phenomenon of biofilm development was previously reported by Seneviratne et al. (2017) and Seneviratne et al. (2011) in plants as well as in soil, respectively.

With the reduction of CFs, the soil fungi showed a significant increase in abundance in comparison to that of the 100% CF treatment ($p<0.05$, Figure 2). A significant increase in endophytic fungi was also observed in 50% CF + BFBF treatment. As such, endophytic colonization of fungi and diazotrophic bacteria exhibited significant increase with the BFBF utilization ($p<0.05$, Figures 1 and 2), suggesting that biofilm-mediated biochemical production has led to a significant increase in diazotrophic abundance (Jiang et al., 2021; Seneviratne & Kulasooriya, 2013). These findings collectively illuminate the intricate interplay between microbial dynamics and fertilization strategies, underscoring the potential of BFBF to enhance agricultural productivity through improved soil quality (Rathnathilaka et al., 2023)

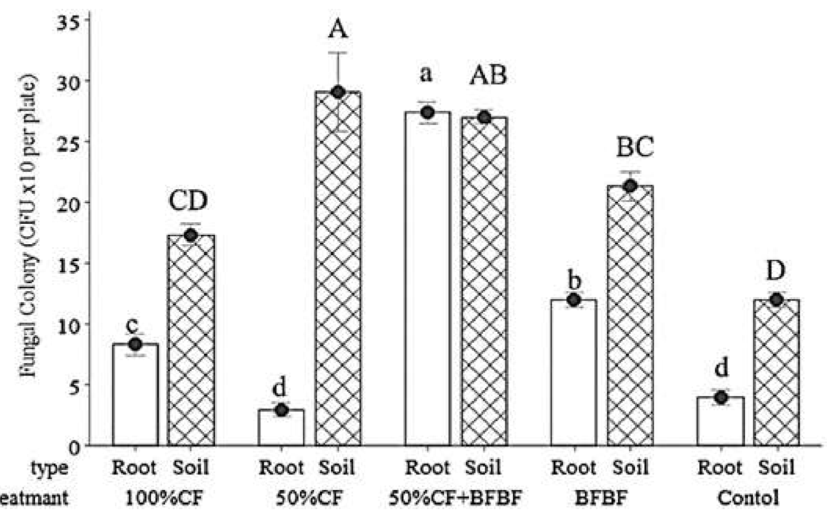

Figure 2. Presence of fungi in the root-zone soil, and in plant roots in the CF alone, CF + BFBF combination and BFBF alone treatments.

Plant growth and soil parameters

Results revealed that the 50% CF + BFBF treatment can produce NT and NP, which were comparable to those of the 100% CF treatment (Figure 3). This may be due to the plant growth-promoting microbial action of the BFBF under the low CF rate (Dewi et al., 2023; Premarathna et al., 2021). It has been reported that the tillering ability has a significant influence on panicle density thus leading to increased grain yield (Mohanan & Mini, 2007; Wu et al., 1998).

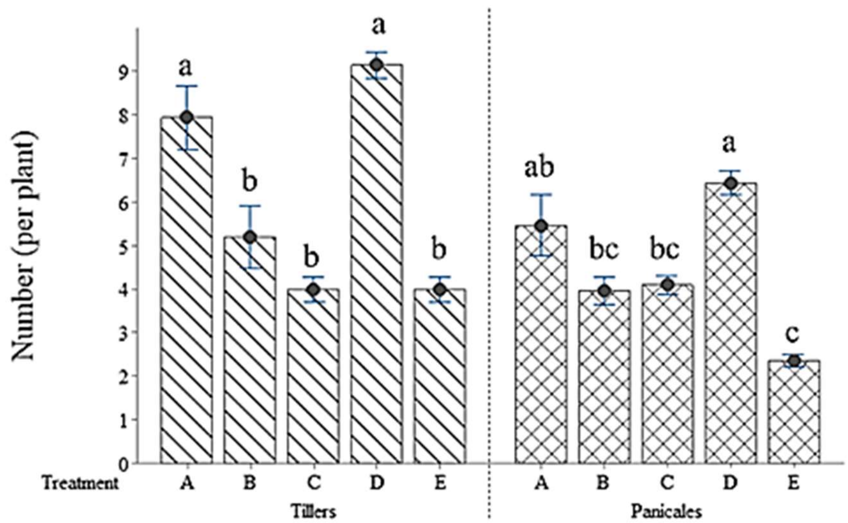

Figure 3. Tiller and Panicle numbers in A: 100% CF (425 kg CF/ha), B: 50% CF, C: BFBF only D: 50% CF + BFBF, and E: control in the pot experiment conducted in 2016 at NIFS.

In the pot experiment conducted during the *Maha*-2016/2017 season, the 65% CF + BFBF treatment showed a significant increase in shoot dry weights at the flowering stage ($p<0.05$, Table 3). Shoot length also significantly increased at the tillering stage in both 80% CF + BFBF and 65% CF + BFBF applications (Table 3). This suggested that the 65% CF + BFBF treatment might have positively impacted on shoot development during the tillering stage. As such, the application of BFBF showed significant positive effects on paddy plants at different growth stages. Generally, BFs like BFBF can enhance paddy plant growth parameters such as plant height, tiller number, and ultimately yield (Shi et al., 2023). The BFs of various microorganisms have shown significantly increases of the number of tillers in paddy plants, suggesting a potential for enhanced growth and productivity compared to CF alone (Sudadi et al., 2022). These findings collectively supported the notion that BFs can play a crucial role in promoting the growth and development of paddy plants, particularly during critical growth stages such as tillering and flowering. This could lead to triggering of plant growth-promoting rhizobacteria (PGPR) with the increased root exudation in the application of BFBF (Hettiarachchi et al., 2018; Rizvi et al., 2015). Tiller and panicle counts, and 100-grain weight were not significantly different between 65% CF + BFBF and 100% CF treatments (Tables 3 and 5).

The soil parameters of this study indicated a significant increase in SOC under the BFBF treatments at both tillering and flowering stages (Table 4). This could be attributed to increased root exudation and incorporation of organic compounds into soil organic matter within the root-zone, driven by the enhanced microbial action, showing a positive impact of BFBF treatments on SOC enhancement (Jayasekara et al., 2022). There were significant increases in STN, STP and SP at the tillering and/or flowering stages under 65% CF + BFBF treatment ($p<0.05$, Table 4). This could have been caused by strengthened network interactions among the soil parameters in paddy cultivation with the BFBF application (Jayasekara et al., 2023). Furthermore, the BFBF has been reported to play a major role in enhancing soil fertility by increasing the pools of essential nutrients in the root-zone (Rathnathilaka et al., 2023). These outcomes underscore the potential of BFs as valuable soil amendments, particularly when combined with reduced rates of CF (Abbasi & Yousra, 2012).

