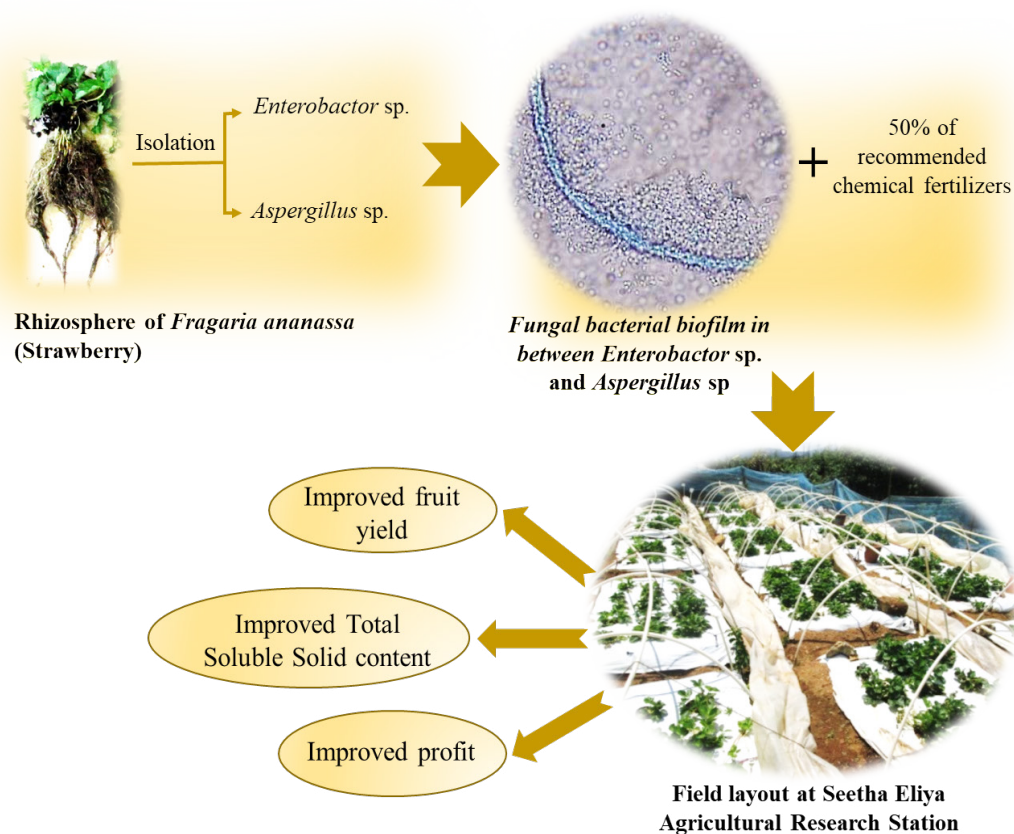


Biofilmed biofertilizers for improved quality and quantity of strawberry (*Fragaria ananassa*) under field conditions

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Highlights

- Strawberry yield under the application of Biofilmed Biofertilizer (BFBFs) was studied.
- The highest fruit yield, count and size per plant were obtained in BFBF with 50% Chemical Fertilizers (CFs).
- The BFBF + 50% CFs practice was ca. 30% more profitable than the 100% CFs alone practice.

RESEARCH ARTICLE

Biofilmed biofertilizers for improved quality and quantity of strawberry (*Fragaria ananassa*) under field conditions

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Abstract: In order to reduce high doses of chemical fertilizers (CFs) used in strawberry, scientists have tested monoculture-based biofertilizers without much success. In recent years, biofilmed biofertilizers (BFBFs) have been used in cash crops successfully while reducing the recommended dosage of CFs up to 50%. A field trial was carried out to evaluate the effects of BFBF, developed using microbial isolates from strawberry rhizosphere (*Aspergillus* sp. and *Enterobacter* sp.), on the quantity and quality of strawberry harvest. Strawberry plants were subjected to BFBF only, BFBF + 50% CFs, 50% CFs and 100% CFs with nine replicates for each treatment arranged in Randomized Complete Block Design. The control was maintained without BFBF or CFs. BFBF diluted 20 times in water was sprayed on to raised planting beds a week prior to introducing strawberry plants followed by repeated application in two-month intervals. Yield was recorded up to the 30th week. The quality of fruits was analyzed using Total Soluble Solids (TSS), titratable acid and ascorbic acid contents. Diameter of the plant spread (crown) and plant dry weight were measured. Flowering was first observed in plants treated with BFBF + 50% CFs. The highest cumulative fruit yield (109 g), mean fruit count (14.77 ± 4.62) and fruit size ($6.39 \text{ cm}^3 \pm 0.86$) per plant were also observed in the same treatment. The plant spread was found to be a good predictor of the yield. The TSS levels and TSS/acid ratios were significantly higher in all treatments over 100% CFs ($p < 0.05$). The BFBF + 50% CFs practice were ca. 30% more profitable than the 100% CFs alone. The results confirmed that the BFBF coupled with 50% CFs can be recommended over the full dosage of CFs alone for more profitable and highquality strawberry production under field conditions tested. Thus, further studies are needed to introduce BFBF for strawberry under variable soil and climatic conditions.

