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Synergism of Industrial and Agricultural Waste as a Suitable Carrier Material for Developing Potential Biofertilizer for Sustainable Agricultural Production of Eggplant

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Abstract: The study investigates biochar from agriculture waste and flyash from coal power station as possible carrier materials for two plant growth-promoting (PGP) bacterial strains *Burkholderia* sp. L2 and *Bacillus* sp. A30 for enhanced eggplant growth and yield. Biochar-based biofertilizers with/without flyash showed higher viability up to 270 days of storage period. The maximum percentage of seed germination was observed in L2-based biochar and flyash + biochar (1:1) bifertilizer. Moreover, the L2 + biochar+flyash produced a maximum percentage increase in fruit yield with significant ($p < 0.05$) improvement in plant growth parameters. Post-harvest soil status also showed enhanced physical (water holding capacity, moisture content), chemical (pH, electrical conductivity, NPK), and dehydrogenase activity. The study suggests that biofertilizer of L2 strain with agriculture waste generated biochar and flyash as carrier materials can tremendously enhance the productivity of eggplant and could act as a substitute for chemical fertilizer thus solving their disposal problem by sustainable waste management.

Keywords: plant growth-promoting rhizobacteria; *Solanum melongena*; biochar; flyash; sustainable waste management; shelf-life

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1. Introduction

Many unsafe and expensive agrochemicals have continuously been used in agriculture for several decades to improve agricultural production. In addition, it further poses a threat to the abiotic and biotic environment. However, less attention has been paid to reducing its use by utilizing industrial and agricultural waste material as a carrier with plant growth-promoting rhizobacteria for sustainable enhanced agricultural production [1,2]. Plant growth-promoting rhizobacteria (PGPR) are valuable microorganisms gaining importance worldwide because of their plant growth-promoting attributes, such as growth-regulating hormones (indole-3-acetic acid, gibberellin), phosphate solubilization, siderophores, and ACC deaminase production which are beneficial for soil fertility and plant growth [3,4]. Moreover, these PGPRs also help in soil nutrient management by supporting micro-floral habitat, thus reducing the use of chemical fertilizers, tolerate various organic and inorganic pollutants. Hence, it can be used as a biofertilizer with suitable carrier materials, enhancing its shelf-life. However, the use of such carrier-PGPR-based biofertilizers has low industrial popularity because of the lack of suitable and economically viable carriers [5].

There are multiple methods generally used for the introduction of bacteria into the soil, such as seed coating [6], root dipping [7,8], bacterial suspension [9], and solid carriers [10–12]. However, after soil inoculation, the cell number of the test inoculants reduces rapidly due to changes in nutrient and environmental conditions [13]. Freshly prepared bacterial inoculum has a low shelf-life and is not convenient for transportation, storage, and application. Carrier-assisted bacterial inoculation enhances enough shelf life and ease of application. It also provides protective habitats and protection from predation, which influences inoculum success [14,15].

Various solid materials such as soils, peat, plant material, such as ground corncob, organic manures and compost, and inert materials, such as perlite, alginate, and vermiculite, have been used as solid carriers for biofertilizer preparation. The use of industrial waste as a carrier for bacterial inoculum could be a promising act to sustain the never-ending creation of industrial waste or by-products. Flyash (FA) is one of the major wastes generated by coal-based industries, like thermal power stations [16,17]. Worldwide fly ash production is approx. 367 MT per year, mainly contributed by China, India, USA, Germany, and UK. India itself produces around 112 MT per year, with its utilization of only 38%. FA particles are usually very fine (surface area, 4000–10,000 cm^2/g) and lightweight (density 1.97–2.89 g cc^{-1}). It is mainly composed of silicates with its highest percentage of 59.38 as SiO_2. Other major chemical components of FA are: Al_2O_3: 23.59%, Fe_2O_3: 6.11%, CaO: 1.94%, MgO: 0.97%, SO_3: 0.76%, and alkalies of 1.41% [18–20]. Apart from this, it also contains an adequate concentration of micro and macro elements and a significant source of plant nutrients; thus its use as carrier material could help in effective waste management in a beneficial manner [21,22]. Another carrier material gaining global importance is biochar. Biochar is generated by thermochemical conversion of biomass in oxygen-limited or anoxic conditions [23,24]. Converting biomass into biochar involves biomass drying, grinding, pyrolysis, and separation [25]. Biochar's highly porous structure can contain significant amounts of extractable humic-like and fluvic-like substances [26]. Moreover, its molecular structure shows a high degree of chemical and microbial stability [27]. The good physical (high specific surface area and high water holding capacity) and chemical (carbon, nitrogen (N), hydrogen, and some lower nutrient elements, such as Na, K, Ca, and Mg) properties of biochar make it suitable to be used as a potential carrier material for biofertilizer preparation. Biochar has several polar or nonpolar substances, which have a strong affinity to inorganic ions such as heavy metal ions, phosphate, and nitrate [28].

