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Original Article

Valorization of Agrowaste into Liquid Biostimulant for Sustainable Agriculture

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Abstract

Chemicals have accumulated in crops and soil as a result of widespread usage of chemical fertilizers, which has impacted the food chain. The purpose of this study was to examine the combined effects of biofertilizers containing microbial consortia of *Azotobacter sp.*, *Bacillus sp.,* and *Trichoderma sp.* on the vegetative growth of wheat plants. The bioreactor built in our lab, fruit waste, such as melon, banana, and papaya peels, potato peels served as the only source of nutrients for the growth of microbial consortia as well as prolong treatment of consortia on agrowaste was studied in case of liquid biofertilizer. The results demonstrated that the microbial inoculants produced using the developed bioreactor can be effectively used as biostimulant for the tested wheat plant. The macro- and micronutrient analysis of the batch 1 biostimulant revealed significant increase ($P \leq 0.05$) in available nitrogen at 600 ± 10.44 mg/kg and potassium at and 1889 ±13.45 mg/kg, respectively, as compared to control. By the Pot culture bioassay and the experimental plants showed better growth than those of the baseline plants. The biostimulant batch 1 showed efficient plant growth-promoting potential, and the average root, shoot, and total heights of the plants were significantly greater ($P \leq 0.05$) than those of the control group. The average root and shoot lengths were 16.6 ± 1.14 cm and 19.0 ± 1.3 cm, respectively. The average height was found to be 36 ± 0.71 cm. Plant height, root length, and shoot length changes were substantially different from control plants by Tukey's Honestly Significant Difference at the 5% significant level. The resultant growth stimulation can be linked to a valuable increase in the N and K content of the treated waste.

Categories: Organic agriculture, Aquaculture for sustainable food production, Use of biostimulants, biofertilizers, bioinoculants in sustainable agriculture

Keywords: biostimulent, agro-waste, bioreactor, biofertilizer, micronutrients

Introduction

A decrease in agricultural income is linked to a decrease in soil fertility as well as unfavorable environmental conditions such as rainfall, drought, floods, and storms. Due to the high cost of inorganic fertilizers, the agriculture sector in the developing countries is under stress and facing new challenges. Chemical fertilizers affect the properties of air, soil, and water as well as the quality and yield of crops [1]. The awareness and popularity of organic fertilizers and biofertilizers is continuously increasing among farmers over chemical and inorganic fertilizers [2]. Biostimulant are substances or products that, when applied to plants or soil, stimulate plant growth, whereas biofertilizers are substances that contain

live microorganisms to enhance the quality of the crop and fertility of soil as well as maintain the micro flora of the soil [3]. The proper production and quality of biofertilizer largely depend on the material that is used to produce the biofertilizer. The encouragement of plant development involves the use of several microbial species. Through ecological interactions, plant growth-promoting microbes including generally includes *Rhizobium leguminosarum*, *Azotobacter chroococcum*, *Bacillus megaterium*, and *Trichoderma viride* improve nutrient uptake and maintain the environment's nutrient cycle. Biofertilizer made from agricultural waste may decrease the harmful effect of soil salinity, which directly effects plant growth and plant development. However, it is crucial to maintain the quality of the materials used to produce biofertilizer [4].

Azotobacter vinelandii belongs to the plant growth-promoting rhizobacteria having free-living nitrogen fixing capacity and also possesses the ability to produce phytohormone, such as cytokinin and indole acetic acid (IAA), with the production of exopolysaccharides [5]. Another organism, *B. megaterium* shows phosphate-solubilizing activity as well as cellulolytic enzyme production [6]. The cellulosic content in agrowaste is beneficially broken down by cellulolytic enzymes such as cellulases, hemicellulases, and pectinases [7]. The fungal species *Trichoderma harzianum* possesses phosphate solubilizing activity, which is an important factor in biofertilizer production. The use of microbial consortia for the production of biofertilizer using agriculture and fruit waste with prolong treatment is a relatively less explored idea. Secondly, India is an agriculture-based economy, and a vast agricultural biomass is generated annually that can be used sustainably for biofertilizer production [8].

In the present study, biofertilizer production was studied using consortia of microorganisms such as *Azotobacter* sp., *Bacillus* sp., and *Trichoderma* sp. The study reveals that the prolong incubation may works for degradation of biomass and increases availability of nutrients. Biofertilizer production was carried out using various agro residues of local fruit crops of the region, such as the peels of watermelon, banana, papaya, and potato. Biofertilizer production using the microbial consortia was also undertaken using a basic bioreactor assembly. The macroelements and microelements of the produced biofertilizer were analyzed. The plant growth promotion ability of the isolated microorganisms was checked using an experimental pot bioassay and results were evaluated by the one-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) at 5% significant level.

