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Employment of agro waste to develop biofertilizer and its effect on *Solanum melongena* var. *depressum* cv. Pragati (Chu Chu)

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Abstract
This study involves the production of biofertilizers from agro-waste under semi-solid-state fermentation. The peels from papaya, banana and carrot were used to produce cheap and eco-friendly biofertilizer to be potentially used in agricultural operations. The biofertilizer prepared from agro-waste was applied to the brinjal plants at 30 days post sowing to determine the efficiency of these biofertilizers. PH & potassium content were estimated for all the three biofertilizer samples. It was found that the carrot batch-2 biofertilizer had the highest pH value (5.89), The banana batch-2 biofertilizer had the highest content of potassium with a content of 402.8 mg/l, The concentration of growth promoting microorganisms were determined by plating over different selective media such as Sabouraud dextrose agar for *Aspergillus sp.*, Pikovskya agar for phosphate solubilizing bacteria, Man, Rogosa and Sharpe agar for *Lactobacillus sp.*, Mannitol yolk neomycin agar for *Bacillus sp*. Followed by colony counting. The performance of Papaya batch-2 biofertilizer was recorded best over the other biofertilizers under study. The maximum plant height (26.66 cm), fresh weight of plant (56.26 gm), no: of leaves (33.66), no: of flowers occurred per week (4.91), no: of fruits (2.33), fruit diameter (1.90 cm), fruit weight (36 gm) were recorded in the plants treated with Papaya batch-2 biofertilizer.

Keywords: agro wastes, semi-solid-state fermentation, biofertilizer

Introduction

All living organisms require nutrients for their growth, be it infants, plants, animals or microorganisms. The traditional knowledge shows that fields have been manured regularly to obtain better yields. Our knowledge of nutrient requirement for plant growth originated from famous "willow tree experiment" conducted by Johann Baptista van Helmont (1579–1644). He concluded that only water is necessary for willow tree growth, not soil. But now it is well established that soil is indispensable for plant growth as it is the source of major nutrients for plants.

Seventeen nutrients are required by the plants in different amounts for their good growth and development. These seventeen nutrients are classified in to three different categories based on their uptake by plants as; major, minor and micro nutrients. Potassium, phosphorous, and nitrogen are the major nutrients; calcium, magnesium, and sulphur are the minor nutrients; molybdenum, iron, boron, copper, manganese, chlorine, nickel, and zinc are the micro nutrients. In addition to these nutrients plants require carbon, oxygen, and hydrogen which they obtain from air and water. The plants take up nutrients as inorganic forms irrespective of the form in which they are present in the soil.

Conventional agriculture plays a vital role in meeting increased food demand of the ever growing population which led to the sole reliance on agrochemicals. Chemical fertilizers are industrially prepared synthetic compounds created specifically to increase crop yield. It causes air as well as groundwater pollution. Efforts have been made to develop agro-waste based biofertilizers to produce "nutrient-rich, high-quality food" in a sustainable manner ensuring biosafety.

The agro-waste from which the biofertilizer is obtained can be collected from the farms where the agricultural activities are on-going. In agriculture, the improvement of soil fertility depends upon the organic addition to the nutrient stockpile and this can be made feasible by utilizing biofertilizer. Thus, the demand for organic fertilizers is expected to increase to serve as an alternative to conventional agrochemicals

Manures, beddings, plant stalks, leaves, hulls and vegetable matter are all forms of agro-wastes. Agro-waste generally carries soluble and insoluble components. Soluble components include various sugars, organic acids, and amino acids, whereas insoluble components include lignin and cellulose and other components like fats, oils, proteins, pigments, minerals, and resins. The decomposing parts of plants act as key sources of organic matter within the soil. After harvesting the crop, the leftover (agro-waste) being useless is discarded by the farmers. As, these farm leftovers (agro-waste) are the economical source and thus can be utilised effectively to enhance fertility of soil.