Previous reports suggest BC from sludge, manure, and wood can be used for amelioration of soil properties; however, limited studies are reported for BC generated from agricultural waste [29,30]. Moreover, FA is always used as an amendment worldwide, and at a higher percentage, it showed a negative effect on plant growth [22,31]; however, its utilization as carrier material is still unknown. Both BC from agricultural waste and FA from industrial waste can compensate for the drawbacks of each other and their consortium study could be interesting in developing a new carrier-based biofertilizer for sustainable crop production. To the best of our knowledge, no synergistic study of FA (inorganic) and biochar (organic) together as carrier material for the preparation of biofertilizer for improved crop production is still studied.

We hypothesize that a consortium of FA (a by-product of coal-powered thermal plants) and BC (from agricultural waste) with PGPRs can improve the biometric growth parameters of the eggplant along with post-harvest soil nutrient status. The major objectives of the study include: (a) preparation of biofertilizer using carrier materials: FA, BC, and their consortium and two PGPRs, *Burkholderia* sp. strain L2 and *Bacillus* sp. strain A30, (b) assessment of shelf-life, pH, and moisture content of these prepared biofertilizers over a nine-month period of time, (c) role of biofertilizers in biometric growth of *Solanum melongena* L. (eggplant) throughout and at the harvest of plant growth experiment, and (d) assessment of post-harvest soil status to justify the efficacy of the inoculated carriers as biofertilizer.

2. Materials and Methods

2.1. Carrier Characterization and Preparation

Solid carriers viz., biochar (BC), flyash (FA), and biochar + flyash (BC + FA, 1:1, *w*/*w*) were selected to conduct the experiment. BC powder, prepared from agricultural waste (mainly leaves of cabbage, spinach, cauliflower, and green gram leaves) pyrolyzed at 250 °C for 2 h, was purchased from the local market of Dhanbad, India, and FA was collected from the thermal power plant of Bokaro, India. Carrier was mixed with distilled water (BC, FA, and BC + FA, 1:5, *w*/*v*) to form a slurry, and pH and EC were determined. Moisture capacity (MC), water holding capacity (WHC), and diethylene triamine penta-acetic acid (DTPA) extractable or plant available metals in each carrier were determined by standard methods [32,33]. Carrier materials were sieved through 300 mesh size, dried for 2 days at 70 °C, and triple sterilized at 110 °C for 20 min for three consecutive days.

2.2. Bacterial Characterization and Plant Growth Promoting Properties

Two rhizobacteria *Burkholderia* sp. strain L2 (Accession No. MZ027310) and *Bacillus* sp. strain A30 (Accession No. MZ027309) isolated from agricultural soil of India were used for the present study [34]. Briefly, the genomic DNA was isolated and 16S rRNA gene was amplified by PCR using the genomic DNA as a template and bacterial primers, 27f (50-AGAGTTTGATCMTGGCTCAG-30) and 1492r (50-TACGGYTACCTTGTTACGACTT-30). For sequencing, the amplified DNA was purified using a Montage PCR Cleanup kit (Millipore). Automated sequencing of the purified PCR products was performed using BigDye Terminator Cycle Sequencing Kit (APPLIED BIOSYSTEM, Foster city, CA, USA). Sequencing products were resolved on an Applied BioSystems-3730XL, an automated DNA sequencing system (Applied Biosystems, Foster city, CA, USA). The 16S rRNA sequences obtained were matched to nucleotide sequences present in GenBank using the BLASTn program. P-solubilization was checked according to the method of Tripti et al. [34]. Indole acetic acid production and siderophore were estimated and checked by the method of Brick et al. [35] and Ma et al. [36], respectively.

2.3. Biofertilizer Preparation

To make the carrier inoculum, each strain was grown in 250 mL of sterilized (121 °C for 20 min) Luria Bertani (LB, Himedia) broth for 2 days in a shaker incubator at 150 rpm at 28 °C. The cells were washed twice with sterile phosphate buffer (pH 6.5), followed by centrifugation at 5000 rpm for 10 min in a cooling centrifuge and finally resuspended in sterile phosphate buffer. About 100 mL of cell suspension of approximately 10^8 colony forming unit (CFU) per mL was added aseptically into 250 g of carrier material with 1% of glucose as carbon source and 1% of guar gum as an adhesive agent, mixed by kneading between the fingers and was spread in a tray for overnight curing to attain the final moisture content of about 25–35%. Prepared biofertilizer was sealed in 75 mm thick low density, UV-sterilized flexible polyethylene bags, leaving two-third vacant space for proper aeration, and stored at ambient room temperature (28 ± 2 °C) in the dark. Each experiment was replicated three times and all the procedures were performed under aseptic conditions.

2.4. Survival Test of Strain L2 and A30 in Carrier Materials

All the carriers were tested for the survivability of strain L2 and A30 over a period of 9 months. Each carrier was examined at the beginning of the experiment, 30 days, and then at 60 days of interval up to 270 days. The cell number of bacterial strain was measured by suspending 5 g of the carrier in a 250 mL flask containing 45 mL of sterile phosphate buffer (pH 6.5). After shaking at 160 rpm for 45 min (28 °C), the suspension was serially diluted up to the required dilution and spread on a PKV agar plate. After 7 days of incubation at 28 ± 2 °C, all halo-zone-forming colonies were counted using a colony counter. Plating was done in triplicates and the mean value was used to represent viable

bacterial count (CFU g^{-1}). The pH (1:5, *w*/*v*) and MC of the inoculated carrier were also measured for the above said time period to check the response of selected strains in each carrier.

