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Effect of biofilmed biofertilizers on potato yield through induced soil -plant interaction

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The current study aimed at developing and examining biofilmed biofertilizers (BFBFs) as an ameliorator for potato cultivation by enhancing soil-plant interaction. Fungal-bacterial biofilms (FBBs), the main ingredient of the BFBFs were developed using soil microorganisms and were screened for their efficacy. The best BFBF coupled with chemical fertilizers (CF) were evaluated for potato yield response and soil amelioration using two field experiments conducted (two seasons) under two different climatic conditions. Results showed that the 50CB treatment significantly ($P < 0.05$) increased tuber yield at both field sites in both seasons compared to the full recommended CF (100C). The treatment 50CB showed significant ($P < 0.05$) improvement in soil chemical properties; soil pH, soil organic carbon (SOC), available phosphorus (available P) and available calcium (Ca^{2+}) in comparison with the 100C at both sites. However, 50CB did not show any significant difference in available nitrogen (available N) and available potassium (K^+) in comparison with 100C. Further, internal biochemical properties of potato plant, e.g., chlorophyll content, stem sucrose content and tuber starch content were significantly ($P < 0.05$) improved by the 50CB. In addition, significant ($P < 0.05$) correlations were observed among soil pH, Ca^{2+}, tuber starch and chlorophyll contents in the 50CB. Thus, it can be concluded that the enhanced soil chemical properties in the rhizosphere , i.e., pH, available Ca^{2+} and K^+, SOC, available P, available N from BFBF induced signals improved plant internal biochemical processes, thus leading to the increased potato yield. The BFBF (150 mL/plant) reduced the CF application by 50% and this is an enormous environmental and economic gain to improve soil health and potato production.

Keywords*: Soil nutrients, rhizosphere, signaling molecules, soil microbes*

INTRODUCTION

Excessive use of chemical fertilizers (CF) leads to the reduction of crop productivity caused by failure of the soil microbial diversity in the rhizosphere and thereby collapsing the soil-plant interactions (Seneviratne *et al*., 2009). As in most crops, large amounts of CF inputs are used in potato (*Solanum tuberosum*) cropping systems to maintain the yield. Since potato tuber is grown under the soil, there is a high possibility to contaminate the tubers with the agrochemicals used in potato cultivation. Therefore, there is an urgent need to reduce the usage of synthetic fertilizers and in turn, increase the usage of eco-friendly alternatives while maintaining soil fertility through sustainable agricultural practices.

Microbial communities in the rhizosphere have a direct influence on the plant to keep the crop production at a pace required for growing demands (Timmusk *et al.*, 2017). Direct application of microbial communities in biofilm mode has been introduced in the recent past and found to be multi-functional in terms of enhancing soil-plant interaction (Henagamage *et al*., 2016). The beneficial biofilms that are developed *in vitro* using rhizosphere-associated beneficial microorganisms, can be effectively used as biofertilizers, known as biofilmed biofertilizers (BFBFs), to enhance plant growth while reducing CF dependency up to 50% by maintaining soil nutrients. (Seneviratne *et al*., 2009).

The diverse forms of fungal-bacterial biofilms (FBBs), the main biofilm ingredient of the BFBFs have been shown to improve the growth of plants by enhancing the rhizosphere environment (Seneviratne *et al.*, 2009; Kanchan, *et al.*, 2019), recycling mineral nutrients in the soil-plant system, producing chemical stimulators which sometimes act as signaling molecules for the growth promotion (Bandara *et al*., 2006). Organic acids produced by phosphorus (P) solubilizing microorganisms are reported lowering the soil pH and thereby enhancing the availability of soil cation such as Ca^{2+} (Bargaz

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et al., 2018), which acts as exogenous inductive signals (Minhas *et al.,* 2004) to enhance crop production. In soils, solubility, mobility and bioavailability of trace elements including Ca^{2+} (Chen *et al*., 2016) and nutrients are strongly affected by hydrogen ion concentration (H^+), which is the measure of soil pH. High soil acidity increases the bioavailability and solubility of most of the soil cations (Edel *et al.,* 2017). Plants take up nutrients from the soil solution in an ionic form which leads to cation reduction in the soil (Tang and Rengel, 2003). To counteract the effect of charge imbalance, plants release H^+ from roots to the rhizosphere, hence lowering soil pH (Msimbira and Smith, 2020). Liu *et al*. (2020) reported the signaling mechanism of Ca^{2+} with the formation of biofilms. Maintaining the required cytosolic Ca^{2+} concentration is done by taking up the Ca^{2+} from the soil solution through plasma membrane channels. The internal Ca^{2+} concentration serves as an internal signal for a variety of processes including plant growth promotion since it acts as a second messenger (Thor, 2019). Field experiments conducted using BFBFs have shown high availability of soil nitrogen and organic acids, which directly increased the synthesis of plant growth stimulants like indole acetic acid (IAA) (Bandara *et al*., 2006). Therefore, the effects of BFBFs on plant growth promotion and soil amelioration open up new avenues in sustainable agriculture, which cannot be observed by the alone application of CF. Therefore, the focus of this study was to investigate the role of BFBFs on potato tuberization, in relation to plant-soil interactions.