'*Biofertilizer'* denotes the fertilizer that fulfils nutrient requirement of plant through microbiological means. The biofertilizers are thus microbial preparations, which are generally based on carriers such as solid carrier as lignite based, liquid based, microorganisms entrapped in sodium alginate matrix and it contains useful microorganisms such as

Rhizobium sp., Aspergillus sp.., Lactobacillus sp., Phosphate solubilizing bacteria etc, in viable condition. These are intentionally produced for seed and soil applications as they amplify the plant growth by enhancing its nutrient uptake and growth hormone production. Nitrogen, phosphorous and plant growth-promoting rhizobacteria are considered chief and favourable microbial inoculants in our country.

These microorganism containing & growth promoting preparations, commercially produced by fermentation. However high energy is required for such a process. Within the look for a more economical process with high efficiency, Solid-state fermentation (SSF) seems to be an appealing approach. It’s a fermentation process during which microorganisms grow over solid substrate in the absence of free water. In recent years, the SSF gained much popularity than semi solid-state fermentation (SmF) because it provides various advantages like higher output, less energy expenditure, less effluent generation, thorough aeration, reduced contamination, and straightforward downstream.

In the present study, the preparation of biofertilizer under semi solid-state fermentation is carried out. This type of process is a variation of SSF during which free water amount is increased to enhance the nutrient availability & fermentation restraint. To the best of my knowledge, the preparation of biofertilizer by semi solid-state fermentation has not been reported yet.

Benefits of Biofertilizer

Currently, the employment of biofertilizers is boosting progressively, especially with more prominence on sustainable agriculture. They are crucial for organic farming and have the following benefits:

- Helps plants to grow under stress condition.
- Low investment technology with high output ratio.
- Improves soil health
- Increases plant growth and crop yield through increased nutrient availability.
- Reduces soil pollution.
- Reduces environmental pollution, which generates as a result of agrochemical manufacturing.
- It protects plants from soil-derived pathogens.
- It conditions the soil.

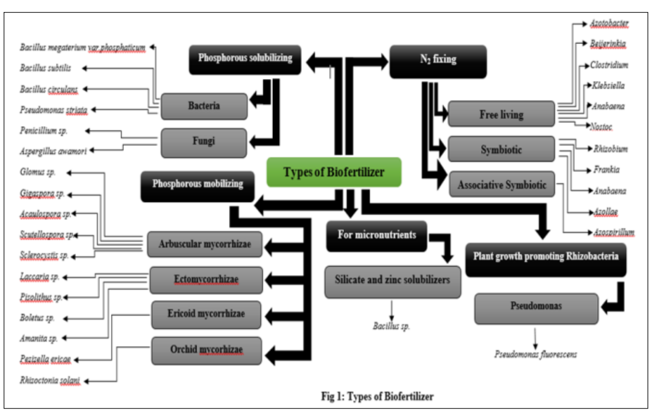

Fig 1: Different microorganisms and their association with plants are being used in production of Biofertilizers. They can be grouped in different ways based on their nature and function (Fig: 01)

Material and Methods

Papaya (*Carica papaya*), Banana (*Musa sapientum*), and Carrots (*Daucus carota*) were obtained from a local market near Jamalpur area and their peelings were retained for use in production of biofertilizer after normal human consumption.

Composition

Papaya peelings are majorly composed of carbohydrates such as glucose, maltose, and fructose. They also contain minor amounts of metallic ions, phenolic compounds, Beta-carotene, anthocyanins, vitamins, and volatile fatty acids, mainly acetic acid, propionic acid, butyric acid and valeric acid (Bharathi and Yashodhara *et al.*,2014; Pathak *et al.*, 2017) [15, 11].

Banana peels consist of lipids, proteins, crude fibers, and carbohydrates mainly glucose, and fructose. They also contain metallic ions (rich in phosphorous), esters, beta-tocopherols, vitamin E, malic acid as the principal acid and traces of citric acid, oxalic acid (Waghmare *et al.*, 2014) [17].