Table 3. Plant growth parameters at tillering and flowering stages in the pot experiment conducted in *Maha*-2016/2017 season at NIFS.

Treatment	Tiller count (per plant)		Shoot length (cm)		Root length (cm)		Shoot dry weight (g/plant)		Root dry weight (g/plant)	
	T	F	T	F	T	F	T	F	T	F
100% CF	9.5^{a}	7.0^{a}	50.6^{ab}	78.4^{a}	15.0^{a}	16.7^{a}	3.56^{abc}	9.41^{bc}	2.90^{ab}	5.40^{abc}
80% CF	9.3^{a}	7.7^{a}	46.1^{abc}	75.2^{ab}	13.2^{a}	12.2^{a}	2.99^{abcd}	7.62^{c}	2.34^{ab}	3.66^{bcd}
80% CF+ BFBF	9.3^{a}	8.5^{a}	56.6^{a}	83.6^{a}	15.7^{a}	13.8^{a}	3.90^{ab}	11.1^{b}	3.87^{ab}	6.01^{ab}
65% CF	8.8^{a}	8.3^{a}	42.8^{bc}	68.9^{ab}	12.2^{a}	16.5^{a}	2.25^{bcd}	6.95^{cd}	2.12^{b}	2.72^{cd}
65% CF + BFBF	10.8^{a}	9.8^{a}	57.5^{a}	82.3^{a}	16.1^{a}	16.1^{a}	4.41^{a}	13.8^{a}	5.67^{a}	7.47^{a}
Control	7.5^{a}	8.5^{a}	33.3^{c}	57.3^{b}	12.7^{a}	13.7_{a}	1.07^{d}	4.58^{d}	1.38^{b}	1.35^{d}
BFBF only	7.5^{a}	5.6^{a}	41.7^{bc}	58.1^{b}	15.2^{a}	12.5^{a}	$1.75c^{d}$	7.056^{cd}	1.79^{b}	2.23^{d}
Pooled SD	2.370	3.344	3.297	4.578	1.767	1.559	0.925	1.108	1.541	1.302
P-value	0.446	0.590	0.001	0.003	0.269	0.087	0.000	0.000	0.012	0.000

Values followed by the same letter in the same column are not significantly different at 5% probability level. T: Tillering stage, F: Flowering Stage, Pooled SD: Pooled standard deviation, CF: Chemical fertilizer, BFBF: Biofilm biofertilizer

Table 4. Root-zone soil organic carbon (SOC), nitrogen (N), phosphorus (P), and exchangeable soil potassium (SP) at tillering and flowering stages in the pot experiment conducted during *Maha*-2016/2017 season at NIFS.

Treatment	STN (%)		STP (%)		Ex. SP (mol/kg)		SOC (%)	
	T	F	T	F	T	F	T	F
100% CF	0.107^{b}	0.136^{bc}	0.177^{a}	0.189^{bc}	31.0^{bc}	12.6^{a}	1.35^{b}	1.60^{c}
80% CF	0.108^{b}	0.127^{bc}	0.179^{a}	0.173^{bc}	31.1^{bc}	11.9^{a}	1.37^{b}	1.66^{bc}
80% CF + BFBF	0.128^{ab}	0.150^{b}	0.226^{a}	0.229^{ab}	40.3^{ab}	14.3^{a}	1.49^{ab}	1.85^{ab}
65% CF	0.111^{b}	0.124^{bc}	0.172^{a}	0.164^{bc}	21.6^{cd}	5.49^{a}	1.18^{b}	1.64^{bc}
65% CF + BFBF	0.193^{a}	0.186^{a}	0.343^{a}	0.294^{a}	48.0^{a}	14.8^{a}	1.81^{a}	1.99^{a}
Control	0.103^{b}	0.112^{c}	0.148^{a}	0.144^{c}	13.2^{d}	1.77^{a}	1.30^{b}	1.59^{c}
Pooled SD	0.035	0.0135	0.1646	0.0322	6.456	7.462	0.1863	0.1055
P-value	0.018	0.000	0.597	0.000	0.000	0.124	0.003	0.000

Values followed by the same letter in the same column are not significantly different at the 5% probability level. T: Tillering stage, F: Flowering Stage, Pooled SD: Pooled standard deviation, CF: Chemical fertilizer, BFBF: Biofilm biofertilizer

100-grain weight

In the pot experiment, there was no significant difference in 100-grain weight among the treatments and the control (Table 5). This suggests that grain filling did not respond to any fertilizer treatment in the pots.

Table 5. Comparison of 100-grain weight at harvest in the pot experiment conducted during *Maha*-2016/2017 season at NIFS.

Treatment	100-grain weight (g)
100% CF	1.978[a]
80% CF	1.999[a]
80% CF+ BFBF	2.273[a]
65% CF	1.810[a]
65% CF+ BFBF	2.506[a]
Control	1.458[a]
Pooled SD	0.769
P-value	0.504

Values followed by the same letter in the same column are not significantly different at the 5% probability level

Field experiments in agriculture research stations and farms

Microbial parameters

At the harvesting stage, the EB counts were significantly higher in the 65% CF + BFBF treatment than that of the 100% CF treatment, but the EB of the former treatment was comparable to that in the control (Figure 6u). Previous studies have also reported that BFs containing beneficial bacteria such as *Pantoea dispersa*, *Burkholderia cenocepacia*(Do et al., 2023), *Paenibacillus alvei*, and

Bacillus cereus(Purwaningsih et al., 2023) have led to enhanced microbial diversity in root-zone soils ultimately resulting in an increase in EDs and EBs (Gupta et al., 2012; Jha et al., 2020). Further, the use of BFs has been found to improve soil properties with an increase in the population of ED, leading to endophytic biological N_2 fixation (BNF) and eventually boosting paddy yields (Rana et al., 2022).

Plant growth and soil parameters

At the flowering stage during *Yala*-2017 season, the 65% CF + BFBF treatment showed an increasing trend in root volume (Figure 4a) compared to other treatments, a phenomenon attributed to the growth-stimulating effect of PGPR (Backer et al., 2018; Ekanayake & Seneviratne, 2024). The panicle number per hill was also significantly higher in the same treatment (Figure 4b), possibly due to the same effect of PGPR (Ariyasena et al., 2022; Shahzad et al., 2017). Interestingly, a significant positive correlation was observed between NP per hill and shoot EB (Figure 4h), highlighting the potential role of these bacteria in panicle development. Additionally, CC increased in the 65% CF + BFBF treatment, particularly at the tillering and flowering stages (Figure 5k and 5l).