Keywords: Strawberry; biofilmed biofertilizer; chemical fertilizer.

INTRODUCTION

In conventional strawberry farming, the use of high doses of CFs is a common practice to gain higher fruit yields. In Sri Lanka, the total fertilizer addition for one cropping season of strawberry is 720 kg/ha of urea, 400 kg/ha of

super phosphate and 400 kg/ha of muriate of potash (Strawberry Growers Manual, Department of Agriculture, Sri Lanka, 2012). The continuous application of CFs can adversely affect the soil fertility and eventually the yield, due to their deleterious effects on the soil fauna including microbes (Medley *et al.*, 1982; Seneviratne, 2009). The adverse impacts on the soil biota can disrupt the nutrient cycles and breakdown of organic matter in soil. While the list of environmental consequences related to conventional agriculture continues, scientists have directed their attention to explore ways to reduce the usage of CFs by coupling CFs with microbial biofertilizers. This practice has demonstrated positive results with many crops. Soya beans showed a better growth with phosphorus solubilizing bacteria, *Bradyrhizobium japonicum* coupled with 33% of CFs (Janagard *et al.*, 2013). Yosefi *et al.* (2011) demonstrated similar results in maize. Strawberry also benefited from the use of biofertilizers in combination with CFs (Zargar *et al.*, 2008; Umar *et al.*, 2010). According to Zargar *et al.* (2008), application of nitrogen (225 kg ha^{-1}), and phosphorus (150 kg ha^{-1}) together with biofertilizer (*Azotobacter*) has increased the average fruit weight of strawberry. The application of 25% nitrogen through ‘subabul’ (green leafy manure of *Leucaena leucocephala*) along with 75% N in the form of urea augmented with biofertilizers has resulted in an improved fruit size and weight in strawberry cv. ‘Chandler’ (Umar *et al.*, 2010). The use of biofilmed biofertilizers (BFBFs) has reduced the recommended dosages of CFs by 50% in tea (Seneviratne *et al.*, 2011). In addition, the application of biofertilizers enhanced soil microbial communities by increasing microbial diversity (Pēsaković, 2013; Seneviratne and Kulasoorya, 2013), restoring conventional agricultural soils (Seneviratne *et al.*, 2011) and bio-controlling effects (Saharan and Nehra, 2011; Seneviratne, 2012).

Strawberry (*Fragaria ananassa*) belongs to the family Rosaceae. Cultivated strawberry, are said to be day-neutrals (Sønsteby and Heide, 2007). Yield, size and shape of strawberry fruit depend mainly on the adequate pollination potential by different insects (Zaitoun *et al.*, 2006).

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Strawberries are cultivated under glasshouse conditions and as well as in open fields. Planted strawberry should be watered in three - day intervals. The fruits are generally harvested 4 – 4½ months after planting.

This study was carried out to evaluate the effects of BFBFs, developed from microbes isolated from the strawberry rhizosphere, on the quantity and quality of strawberry harvest under field conditions.

MATERIALS AND METHODOLOGY

Locations and treatments

The field experiment was conducted in Seetha Eliya Agricultural Research Station located in the upcountry of Sri Lanka (location: 6° 93' – 80° 81', altitude: 1868 m, day and night temperature: 20.0 and 11.6 °C, relative humidity: 77% and light intensity: 1600 lux). Treatments used in this study were fungal-bacterial BFBF only, BFBF coupled with 50% of recommended dosage of chemical fertilizers (BFBF + 50% CFs, this treatment was selected based on the findings of previous biofertilizer experiments), 100% (reference treatment) and 50% CFs only. A control was maintained without BFBF or CFs. The BFBF were developed in a low cost medium (LCM) formulated by the Microbial Biotechnology Unit (MBU) of the National Institute of Fundamental Studies (NIFS), Sri Lanka using microbial isolates (*Aspergillus* sp. and *Enterobacter* sp.) from the strawberry rhizosphere carried out in a previous study (Singhalage *et al.*, 2019).