2.5. Plant Development Assay

A pot assay was carried out to examine the effect of carriers inoculated with strain L2 and A30 on eggplant growth. Two times sterile (100 °C for 30 min) garden soil (EC: 0.39 ± 0.02 dS m^{-1}; pH: 6.7 ± 0.10 (1:2.5; *w*/*v*)); WHC: 45.62 ± 4.93 %; OC: 0.95 ± 0.03 %; Available N: 101.73 ± 4.93 mg kg^{-1}, Available Phosphorous (P): 1.98 ± 0.08 mg kg^{-1};) was used for performing the growth experiment. The non-sterile garden soil showed a dehydrogenase activity (DHA) of 3.81 ± 0.11 μg TPF g^{-1} h^{-1}.

Seeds of eggplant (F-1 hybrid Magadh Long) were surface sterilized by immersing in 10% sodium hypochlorite solution for 10 min, followed by rinsing 4 times with sterile distilled water. The seeds were further soaked in a homogenous mixture of biofertilizer and sterile double distilled deionized water (1:1, *w*/*v*) in a closed sterile Petri plate. After 2 h of soaking, the bacterized seeds were dried with air in a laminar hood for about 30 min and thirty seeds were transferred using forceps in each pot (diameter 20 cm and height 16.5 cm), having a capacity of 5 kg. Seeds without inoculated carriers were considered as control. The treatments used to study the pot assay are described in Table 1. The pots were regularly watered as per the requirement to maintain an optimum moisture level of 70% of field capacity. The seed germination rate was monitored by following the method of Wijewardana et al. [37]. Germinations were recorded on alternate days till the 14th day after sowing. After that, thinning was done by hand and four healthy and matured seedlings were transplanted in the same pot and allowed to grow for another 90 days. Five grams of one-month-old biofertilizer was applied near the rooting zone of each transplanted plant. To nullify the environmental effect, the present work was done in a completely randomized block design and each was replicated four times. However, to remove the plant after every 30 days, separate pots with four seedlings were also established.

Table 1. Description of treatments used for pot experiments.

Treatment	Composition
Control	Soil
L2 + FA	Flyash + Soil + *Burkholderia* sp. LRS02
L2 + BC	Biochar + Soil + *Burkholderia* sp. LRS02
L2 + FA + BC	Flyash + biochar (1:1) + Soil + *Burkholderia* sp. LRS02
A30 + FA	Flyash + Soil + *Bacillus* sp. A30
A30 + BC	+Biochar + Soil+ *Bacillus* sp. A30
A30 + FA + BC	Soil + Flyash + biochar (1:1) + *Bacillus* sp. A30

L2: *Burkholderia* sp. LRS02; FA30: *Bacillus* sp. A30; FA: flyash; BC: Biochar.

Single plant from each experimental pot was taken out carefully for biometric observations every 30 days of interval after their transplantation. The dry weight of root and shoot was determined by thoroughly washing with tap water followed by air drying (for a day) and then oven drying at 70 °C for 72 h. The number of flowers was recorded at 60 days after transplantation (DAT), whereas the yield of fruit was estimated at 90 DAT.

At the end of the experimental duration (post-plantation), different physico-chemical properties of soils were analyzed to understand its fertility condition driven by inoculated carrier as described in the previous section, post plantation soil MC, WHC, pH and EC were determined. Furthermore, OC [38], Available N [39], Available P [40], and soil DHA [41] were determined according to standard methods.

2.6. Statistical Analyses

Logarithmic transformation was applied to calculate bacterial counts (CFU g^{-1}). Both normality of data and homoscedasticity were tested and significant differences in the mean values of shelf-life of inoculated strain in carriers, seed germination, plant growth parameters, fruit yield, and post-harvest soil parameters were analyzed by Tukey's test when one-way analysis of variance was found significant at $p < 0.05$. Kruskal–Wallis test was used to analyze non-normal data. All statistical analyses were performed using SPSS version 20.0 (Chicago, IO, USA) software package.

3. Results

3.1. Carrier Characterization and Shelf Life, pH and MC of Biofertilizers

All carrier materials had a pH value near neutral (Table 2). BC showed maximum WHC and inherent MC of 80.25% and 15.30%, respectively, followed by FA + BC and FA. BC depicted a comparatively lower concentration of plant/bioavailable heavy metals than other carriers and all metals were found much below the critical plant toxicity limit. The shelf life of L2 and A30 was studied in three prepared solid carriers for a period of nine months. After one month of incubation, there was a slight decrease in the initial cell load (10^9 CFU g^{-1}) in all carriers was observed except A30 + FA carrier (Figure 1a). However, there was no significant difference in viable cell number (10^7 CFU g^{-1}) of L2 and A30 inoculated in BC and FA + BC based carrier observed after five months of incubation. Biochar based formulations showed better cell viability than FA alone. The pH was reduced from neutral to slightly acidic after nine months of incubation (Figure 1b) and very slight changes were observed at five months of incubation. Higher moisture content was observed in BC followed by FA + BC mixture and FA alone (Figure 1c).