Soil moisture (Figure 5i) and SOC content (Figure 5j) significantly increased in the 65% CF + BFBF treatment, underscoring the positive impact of this fertilizer combination on soil moisture and C sequestration (Jayasekara et al., 2022). These findings collectively demonstrate the influences of applying BFBF with a reduced rate of CFs on plant growth and soil parameters, indicating the potential for enhancing agricultural productivity and sustainability. Moreover, it was observed that the soil, plant, and microbial parameters were consistently comparable or higher in the 65% CF + BFBF treatment compared to the other treatments having different rates of CF, with or without BFBF (Figures 4 and 5).

During the *Maha*2017/2018 season, the initial soil parameters of Thoda farm and RRDI Bathalagoda research station showed no significant differences (Table 6).

Table 6. Initial soil analyses during *Maha*2017/2018 season at Thoda farm, Dehiaththakandiya, and Rice Research and Development Institute (RRDI), Bathalagoda.

Soil Parameters	Thoda farm	RRDI
pH	$6.1^{a}\pm1.41$	$6.4^{a}\pm0.06$
SOC (%)	$1.26^{a}\pm0.10$	$1.33^{a}\pm0.03$
STN (%)	$0.11^{a}\pm0.005$	$0.09\pm0^{a}.002$
STP (%)	$0.22^{a}\pm0.08$	$0.07^{a}\pm0.002$
Exchangeable K (µg/g)	$1.13^{a}\pm0.002$	$1.51^{a}\pm0.13$

Values followed by the same letter in each row are not significantly different at 5% probability levels

In RRDI Bathalagoda, EB counts showed a significantly increasing trend from 100% CF treatment to 65% CF + BFBF treatment, including the control ($p<0.05$, Figure 6u). It is clear from this that the CF rate has influenced the EB colonization, higher the CF rate lower the colonization (Adeleke et al., 2021; Bueno et al., 2022). Shoot growth and NT decreased with the increased EB colonization (Figure 6q and 6r). This might have been caused by the increased utilization of photosynthate by the EB. However, the NP did not change (Figure 6s).

In the same *Maha*-2017/2018 season at Thoda farm Dehiaththakandiya, the 65% CF + BFBF treatment showed significantly higher SOC, STN, and soil moisture than that of the 65% CF alone treatment ($p<0.05$, Fig. 7a, 7b and 7d). This clearly shows that the effect of BFBF is real and significant in terms of basic soil parameters such as the above. It has been reported that the reduced amount of CF application with BFBF facilitates BNF in non-legumes, root-associated biofilms acting as pseudo nodules (Seneviratne et al., 2009). This could ultimately lead to higher grain yield. It has been reported that the BFBF application increases the nutrient availability within the root-zone, which facilitates higher nutrient uptake for plants (Artursson et al., 2006; Seneviratne et al., 2008). The STP did not change from 65% CF to 100% CF treatments (Figure 7c and 7e). This is because the paddy crop does not respond to P fertilizer in a majority of field soils in Sri Lanka (Sirisena & Suriyagoda, 2018). Interestingly, the 65% CF + BFBF treatment showed higher magnitudes in numerous soil, plant, and microbial parameters than the other treatments of almost all field locations (Figures 4, 5, and 7f). This is a good proof to conclude that the optimum level of CF for the BFBF action is 65%. Therefore, this fertilizer level was used for further research in large-scale farmers' fields.

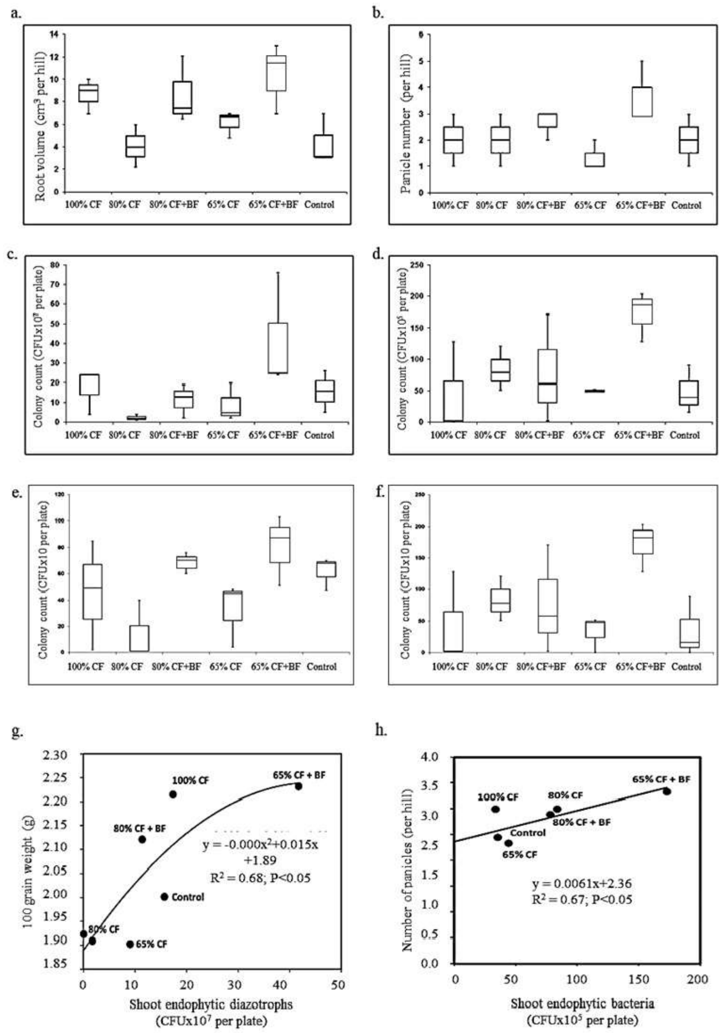

Figure 4. Multiple graphs including plant and microbial parameters and relationships of these parameters in Yala-2017 season, Ambalantota, Sri Lanka. (a) Root volume, (b) panicle number per hill, (c) shoot endophytic diazotrophs, (d) shoot endophytic bacteria, and in the same season at Mahailluppallama, Sri Lanka, (e) shoot endophytic diazotrophs, and (f) shoot endophytic bacteria, at 50% flowering stage. (g) Relationship between 100-gain weight and shoot diazotrophs, (h) relationship between the number of panicles per hill and shoot endophytic bacteria. The 100% CF rate was the recommended amount of the Department of Agriculture, Sri Lanka in 2013, i.e. 340 kg CF/ha, and BFBF was applied at 2.5 L/ha. Standard error bars are on the boxes in the box-plots