MATERIAL AND METHODS

***Development and screening of biofilms*:** Microbial isolations were carried out using soil samples obtained from potato rhizosphere in an unfertilized and organically grown potato cropland at Regional Agriculture Research and Development Center, Bandarawela, (6° 48' 0" N,80° 58' 0" E) [up country intermediate zone, IU3, elevation 1,506m amsl, temperature 22 ºC, and annual rainfall 1,100 mm - 1,400 mm, Red Yellow Podsolic soil type], Sri Lanka. Biofilms were developed by combining isolated fungi and bacteria, according to the method described in Seneviratne and Jayasinghearachchi (2003). Briefly, both fungi and bacteria were cultured separately in yeast mannitol broth (YMB) without agar for 7 days and they were combined into one culture at day 14. The best FBB was selected after several laboratory screening experiments on pH_s of the broth media, lettuce seed germination assay (Mia *et al.,* 2012), nitrogenase activity by acetylene reduction assay (Piromyou *et al*., 2011) and production of IAA (Seneviratne *et al*., 2009). The best biofilm with different rates of CF was evaluated for the responses of potato yield and several soil parameters i.e., SOC, pH, available P, available Ca^{2+}, available K^+, available N in two field experiments one with favorable and the other with unfavorable conditions for tuberization, conducted in the year 2015 and 2016.

***Field experiments*:** Two locations were selected based on their average daytime temperatures (day temp.) and nighttime temperatures (night temp.), which are shown as follows.

- Bandarawela (6° 48' 0" N,80° 58' 0" E) [up country intermediate zone (IU_3) 1506 m amsl], Sri Lanka. – (average day temp. 30 ºC, average night temp. 19 ºC, Red Yellow Podsolic soil type): favourable condition for potato tuberization.
- Bibile (7°09'20.5"N, 81°13'26.4"E) [low country intermediate zone, 305 m amsl], Sri Lanka – (average day temp. 30 ºC, average night temp. 24ºC, Red Yellow Podsolic soil type): unfavorable condition for potato tuberization.
- Bed preparation of each location was performed according to the standard dimensions for potatoes. Treatment combinations were 100% CF (100C), 50% CF (50C), 50% CF + BFBF (50CB), BFBF alone (B), and no amendments (0CB) with five replications for each treatment, arranged according to randomized complete block design (RCBD). Government-certified disease-free potato seed tubers ('Granola' variety) were obtained from Regional Agriculture Research and Training Center, Bandarawela, Sri Lanka. Plot size was 25 m^2 (5 m × 5 m) and contained 144 plants at inter-and intra-row spacings of 60 cm and 25 cm, respectively. Plots were separated by an alley of 1 m from each other. After two weeks from the planting, a blend of urea (17 g/m^2), triple super phosphate (TSP) (27 g/m^2), and muriate of potash (MOP) (12.5 g/m^2) was mixed with surface soil as basal fertilizers for the treatment 100C. Half (50%) of the recommended fertilizer was applied as a basal mixture to the 50% CF treatment plots (50C and 50CB). Further, 150 mL of the diluted biofilm (diluted 250 times in water) was sprayed per potato plant using sprayed tank directly near the root zone for the 50 CB and B treatments respectively. The fertilizer application (17 g/m^2 of urea and 12.5 g/m^2 of MOP) was repeated after five weeks from planting, simultaneously with the BFBFs application. Irrigation was carried out twice a day (morning and the evening) and an equal amount of water supply was maintained starting from planting till harvesting. After 90 days from planting, plants were uprooted and brought to the laboratory to measure underground and above-ground biomass. Fresh weights of tubers were recorded and tuber yield was calculated in metric tons per hectare to evaluate the growth responses for the treatments. Three soil samples (up to 10 cm depth from the surface) were collected from each plot for analysis. The effect of different CF and BFBF treatments on physicochemical parameters of the soil and also plant biochemical properties were evaluated using the following methods.

Soil and tuber physicochemical properties

***Soil chemical properties*:** Soil pH was measured using the following method. Soil (20g) was mixed with 50 mL of distilled water and stirred for 10 minutes followed by keeping

it for 30 minutes without stirring. Subsequently, pH was measured using a glass electrode connected to a pH meter (Trans Instruments, BP3001). Soil organic carbon (SOC) was determined using the wet oxidation method explained in Anderson and Ingram (1998). Finely ground soil (1g) was oxidized with 1.0 *M* potassium dichromate and concentrated sulfuric acid, diluted and titrated against 0.5 *M* acidified ammonium ferrous sulphates till the color changes from violet to blue and finally bright green. Soil available P content was measured according to Olsen et al. (1954) method. Finely ground soil (2.5g) was mixed with 50 mL of 0.5 M sodium bicarbonate (pH 8.5) solution and shaken for 30 minutes. The mixture was then filtered and ortho-phosphate in the filtrate was determined by molybdenum blue method using UV spectrophotometer (Thermo Scientific Multiskan Go, 1510) at 880 nm. Soil available N content was measured according to Anderson and Ingram (1998) method. The soil (10g) sample was mixed with 20 ml of potassium sulfate solution and filtered. Then 0.5 mL filtrate was mixed with 1mL of salisylic acid solution followed by the addition of 10 mL of sodium hydroxide. The absorbance of the sample was measured after the color development at 410 nm using UV visible spectrophotometer. Soil available Ca^{2+} content was measured according to Plank (1992) method. Finely ground soil (1g) was converted to ash by heating and was treated with concentrated nitric acid followed by heating for 4 h. Subsequently, the product was wetted with deionized water and treated with concentrated hydrochloric acid. The diluted product was treated with lanthanum chloride, and Ca^{2+} concentration of the solution was determined using atomic absorption spectrophotometer (Varian AA240) with calcium carbonate standard. Soil available K^+ was determined according to Anderson and Ingram (1998) method. Briefly, 5g of finely ground soil was digested with nitric and perchloric acid solution followed by vigorous shaking. Soil available K^+ was measured using flame photometer (FlameCal 50).