Carrot are mainly composed of water and carbohydrates, the peels of carrot are rich in vitamin C and niacin. It contains various phytonutrients and metallic ions.

Substrate preparation

Substrate preparation was classified into two batches, the two batches were selected to know the efficiency of batch-1 (i.e: primary batch) which developed as a result of natural fermentation, batch-2 (i.e: secondary batch) which developed by having inoculum from batch-1. Peels of papaya, banana, and carrot were collected separately, chopped, and thoroughly washed with distilled water. These chopped peels were transferred into a 1000 ml cotton-plugged Erlenmeyer flask containing autoclaved tap water. This constitutes the preparation of Batch-1. Batch-2 substrates were prepared following similar procedure except was autoclaved at 121°C at 15 psi for 20 minutes.

Biofertilizer preparation through Semi-solid-state fermentation

Batch-1 experiment was conducted in a1000 ml cotton-plugged Erlenmeyer flask containing 300 grams of finely chopped papaya peels. 400 ml of autoclaved tap water was added into it. The flask was then kept in room temperature for natural fermentation for 25-30 days until a soluble product was formed. The fermented end-product was then filtered with a fabricated filter. The fabricated filter was prepared by using four layers of muslin cloth and one layer of cotton cloth. The collected fermented solution was Batch-1 papaya biofertilizer.

Batch-2 experiment was conducted in three different 1000ml cotton plugged Erlenmeyer flask containing 300 grams of finely chopped peels of each papaya, banana, and carrot. 400 ml of autoclaved tap water was added into all the Erlenmeyer flasks, which were then, autoclaved for 20 minutes. After autoclaving, the flasks were kept in pre-sterilized laminar airflow hood, to bring them to room temperature. 50 grams of soluble product from Batch-1 was used as inoculum for Batch-2 fermentation. The inoculated Erlenmeyer flasks (Batch-2) were kept undisturbed at room temperature for 15-20 days. The inoculum increased the rate of fermentation. The downstream process for batch-2 biofertilizers were performed as same as for batch-1 biofertilizer.

Analytical determinations

PH and potassium content in the biofertilizer was assessed. PH test was performed to analyze the acidity of biofertilizer. The pH was analyzed with the help of digital pH meter. Potassium was determined to analyze the potassium species in the biofertilizer as it supports the growth of plant, with the help of atomic absorption spectroscopy.

Quality assessment

Quality was assessed based on microbial concentration and its phenotypic or functional traits. These biofertilizers were theoretically proposed as plant growth enhancers as they contain nitrogen-fixing bacteria, phosphorous solubilizing microorganisms and that they modify the soil fertility and enhances the crop production.

Estimation of total viable numbers of microorganisms was conducted by dilution plate count method. Physiological Saline Solution (0.9% NaCl) was used as diluent for dilution. The tubes of each dilution were spread over the selective. The number of colony-forming units (CFU) after the incubation period (24-192 hours) constitutes microbial concentration (CFU per ml of biofertilizer).

The selective media Sabouraud Dextrose Agar (SDA) for *Aspergillus sp*, Pikovskaya agar for P-solublizing bacteria, MRS (Man, Rogosa and Sharpe) agar for *Lactobacillus sp*, Mannitol yolk Neomycin agar for *Bacillus sp*.

Application of biofertilizer on Brinjal Plant

Comparative study of biofertilizers was carried out for Brinjal plant (*Solanum melongena* var. *depressum* cv. Pragati (Chu chu). Its growth was studied under the Faculty of Biotechnology at Punjab College of Technical Education, Jhande (Punjab), India during February to April of year 2020. Geographically this area falls under a humid subtropical climate. Variety of brinjal var., Pragati was selected for the present study. The seeds of the cultivar were obtained from the seed store of Punjab Agricultural University, Ludhiana.