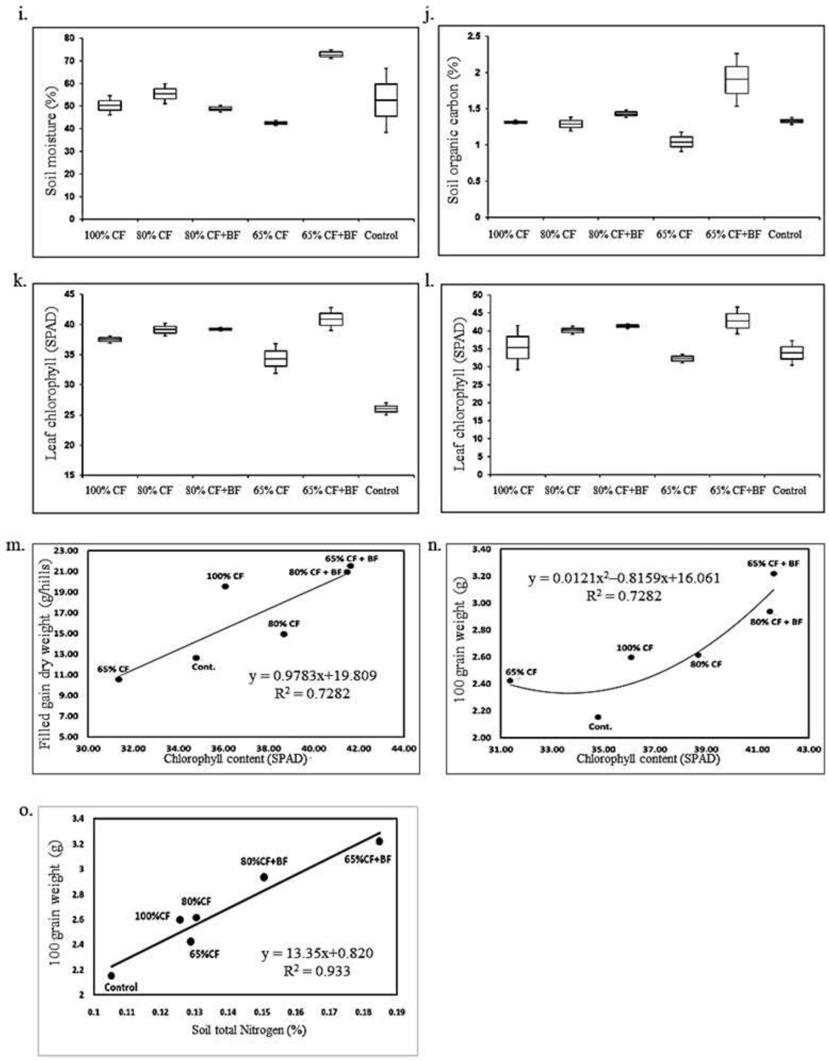

Figure 5. Multiple graphs including soil and plant parameters and their relationships with yield in Maha 2017/2018 season, Ambalantota, Sri Lanka. (i) Soil moisture, (j) soil organic carbon, and (k) leaf chlorophyll content in the tillering and (l) 50% flowering stages, (m) relationships between chlorophyll content and filled grain weight, (n) between chlorophyll content and 100-gain weight, and (o) soil total nitrogen and 100-gain weight. The 100% CF rate was the recommended amount of the Department of Agriculture, Sri Lanka in 2013, i.e. 340 kg CF/ha, and BFBF was applied at 2.5 L/ha. Standard error bars are on the boxes of box-plots

Grain yield parameters

The positive correlation between plant growth, yield, and shoot EDs suggests that the presence of EDs in plant tissues has contributed to enhanced panicle number and grain yield (Figures 4g and 4h). Endophytes play a vital role in promoting plant growth and increased nutrient uptake, especially N, which is essential for grain production (Ferreira et al., 2023; Jaiswal et al., 2023; Watts et al., 2023). Furthermore, there was a positive correlation between CC, filled grain weight, and the 100-grain weight (Figures 5m and 5n). Previous studies have shown that CC is closely related to grain yield (An et al., 2020; Liu et al., 2017). Moreover, there was a positive correlation between STN and the 100-grain weight (Fig. 5o). These numerous relationships suggest that the BFBF enhances soil nutrient cycling, nutrient uptake, and grain production via the network interactions among soil, plant and microbial parameters (Premarathna et al., 2021).

During *Maha*-2017/2018, there was a significant increase in 100-grain weight (Fig. 7f) and grain yield in the 65% CF + BFBF treatment in comparison to 65% CF, 80% CF, and 100% CF treatments at the Mahaweli Thoda Farm (Fig. 8a). However, this was not observed at the RRDI, Bathalagoda (Fig. 6v and 8b). This may probably be due to resource-rich conditions in the research station at RRDI in comparison to the agricultural farm (Chambers & Ghildyal, 1985; Morgan & Connolly, 2013). These inconsistent results prompted us to further investigate this to confirm the BFBF action on various soil conditions. Thus, extensive field trials were conducted from 2018 to 2023 in collaboration with the IMD, MASL, and the DoA to examine this.