***Tuber physicochemical properties*:** Chlorophyll content was determined spectrophotometrically from fresh leaf samples by preparing their acetone extracts by the method explained in Sheikh *et al*., (2017). The extracts of the plants were prepared with 80% acetone by macerating 1 g of fresh leaves taken from the 3rd and 5th fully expanded leaves separately. After centrifuging the decanted suspension for 3 minutes at 1320 rpm, the upper green clear solution was decanted from the colorless residue and the volume was increased to 10 ml with 80 percent acetone. The solution was then subjected to centrifugation at 10,000 rpm for 10 min. The absorbance of the solution was determined using a UV visible spectrophotometer (Thermo Scientific Multiskan Go, 1510) at wavelengths of 665 and 649 nm, respectively. The samples were taken in triplicates and the results expressed as µg/ml was calculated by the following formulae: Total chlorophyll (µg/ml) = 6.45 × A665 + 17.72 × A649 (A-absorbance). The starch content of the potato tubers was measured according to the method explained by Luo and Huang, (2011). Finely ground tissue sample (0.5g) was heated in an 80 ºC water bath after treating it with 80% ethanol. The residue obtained after centrifugation (3000 rpm) was washed repeatedly with hot ethanol (80%) and was treated with perchloric acid followed by centrifugation (3000 rpm). The supernatant of the extract was diluted and allowed for color development with anthrone reagent. Color intensity of the solution was measured using UV visible spectrophotometer (Thermo Scientific Multiskan Go, 1510) at 630 nm, using distilled water as the blank. The total sugar content of the stem of the potato plant was measured according to Luo and Huang, (2011) method. Dried tissue sample (0.1g) was mixed with 6-7 mL of 80% ethanol. The sample was heated at 80 ºC for 30 min and centrifuged (3000 rpm) for 5 min. The supernatant was mixed with 80 % ethanol and the extracted solution was heated in a boiling water bath. Next, 0.1 mL of 30% potassium hydroxide was added and was incubated for 10 min. Subsequently, 3 mL of anthrone reagent was added, and the solution was incubated at 40 ºC for 10-15 min. Absorbance was measured using UV visible spectrophotometer (Thermo Scientific Multiskan Go, 1510) at 630 nm with distilled water as a blank. Glucose equivalents were calculated from a standard curve obtained with pure analytical grade glucose.

***Molecular identification of microbial components in the responsive biofilm*:** Genomic DNA of the fungal component of the biofilm was extracted from 5-day old fungi cultures grown on plates using DNeasy Plant Mini Kit (Supplied by QIAGEN). Fungal DNA was amplified using universal primers of fungal DNA ITS1 (5'TCC GTA GGT GAA CCT GCG G3') and ITS4 (5'TCC TCC GCT TAT TGA TAT GC3') (White *et al*., 1990). PCR products were purified using the QIA quick PCR purification kit (Bao *et al.,* 2012). The PCR products were sent for sequencing and obtained sequences were compared with the other related sequences using BLAST search in GenBank (NCBI).

Identification of the bacterial component in the responsive biofilm was done through 16S rDNA sequence analysis. The genomic DNA of each biofilm-forming isolate grown in TSB for 24 to 48 h was extracted using ZR Fungal/Bacterial DNA Kit™ (Zymo Research California USA) according to the manufacturer's protocol. PCR procedure was done using primers 11F (5'GTTTGATCMTGGCTCAG3') and 1492R (5'TACGGCTACCTTGTTACGACTT3') with 1 µL of undiluted genomic DNA extract as a template. Agarose gel electrophoresis was employed to detect amplification of the 16S rDNA of the biofilm-forming isolates. The amplified products were sequenced at the Macrogen Sequencing facility in Korea and sequences were compared with those stored in the GenBank databases of the National Center for Biotechnology Information available online using pairwise alignment or BLAST algorithm.

***Data Analysis*:** Statistical data analyses were performed on all data collected using the one-way Analysis of Variance (ANOVA) Model in MINITAB 16 Statistical Software. The mean values of each soil and plant parameter were compared on a treatment basis using Tukey's simultaneous test at 5% significance level. Correlations between different soil and plant biochemical properties were constructed by using correlation analysis.

RESULTS

***Potato plant response to different CF and BFBF treatments*:** Out of all treatments, the significantly ($P < 0.05$) highest mean tuber yield was recorded under 50CB at both locations in both seasons (Table 1). Treatment 50CB increased the mean tuber yield by approximately 220% in Bibile and 80% in Bandarawela, in comparison to 50C in the first growing season. Further, a similar trend was observed in the mean tuber yield in the second growing season at both locations. Interestingly, 50CB boosted mean tuber yield even over 100C, the increases being 77% in Bibile and 17% in Bandarawela in the first growing season whereas 68% in Bibile and 19% in Bandarawela in the second growing season.