Table 2. Physicochemical properties and concentration of DTPA-extractable metals in two different carrier materials and in their mixture. (Mean ± SD; $n = 3$); <dL: detection limit; * Flyash + Biochar (1:1; *w*/*w*). MC: moisture content; WHC: water holding capacity; EC electrical conductivity; DTPA: diethylenetriaminepentaaceticacid

Properties	Flyash	Biochar	Flyash + Biochar *
Physico-chemical characteristics			
MC (%)	2.3 ± 0.21	15.30 ± 0.87	10.2 ± 0.47
WHC (%)	60.9 ± 1.05	80.25 ± 2.10	64.28 ± 3.24
pH (1:2.5; *w*/*v*)	6.57 ± 0.91	7.28 ± 0.32	7.03 ± 0.29
EC (dS m^{-1})	0.43 ± 0.05	0.33 ± 0.05	0.39 ± 0.03
DTPA extractable heavy metals (mg kg^{-1})			
Pb	3.28 ± 0.05	6.82 ± 0.21	4.99 ± 1.21
Zn	15.91 ± 0.09	15.02 ± 1.10	15.19 ± 1.93
Cu	7.10 ± 0.05	2.15 ± 0.93	3.55 ± 0.79
Fe	9.16 ± 0.06	7.08 ± 1.47	8.20 ± 1.20
Mn	5.12 ± 0.19	0.29 ± 0.19	4.60 ± 0.33
Ni	0.20 ± 0.03	<dl	<dl
Cd	0.05 ± 0.01	<dl	<dl
Co	0.10 ± 0.03	1.54 ± 0.23	0.31 ± 0.12

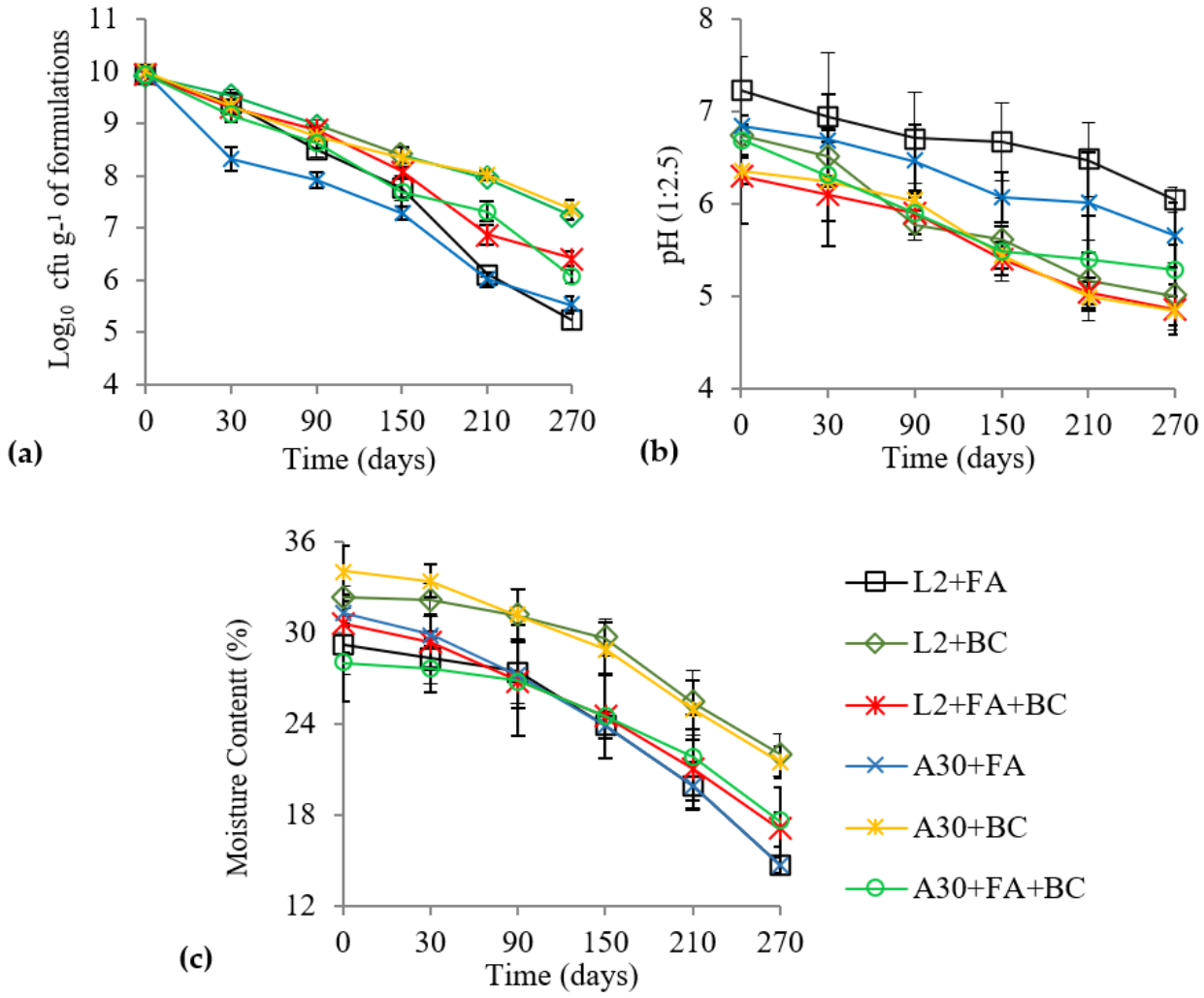

Figure 1. Changes in (**a**) logarithmic value of CFU g^{-1} determined by plate-count method, (**b**) pH, and (**c**) moisture content of three biofertilizers (carrier with strain L2 and A30) during the different storage period. Mean ± SE (n = 3). FA: flyash; BC: biochar.