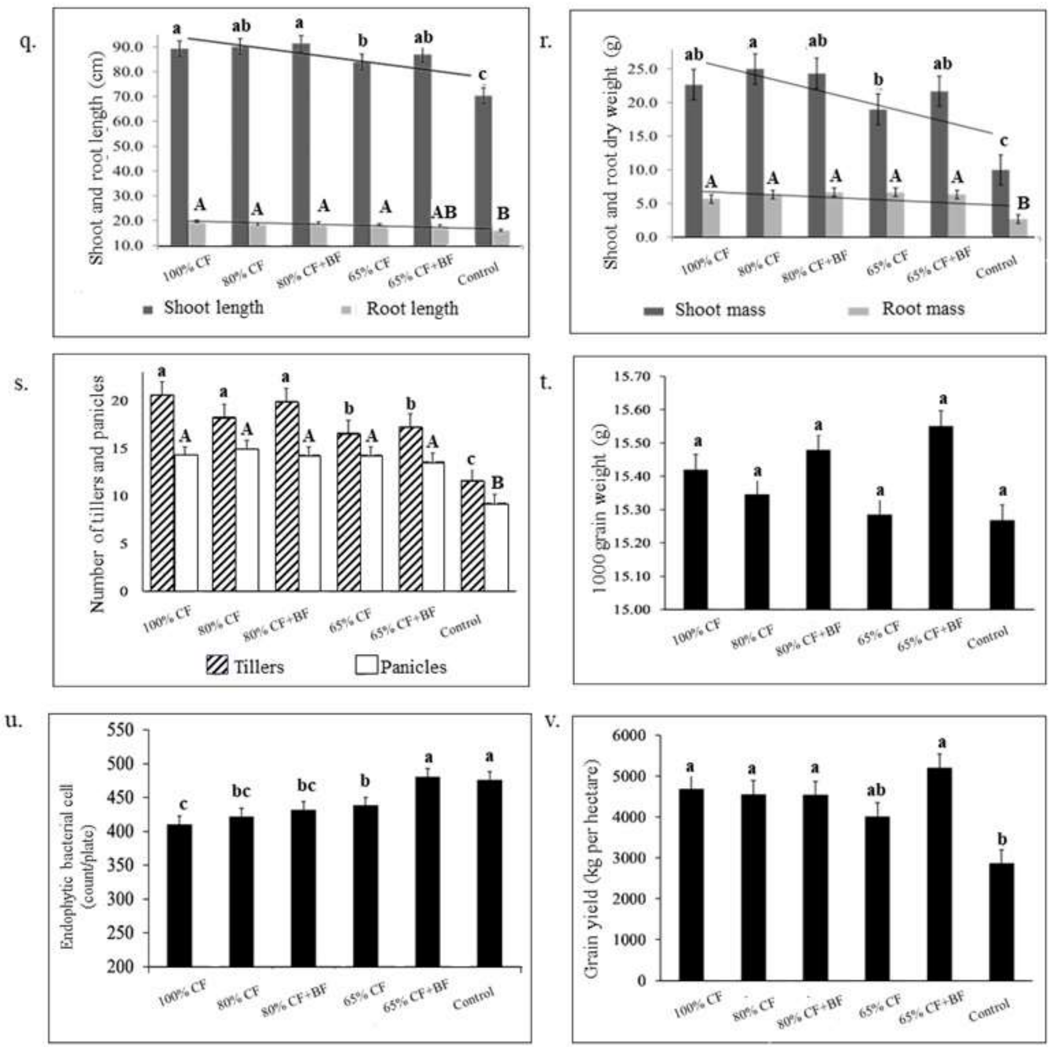

Figure 6. Multiple graphs of paddy plant growth and yield parameters in at RRDI Bathalagoda, Sri Lanka, during the Maha 2017/2018 season. (q) Shoot and root length, (r) shoot and root dry weights, (s) number of tillers and panicles (u) endophytic bacterial count at 50% flowering stage, and (t) thousand-grain weight, (v) grain yield at harvesting stage. The 100% CF rate was the recommended amount of the Department of Agriculture, Sri Lanka in 2013, i.e. 340 kg CF/ha, and BFBF was applied at 2.5 L/ha. Treatments of the colums with the same letter are not significantly different at 5% probability level. Standard error bars are on the columns

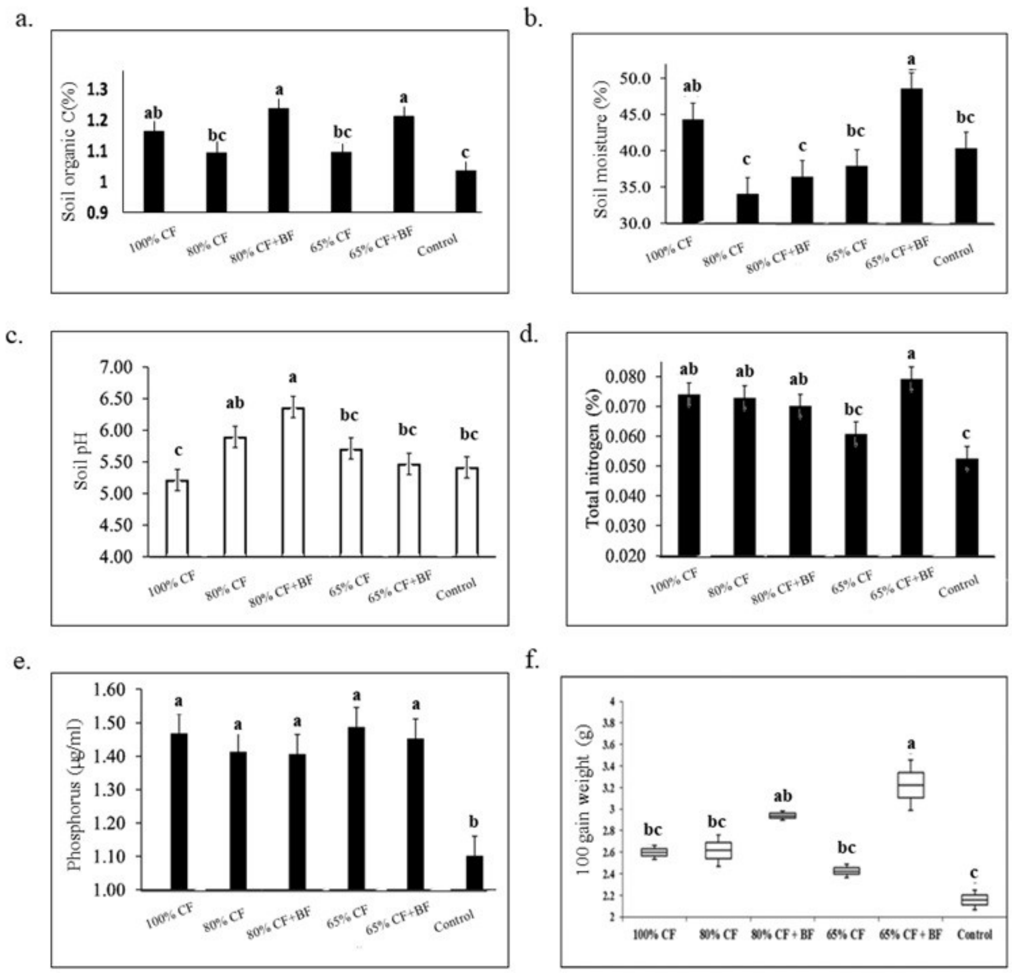

Figure 7. The multiple graphs for the comparison of soil parameters. (a) Soil organic C (%), (b) soil moisture (%), (c) soil pH, (d) total nitrogen (%), (e) total phosphorus (in soil extract), and (f) 100-grain weight. The data were collected in Thoda Farm, Dehiattakandiya, Sri Lanka, during *Maha* 2017/2018 season. The 100% CF rate was the recommended amount of the Department of Agriculture, Sri Lanka in 2013, i.e. 340 kg CF/ha, and BFBF was applied at 2.5 L/ha. Treatments of the columns or boxes with the same letter are not significantly different at 5% probability level. Standard error bars are on the columns and boxes of the box-plot.