Bedding, planting and maintaining treatments

Fifteen strawberry (*Fragaria ananassa* var. Chandler) plants from generation 1 (raised in a nursery) were planted in mulched, raised beds (1.5 × 0.9 × 0.2 m, soil pH 6.65 ± 0.19, moisture 29.67 ± 1.50 g/g soil, available NO₃⁻ 71.76 ± 0.00 µg/g soil, available NH₄⁺ 0.05 ± 0.00 µg/g soil and available PO₄³⁻ 5.46 ± 1.00 µg/g soil) with a spacing of 0.3 x 0.3 m. This unit is considered as a plot. The growth data were collected from the three plants in the middle of each bed to avoid any edge effects (Figure 1a and 2). There were three main blocks each having five beds with treatments allocated to them randomly. BFBF in LCM, was diluted 20 times in water and sprayed on to raised planting beds at a rate of 500 mL/ha a week prior to planting followed by repeated applications in two-month intervals up to 40 weeks. There were nine replicates for each treatment. Arch-shaped mini tunnels (with 0.75 m maximum middle point height) were established for each block using white colour polythene (recommended for mini tunnels) to maintain a higher temperature inside the tunnels especially during the nights (Figure 1b). Mini tunnels were kept open during the daytime to facilitate the pollinators (Figure 1c). Plants were watered in 3-day intervals. The plots were arranged in Randomized Complete Block Design (RCBD). Field experiment was maintained for 10 months to make sure that the fruiting period was over after the 30th week.