The brinjal seeds were sown in the field ploughed soil in a tray and watered once daily. After that fifteen polyethylene plant bags were filled with field ploughed soil up to a height of 20 centimetre. After 25 days, 15 healthy seedlings of uniform height were transplanted from a tray into the polyethylene plant bags at the rate of 1 plant per polyethylene plant bag. Biofertilizer samples were applied in plant bags containing the transplanted seedlings. The experiment consists of the following treatments:

- 3 plant bags applied with water (Control)
- 3 plant bags applied with Papaya biofertilizer (Batch-1)
- 3 plant bags applied with Papaya biofertilizer (Batch-2)
- 3 plant bags applied with Banana biofertilizer (Batch-2)
- 3 plant bags applied with Carrot biofertilizer (Batch-2)

The effect of Biofertilizer on plant was recorded for growth parameters.

Results & Discussions

The waste from papaya was first fermented using semi-solid-state fermentation method**,** which yielded batch-1 soluble product after 25-30 days. The filtrate from batch-1 was used as inoculum precursor for batch-2 fermentation, which took place in 15–20 days, faster than batch-1 The observation was similar as result published by Soh-Fong Lim and Vidhya Devi. Table-1 shows application of different biofertilizers on different plant samples of Brinjal. Table-2 shows the pH level and potassium content of developed bio fertilizers. Although all bio fertilizers are acidic in nature, but the biofertilizer developed from papaya have the lowest pH i.e: 4.19. The biofertilizer developed from banana and carrot have the pH value 4.60 and 5.89 respectively. The result represented in table-1 shows that the papaya biofertilizer has the high acidic nature, whereas the carrot biofertilizer has the low acidic nature.

Banana biofertilizer has the highest potassium content of about 402.3 mg/l, whereas papaya biofertilizer has 238.2 mg/l of potassium in it, which was followed by carrot bio fertilizer containing 31.1 mg/l of potassium, which was lowest among all the bio fertilizer.

Table-3 indicates the increase in height of individual brinjal plants on treatment with different bio fertilizers, after the transplantation of plantlets in to the plant bags. The observation in table was recorded at an interval of after every 10 days.

Table-4 indicates the mean heights of the plants. The brinjal plants treated with papaya batch-2 biofertilizer was recorded with maximum mean height of 26.66 cm, which was followed by brinjal plants treated with carrot batch-2 biofertilizer recorded with mean height of 21.67 cm. The Brinjal plants treated with papaya batch-1 and banana batch-2 were recorded with mean height of 21.67 cm and 20.33 cm respectively. The lowest recorded height among all the plants was 16.83 cm of control plants. Noteable differences were observed in plant height, fresh weight, No. of leaves, No. of flowers per plant due to the application of different bio fertilizers on different plants (Table-8).

Maximum plant height (26.66 cm), fresh weight (58.26 gm), no: of leaves (33.66 cm), no: of flowers (4.91) were recorded at the harvest with the application of Papaya batch-2 biofertilizer. Whereas plants with no treatment (i.e: control) were recorded with the lowest height (16.83 cm), fresh weight (29.55 gm), no: of leaves (19), and no: of flowers (1.16). Significant increase in biofertilizer level leads to increase nutrient uptake by the plants which caused the significant increase in growth parameters of different plants.

Data on the flowering (table-5) shows the maximum no: of flowers in the first flowering period was observed in plants treated with carrot biofertilizer, whereas no flowers were observed in banana biofertilizer treated and control plants. This attributes that bio fertilizers developed from papaya and carrot have some supporting compounds in the form of hormones which support early flowering in the papaya & carrot treated plants as compared to control plants.