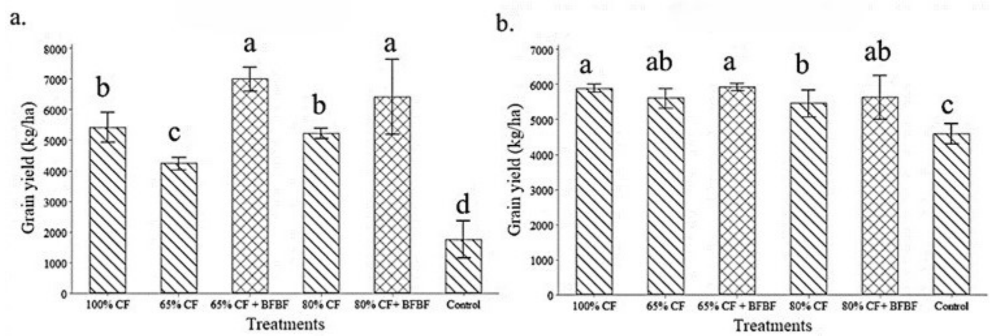

Figure 8. Paddy grain yield ($p<0.05$) at (a) Mahaweli Thoda farm and (b) RRDI at Batalagoda, Sri Lanka during *Maha* 2017/2018. Three replicates in an RCBD, 5 × 4 m plots. 100% CFP (340 kg CF/ha), BFBF (2.5 L/ha). The 100% CF rate was the recommended amount of the Department of Agriculture, Sri Lanka in 2013, i.e. 340 kg CF/ha, and BFBF was applied at 2.5 L/ha. Treatments of the columns with the same letter are not significantly different at the 5% probability level. Standard error bars are on the columns.

Collaborative trials with the Irrigation Management Division and the Department of Agriculture conducted in farmers' fields

Soil-plant-microbial parameters

During *Yala*-2020 season, the BFP showed significantly higher magnitudes of the EDs and EBs than those of the CFP. The SOC, moisture, and STN showed significantly higher values in the BFP than in the CFP. The shoot and root dry weights also increased significantly with the BFBF application (Table 7)(Ekanayake & Seneviratne, 2024). This may be attributed to the action of BFBF, which triggers dormant microbes in the soil and enhances soil-plant-microbe interactions (Premarathna et al., 2021).

Table 7. Comparison of BFP and CFP microbial, soil and plant parameters in the *Yala*-2020 season conducted in large-scale farmers' field trials established in Ampara and Polonnaruwa districts.

Parameters	Practices	
	CFP (n=12)	BFP (n=12)
Microbial		
ED (cfu/plate)	$39.20^{b} \pm 7.8$	$69.38^{a} \pm 8.9$
END (cfu/plate)	$76.4^{b} \pm 13.9$	$139.4^{a} \pm 21.7$
Soil		
Moisture (%)	$43.55^{b} \pm 3.63$	$55.08^{a} \pm 3.34$
pH	$6.35^{a} \pm 0.15$	$5.99^{a} \pm 0.24$
SOC (%)	$1.418^{b} \pm 0.098$	$1.727^{a} \pm 0.066$
STN (%)	$0.155^{b} \pm 0.009$	$0.201^{a} \pm 0.012$
STP (%)	$0.039^{a} \pm 0.006$	$0.039^{a} \pm 0.006$
Ex. SP (C mol/kg)	$0.63^{a} \pm 0.081$	$0.71^{a} \pm 0.078$
Plant		
Shoot DW (g)	$7.79^{b} \pm 0.83$	$11.88^{a} \pm 1.45$
Root DW (g)	$2.25^{b} \pm 0.43$	$4.25^{a} \pm 0.71$

Mean ± SE. Values in each column followed by the same letter are not significantly different at 0.05 probability level according to the t-test. CFP: Chemical fertilizer practice, BFP: Biofilm biofertilizer practice, ED: Endophytic diazotrophs, END: Endophytic nun-diazotrophs, SOC: Soil organic carbon, STN: Soil total nitrogen, STP: Soil total Phosphorous, Ex. SP: Exchangeable soil phosphorous. DW: Dry weight.

The research conducted during the *Maha*-2018/2019 season demonstrated that the application of BFBF significantly enhanced soil, plant and microbial parameters. This showed the potential to restore the degraded agroecosystem, enabling crops to achieve higher yields (Premarathna et al., 2021). Furthermore, a subsequent study conducted in the *Maha*-2021/2022 season revealed that BFBF improved soil quality, thereby allowing the crop to break the yield barriers (Rathnathilaka et al., 2023). Collectively, these findings underscore the significant potential of BFBF in optimizing the productivity of the paddy agroecosystems.

Grain yields

In the field research conducted collaboratively with the IMD during *Yala*-2018 season, the yields of the BFP averaged 5174 kg/ha, compared to 4285 kg/ha achieved through the CFP, showcasing a difference of 889 kg/ha (Figure 9). Notably, the BFP demonstrated ca. 50% reduction (Table 8) in CF usage, while achieving ca. 20% increase in grain yield. Furthermore, the farmer-friendly use of BFBF together with dry fine sand and/or biochar highlighted the economic viability of the BFP due to the removal of the spraying cost of BFBF.

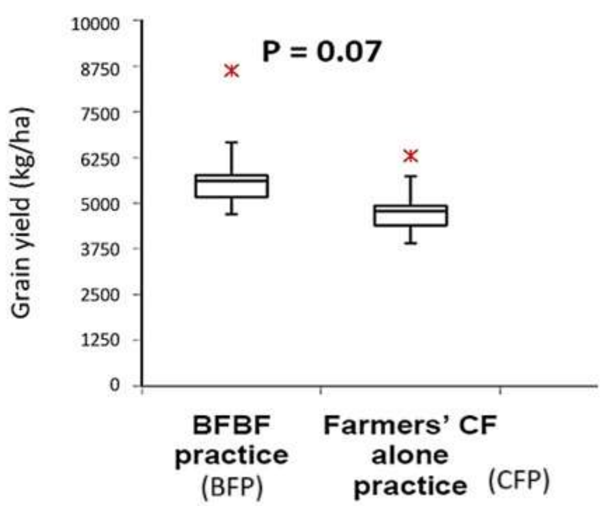

Figure9. The comparison of averaged paddy yields between the BFP (225 kg CF/ha + 2.5 L/ha BFBF) and the CFP (425 kg CF/ha) in Ampara, Kurunegala and Polonnaruwa districts during *Yala*-2018 season.