Materials And Methods

Isolation and preliminary identification of bacteria and fungi to produce biofertilizer

For isolation, rhizospheric soil (50 g) was collected from cotton field. Serially diluted with sterile distilled water up to 10^{-9} dilutions. From that 0.1 ml suspension were spread aseptically onto agar surface of the nutrient agar media plates for bacteria and the plates were incubated at 37°C for 24 hours. After incubation, bacterial colonies were studied for morphology, colony characteristics, Gram character, and different biochemical tests.

For the isolation of fungi, diluted samples (0.1 ml) were spread on sterile potato dextrose agar (PDA) plates incubated at room temperature for 3 to 4 days. Morphological identification of fungal isolates was carried out by slide culture techniques. For the isolates, sterile PDA medium agar blocks were prepared and placed on sterile slide culture assembly. The agar blocks were inoculated with the respective fungal cultures from preliminary PDA plates coverslip was placed on agar block. This moisture containing assembly were incubated at 28^{O}C for 3 to 5 days [9]. After incubation coverslip was removed and staining were done by the Lactophenol cotton blue and observed under microscope [10]. Hyphae, conidiophores, phialides, and conidia of the isolates were studied and compared with literature study [11,12]

Qualitative screening of isolates for plant growth-promoting activities

Phosphate solubilizing activity

The bacterial and fungal isolates were screened for phosphate solubilization activity using Pikovskaya's agar medium. The bacterial and fungal isolates were inoculated on sterile Pikovaskaya's agar media plates for the phosphate solubilization activity [13]; phosphate solubilizing potential were checked [14].

N2 fixation assay

For the qualitative determination of nitrogen fixation potential, isolates were inoculated in nitrogen-free sterile *Azotobacter*-selective media with 0.05% (w/v) bromothymol blue indicator. Changes in the color of the media indicated the nitrogen fixation activity of the isolates [15].

Cellulase assay

Screening of isolated microorganisms for cellulase activity was carried out by the Congo red assay. The isolates were spot inoculated on sterile carboxy methyl cellulose (CMC) agar media. After incubation, a zone of hydrolysis was observed when the plates were flooded with 1% Congo red solution for 15 minutes and washed thoroughly with 1 M NaCl. The isolates showing the zone of clearance around the colony were considered cellulase producers [16].

Biocontrol activity

The antagonistic activity of the isolates against the common plant pathogen *Aspergillus niger* (MCC 1374) was studied. The spore suspension of the test fungi was adjusted to 10^6 spores/ml and used for the experiment. For the test assay, an aliquot of a suspension of isolate KCP7 and test pathogens with similar spore counts were inoculated on PDA plates and incubated at 25°C for 4 to 5 days with an adequate control [17]. The test isolates inhibiting the growth of the pathogen, showing positive antagonistic activity, were selected [18].

Preparation of consortia of different organisms

Based on qualitative screening, the test bacteria and fungi that possessed plant growth-promoting activities were selected for the preparation of consortia. Pure cultures of selected isolates (KCP7 and KCP4) were inoculated in 100 ml of Pikovaskys broth and incubated for 5 days on a rotary shaker at 120 rpm at room temperature. This consortium of two isolates was prepared as per the published reports [19].

Simultaneously, the isolate KCP1 was inoculated in 100 ml of nitrogen-free *Azotobacter* media and incubated at room temperature on a rotary shaker at 120 rpm for 4 to 5 days. To produce liquid biofertilizer, the consortium of the KCP7 and KCP4 isolates was simultaneously used with a pure culture of the isolate KCP1 [20].

Construction/design of the basic bioreactor

For the fermentation of agrowaste for the production of biofertilizers, a basic bioreactor was constructed [21]. To construct the basic bioreactor, 2500 ml of the waste plastic jar was used with dimensions of 12 × 30 cm in diameter and height. The minimum electricity-consuming motor with the capacity to move 60 RPM continually and work at 350 mA current was fixed on top of the lid. Aluminum agitators were fixed below the lid. The motor and agitator were coupled with the lid of the jar to move it properly for mixing. To withdraw the biofertilizer sample for analysis, a basic bioreactor was equipped with a sampling port to avoid contamination. The bioreactor is also equipped with an aeration port. Regulation of factors for the basic bioreactor, such as temperature, pH, feeding of nutrients, and level of liquid, was performed manually. The proper fermentation activity was carried out in the basic bioreactor with a minimum cost of electricity [22].