3.2. Biometric Growth Parameters

Effects on seed germination: Seeds treated with biofertilizer demonstrated significantly ($p < 0.05$) increased rate and percent of germinations compared to untreated control seeds (Table 3). Treatment L2 + FA + BC outperformed amongst other treatments and showed a maximum increase (17.65%) in seed germination over control, whereas L2 + BC showed the maximum rate of seed germination followed by L2 + FA + BC.

Table 3. Seed germination details of eggplant treated with different treatments. (Mean ± SD)

Treatments	Seed Germination (%)	Seed Germination Increased over Control (%)	Rate of Seed Germination *
Control	75.56 ± 5.25 d	-	7.30 ± 0.80 c
L2 + FA	83.33 ± 2.80 bc	10.29	8.64 ± 0.37 ab
L2 + BC	87.78 ± 3.50 a	16.18	9.08 ± 0.50 a
L2 + FA + BC	88.89 ± 3.24 a	17.65	8.91 ± 0.69 a
A30 + FA	84.44 ± 6.85 b	11.76	7.67 ± 0.30 bc

A30 + BC	84.44 ± 6.85 b	11.76	8.39 ± 0.54 abc
A30 + FA + BC	82.22 ± 4.60 c	8.82	7.94 ± 0.76 abc

* Means followed by a common letter in a column are not significantly different ($p < 0.05$) according to Tukey's test.

Effects on plant growth and fruit yield: Among seven treatments, L2-based biofertilizer produced significantly (p <0.05) improved results for length and biomasses as compared to A30. At 30 DAT, no significant difference in plant growth parameters was observed when treated with L2 and A30-based biofertilizer. At 60 DAT, in general, significantly higher growth was observed for L2 + BC and L2 + FA + BC. At harvest, maximum root length was observed for L2 + FA + BC; however, no significant difference was observed between L2 + BC (Figure 2a). The L2 + FA + BC treatments also produced the utmost results for root fresh and dry weight. Similarly, in the case of length, dry and fresh shoot biomass, maximum growth was observed for L2 + FA + BC (Figure 2b,c). Among all the treatments, an insignificant difference in the number of leaves was observed at 30 DAT, which were significantly varied at 60 DAT and 90 DAT as compared to control (Figure 3). At harvest, the maximum and minimum percent increase in leaf numbers were 60.7% and 3.6% for L2 + BC and A30 + FA + BC, respectively, as compared to control, which were relatively less in comparison to 60 DAT. However, no significant difference was observed between L2 + BC and L2 + FA + BC for the number of leaves at 90 DAT. Moreover, among L2-based biofertilizers at 60 DAT, the maximum number of flowers per plant (6.0) was observed for L2 + FA + BC followed by L2 + BC > L2 + FA.

Strain L2-based biofertilizer produced a higher fruit yield as compared to strain A30 (Figure 4). FA + BC inoculated with L2 attained maximum fruits biomass (4025.04 g pot^{-1}) and fruit yield (2012 g $plant^{-1}$). After L2 + FA + BC the fruit yield production (g $plant^{-1}$) was in the order of L2 + BC (1980) > L2 + FA (1714) > A30 + FA + BC (1366) > A30 + BC (1348) > A30 + FA (1323).

Post-harvest status of soil: Soil properties of different treatments were statistically evaluated at harvest (Table 4). Overall, it was found that soil properties were improved due to the application of biofertilizer as compared to control. At the start of the experiment, the pH of the soil decreased significantly but remained slightly acidic after the experiment. Whereas the average MC, WHC, OC, EC, Available N, Available Phosphorous and dehydrogenase of the harvested soil of L2 + BC + FA based biofertilizer were significantly increased by 11%, 28%, 15%, 16%, 50%, 16%, and 38%, respectively, compared to control soil.