The outcomes of those field trials were subsequently presented to the DoA, igniting their keen interest in conducting research on BFBF in collaboration with the NIFS which led to the start of a series of trials Island-wide. With regard to the rate of fertilizer application, the total CF reduction of the BFP was 115 kg/ha, a cutdown of 34% in comparison to the 2013 DoA recommendation (Table 8). The advantages of reducing CF in the agroecosystems are well-established. The CF is associated with several disadvantages that impact agricultural productivity, and human and environmental health. Firstly, they often reduce the diversity of micronutrients found in naturally decomposing materials, predominantly consisting of the essential N, P, and K (Ahmed & Shahab, 2011). The CF can also lead to pollution by contaminating waterways, resulting in algal blooms, unpleasant odors, and oxygen depletion, jeopardizing aquatic life (Glibert & Burkholder, 2018; Sellner et al., 2003).

Table 8. Different fertilizer application rates for the trials conducted, including the recommended rate of the DoA (2013).

Fertilizer type	CFP (kg CF/ha) (DoA 2013)	65% CFP (kg CF/ha) (DoA 2013)	BFP (kg CF/ha & BFBF L/ha)	Difference between CFP and BFP (kg CF/ha)
Urea	225	150	150	75
TSP	55	33	33	22
MOP	60	42	42	18
BFBF	-	-	2.5	-
Total CF	340	225	225	115
Fertilizer reduction rate				34%

CFP: 100% DoA Recommended (2013) Chemical fertilizer practice, 65% CFP: 65% of DoA Recommended (2013) Chemical fertilizer practice (225 kg CF/ha), BFP: Biofilm biofertilizer practice (225 kg CF/ha+2.5L)

Table 9. Fertilizer application rate of the Biofilm biofertilizer practice in comparison to the National Fertilizer Secretariats' fertilizer distribution rate.

Fertilizer type	NFS fertilizer rate (kg CF/ha)	BFP (kg CF/ha and L/ha for CF and BFBF, respectively)
Urea (N)	205	150
TSP (P)	53	35.5
MOP (K)	25	12.5
BFBF		2.5
Total CF	283	198
Fertilizer reduction rate		30%

NFS: National Fertilizer Secretariat, BFP:Biofilm biofertilizer practice

The pH imbalances caused by over-fertilization are potential risks as well (Pahalvi et al., 2021). The concentrated nature of CF can overwhelm agroecosystems if over-applied, causing immediate damage such as root burn and long-term alterations in soil pH and nutrient accumulation (Pahalvi et al., 2021; Shaji et al., 2021). Additionally, CF requires frequent applications due to its rapid loss, which often compromises plant growth. Conversely, these disadvantages are nonexistent in the BFBF with low rates of CF, which possesses microbial functions to effectively supply nutrients to plants (Premarathna et al., 2022). The combined use of minimal CF (225 kg/ha) with sufficient BFBF (2.5 L/ha) can mitigate these disadvantages, by reinstating microbes as key agents in the soil biochemical process (Premarathna et al., 2022; Ye et al., 2020). The unique biochemical composition of BFBF acts as a catalyst, awakening dormant native microbes and enhancing their beneficial functions (Premarathna et al., 2022).

This strengthens the network interactions between soil, plants, and microbes, positioning BFBF as an environmentally sustainable and economically viable alternative to prevailing practices of using CF in isolation. Rigorous testing ensures that BFBF poses no toxicity or pathogenicity risks, making it safe for plants, animals, and water bodies. Additionally, the buildup of soil organic matter facilitated by BFBF helps retain soil nutrients in the root-zone, ensuring their availability to plants as needed (Jayasekara et al., 2022; Lehmann & Kleber, 2015). The overarching goal of BFP is to effectively nourish plants while minimizing the potential adverse effects associated with conventional, intensive CF applications. This holistic approach demonstrates BFBF's potential to revolutionize agricultural practices by prioritizing environmental stewardship and sustainable resource management. This approach capitalizes on BFBF's potential to optimize nutrient delivery and foster balanced nutrition, benefiting crop yields and ecological wellbeing.

Table 10. Paddy grain yields produced by the BFBF and CF practices during 2018-2023 in 13 districts of Sri Lanka.

Fertilizer practice & the rate of fertilizer application [(kg/ha) in CF and (L/ha) in BFBF)]	Season	Number of locations	Yield (kg/ha)			Yield increase of BFP over CFP (%)
			BFP	CFP	Difference	
CFP (425 kg), BFP (225 kg + 2.5 L BFBF)	*Yala*-2018 – *Maha*-2018/2019	61	4568[a]±147	3542[b]±153	1026	24%
CFP (340 kg), BFP (225 kg + 2.5 L BFBF)	*Yala*-2019 – *Maha*-2022/2023	112	5249[a]±121	4204[b]±107	1045	25%
CFP (283 kg), BFP(198 kg + 2.5 L BFBF)	*Maha*-2020/2021 – *Yala*-2021	59	5997[a]±156	4840[b]±136	1157	24%
Total/Average		232	6430[a]±106	5197[b]±91	1233	24%

Mean ± SE. BFP: biofilm biofertilizer practice, CFP: Chemical fertilizer practice. Values in the same row with different letters differ significantly at P<0.05. Rice varieties used, Bg 94-1, Bg 352, Bg 366, Bg 357, Bg 300, Bg 360, and At 302.

Table 11: Paddy grain yields produced by the biofilm biofertilizer practice in comparison to chemical fertilizer alone practices (225 & 340 kg CF/ha alone) during 2019-2020 in five districts of Sri Lanka.

Season	No. of trials	Yield (kg/ha)				Yield increase of BFP over CFP (225 kg CF/ha) (%)
		BFP (225 kg CF/ha + 2.5 L BFBF)	CFP (225 kg CF/ha)	CFP (340 kg CF/ha)	P value	
Maha-2019/2020	76	6509[a] ± 195	5519[b] ±161	5407[b] ±164	0.000	18%
Yala-2020	19	6421[a] ± 247	5413[b] ±231	4957[b] ±169	0.000	19%
Total/Average	95	6494[a] ± 166	5509[b] ±147	5321[b] ±138	0.000	18%

Mean ± SE. BFP: biofilm biofertilizer practice, CFP: chemical fertilizer practice. Values in the same row with different letters differ significantly at P<0.05.