Production of biofertilizer using microbial consortia

Fruit waste of Banana, Water melon, Papaya and potato waste was taken in equal quantity. Clean manually and grounded it. The biomass then mixed with water with 1:1 ratio to form semisolid suspension and pH at 6.5-7.5 was adjusted. To remove the microbial load, Slurry was heat up to 70-80 °C for 30 minutes. Cool the slurry up to room temperature. Five percent (100 ml) of the consortia of two isolates (KCP4 and KCP7) and Five percent (100 ml) of the pure culture of isolate KCP1 were utilized to inoculate the agro-waste in the basic bioreactor [19]. This experiment was performed in triplicate. The incubation was carried out at room temperature times were 20, 40 and 60 days because of fungal strain used in consortia for first, second and third bioreactors, respectively. Agitation was carried out at 60 RPM [23]. for the stabilization of species two percent of glycerol was added. Which works for maximize CFU retention with shelf life [24]. The batch time was selected considering the presence of complex polysaccharides in the selected waste material. After the completion

of the incubation time of each batch, the biofertilizer was harvested, filtered by sieving and used for further analysis. Extensive incubation required for lignocellulosic degradation [23]. The fermentation time used according to the lignocellulosic waste degradation [25, 26].

Determination of macro- and micronutrients

After harvesting, the biofertilizer generated was compared to a control sample of untreated waste. Numerous factors, including pH, N, P, K, Cu, Fe, Zn, Mn, Ca, Mg, and Na, were analyzed [27]. pH was analyzed by a digital pH meter; total nitrogen was estimated as per the Kjeldahl method [28]. The determination of phosphorus was carried out by the quinoline phosphomolybdate gravimetric method [29]. The potassium was analyzed by a flame photometer (atomic emission spectroscopy). Other parameters, such as Cu, Fe, Zn, Mn, Ca, Mg, and Na, were analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP–OES) [30].

Plant growth promotion study

Healthy seeds of wheat were selected for the bioassay. Seeds were surface sterilized using 1% sodium hypochlorite solution for three to five minutes and the seeds were rinsed with distilled water. The plastic pot of 20 cm diameter was filled with two kilograms of sterile soil. The biofertilizer inoculation was done through seed treatment method by coating the biofertilizer (approximately 10^8 CFU/ml) using 1% CMC as an adhesive agent followed by drying room temperature. In test group, seeds were treated with a biofertilizer batch I and sown at a depth of 2-3 cm of depth. the experiment was performed in duplicate and the pots were labelled as 1A and 1B. The same procedure was followed for bioassay using batch II (pots 2A and 2B) and batch III (pots 3A and 3B) biofertilizers. Control pots (not treated with biofertilizer) were inoculated and monitored. Pots were maintained under the natural environmental condition and irrigated regularly. The soil was treated with a mixture of 10 ml of each biofertilizer and 10 ml of water at five days of interval. Plants were harvested after three weeks and various parameters such as shoot length and root length were measured from the base to the tip of the plant using a meter scale [31].

Statistical analysis

The micro and macronutrient data were evaluated by analysis of variance (one-way ANOVA). The results of the pot assay were also analyzed by ANOVA [32] and Tukey's HSD at the 5% significant level in order to determine the significance of the measured parameters by using R software [33].

Results And Discussion

Isolation of organisms and qualitative screening for biological activity

A total of eight strains were isolated and designated with codes from KCP1 to KCP8, with KCP1 to KCP6 representing bacterial isolates, whereas KCP7 and KCP8 representing fungal isolates. The isolates were screened for plant growth-promoting activity. Isolates KCP1, KCP2, and KCP3 formed colonies on nitrogen-free *Azotobacter*-selective media. These isolates showed large, flat, irregular, watery, and mucilaginous transparent colonies having 2-3 mm in diameter. The microscopic characteristics revealed cocci-shaped, Gram-negative bacteria, and negative staining showed capsular characteristics (Figure *1A*). Based on the morphological colony and biochemical characteristics, the isolates KCP1, KCP2, and KCP3 were identified as *Azotobacter* sp. (Table *1*) [15].