Biofertilizers notably influenced fruit yield, fruit no: per plant, fruit diameter. Notable increase in fruit yield (2.33 fruit per plant), fruit diameter (1.90 cm), fruit weight (36 gm per fruit) were recorded with the application of Papaya batch-2 biofertilizer, followed by the plants treated with carrot batch-2 biofertilizer which were recorded fruit yield (1.75 fruit per plant), fruit diameter (1.51cm), fruit weight (25.33 gm per fruit). Increase in fruit yield and its various attributes may be due to the increased no: of leaves which brought photosynthesis at maximum rate, thus lead to the production of carbohydrates at good level in the plants. Whereas the lowest fruit parameters were recorded fruit yield (0.41 fruit plant^{-1}), fruit diameter (1.13 cm) and fruit weight (9.0gm per fruit) in the control plants. This decrease in the fruit growth parameters may be due to lack of nutrient, growth supporting microorganisms.

Analyses on microbial densities based on genus and functional group approaches showed that papaya batch-2 biofertilizer had the highest density of microorganisms (Table-9). Maximum quantity of *Aspergillus sp.* (1.78 x 10^9 cfu), Lactobacillus *sp.* (3.1 x 10^8 cfu), Bacillus *sp.* (1.02 x 10^9cfu) was found in papaya batch-2 biofertilizer, whereas maximum PSB concentration (2.6 x 10^8 cfu) was found in carrot batch-2 biofertilizer.

Table 1: Mode of treatment: Different types of Biofertilizer

Plant Bag	Control	Papaya -1[1]	Papaya-2[2]	Banana-2	Carrot-2
1	+	-	-	-	-
2	+	-	-	-	-
3	+	-	-	-	-
4	-	+	-	-	-
5	-	+	-	-	-
6	-	+	-	-	-
7	-	-	+	-	-
8	-	-	+	-	-
9	-	-	+	-	-
10	-	-	-	+	-
11	-	-	-	+	-
12	-	-	-	+	-
13	-	-	-	-	+
14	-	-	-	-	+
15	-	-	-	-	+

[1]Papaya-1: First batch Papaya biofertilizer.
[2]Papaya-2: Second batch Papaya biofertilizer.

Table 2: pH level and potassium content of Biofertilizer

Type of Biofertilizer	pH	Potassium content (mg/l)
Papaya Biofertilizer	4.19	238.2
Banana Biofertilizer	4.60	402.8
Carrot Biofertilizer	5.89	31.1

Table 3: Effect of biofertilizer on plant height (cm)

Treatment	Plant bag no:	30 Day	40 Day	50 Day	60 Day	70 Day	80 Day	90 Day	100 Day	110 Day
Control	1	2	3.5	5.2	7.9	9.4	11.1	13.3	14.8	16
	2	2	3.8	5.5	8.0	9.5	11.1	13.2	15.3	16.5
	3	2.1	4.1	6.4	8.4	9.9	11.3	13.5	15.8	18
Papaya Batch-1	4	2.1	4.2	5.2	8.5	10	11.4	13.8	16.1	19
	5	2.2	4.4	6.5	8.6	10.1	11.5	13.9	17.2	20
	6	2.4	5.0	6.7	8.7	10.3	12.8	15.4	17.4	22
Papaya Batch-2	7	2.4	5.0	7.4	10.9	13.9	16.7	19.8	22.6	25
	8	1.8	4.0	7.6	10.8	13.8	15.5	19.8	23	26
	9	2.3	5.3	8.3	11.1	14.2	19.9	22.1	26.4	29
Banana Batch-2	10	2	4.5	5,4	8,4	9.9	11.3	13.4	15.4	18
	11	2.3	5.0	5.8	8.5	10.4	11.4	13.9	15.8	19
	12	2.6	5.5	6.3	8.4	10.1	13.1	16.5	18.6	20
Carrot Batch-2	13	2.5	5.3	7.8	9.6	11.5	13.6	16.4	18.3	20
	14	2	4.2	6.8	8.7	10.8	13.3	16.2	19.1	22
	15	1.8	4.1	6.5	8.8	10.7	13.4	16.5	19.4	23

Note: Height measured from the base of the plant to the tallest.