The BFBF is engrained in its user-friendly nature with a simple application process. Importantly, BFBF has demonstrated a positive impact on the environment, human health, and the broader ecosystem (Meepegamage et al., 2021; Premarathna et al., 2022). Thus, the BFBF brings promising outcomes to farmers. The current BFP has been tested thoroughly for BFBF's effectiveness in paddy cultivation, with reduced rates of CF. All in all, the average paddy yield increase achieved through the BFP over that of CFP was up to ca. 25% (Table 10).

Due to the reduction in fertilizer distribution by the NFS from *Maha*-2020/2021 to *Yala*-2021, the application rate of CF was decreased from 340 kg CF/ha to 283 kg CF/ha (Tables 8 and 9). In this context, the rate of CF of the BFP was further reduced to 198 kg CF/ha i.e. a 30% reduction of the NFS's fertilizer distribution rate (Table 9). Interestingly, the yields of both practices increased as the CF rate was decreased because the reduced CF promotes soil microbial development and nutrient use efficiency in paddy cultivation (Liu et al., 2009). Another important finding is that there was no response of the paddy crop to the fertilizer treatments in pot experiments, but when it comes to small plots in experimental stations there was an inconsistent crop response to the fertilizer treatments. Intriguingly, in large plots of farmers' fields, the response of crop yield in particular, for the fertilizer treatments was the highest and consistent, with BFP being significant over CFP in almost all locations. This could be attributed to increased fertilizer use efficiency with BFBF application in larger plots than smaller plots (Meepegamage, 2024; Wang et al., 2015), irrespective of rice varieties, farmers' practices, and soil and climatic conditions (Table 10). Those results and observations made the farmers trust the BFP. Thus, the BFBF has been adopted in ca. 0.11 Mha of paddy cultivation in Sri Lanka by now, showing the social acceptance by offering a sustainable and economically viable solution to farmers while contributing to agricultural advancement (Ekanayake et al., 2023).

The field trials conducted during both the *Maha*-2019/2020 and *Yala*-2020 seasons in 95 locations revealed that the BFBF effect was real and significant with an 18% yield increase over the 225 kg CF/ha alone ($p < 0.05$, Table 11).

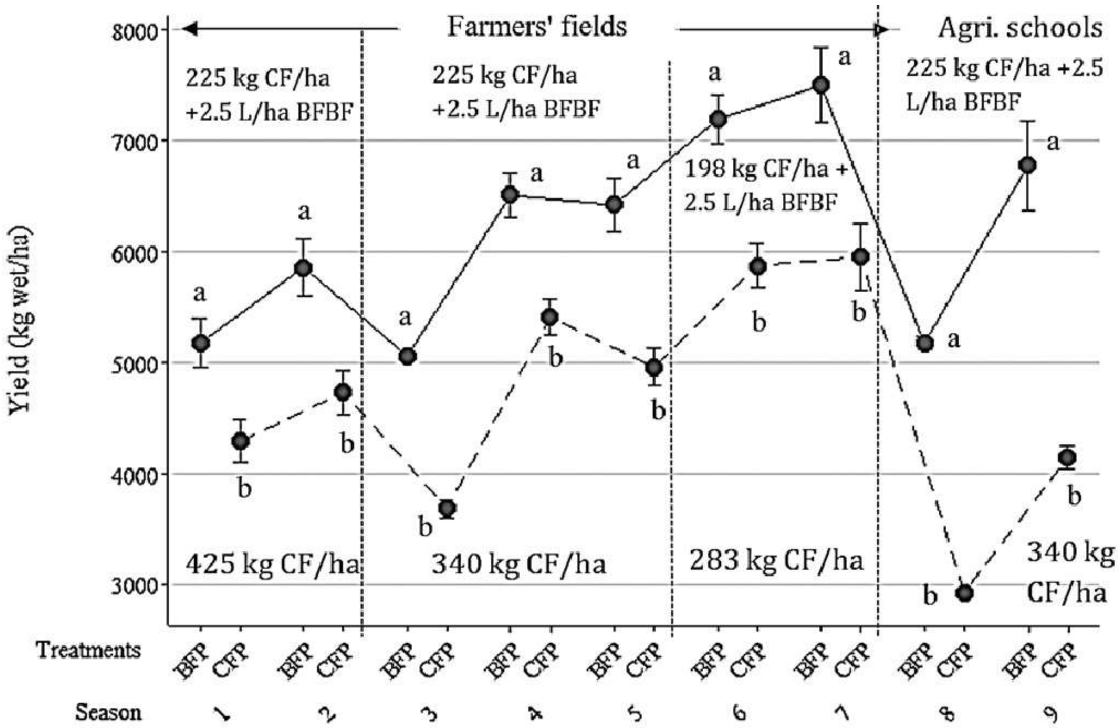

Figure 10. Paddy grain yields produced by the BFP and CFP during 2018-2023 in all the field trials conducted in 13 districts of Sri Lanka. BFP: biofilm biofertilizer practice [198 or 225 kg CF/ha + 2.5 L/ha of BFBF (continuous line)], CFP: chemical fertilizer practices [283, 340 or 425 kg CF/ha (broken line)]. Grain yields of the same season with different letters differ significantly at P<0.05. Seasons: 1- *Yala*-2018, 2- *Maha*-2018/2019, 3- *Yala*-2019, 4- *Maha*-2019/2020, 5-*Yala*-2020, 6-*Maha*-2020/2021, 7-*Yala*-2021, 8-*Yala*-2022, 9-*Maha*-2022/2023.

On the whole, the BFP showed significantly higher grain yields with reduced rates of CF throughout the study period (p<0.05, Figure 10).

Conclusion

The study emphasizes the essential role of BFBF in promoting sustainable agriculture. The application of BFBF has been shown to enhance the endophytic microbial diversity in plants, thereby improving plant growth and productivity. Moreover, BFBF has proven to be environmentally friendly, playing a significant role in restoring degraded ecosystems. It is economically viable since it reduces input costs and increases yields. Furthermore, BFBF has gained wide acceptance, as reflected by the vast extent of its adoption due to its user-friendly nature and increased yields. Finally, applying BFBF improves the sustainability and functionality of ecosystems, providing compelling evidence of practical implementation of fundamental science leading to real-world applications, specifically in sustainable farming practices.

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Declaration of Conflict of Interest

Authors have no conflict of interest to declare.

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