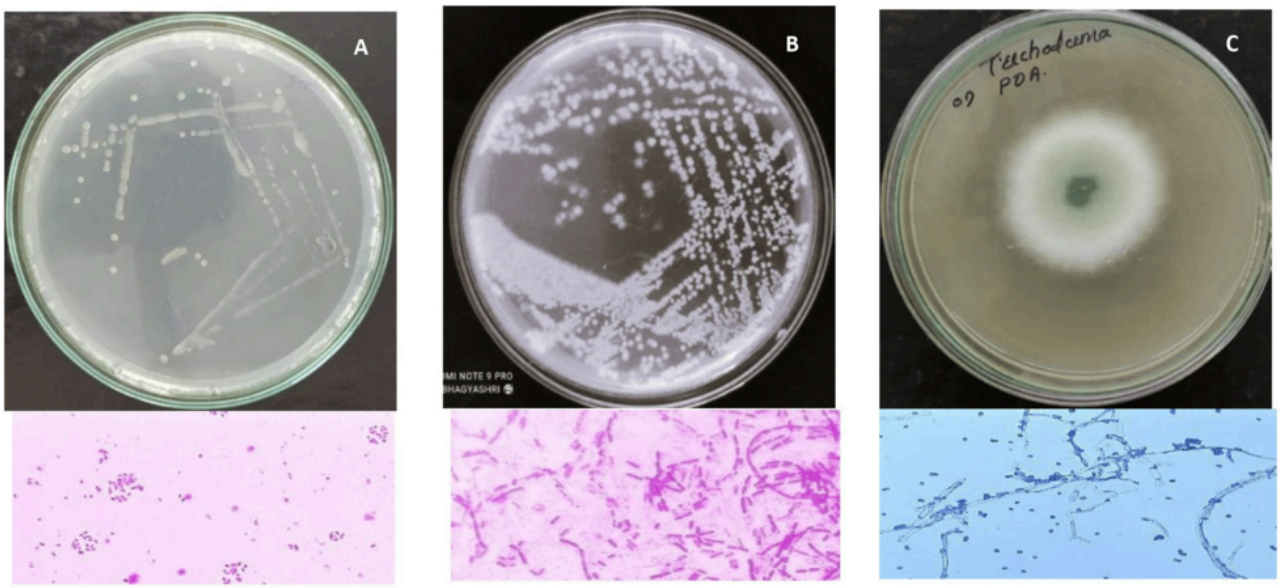

FIGURE 1: Morphology and microscopic characters of the bacterial and fungal isolates (A) Azotobacter sp., (B) Bacillus sp., and (C) Trichoderma sp.

Sr. No	Biochemical characteristics	KCP 1	KCP 2	KCP 3	KCP 4	KCP 5	KCP 6
1	Gram staining	Gram-negative	Gram-negative	Gram-negative	Gram-positive	Gram-positive	Gram-positive
2	Glucose	+	+	+	+	+	+
4	Mannitol	+	+	+	+	+	+
5	Maltose	+	+	+	+	+	+
6	Indole production	–	–	–	–	–	–
7	Methyl red test	+	+	+	+	+	+
8	Voges-Proskauer test	–	–	–	–	–	–
9	Citrate utilization test	+	+	+	+	+	+
10	Starch hydrolysis	+	+	+	+	+	+
11	Urease test	+	+	+	+	+	+
12	Catalase test	+	+	+	+	+	+
13	Phosphate utilization	–	–	–	+	+	+
14	Triple sugar iron test	–	–	–	+	+	+

TABLE 1: Biochemical characteristics of selected bacterial isolates under the study.

+, Positive; –, Negative

The morphological, biochemical, and colony characteristics of the KCP4, KCP5, and KCP6 showed white, large (up to 2-3 mm) colonies with irregular, flat, opaque characteristics on Mannitol Yolk Polymyxin (MYP) agar. It appeared as Gram-positive, rod-shaped bacteria (Figure *1B*). Based on biochemical test the isolates KCP4, KCP5, and KCP6 were identified as

Bacillus sp. (Table *1*) [34].

The fungal isolate KCP7 initially showed a white, fluffy appearance for 1 to 2 days on potato dextrose agar. It required 3 to 4 days for complete growth at 25-30°C. The color of the upper surface is watery white to bluish green, which turns green in the middle after 4 days of incubation [17]. The surface of mycelium was white to green in color and floccose in nature with compact and smooth conidiophores all over on media. Conidia were hyaline, subglobus with smooth walls. The microscopic appearance of the species indicated a septate hyaline hypha, conidiophores, phialides, and conidia as well as spores. Based on these findings, the traits of KCP7 resembled those of *Trichoderma* sp. Hence, it was identified as a member of the genus *Trichoderma* (Figure *1C*) [35].

The isolate KCP8 showed luxurious growth on Czapek Dox agar. The isolate showed characteristics of filamentous fungi, such as filamentous hyphae, irregular conidiation, a dense arrangement of conidiophores with spherical conidia, and black coloration. Based on these characteristics, the isolate KCP8 resembled *Aspergillus* sp. and therefore it was identified as a member of the genus *Aspergillus* [36].

In the qualitative screening of all isolates for plant growth-promoting activities, it was observed that the bacterial isolates KCP1, KCP2, and KCP3 could effectively grow on nitrogen-free *Azotobacter* media, indicating their nitrogen-fixing activity. The isolates KCP4, KCP5, and KCP6 produced a clear zone around the colony when grown on Pikovaskys agar media and showed phosphate solubilization activity [37]. KCP4 produced a zone of hydrolysis around the colony on carboxy methyl cellulose (CMC) agar media when the plate was flooded with 1% Congo red and NaCl (Figure *2C*). These observations confirmed that the bacterial isolates have the ability to produce cellulase extracellularly (Table *2*).