Table 4: Mean height of the brinjal plant (in cm) treated with Biofertilizers.

Treatment	30 Day	40 Day	50 Day	60 Day	70 Day	80 Day	90 Day	100 Day	110 Day
Control	2.03	3.8	5.7	8.1	9.6	11.16	13.33	15.3	16.83
Papaya Batch-1	2.23	4.53	6.13	8.6	10.13	11.9	14.36	16.9	20.33
Papaya Batch-2	2.16	4.76	7.76	10.93	13.96	17.36	20.56	24	26.66
Banana Batch-2	2.3	5.0	5.83	8.43	10.13	11.93	14.6	16.6	19
Carrot Batch-2	2.1	4.53	7.03	9.03	11	13.43	16.36	18.93	21.67

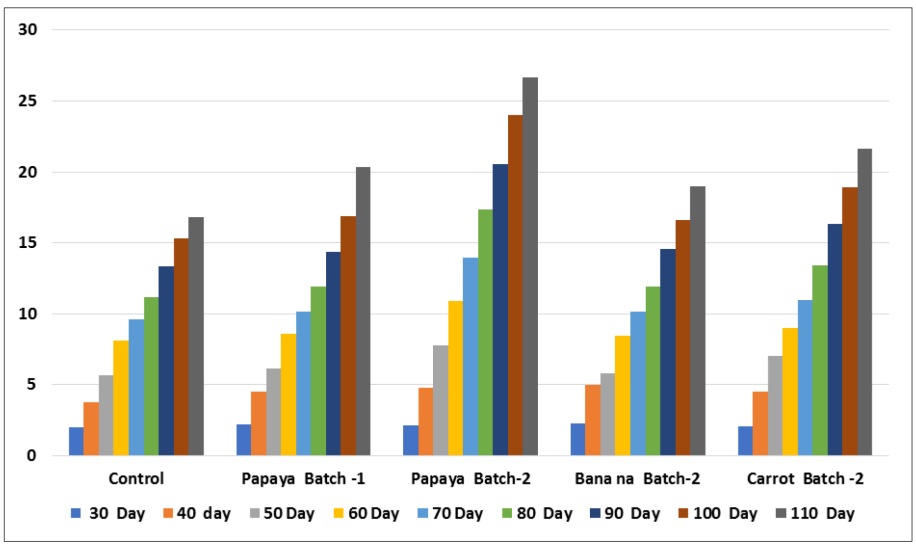

Fig 1: Mean heights of the plants following different biofertilizer treatment. 3 plant samples per treatment and control. The bars show height of plants recorded at interval of 10 days.

Table 5: Effect of Biofertilizer on Flowering.

Treatment	Plant bag no:	First week[1]	Second week	Third week	Fourth week
Control	1	-	1	2	-
	2	-	3	2	1
	3	-	2	2	1
Papaya Batch-1	4	3	2	2	1
	5	2	1	1	1
	6	3	4	1	2
Papaya Batch-2	7	4	5	2	6
	8	4	9	2	8
	9	5	3	5	6
Banana Batch-2	10	-	2	2	-
	11	-	2	1	-
	12	-	1	3	1
Carrot Batch-2	13	5	8	5	7
	14	6	6	7	8
	15	5	7	6	9

[1] The time period (week) in which first flowering appears.

Table 6: Fruits harvested per plant per week

Treatment	Plant bag no:	First week	Second week	Third week	Fourth week
Control	1	-	-	1	1
	2	-	-	-	1
	3	-	1	-	1
Papaya Batch-1	4	-	-	1	-
	5	-	1	1	1
	6	-	1	1	2
Papaya Batch-2	7	1	-	4	2
	8	1	2	5	3
	9	1	3	2	4
Banana Batch-2	10	-	-	-	1
	11	-	1	2	1
	12	-	-	2	2
Carrot Batch-2	13	1	3	2	3
	14	2	1	1	2
	15	2	1	2	1

Table 7: Effect of biofertilizers on plant growth and its parameters.