***Effect of different CF and BFBF treatments on soil chemical properties*:** In comparison with all other treatments, 50CB significantly reduced ($P < 0.05$) soil pH at both locations in both seasons (Tables 2 and 3). Treatment 50CB showed the significantly ($P < 0.05$) highest mean SOC content at both field sites in both seasons. Treatment 50CB increased the mean available N content by 3% in Bandarawela and 2.5% in Bibile even over 100C in the first growing season whereas the same was increased by 10% in Bandarawela and by 8% in Bibile in the second growing season.

Table 1. Potato yield responses of different treatment combinations in Bandarawela and Bibile for two growing seasons.

	Year 1/Season 1		Year 2/Season 2	
	Bandarawela	Bibile	Bandarawela	Bibile
Treat.	Tuber yield (MT/ha)			
100C	$17.6^{a}\pm2.6$	$4.2^{b}\pm0.8$	$17.1^{a}\pm2.2$	$3.9^{b}\pm0.8$
50C	$13.7^{b}\pm1.8$	$2.3^{c}\pm0.6$	$12.5^{b}\pm1.6$	$1.8^{c}\pm0.3$
50CB	$20.8^{a}\pm2.2$	$8.1^{a}\pm1.1$	$19.7^{a}\pm1.8$	$6.9^{a}\pm0.8$
B	$8.0^{c}\pm0.8$	$1.4^{c}\pm0.2$	$7.4^{c}\pm0.8$	$1.1^{c}\pm0.1$
0CB	$2.3^{d}\pm0.3$	$0.6^{d}\pm0.1$	$3.1^{d}\pm0.8$	$0.5^{d}\pm0.07$

Mean ± Slandered Deviation (SD). Treatments are 100% chemical fertilizer (100C), 50% chemical fertilizer (50C), 50% chemical fertilizer with biofilmed biofertilizer (50CB), biofilmed biofertilizer alone (B) and no amendments (0CB), respectively. Means in the same column followed by the same letter are not significantly different at 5% probability level according to Tukey's test.

Moreover, the significantly ($P < 0.05$) highest mean soil available P content was recorded by 50CB in both locations and the increases being 28% in Bandarawela and 16% in Bibile even over 100C. In comparison with all treatments, the significantly ($P < 0.05$) highest mean soil Ca^{2+}content was observed from 50CB at both field sites in both growing seasons and the increments being 50% in Bandarawela and 22 % in Bibile in the first growing season whereas the enhancements being 76% in Bandarawela and 24% in Bibile compared to 50C. Interestingly, 50CB boosted the mean soil Ca^{2+} level approximately by 26 % in Bandarawela and 14 %

Table 2. The effect of different chemical fertilizer (CF) and biofilmed biofertilizer (BFBF) treatments on soil chemical properties in Bandarawela and Bibile field sites in the first growing season.

Treatment	pH	SOC (mg/g)	Avail. P (mg/kg)	Avail.Ca^{2+} (mg/g)	Avail. N (mg/kg)	Avail. K^{+}(mg/kg)
Bandarawela						
100C	$4.8^{c}\pm0.03$	$10.8^{b}\pm0.19$	$44.0^{b}\pm1.3$	$0.38^{b}\pm0.019$	$59.7^{a}\pm0.9$	$97.3^{a}\pm3.6$
50C	$4.9^{b}\pm0.04$	$10.1^{b}\pm0.09$	$37.5^{bc}\pm6.1$	$0.32^{c}\pm0.005$	$48.1^{b}\pm1.6$	$68.2^{b}\pm3.1$
50CB	$4.6^{d}\pm0.03$	$12.1^{a}\pm0.38$	$56.5^{a}\pm4.8$	$0.48^{a}\pm0.006$	$61.4^{a}\pm0.8$	$101.1^{a}\pm4.2$
B	$4.9^{b}\pm0.02$	$9.6^{c}\pm0.73$	$21.0^{d}\pm3.1$	$0.30^{d}\pm0.007$	$49.9^{b}\pm0.5$	$61.4^{b}\pm2.5$
0CB	$5.0^{b}\pm0.05$	$7.3^{b}\pm0.12$	$10.0^{e}\pm3.5$	$0.29^{d}\pm0.005$	$30.8^{d}\pm0.8$	$40.8^{c}\pm3.3$
Initial	$5.2^{a}\pm0.02$	$7.7^{c}\pm0.11$	$31.5^{c}\pm2.3$	$0.29^{d}\pm0.002$	$43.0^{c}\pm0.5$	$47.4^{c}\pm3.7$
Bibile						
100C	$5.5^{cd}\pm0.04$	$8.9^{b}\pm0.17$	$56.5^{b}\pm3.7$	$0.37^{b}\pm0.004$	$41.2^{a}\pm0.8$	$81.3^{a}\pm4.1$
50C	$5.6^{c}\pm0.05$	$8.3^{c}\pm0.05$	$28.5^{c}\pm5.4$	$0.34^{c}\pm0.003$	$26.2^{c}\pm1.9$	$51.0^{b}\pm2.7$
50CB	$5.4^{d}\pm0.06$	$10.1^{a}\pm0.25$	$65.4^{a}\pm1.9$	$0.42^{a}\pm0.004$	$42.1^{a}\pm0.7$	$89.2^{a}\pm3.4$
B	$5.6^{c}\pm0.03$	$8.6^{bc}\pm0.09$	$20.0^{d}\pm2.3$	$0.33^{c}\pm0.005$	$32.4^{b}\pm0.3$	$48.4^{b}\pm2.8$
0CB	$6.0^{b}\pm0.03$	$6.9^{d}\pm0.14$	$9.5^{e}\pm1.3$	$0.28^{d}\pm0.002$	$18.8^{d}\pm0.9$	$31.3^{c}\pm2.1$
Initial	$6.1^{a}\pm0.02$	$7.2^{d}\pm0.06$	$29.0^{c}\pm2.4$	$0.29^{d}\pm0.003$	$28.2^{c}\pm0.9$	$44.4^{b}\pm2.4$