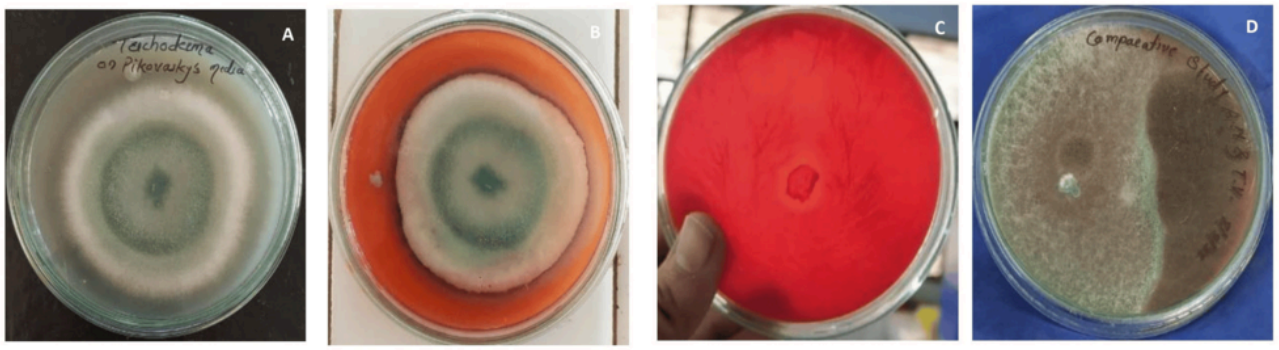

FIGURE 2: Biological and plant growth promoting potential of the isolates; phosphate solubilization activity of KCP7 (A), cellulolytic activity of KCP7 and KCP4 (B and C, respectively), biocontrol potential of isolate KCP7 (D).

Sr.No.	Isolates	N2 fixation	Phosphate solubilization	Cellulolytic activity	Biocontrol potential
1	KCP1	+	–	–	–
2	KCP2	+	–	–	–
3	KCP3	+	–	–	–
4	KCP4	–	+	+	–
5	KCP5	–	+	+	–
6	KCP6	–	+	+	–
7	KCP7	–	+	+	+
8	KCP8	–	–	+	–

TABLE 2: Screening of biological activity of isolates.

+, Positive; –, Negative; KCP1 to KCP6, bacterial isolates; KCP7 and KCP8, fungal isolates

The fungal isolate KCP7 showed a zone of clearance around the colony when grow on Pikovskaya's agar media (Figure *2A*) and also show zone of hydrolysis around the colony when grow on CMC agar media, indicating its cellulase producing capacity (Figure *2B*). Isolate KCP7 also shows biocontrol activity where it inhibited the growth of *A. niger* "MCC 1374," which is a well-known phytopathogen (Figure *2D* and Table *2*).

Preparation of consortia of different organisms and bioreactor studies

The isolates KCP4 and KCP7 could effectively grow on Pikovskaya's medium, indicating their phosphate solubilization ability. Phosphate-solubilizing bacterial isolate KCP4 and fungal isolate KCP7 could effectively grow on Pikovskaya's liquid media, resulting in the formation of productive consortia. Simultaneously, the free-living nitrogen fixer isolate KCP1 could luxuriously grow on *Azotobacter* media. The developed microbial consortia in liquid media were ready to be applied to agrowaste with effective consortia.

Constructed bioreactor vessels aimed to avoid contamination and to maintain aseptic conditions throughout the bioconversion cycle. The constructed bioreactors were modified to a fermentation vessel that favors some chemical as well as biological reactions under controlled conditions. A total of 5% of the consortia of KCP4 and KCP7 and 5% of the culture of KCP1 were used to inoculate the agrowaste. After inoculation, the microbial consortia were allowed to multiply under preoptimized conditions. Based on incubation time, three biofertilizer samples were assigned batch numbers one, two and three indicating incubation times of 20, 40, and 60 days, respectively. The assay demonstrated varying results for all three biofertilizer batches based on incubation time [23,38].

Analysis of various parameters

To evaluate the plant growth promotion efficiency of the biofertilizer, the samples from all three batches were evaluated for the presence of various macro- and microelements and compared with the control sample (Table *3*). The pH of the biofertilizer ranged from 3 to 4.5, which is nearly acidic, similar to the control sample. The availability of macroelements

such as nitrogen, and potassium increased significantly in Batch 1 biofertilizer as compared to the control sample ($P \leq 0.05$) [39,40]. Batch 2 and Batch 3 showed relatively lower levels of parameters. Batch 1 biofertilizer also showed increased level of microelement, especially P, Cu, Fe, Zn, Mn, Ca, and Mg than the control sample ($P < 0.05$). The biofertilizer from Batch 2 and Batch 3 showed more variation in the level of microelements (Table *3*) [41].