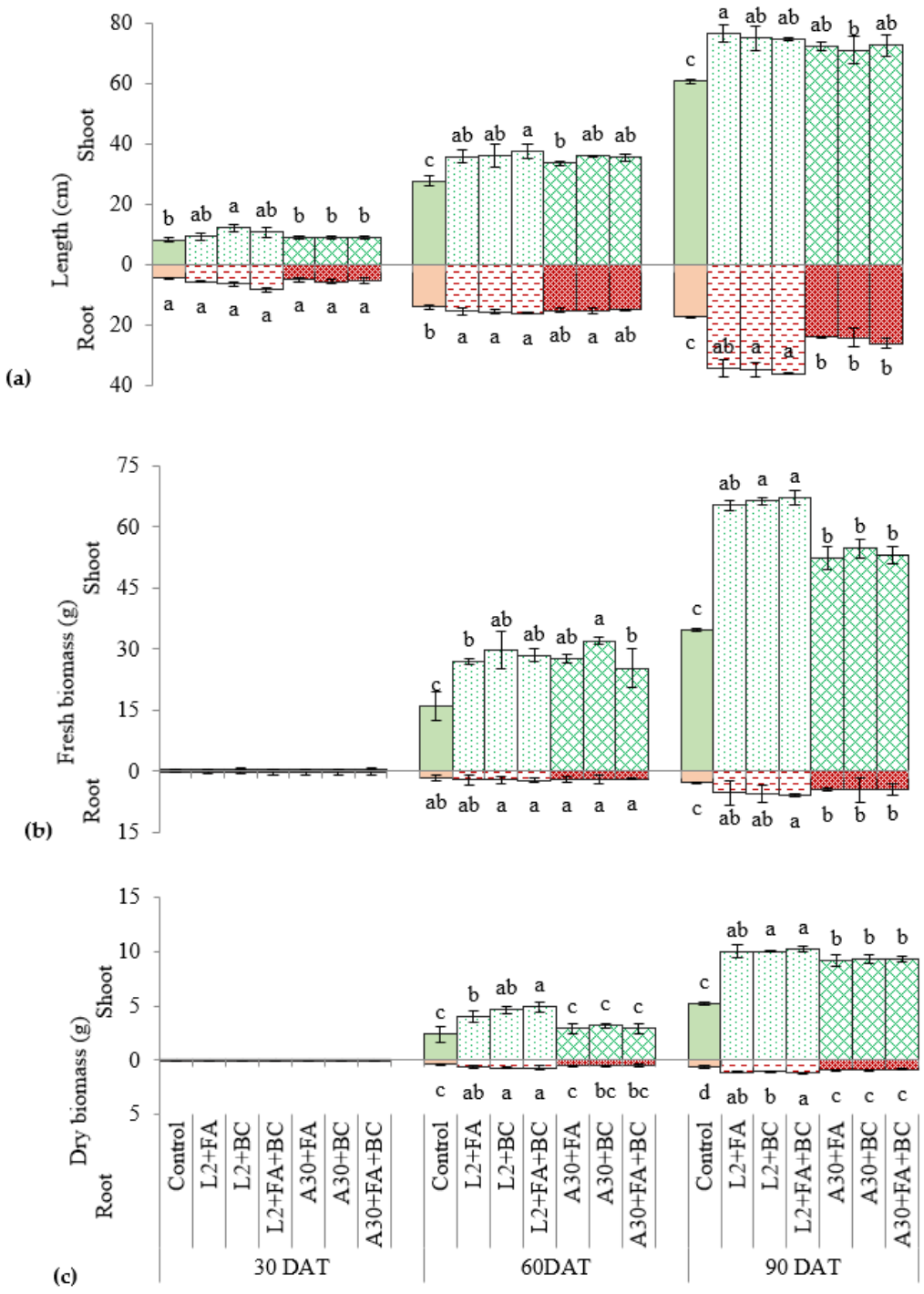

Figure 2. Influence of biofertilizers prepared using strain L2 and A30 on biometric growth parameters of eggplant root and shoot: (**a**) length (cm), (**b**) fresh wt. (g), and (**c**) dry wt. (g), at the end of the pot experiment at an interval of 30 days till 3 months after transplantation (DAT) (Mean ± SD; n = 4). Different alphabetical letters represent a significant difference at $p < 0.05$ for the same DAT and parameters. No significant difference at 30 DAT for root and shoot fresh and dry weight was observed.

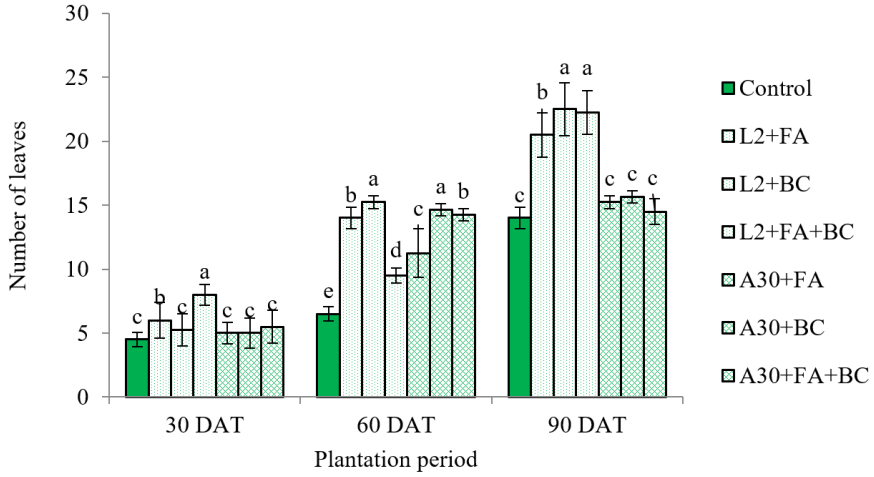

Figure 3. Influence of carrier-based biofertilizer prepared using strain L2 and A30 on the number of leaves per plant of eggplant at an interval of 30 days until 90 days after transplantation (DAT) (Mean ± SD; $n = 4$). The difference in values of the same DAT were shown with different alphabetical letters at a $p < 0.05$ level of significance.