Mean ± Slandered Deviation (SD). Treatments are 100% chemical fertilizer (100C), 50% chemical fertilizer (50C), 50% chemical fertilizer with biofilmed biofertilizer (50CB), biofilmed biofertilizer alone (B) and no amendments (0CB), respectively. SOC- soil organic carbon, Avail. P- available phosphorus, Avail. Ca^{2+} - available calcium, Avail. N- available nitrogen and Avail. K^{+}- available potassium. Means in the same column followed by the same letter are not significantly different at 5% probability level according to Tukey's test.

Table 3. The effect of different chemical fertilizer (CF) and biofilmed biofertilizer (BFBF) treatments on soil chemical properties in Bandarawela and Bibile field sites in the second growing season

Treatment	pH	SOC (mg/g)	Avail. P (mg/kg)	Avail. Ca^{2+} (mg/g)	Avail. N (mg/kg)	Avail. K^+ (mg/kg)
			Bandarawela			
100C	4.9^{b}±0.02	11.2^{b}±0.12	42.5^{b}±2.4	0.37^{b}±0.011	57.7^{a}±0.3	91.3^{a}±3.8
50C	4.9^{b}±0.04	09.1^{c}±0.11	39.7^{b}±4.7	0.30^{c}±0.005	44.1^{b}±1.1	69.1^{b}±3.1
50CB	4.5^{c}±0.02	14.6^{a}±0.24	58.3^{a}±2.8	0.53^{a}±0.002	63.6^{a}±0.2	93.1^{a}±2.7
B	4.9^{b}±0.02	8.4^{d}±0.53	23.5^{d}±1.5	0.27^{d}±0.005	47.9^{b}±0.9	51.9^{c}±2,1
0CB	4.9^{b}±0.03	7.0^{e}±0.10	12.0^{e}±3.1	0.23^{d}±0.005	31.2^{d}±0.3	41.2^{c}±2.4
Initial	5.3^{a}±0.02	7.8^{e}±0.09	34.0^{c}±5.2	0.26^{d}±0.004	42.3^{c}±0.1	48.3^{c}±1.8
			Bibile			
100C	5.5^{c}±0.03	8.5^{b}±0.10	46.3^{b}±5.6	0.38^{b}±0.002	40.0^{a}±0.3	80.7^{a}±3.7
50C	5.6^{b}±0.05	8.0^{b}±0.15	23.5^{d}±2.4	0.33^{c}±0.001	27.0^{c}±1.2	64.1^{b}±2.4
50CB	5.3^{d}±0.03	11.2^{a}±0.07	60.4^{a}±4.3	0.41^{a}±0.001	43.3^{a}±0.1	83.1^{a}±3.4
B	5.6^{b}±0.03	8.0^{b}±0.02	16.0^{e}±2.0	0.30^{c}±0.004	30.1^{b}±0.7	47.9^{c}±1.6
0CB	6.0^{a}±0.04	5.9^{d}±0.14	14.5^{e}±3.0	0.27^{d}±0.002	19.1^{d}±0.3	36.3^{d}±1.3
Initial	6.0^{a}±0.02	6.4^{c}±0.04	30.5^{c}±1.4	0.29^{d}±0.004	29.9^{c}±0.2	49.1^{c}±2.4

Mean ± Slandered Deviation (SD). Treatments are 100% chemical fertilizer (100C), 50% chemical fertilizer (50C), 50% chemical fertilizer with biofilmed biofertilizer (50CB), biofilmed biofertilizer alone (B) and no amendments (0CB), respectively. SOC- soil organic carbon, Avail. P- available phosphorus, Avail. Ca^{2+} - available calcium, Avail. N- available nitrogen and Avail. K^+- available potassium. Means in the same column followed by the same letter are not significantly different at 5% probability level according to Tukey's test.

Table 4. The effect of different chemical fertilizer (CF) and biofilmed biofertilizer (BFBF) treatments on plant physicochemical properties in Bandarawela and Bibile field sites in two growing seasons.