Sr. No.	Parameters	Control	Batch 1	Batch 2	Batch 3	ANOVA *P*-value
1.	pH	4.15 ± 0.31	3.91 ± 0.34	4.26 ± 0.36	4 ± 0.1	>0.05
2.	Available Nitrogen, as N (mg/kg)	400 ± 86.97	600 ± 10.44	421 ± 22.27	405 ± 16.52	<0.05
3.	Available Phosphorus as P (mg/kg)	213 ± 15.13	250 ± 36.05	202 ± 7.21	204 ± 18.33	<0.05
4.	Available Potassium as K (mg/kg)	1,398 ± 30.64	1,889 ± 13.45	1,470 ± 50.23	1390 ± 19.07	<0.05
5.	Copper, as Cu (mg/kg)	1.03 ± 0.15	0.93 ± 0.2	0.67 ± 0.15	0.63 ± 0.04	<0.05
6.	Iron, as Fe (mg/kg)	49.22 ± 2.14	58.58 ± 1.2	58.05 ± 2.5	42.41 ± 2.7	<0.05
7.	Zinc, as Zn (mg/kg)	4.76 ± 0.8	5.1 ± 0.7	6.21 ± 0.81	6.43 ± 0.63	<0.05
8.	Manganese as Mn (mg/kg)	1.83 ± 0.89	3.16 ± 0.33	2.14 ± 0.76	2.09 ± 0.34	<0.05
9.	Calcium, as Ca (mg/kg)	215 ± 14.79	260 ± 9.0	280 ± 27.78	237 ± 12	<0.05
10.	Magnesium, as Mg (mg/kg)	179 ± 8.66	186 ± 8.54	218 ± 18.33	210 ± 6.92	<0.05
11.	Sodium as Na (mg/kg)	128 ± 18.33	145 ± 18.68	120 ± 7.21	132 ± 10.58	>0.05

TABLE 3: Analysis of different parameters of the produced biofertilizer.

Micronutrients data represented as the mean ± standard deviation of three sets. represent significant difference ($P \leq 0.05$) in most of micronutrients in comparison with control (untreated).

In this study, the effect of biofertilizer on the growth of the plant was noted and compared with the control. After 3 weeks, the total height of the plant, root elongation, shoot elongation were checked, and the results are summarized in Table *4*. The data represent the average value of root length, shoot length, and total height of the five plantlets in the pot culture experiment in each group. The one-way ANOVA revealed that significant variation among treatments for all parameters ($P < 0.05$). Nitrogen, phosphorous, and potassium content increases significantly compared to control. Cu, Fe, Zn, Mn, Ca, and Mg also increases compared to control [42].

The Experimental pot (Expt. Pot) bioassay experiments demonstrated that Expt. Pot 1A of biofertilizer had a positive impact on plant growth when compared with the control pot (Table *4*).

Biostimulant batch	Root length (cm)	Shoot length (cm)	Total height (cm)
Control	6.8 ± 0.84	12.8 ± 0.84	19.6 ± 1.34
Expt. pot 1A	16.6 ± 1.14	19 ± 1.3	36 ± 0.71
Expt. pot 1B	14 ± 1.22	17.8 ± 0.84	31.8 ± 1.79
Expt. pot 2A	12.4 ± 1.14	15.6 ± 0.89	28 ± 2.0
Expt. pot 2B	14.2 ± 1.3	15.6 ± 0.89	29.8 ± 2.17
Expt. pot 3A	12.4 ± 1.14	14.6 ± 0.55	27 ± 1.41
Expt. pot 3B	13.4 ± 0.89	16.6 ± 1.14	30 ± 1.0
P-value as per ANOVA table	4.74E-12	6.55E-10	7.7E-14

TABLE 4: Average root, shoot length, and total height of the plantlets in the pot assay experiment.

Data represented are the mean ± standard deviation of five independent variables treated with biofertilizer. represent significant difference ($P \leq 0.05$) in root length, shoot length, and total height, respectively, in plantlets treated with Expt. pot 1A to Expt. pot 3B in comparison with the control (untreated) by Tukey's test.

The results of ANOVA revealed significant differences in root length, shoot length, and total height of the plant among the treatment ($P \leq 0.05$). Tukey's HSD test indicates that there is a significant difference observed by comparing all possible pairs (Figure *3*). Liquid biofertilizer Batch 1A treated plants had significant higher root length, shoot length, and total height compared to control and other Expt. pots.