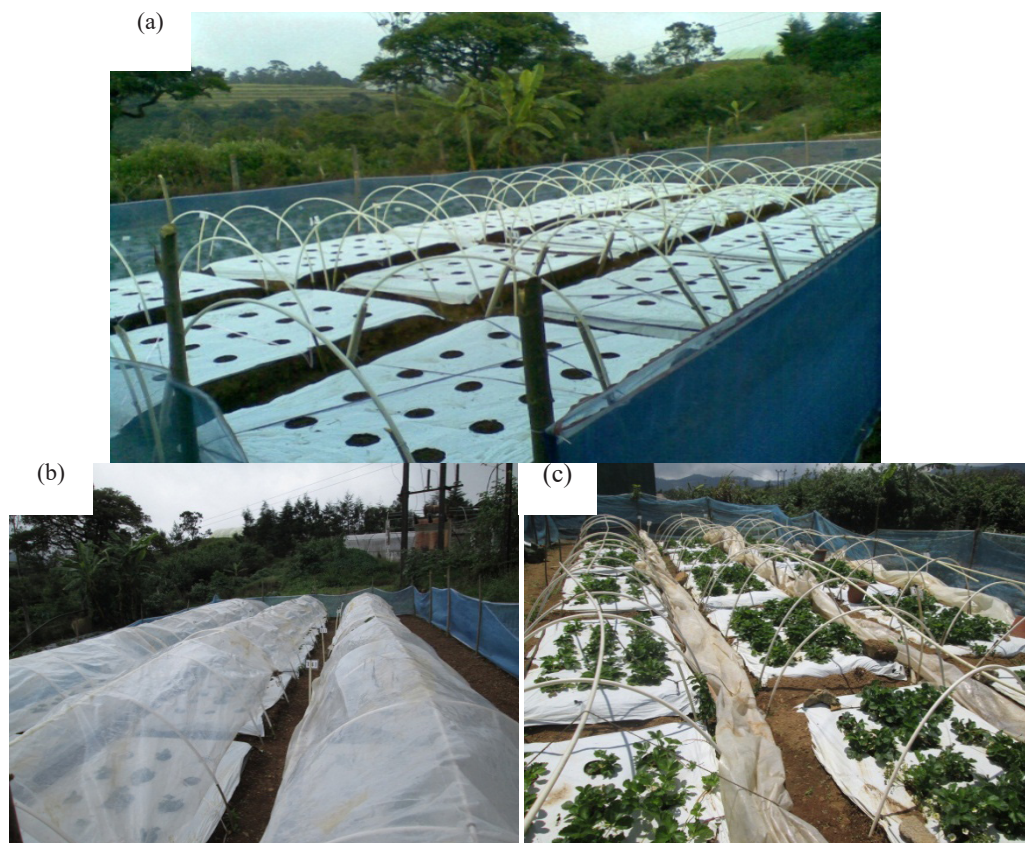


Figure 1: Cultivation practices of strawberry in the field trial. (a) Planting beds prepared at Seetha Eliya Agricultural Research Station after mulching and mini tunneling. Each bed contained fifteen strawberry plants. (b) Covered mini tunnels by polythene. (c) Uncovered mini tunnels during the day time to facilitate the pollinators.

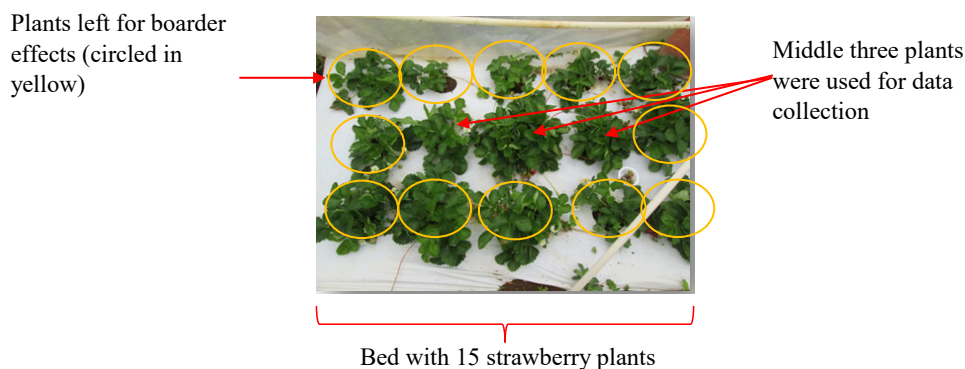


Figure 2: A planting bed showing the 15 strawberry plants. Only the three plants in the middle were used for data collection (in each bed) to avoid the edge effect.

Data collection and analysis

Growth and yield data

Diameter of the plant crown (spread) was measured after 4 months and the diameter data were used to calculate the crown area as, $\{\pi (\text{diameter}/2)^2\}$. After 6 weeks of planting, flowering and fruiting commenced. The yield data were recorded weekly (number of fruits, their fresh weights and fruit volume) during the first (8th to 17th week) and second (26th to 30th week) harvesting cycles. At the end of the field experiment (after 10 months), plants were harvested and dry biomass was recorded to determine the growth performances of plants under each treatment. The yield increase over the control (%) was calculated as, $\{(\text{yield under the treatment} - \text{yield under the control})/\text{yield under the control}\} \times 100$.

Quality of fruits

Samples taken from each harvest were used to analyze total soluble solids (TSS), titratable acids (TA) and ascorbic acid using standard methods. TSS was determined using a hand-held refractometer (Atago), while TA and ascorbic acid were determined after extracting to distilled water from fruits with equal stages of colour development, and titrated against 0.1 M NaOH (phenolphthalein as the indicator) and 0.005 mol/L iodine solution (starch as the indicator), respectively. The results were presented as parts per million (ppm) and expressed in fresh weight basis (Ali *et al.*, 2011). The fruit volume was determined by measuring the amount of water replaced in a measuring cylinder after immersing the fruit.

Data analysis

The growth (plant spread and biomass) and fruit quality (TSS, TA and ascorbic acid) parameters were analyzed using Analysis of Variance (ANOVA) and means were separated by Tukey's simultaneous mean separation test. Strawberry yield was correlated with the growth parameters using Pearson correlation coefficient. Net profits of strawberry production were calculated under each treatment. Statistical analyses were performed in Minitab statistical package (Minitab® 16.2.1, 2010).

RESULTS

Plant growth

A higher spread was observed in plants treated with BFBF + 50% CF in comparison to the control (Figure 3a). Plants provided with 100% CFs or BFBF + 50% CFs showed significantly improved ($p < 0.05$) plant dry mass than the control (Figure 3b).

Strawberry yield

Count, weight, and the volume of the strawberry fruits under different fertilizer treatments are given in Table 1. The BFBF + 50% CFs treatment showed a yield increase of 92% over the control. It was mainly attributed to fruit volume increase, and hence weight increase. The yield increase of 100% CFs alone was only 74%.