Treatment	Chlorophyll Content 5^{th} leaf (µg/ml)	Chlorophyll Content 3^{rd} leaf (µg/ml)	Tuber starch content (mg/g)	Stem Sucrose content (mg/g)
		Year 1/Season 1		
		Bandarawela		
100C	18.4^{b}±2.3	22.2^{b}±1.3	18.1^{b}±0.4	5.87^{b}±0.12
50C	17.5^{b}±1.6	21.7^{b}±1.7	16.5^{b}±1.9	4.13^{c}±0.25
50CB	24.6^{a}±1.1	28.5^{a}±1.3	24.1^{a}±0.9	9.23^{a}±0.34
B	14.6^{c}±1.0	17.6^{c}±1.3	14.2^{c}±0.6	5.12^{b}±0.67
0CB	14.3^{c}±1.2	16.1^{c}±1.6	13.6^{c}±0.6	2.34^{d}±0.37
		Bibile		
100C	42.4^{b}±1.2	45.4^{b}±2.5	12.1^{b}±1.0	4.12^{b}±0.43
50C	41.3^{b}±1.5	45.1^{b}±2.1	11.6^{b}±0.3	2.18^{c}±0.11
50CB	46.7^{a}±1.1	58.3^{a}±1.6	14.2^{a}±0.3	8.56^{a}±0.54
B	36.4^{c}±0.6	38.5^{c}±1.6	10.9^{b}±0.2	4.07^{b}±0.22
0CB	24.7^{d}±0.7	33.0^{d}±1.9	10.7^{b}±0.5	1.93^{c}±0.12
		Year 2/Season 2		
		Bandarawela		
100C	20.5^{b}±1.3	25.7^{b}±1.4	21.7^{b}±0.8	5.12^{c}±0.72
50C	13.5^{c}±1.1	19.2^{c}±1.1	15.1^{c}±1.2	4.88^{c}±0.87
50CB	28.0^{a}±1.6	38.5^{a}±1.4	33.5^{a}±0.6	8.74^{a}±0.58
B	13.2^{c}±0.5	14.7^{d}±1.1	11.2^{d}±0.9	5.04^{b}±0.66
0CB	7.8^{d}±0.8	13.8^{d}±1.1	10.1^{d}±0.6	3.12^{d}±0.54
		Bibile		
100C	42.6^{b}±1.8	45.7^{b}±1.7	10.9^{b}±0.5	3.67^{b}±0.22
50C	41.2^{b}±1.2	44.2^{b}±1.8	10.5^{b}±0.9	3.44^{b}±0.43
50CB	51.6^{a}±1.1	60.5^{a}±2.4	18.7^{a}±0.4	6.13^{a}±0.12
B	39.4^{bc}±1.8	41.7^{bc}±1.9	9.5^{b}±0.5	3.56^{b}±0.32
0CB	20.3^{d}±1.2	34.3^{d}±1.5	9.3^{b}±0.3	1.54^{c}±0.15

Mean ± Slandered Deviation (SD). Treatments are 100% chemical fertilizer (100C), 50% chemical fertilizer (50C), 50% chemical fertilizer with biofilmed biofertilizer (50CB), biofilmed biofertilizer alone (B) and no amendments (0CB), respectively. Means in the same column followed by the same letter are not significantly different at 5% probability level according to Tukey's test.

in Bibile even over 100C in both growing seasons. Significant differences were not observed in soil K^+ content between 50CB and 100C in both growing seasons. However, the highest soil K^+ content was observed in 50CB.

***The effect of CF and BFBF treatments on plant biochemical properties*:** In comparison with all other treatments, the significantly ($P < 0.05$) highest mean chlorophyll contents in 3^{rd} and 5^{th} leaves were observed from 50CB at both field sites

in both growing seasons (Table 4). Treatment 50CB increased the mean chlorophyll content in the 3rd potato leaf by 20% in Bandarawela and 19% in Bibile location in the first growing season whereas the same was increased by 26 % in Bandarawela and by 32% in Bibile in the second growing season even over 100C. Further, it was observed that the mean chlorophyll content of the 3rd leaf was higher than that of the 5th leaf at both locations in both growing seasons. Treatment 50CB showed the significantly highest tuber starch content at both locations in both growing seasons. Further, 50CB increased the tuber starch content by 33 % in Bandarawela and 18% in Bibile in the first growing season whereas the same was increased by 54% in Bandarawela and by 72% in Bibile in the second growing season even over 100C. In comparison with all other treatments, the significantly ($P < 0.05$) highest mean total sugar content in the stem tissues was observed from 50CB at both field sites in both growing seasons (Table 4). It was noted that the stem sugar content was higher in treatment B even over 100C except 50CB at both field sites in both growing seasons.

Significant negative correlations ($P < 0.05$) were observed from mean tuber yield, chlorophyll content of 5th and 3rd leaves and mean tuber starch content with mean soil pH at both locations in both growing seasons (Fig. 1). Further,