Plant bag no:	Fresh weight of plant (gm)	No: of leaves per plant	Approx. fruit diameter (cm)	Approx. fruit weight (gm)
1	24	16	1.10	9
2	25.5	18	1.20	10
3	30.16	23	1.10	8
4	32.16	15	1.20	10
5	39.9	20	1.23	12
6	42.72	21	1.30	14
7	54.44	30	2.00	40
8	55.47	32	1.90	35
9	64.88	39	1.80	33
10	36.16	20	1.40	20
11	38.43	21	1.42	18
12	36.41	19	1.44	22
13	40.66	21	1.45	25
14	45.91	23	1.60	23
15	48.74	28	1.50	28

Table 8: Effect of biofertilizer on plant height, fresh weight, no: of leaves, no: of flowers, no: of brinjal harvested, and their characteristics in brinjal.

Treatment	Plant height	Fresh weight of plant	no: of leaves	no: of flowers	no: of fruits harvested	Fruit diameter (approx...)	Fruit weigh (approx.)
Control	16.83	29.55	19	1.16	0.41	1.13	9.0
Papaya Batch-1	20.33	38.40	18.67	1.91	0.66	1.25	12
Papaya Batch-2	26.66	58.26	33.66	4.91	2.33	1.90	36
Banana Batch-2	19	37	20	0.83	0.75	1.42	20
Carrot Batch-2	21.67	45.10	24	6.58	1.75	1.51	25.33

Note: Mean value of every parameter is represented in the above table.

Table 9: Analyses of microbial density in biofertilizer.

Type of Biofertilizer (Batch)	Analyses of microbial density		
	Genus & functional groups	Media agar used	Cfu ml^{-1}
Papaya Batch-1	Aspergillus	SDA	4.15×10^{6}
	PSB[1]	Pikovskya	2.5×10^{5}
	Lactobacillus	MRS[2]	3.7×10^{7}
	Bacillus	Mannitol yolk neomycin	2.81×10^{8}
Papaya Batch-2	Aspergillus	SDA	1.78×10^{9}
	PSB[1]	Pikovskya	1.40×10^{8}
	Lactobacillus	MRS[2]	3.1×10^{8}
	Bacillus	Mannitol yolk neomycin	1.02×10^{9}
Banana Batch-2	Aspergillus	SDA	1.22×10^{8}
	PSB[1]	Pikovskya	4.8×10^{6}
	Lactobacillus	MRS[2]	2.4×10^{7}
	Bacillus	Mannitol yolk neomycin	2.33×10^{7}
Carrot Batch-2	Aspergillus	SDA	2.28×10^{7}
	PSB[1]	Pikovskya	2.6×10^{8}
	Lactobacillus	MRS[2]	1.28×10^{6}
	Bacillus	Mannitol yolk neomycin	1.15×10^{5}

[1]PSB = Phosphate solubilizing bacteria.

[2]MRS = Man, Rogosa & Sharpe agar.

Conclusion

The current study showed that SmF is better method over SSF to produce productive & potent biofertilizer, which could support the crop growth and increases crop output. Effective role of biofertilizer in crop development can easily be observed by comparing the plants growth parameters. The study showed that of all the three biofertilizers (i.e: papaya, banana & carrot) developed by SmF. As, per observations recorded for various parameters such as plant height, fresh weight, no: of flowers, fruit yield, fruit diameter, fruit weight. It was observed that the maximum recorded values for every parameter was observed in the plants treated with papaya batch-2 biofertilizer. The result recorded from the microbial density analysis revealed that the maximum concentration of growth promoting microorganisms was observed in papaya batch-2 biofertilizer. On the basis of result observed above we can conclude that papaya batch-2 biofertilizer is the best. Thus, the study revealed the waste obtained from papaya, banana, and carrot are suitable for developing biofertilizer by employing smF.

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