The fruit counts were nearly equal in all treatment and the control. However, the fruit sizes were larger in treatments of 100% CFs, BFBFs and BFBFs + 50% CFs.

Fruit mass of strawberry showed linear relationships with plant spread ($p = 0.000$) and plant dry mass ($p = 0.044$) (Figures 4a and 4b). It is clear from this that plant spread, a non-destructive measurement is a better predictor of fruit mass than plant dry mass.

Fruit quality

According to the measured fruit quality parameters, TSS was significantly higher in all treatments than that of 100% CFs (Figure 5a). Although not significant, concentration of TA was higher in the BFBFs + 50% CFs treatment in comparison to other treatments except 50% CFs (Figure 5b). The TSS/TA ratio that determines the organoleptic properties of fruit was the lowest in 100% CFs (Figure 5c). This adversely affects consumer demand of the produce. Higher ascorbic acid contents were detected in fruits of 50% CFs and BFBFs + 50% CFs (Figure 5d). It is worthy to mention that the organoleptic qualities of the control were similar to 50% CFs and BFBFs + 50% CFs at all parameters except in the case of ascorbic acid content.

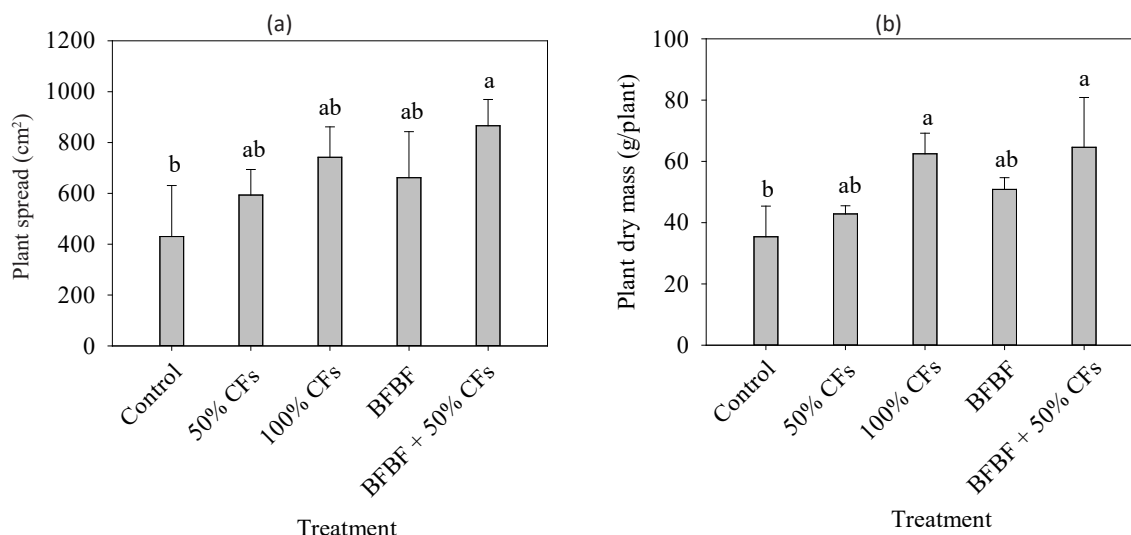


Figure 3: Plant spread (a) and dry mass (b) of strawberry under treatments 50% CFs, 100% CFs, BFBFs, BFBFs + 50% CFs and the control. Treatment columns with different letters are significantly different at the 5% probability level according to the ANOVA.

Table 1: Mean fruit count, mean weight and mean volume (\pm SEM) of strawberry of different treatments

Treatment	Fruit count/plant	Fruit weight (g/plant)	Fruit volume (cm ³ /fruit)	Yield increase over the control (%)
Control	14.33 ^a (\pm 3.27)	58.49 ^a (\pm 6.75)	4.71 ^a (\pm 0.81)	-
50% CFs	10.78 ^a (\pm 2.00)	52.87 ^a (\pm 10.4)	3.90 ^a (\pm 0.94)	13.10
100% CFs	14.17 ^a (\pm 1.85)	96.04 ^a (\pm 8.67)	4.81 ^a (\pm 0.51)	74.05
BFBFs	14.11 ^a (\pm 3.94)	89.09 ^a (\pm 17.3)	4.71 ^a (\pm 0.37)	50.42
BFBFs + 50% CFs	14.77 ^a (\pm 4.62)	108.78 ^a (\pm 17.0)	6.39 ^a (\pm 0.86)	92.47