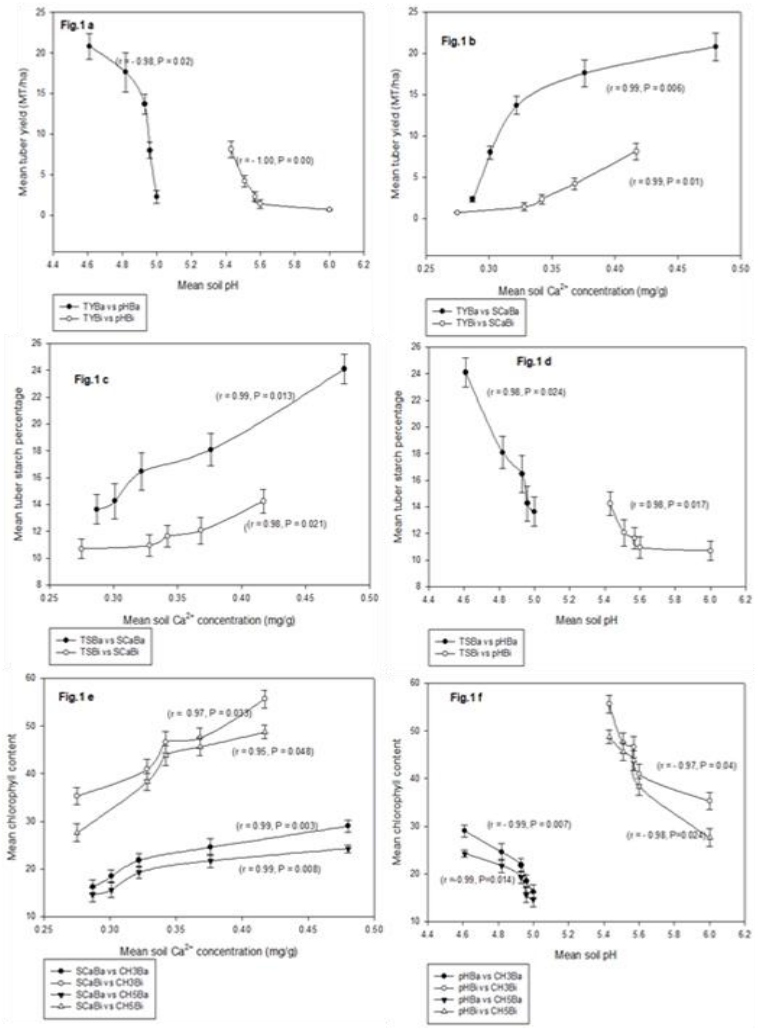

Figure 1. a) The effect of soil pH on tuber yield of potato in two field sites. b) The effect of soil calcium (Ca^{2+}) concentration on tuber yield of potato in two field sites. c) The effect of soil Ca^{2+} concentration on tuber starch percentage of potato in two field sites. d) The effect of soil pH on tuber starch percentage of potato in two field sites. e) The effect of soil Ca^{2+} concentration on chlorophyll content of 5th and 3rd leaves of potato in two field sites. f) The effect of soil pH on chlorophyll content of 5th and 3rd leaves of potato in two field sites. (Ba-Bandarawela, Bi- Bibile field sites TW- tuber weight, SCa- soil Ca^{2+} concentration, TS- tuber starch content, CH5- chlorophyll content (µg/ml) of 5th leaf µg/ml, CH3- chlorophyll content (µg/ml) of 3rd leaf)

Table 5. Molecular identification of fungal and bacterial components of the responsive biofilm.

Sr.	Microorganism type	Length of the fragment (bp)	Closest Relative	Similarity (%)	Accession No.
1	Bacteria	731	*Bacillus pumilus*	99	NZ_CP011007
2	Bacteria	842	*Bradyrhizobium japonicum*	100	NC_017249
3	Bacteria	621	*Bacillus subtilis*	100	NC_000964
4	Fungi	725	*Trichoderma harzianum*	100	KR868300

significant positive correlations ($P < 0.05$) were observed from mean tuber yield, mean chlorophyll contents of 5th and 3rd leaves and mean tuber starch content with mean soil Ca^{2+} content at both locations in both growing seasons.

***Molecular identification of microbial components in the responsive biofilm*:** Nucleotide sequence analysis of the responsive microbial components through GenBank search revealed that the isolates had high sequence similarity to the species *Bacillus pumilus* (NZ_CP011007.1), *Bradyrhizobium japonicum* (NC_017249.1), *Bacillus subtilis* (NC_000964.3) and *Trichoderma harzianum* (KR868300.1), respectively (Table 5) among the nucleotide sequences available in the National Center for Biotechnology Information (NCBI) database.

DISCUSSION

The data extracted from agricultural field stations of the Department of Agriculture (DOA), Sri Lanka showed that the average potato tuber yield obtained at Bandarawela cultivated area during the past ten years was approximately 18-22 MT/ha under the standard recommended CF rates (urea- 330 kg/ha + TSP- 270 kg/ha + MOP- 250 kg/ha). The current study also confirmed that the average yield of potato by 100C is within the expected range (20 MT/ha). However, it is noteworthy that our results showed a yield of 26.8 MT/ha and 27.2 MT/ha by 50CB in Bandarawela location in both growing seasons, respectively. It was reported that the Bibile climatic area has lower or zero potential to establish commercial potato cultivation since the climatic condition is not favorable for tuberization. The current study also confirmed that the Bibile cultivated area has very low potential (approximately 4 MT/ha) for potato cultivation even with the DOA recommended CF. However, it is noteworthy that the average potato tuber yield could be enhanced up to 8.1 MT/ha and 6.9 MT/ha by 50CB over 100C in Bibile location in both growing seasons respectively. These findings imply that the input of CF can be reduced by 50% with the use of developed BFBFs while enhancing the potato yield, which could be a huge gain in terms of fertilizer saving. Similar yield increments were reported in potato and other crops by the applications of beneficial microbial biofertilizers. It was reported that the BFBFs can produce equal or even relatively high yields with only 50% CF in several crops in comparison to 100% CF application (Seneviratne *et al*., 2009). Further, the addition of beneficial soil microorganisms has been proved to improve fertility, quality, yield and size of potato tuber through their beneficial activities (Sugiarto *et al*., 2013).