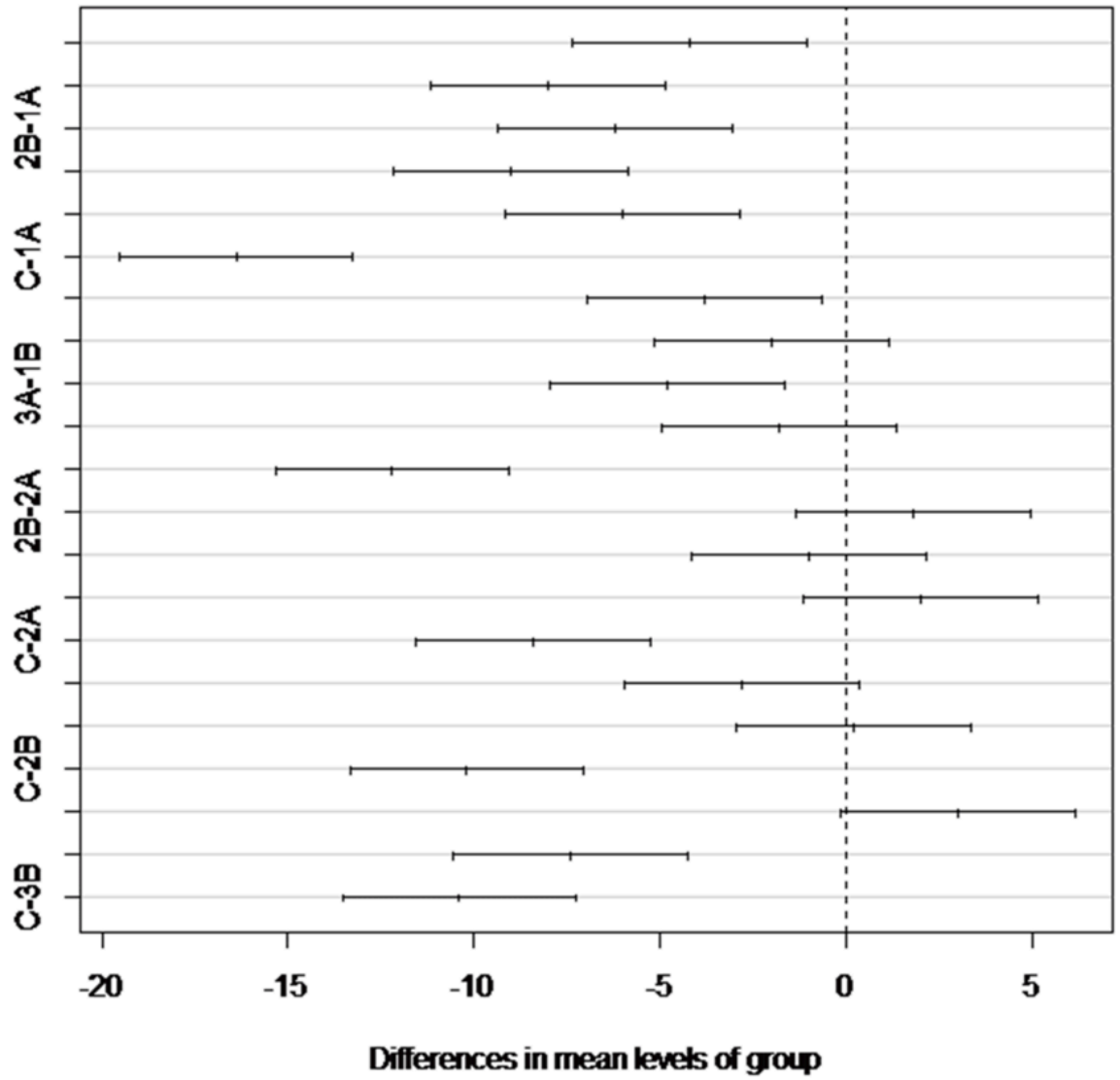

FIGURE 3: Tukey's test followed by ANOVA on total height of the plant showing 95% family-wise confidence level.

The figure describes the 95% family-wise confidence intervals of the difference in means between total height of different Expt. Pots-plantlets from 1A to 3B and control (pair-wise comparison by Tukey's test followed by ANOVA) is evident that all group means are significantly different at the 5% level of significance. Post hoc analysis using Tukey's HSD showed that Group 1A had significantly higher mean values than all other groups. Groups 1B, 2B, and 3B formed a homogeneous subset with no significant differences among them. Groups 2A and 3A were statistically similar but significantly lower than the 1B group. Group C had the lowest mean and differed significantly from all other groups (Figure *3*).

Those highest value observed in Expt. pot 1A promoted maximum root and shoot length and plant height (16.6 ± 1.1, 19.0 ± 1.3 and 36.0 ± 0.71), respectively, compared to Expt. pot 2A, 2B and 3A, 3B. The parameters of the treated plants were significantly greater than that of the control plants. Therefore, it can be concluded that the application of biofertilizer had a positive influence on all the three parameters compared to the control plants (Table *4* and Figure *3*) [31,43].

India is one of the largest producers of agricultural waste. There is an opportunity to use these agro residues to produce liquid biofertilizers or biofertilizer due to their plentiful composition in plant and soil nutrients. Moreover, recycling agricultural waste products and demonstrating their reuse can play an important role in a sustainable environment. The use of waste materials for the production of liquid fertilizers seems to be an alternative to chemical fertilizers, as the process is less energy incentive and does not require other mineral resources.