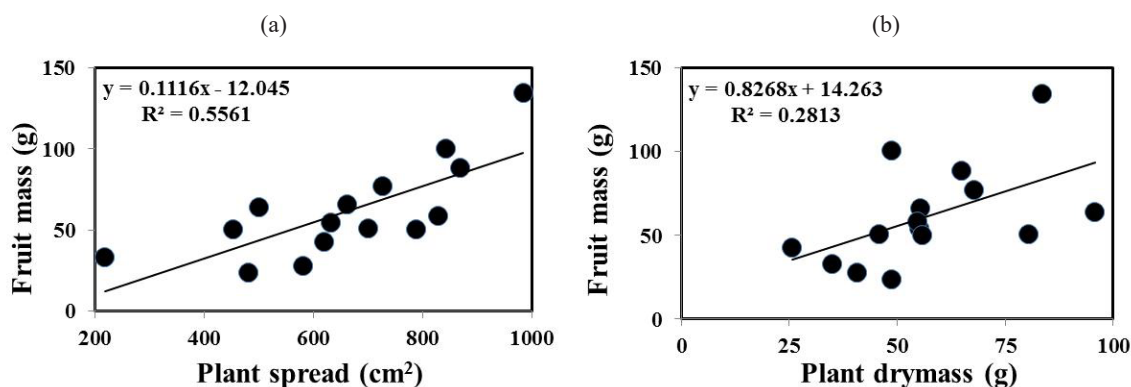


Figure 4: Relationships between fruit mass and (a) plant spread, and (b) plant dry mass.

Profitability of strawberry production using BFBFs under field conditions

The calculated net profits of strawberry under different treatments are given in Table 2. The net profit of BFBFs + 50% CFs treatment is LKR 164,722.00 and it was only LKR 125,444.00 of 100% CFs. The net profit increases of BFBFs + 50% CFs over the control and 100% CFs were 52% and 31%, respectively.