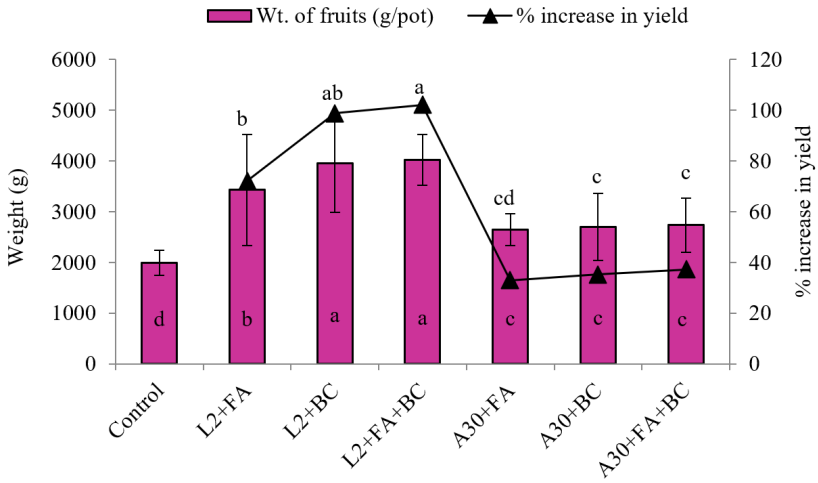

Figure 4. Influence of carrier-based biofertilizers of strain L2 and A30 on average fruit weight (g pot^{-1}) and yield (g plant^{-1}) at harvest. (Mean ± SD; $n = 4$). Different alphabetical letters represent significant differences at $p < 0.05$.

Table 4. Post-harvest study of nutrient and fertility status of the soil. (Mean ± SD; $n = 4$)

Treatments	MC (%)	WHC (%)	pH (1:2.5; *w/v*)	EC (dS m^{-1})	OC (%)	Avl. P (mg kg^{-1})	Avi. N (mg kg^{-1})	DHA (µg TPF g^{-1} h^{-1})
Control	11.56 ± 1.05	49.13 ± 9.94	6.60 ± 0.10	0.56 ± 0.03	0.96 ± 0.03	1.59 ± 0.13	92.40 ± 3.70	3.85 ± 0.33
L2 + FA	12.98 ± 0.79	53.66 ± 19.53	6.40 ± 0.10	0.63 ± 0.03	1.11 ± 0.08	2.76 ± 0.19	103.13 ± 9.32	4.56 ± 0.26
L2 + BC	11.83 ± 0.97	58.07 ± 24.03	6.30 ± 0.10	0.64 ± 0.02	1.04 ± 0.14	3.29 ± 0.29	118.07 ± 4.04	5.27 ± 0.16
L2 + FA + BC	12.90 ± 0.34	59.22 ± 9.76	6.25 ± 0.06	0.66 ± 0.02	1.15 ± 0.11	3.19 ± 0.31	110.60 ± 4.20	5.38 ± 0.47
A30 + FA	13.87 ± 0.21	52.35 ± 26.27	6.32 ± 0.03	0.65 ± 0.05	1.09 ± 0.05	2.83 ± 0.16	103.60 ± 12.60	5.24 ± 0.75
A30 + BC	12.94 ± 1.29	55.36 ± 11.60	6.20 ± 0.10	0.64 ± 0.01	1.19 ± 0.06	3.04 ± 0.11	110.60 ± 6.10	5.20 ± 0.30
A30 + FA + BC	12.10 ± 1.88	54.26 ± 22.11	6.42 ± 0.11	0.63 ± 0.03	1.16 ± 0.11	2.65 ± 0.23	112.47 ± 5.83	4.67 ± 0.43

MC: content of moisture; WHC: water holding capacity; EC: electrical conductivity; OC: organic carbon; Avl: Available; P: phosphorous; N: nitrogen; DHA: dehydrogenase activity; TPF: triphenyl formazan.

4. Discussion

4.1. Shelf Life of Biofertilizer

A good shelf-life of any inoculants depends on the carrier materials used that could ensure the survivability of inoculum before its application into the soil. Carrier materials provide defensive habitats which encourage inoculum success and also provide protection from predation [14,15]. Commonly used carrier materials are powders, granules, and liquids. Peat moss is commonly used as a microbial carrier, whereas many other carrier materials have been studied, like vermiculite, lignite, and sodium alginate encapsulation, an alternative to peat [42,43]. While this information has existed for years, very little work has been continued to examine the suitability of waste generated economically available BC and FA as carriers for microorganisms. Hence, there was a need to make biofertilizers for improved shelf-life, easy and controlled distribution of the tested microorganisms. In this study, BC, FA, and a mixture of both were used as a carrier to support the PGP bacterial species.

The shelf life of L2 inoculated in BC-based carriers showed better CFU up to nine months compared to FA alone based carrier. Despite a lower CFU after 9 months, the FA-based carrier held a significant number of CFU. The continued existence of high PGP bacterial density in BC and BC + FA for a long period of nine months seems to be a novelty, as we found out in our results. The beneficial effects of charcoal on the growth of rhizobacteria have also been observed by Rasool et al. [44]. Biochar is a recalcitrant carbonaceous material that can be incorporated into soils, thereby acting as a stable carbon sink and climate change mitigation strategy [45].