The results of the current study confirmed that the application of microbial biofilm enhances the soil nutrient level, i.e., SOC, available P, available Ca^{2+} level, available K^{+} level and available N, even compared to 100% CF application (Tables 2 and 3). It has been well documented that the soil microbial communities like biofilms directly or indirectly contribute to the plant growth and development (Seneviratne *et al*., 2009) through the enhancement of soil nutrient level, i.e; SOC (Ekin *et al.,* 2009), available P (Wang *et al*., 2020) and available Ca^{2+} level (Mohamed and Basala, 2015). It has been reported that a wide variety of climatic, physiological and soil biochemical cell signaling ions are known to be involved either directly or indirectly as stimuli in the induction of potato tuberization through starch deposition (Nookaraju *et al*., 2012). Suárez-Lopez (2013) reported that potato tuberization is induced by several phloem-mobile signaling molecules. Like other biochemical and physiological changes, phloem translocation and the starch/sugar movement through the plant body are induced by different cell signaling molecules (Koenig and Benning, 2020). Therefore, it is important to study the effect of exogenous stimuli on the endogenous levels of different biochemical processes which induce tuberization.

According to previous studies, it has been confirmed that potato tuberization is sensitive to different external inductive signals (Suárez-Lopez, 2013). Among the soil factors, Ca^{2+} plays important role in potato tuberization (Kleinhenz and Palta, 2002). The Ca^{2+} is an intracellular second messenger used in living organisms including microorganisms, coordinating extracellular stimuli for their characteristic intracellular responses (Edel *et al.,* 2017). It has been reported that soil Ca^{2+} near the rhizosphere in potato influences tuberization by altering the hormonal and biochemical balance at the stolon tip (Nookaraju *et al*., 2012). This might be due to the induction activity of exogenous Ca^{2+} on the starch accumulation process (Edel *et al.,* 2017), thereby enhanced the tuber weight. Calcium ions affects the opening of K^{+} channels in leaves, especially guard cells by working as a secondary messenger (Helal and AbdElhady, 2015). This indicates the induction activity of Ca^{2+} on tuberization signal. For instance, an external stimulus causes the rapid increment of cytosolic Ca^{2+}concentration (Nookaraju *et al*., 2012) through the massive influx of Ca^{2+} from Ca^{2+} channels

enhances the tissue sucrose level which ultimately serves as an internal signal for tuberization (Edel *et al.*, 2017). In the current study, the lowest mean soil pH (Table 2), the highest tuber yield (Table 1), the highest chlorophyll content, the highest tuber starch content and stem sucrose contents (Table 4) were recorded from BFBF treatment along with 50% CF at both field sites and in both growing seasons. This implies that potato yield and tuber starch accumulation are greatly affected by the soil pH and this might be due to the introduction of beneficial microorganisms in the form of BFBFs which creates low pH around the rhizosphere.

It was recorded in the current study that the application of BFBF along with 50% CF enhanced the soil Ca^{2+} and H^{+} content, tuber starch content and ultimately tuber yield irrespective of the climatic factors at both field sites in both seasons. Though Bandarawela has the potential to cultivate potatoes, favorable climatic conditions (thermoperiod/ photoperiod) for commercial potato cultivation have not been recorded in Bibile region. Therefore, this region is considered a potato non- inducing region. However, in the current study, high potato yield was observed by the application of beneficial microorganisms in the form of BFBFs treatments irrespective of the climatic conditions even at the Bibile field site in both growing seasons. Generally, photoperiodic signal is integrated with other environmental factors, such as nutrient availability, temperature, and light intensity, as well as with the overall metabolic status of the plant (Timlin *et al*., 2006). Tuber inducing potential of exogenous stimuli has been well documented and the production of those signaling compounds and ions by beneficial microorganisms has also been recorded (Spaepen *et al*., 2007).

Therefore, this implies that the elevated soil Ca^{2+} and H^{+} content by the treatment 50CB (BFBF along with 50% CF) might act as potential stimulators for the tuber yield enhancement by altering the essential biochemical processes in potato tissues irrespective of the climatic conditions. However, the exact molecular and biochemical mechanism of Ca^{2+} and H^{+}- induced signal pathways controlling tuberization is needed to be further studied.

***Conclusions*:** It is noteworthy that the treatment 50CB responded positively to create favorable soil biochemical (SOC, P and Ca^{2+}, pH) conditions, leading to the enhanced tuber yield. Such exogenous microbial signals created by the application of the microbial biofilm along with 50% CF induced the required internal biochemical and physiological conditions for increased tuber yield of potato. Thus, it seems that the plant biochemical and physiological cycles required for tuber yield, generally induced by the specific climatic factors like day/night temperature difference have been stimulated by the microbial signals of the biofilm. As such, the climatic requirements for potato tuberization have been compensated by the biofilm microbial actions. This finding opens a new avenue for potato cultivation in regions where there are no suitable climatic requirements.

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