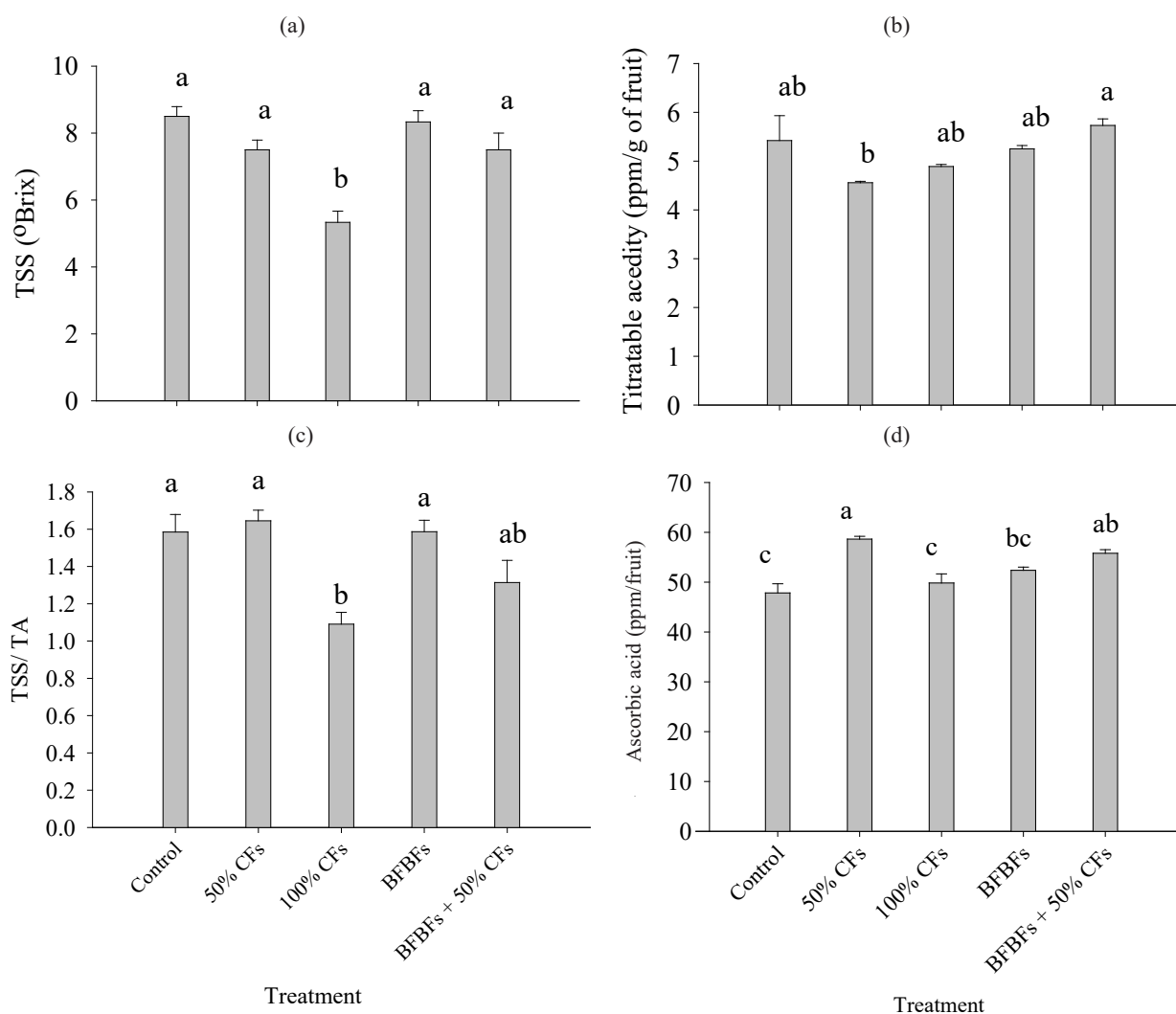


Figure 5: Total Soluble Solids – TSS (a), Titratable acids – TA (b), TSS/TA ratio (c) and Ascorbic acid content (d) of strawberry fruits of different treatments. Bars with different letters indicate significant differences at 5% probability level. Vertical bars on the columns show standard errors of mean (SEM).

Table 2: Net profit of strawberry production for a 1000-plant strawberry crop

Treatment	CFs (%)	Yield (kg)	Earning (LKR 2,500 (13.79\$/kg of Strawberry)	Approximate Expenditure: fertilizer + labor costs (LKR)	Net profit (LKR)	Profit increase over the control (%)	Profit increase over the 100% CFs (%)
Control	0	47.9	119,750 (660.32 \$)	11,111 (61.27 \$)	108,639 (599.05 \$)	-	-13.4
50% CFs	50	48.9	122,250 (674.11\$)	14,778 (81.49 \$)	107,472 (592.62 \$)	-1.1	-14.3
100% CFs	100	62.0	155,000 (854.69 \$)	29,556 (162.98 \$)	125,444 (691.72 \$)	15.5	-
BFBFs	0	57.0	142,700 (786.87 \$)	11,111 (61.27 \$)	131,589 (725.61\$)	21.1	4.8
BFBFs + 50% CFs	50	71.8	179,500 (989.79 \$)	14,778 (81.49 \$)	164,722 (908.30 \$)	51.6	31.3

1\$ = LKR 181.35 (02.01.2020); LKR = Sri Lankan Rupees

Labor cost: payment for field preparation, planting, weeding and watering.

DISCUSSION

Many studies have been conducted in the past to evaluate the improvement of vegetative growth and yield of strawberry under chemical and biofertilizer applications. Amongst, Abu-Zahra and Tahboub (2008) showed an improved vegetative growth in strawberries under the chemical fertilizer application, but it delayed the onset of flowering. According to the present study, an improved vegetative growth and the highest yield were shown by plants treated with BFBF + 50% CFs (Figure 3 and Table 1). Previous studies have demonstrated improved yields in strawberry var. Chandler with the application of mono and co-culture based biofertilizers (Umar *et al.*, 2009; Esitken *et al.*, 2010; Pèsaković *et al.*, 2013). However, the present results demonstrated the highest yield increase over the control (92%) in comparison to previous studies (Table 3). Under experimental conditions of Esitken *et al.* (2010), the best monoculture biofertilizer for strawberry production was *Bacillus* OSU 142 strain with a yield increase of ca. 3% over the control. *Azotobacter* improved the strawberry yield by 37% over the control, but it was in combination with 100% chemical fertilizer dosage (Umar *et al.*, 2009), highlighting no economic benefit of the practice. The findings of the present study in comparison to previous studies clearly showed the improved strawberry yield under the mixed-culture biofilms over conventional monoculture biofertilizers. Bandara *et al.*, (2006) and Seneviratne *et al.*, (2009) showed increased production of plant growth hormones like substances due to improved metabolic activities of fungal-bacterial biofilms over the monocultures of fungi or bacteria. Biofilm developed from *Penicillium* and *Bradyrhizobium elkanii* SEMIA 5019 generated higher number and concentrations of detectable monosaccharides than its respective monocultures (Zavahir and Seneviratne, 2007). Another biofilm developed from *Azotobacter* and *Colletotrichum* secreted carboxylic acids and carboxylic salts, which contributed significantly to enhance germination and plant height of lettuce (Herath *et al.*, 2013). Thus, the improved growth and yield of strawberry in the present study may also have been attributed to the production of such bioactive compounds.