All tested solid carriers portrayed great variance in their physical and chemical properties, which affect their abilities to serve as carriers for introducing bacteria into soils. High WHC describes the characteristic of good carrier materials. BC pores may serve as an ideal micro-environment for biological activity and acts as a water retention agent in the biofertilizer, which may help it perform well [46,47]. BC and FA + BC both have high porosity and may influence a better niche for a higher population count of inoculated strains. FA is also a suitable source of plant nutrients (micro and macro-nutrients), and thus, its application as a carrier could be an efficient way for its management in a purposeful manner and simultaneously will reduce its disposal problem and cost of formulation material [34]. Thus, the mixture of BC + FA together created a synergistic effect by balancing the micro and macro environment for the survival of inoculant and plant growth. Metal concentration in both the carrier materials for most of the analyzed metals was below the permissible limit, further enhancing its applicability. Gaind and Gaur [48] observed that FA or soil: FA (1:1) combination is most suitable as a carrier for diazotrophs. A positive result has also been observed in the present study for FA-based treatments, which will give a new area of research for its plausible utilization in the field of waste management and preparation of efficient biofertilizers when used in limited quantity.

4.2. Plant Development and Fruit Yield

We hypothesized that waste generated economically available carrier material-based biofertilizer would improve the long duration survival ability of two test inoculants in the carrier as well as enhance plant growth and development after its application in soil. The results satisfied our hypothesis and indicated that BC + FA was the best material for the carrier when mixed with L2 and gave the best results for plant biometric growth and yield parameters significantly. In general, all the L2-based treatments showed better results than A30. At harvest, all plant growth parameters, root and shoot length, dry fresh, and biomass were maximum in FA + BC ($p < 0.05$) inoculated with L2. This may be due to better phosphate solubilization and IAA production of L2 than the A30 strain. The study has also indicated that FA can be more beneficial when combined with organic additives [21]. Masto et al. [47] also reported that mixing lignite FA with biochar enhanced plant growth compared to BC or lignite FA alone as an amendment. The result elucidated that

plants achieved a maximum percentage of increase in shoot length at 60 DAT, whereas maximum root measurement was achieved at 90 days of transplantation. This indicates that nitrogen phosphorous and potassium were essential and required by the plants for the flowering and seed setting stage. Moreover, uptake of nutrients depends on the ability of plant roots to absorb essential components from soil [48,49]. The results received in our research are in affirmation with all these observations.

The present study also revealed, the addition of biofertilizer ameliorated the fertility of soil along with the texture of post-plantation soil as compared to control. Both FA and BC reduce bulk density, improve WHC and soil aeration, and provide macronutrients [21,34]. Change in the pH to circumneutral was found to be favorable for plant growth. Brady and Weil [50] found a pH range of 6.5–7.5 optimal for plant nutrient availability. The increase in EC, K, P, and Ca could be due to the presence of FA in soil, which enhances microbial activity and organic matter decomposition. Moreover, FA may increase available micronutrients (Zn, Cu, Fe, and Mn), which helps plants in their growth [21]. A decline in nutrient content such as available P, available N, and OC towards the maturity of the crop in control pots were observed, whereas an increase in OC was found in the biofertilizer-treated soil. Phosphorous being a vital primary nutrient provides helps for root development and crop growth and thereby enhances final yield [47,51]. Post-harvest soil analysis showed higher available P after the application of biofertilizer compared to control. The treatments receiving biofertilizers indicated further enhancement in phosphorous and nitrogen availability because of their potential to solubilize inorganic phosphorus. Liu et al. [52] also suggested that available P in post-harvest soil is directly related to the rate of P fertilizer. Chinnusamy et al. [53] found the addition of biofertilizers considerably improved the fertility status of peat by increasing the P and N content. In addition, the enzymatic activity of dehydrogenase also plays a key role in soil amelioration [34]. Soil inoculation with our PGPR strains had significantly enhanced the activities of dehydrogenase enzyme of post plantation soil compared to that of post-harvest control soil, may be due to an increase in the rhizosphere microbial population as a consequence of the inoculation treatments [54].

5. Conclusions

Both flyash (a freely available industrial waste) and biochar (from agricultural waste) have a great impact on the prolongation of shelf-life of *Burkholderia* sp. strain L2 up to 9 months. Prepared biofertilizer of biochar + flyash inoculated with L2 strain has effectively increased the rate of seed germination, efficiently promoted eggplant growth (leaves, root, shoot, leaves) and fruit yield, and ameliorated the soil physical (water holding capacity, moisture content) and chemical properties (pH, electrical conductivity, and available nitrogen, phosphorous and potassium) along with dehydrogenase activity compared to single carrier based biofertilizer and control. The availability of a minute proportion of essential plant-available metals in biochar and flyash (much below the toxicity limit) further helped plants in their growth and development. Overall, both biochar and flyash have significant beneficial properties and can be used as carrier materials to prepare biofertilizers. Thus, utilization of a small proportion of flyash and biochar together with *Burkholderia* sp. strain L2 could act as a sustainable substitute over chemical fertilizers for enhanced growth and yield of brinjal and can help in sustainable waste management, thus also solving the disposal problem of both flyash and agricultural waste.

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