The organisms isolated in this study efficiently transformed the tested agrowastes into biofertilizer that promoted the growth of the tested plants. The bacterial isolates KCP1, KCP2, and KCP3 have the potential for nitrogen fixation. The morphological and biochemical characteristics confirmed the genus-level identification of *Azotobacter*, which is known for nitrogen fixation [15]. The other isolates, KCP4, KCP5, and KCP6, possessed phosphate solubilization activity as well as cellulose degradation activity so the species may belong to *Bacillus* [34]. The fungal isolate KCP7 was found to be a member of the genus *Trichoderma* that has been explored for phosphate solubilization and biocontrol activity against phytopathogenic fungi [18]. The biofertilizer requires an aerobic environment with continuous shaking of media aseptically for the rapid growth of the microorganisms. The basic bioreactor designed in the study supports the growth and multiplication of the microbial consortium. The aerator of the bioreactor allowed continuous oxygenation for microorganisms, and an agitator with a motor of 60 rpm mixed the biomass and heat evenly. A sampling port is provided to remove the microbial culture or biofertilizer aseptically for analysis. Therefore, the designed bioreactor is cost-effective and may be beneficial for small and marginal farmers [21].

Analysis of biofertilizer from a basic bioreactor showed decrease in pH in first 20 days of Batch 1 ($P > 0.05$), which is due to acidification by acetic acid, lactic acid, and humic acid produced in early microbial metabolism; again, it stabilizes in Batches 2 and 3, which indicates maturation of the fermentation process. Similarly, there is an increase in the levels of nitrogen, phosphorus, and potassium compared with those of the control sample. Likewise, it showed an increase in some micronutrients, such as Ca, Mg, Fe, Cu, Zn, and Mn ($P < 0.05$). The primary increase in N, P, K content may be due to active mineralization of microbes in first 20 days but prolonged incubation of 40 to 60 days resulted in decrease in nutrients because of microbial immobilization, volatilization, or denitrification leading to decline in nutrients. Long-term incubation results in nutrient loss or volatilization in liquid biofertilizer; nevertheless, fungal culture used in consortia may produce secondary metabolites and enzymes that effectively degrade the lignocellulosic agrowaste in the first 20 days.

The experimental pot bioassay demonstrated that the biofertilizer produced in Batch 1 enhanced the germination index, total height, root length, and shoot length of the plant compared to the control. Based on these findings, it can be concluded that the liquid biofertilizer from Batch 1 obtained through the basic bioreactor with an incubation time of 20 days gives significant results. Moreover, the application of Batch 1 liquid biofertilizer also shows collective effectiveness on the vegetative growth of wheat plants. There was a significant increase in root length, shoot length, and total height of the plant in agreement with a previous report [31]. Statistical analysis of the data confirmed that the application of biofertilizer had a positive influence on plant root length, shoot length, and total height. Significant differences between the treatments were observed for plant growth parameters, with Batch 1A performing best as confirmed by Tukey's HSD test at $P \leq 0.05$ followed by ANOVA [43].

Conclusions

The present study demonstrates the successful valorization of locally available fruit and agricultural wastes, including banana, papaya, watermelon, and potato residues, into a nutrient-rich liquid biofertilizer through the action of a microbial consortium comprising *Azotobacter* sp., *Bacillus* sp., and *Trichoderma* sp. The designed microbial consortia exhibited plant growth-promoting activities, such as nitrogen fixation, phosphate solubilization, cellulolytic activity, and biocontrol potential, enabling efficient biodegradation and nutrient transformation of agrowaste substrates. The low-cost bioreactor developed in this study effectively supported microbial growth and fermentation, providing a practical and economical approach for biofertilizer production. Among the three fermentation periods evaluated, the 20-day fermentation batch (Batch 1) proved to be the most effective, showing significantly higher concentrations of essential plant nutrients, particularly nitrogen, phosphorus, and potassium along with improved levels of several micronutrients.

The enhanced nutrient profile resulted into enhanced plant growth-promoting effects in test group wheat with greater root length, shoot length, and total plant height as compared with the untreated control. Statistical analyses confirmed that these improvements were significant, indicating that the observed growth stimulation was strongly associated with increased nutrient availability generated during microbial bioconversion. Overall, the study established that bio-organic liquid biofertilizer can be produced from locally available substrates such as banana peels, papaya, watermelon, and potato wastes. Small and marginal farmers can benefit from using this technology. It will also reduce their dependence on the use of chemical fertilizer, which is expensive and not environmentally friendly. However, further studies are necessary for complete characterization of the bioprocess for the different groups of wastes and the possibilities for their transformation and reuse. However, future investigations on molecular characterization of the microbial consortium, shelf-life assessment, large-scale field validation across different crops and soil types, and optimization of fermentation parameters are necessary to facilitate commercial application of this technology.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Kalyani C. Patil

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Drafting of the manuscript: Kalyani C. Patil

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Disclosures

Plant subjects: All authors have confirmed that this study did not involve any herbarium specimen. **Human subjects:** All authors have confirmed that this study did not involve human participants or tissue. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Data Availability Statements

The datasets (and/or code) supporting this study are available from the corresponding author upon reasonable request.

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