Biofilm mediated growth improvements have also been reported in other crops such as rice (*Oryza sativa*), tea (*Camellia sinensis*), wheat (*Triticum aestivum*) and in Anthurium (*Anthurium andraeanum*) (Seneviratne *et al.*, 2009).

Sugars (TSS) and acid contents (TA) are considered as important quality attributes in strawberries. Results suggested that the addition of full dosage of chemical fertilizers can reduce the quality of fruits drastically (Figure 4). Thus, the addition of BFBFs with a reduced dosage of fertilizer can help maintain the quality of fruits in strawberry by increasing the aforementioned parameters, including vitamin C or ascorbic acid, which is an efficient oxygen radical scavenger with a strong antioxidant power (Zhang and Hamauzu, 2004). Strawberries have been reported to have the potential to contain a greater antioxidant capacity compared to other fruits (Giampieri *et al.*, 2012). Citric acid, which determines TA, is the major organic acid in strawberries (Moing *et al.*, 2001). In addition to citric acid, other organic acids such as malate, quinate, shikimate, succinate, and fumarate may also present in strawberries (Moing *et al.*, 2001). Thus, the results of the present study suggested that 100% CFs treated plants produced fruits with the lowest quality, whereas BFBF amended soils with greater microbial activity and resilience to stress improved the fruit quality. Jiménez-Gómez *et al.*, (2017) also explained the ability of plant probiotic bacteria to enhance the quality of fruit and horticultural crops. However, the best treatment of the present study (BFBFs + 50% CFs) did not show significant fruit quality improvement, possibly due to the soil condition of the study. This warrants further evaluations under different soil conditions.

Previously, Singhalage *et al.*, (2019) showed an increased profitability (152% over the 100% CFs usage) of strawberry production with BFBFs containing *Aspergillus* sp. and *Enterobacter* sp. coupled with 40% of CFs in a pot experiment, further highlighting the promising potential of BFBFs in strawberry cultivation. The present study confirms that the BFBF between *Aspergillus* sp. and *Enterobacter* sp. with 50% of CFs can be introduced as the best fertilizer combination for strawberry production under

Table 3: Yield increases over the control under different bio- and chemical fertilizer applications, as reported in previous studies in comparison to the present study

Reference	Type of experiment	Control	Best performed treatment	Yield increase over the control (%)
Esitken <i>et al.</i> , (2010)	Field	Without microbes	<i>Bacillus</i> OSU 142	2.8
Umar <i>et al.</i> , (2009)	Field	Without microbes or chemical fertilizers	<i>Azotobacter</i> + 100% of N as urea	36.5
Pèsaković <i>et al.</i> , (2013)	Green house	Without microbes or chemical fertilizers	<i>Klebsiella planticola</i>	34.0
Present study	Field	Without microbes or chemical fertilizers	BFBF between <i>Aspergillus</i> sp. and <i>Enterobacter</i> sp.+ 50% recommended chemical fertilizers	92.5

field conditions of the tested site.

CONCLUSIONS

At present, profitability and fruit quality are major concerns in the strawberry cultivation due to heavy chemical inputs. Present study demonstrates that the use of BFBF can address these issues successfully. The use of BFBFs has reduced the usage of recommended chemical fertilizers up to 50% while improving the fruit quality parameters. Thus, the usage of BFBFs together with 50% of chemical fertilizer dosage can provide the growers a higher profit margin and consumers able to receive a high quality fruits. However, further studies are needed to test BFBF under variable soil and climatic conditions.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no competing interests.

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