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BIOFERTILIZER PRODUCTION FROM AGRO - WASTES

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ABSTRACT

A Bio fertilizer is a substance which contains living microorganisms which, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus and stimulating plant growth through the synthesis of growth-promoting substances. Bio-fertilizers can be expected to reduce the use of chemical fertilizers and pesticides. The microorganisms in bio-fertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of bio-fertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Since they play several roles, a preferred scientific term for such beneficial bacteria is "plant-growth promoting rhizobacteria" (PGPR). Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their byproducts. Hence, bio-fertilizers are much preferred over other Fertilizers as they do not contain any chemicals which are harmful to the living soil. The present study is focused on producing Biofertilizer from Agro – wastes using Solid State Fermentation (SSF).

KEYWORDS: Biofertilizer, Microorganisms, Rhizobacteria, Agro - wastes and Solid State Fermentation.

INTRODUCTION

India's agriculture is composed of many crops such as wheat and rice besides pulses, potatoes, sugarcane, coffee, oil seeds and jute **(Shah Alam and Rajendra Kumar Seth,**

2012). Currently, the total agricultural output is lost due to inefficiencies in harvesting, transport and storage of government subsidized crops. (**Abdullahi *et. al.*, 2012**). Decline of agriculture is due to depletion of soil fertility and also partially associated with unfavourable distribution of rainfall, drought, storm and floods. The major problem faced by the farmers are high cost of inorganic fertilizers required for the plant growth **(Anita Khanafari *et. al.* 2012)**. The chemical fertilizer pollutes the air, soil and water polluting agents during the production of crops. To overcome this, researchers have found the term "Bio fertilizer" an alternate to chemical fertilizer, especially in the developing countries **(Soh-Fong Lim and Sylvester Usan Matu, 2015)**. Fertilizers improve soil fertility and lead to increases in yield of crops. Fertilizer is made up of two forms, organic and inorganic **(José Thyago Aires Souza *et. al*. 2016)**. High amount of fertilizers use may also reduce the nutrient produced by beneficial microorganism present in the soil **(Aher *et. al*. 2015)**. Proper use of fertilizer is also required to retain the soil fertility. Organic fertilizer is also another way of supplying nutrients to the soil. An organic material present in the soil may improve the soil fertility and also slow down the release of nitrogen and thus help to control the depletion of the soil and increase the other nutrient supply **(Kanmani, P and Karuppasamy, P, 2009)**.

Biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants' uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil **(Laditi *et. al*. 2012)**. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants **(Moola Ram1 *et. al*. 2014)**. Very often microorganisms are not as efficient in natural surroundings as one would expect them to be and therefore artificially multiplied cultures of efficient selected microorganisms play a vital role in accelerating the microbial processes in soil **(Muhammad Yasin *et. al*. 2012)**. Use of biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture **(Bákonyi *et. al*. 2013)**. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers **(Rakesh Kumar Meena *et. al*. 2014)**. They can be grouped in different ways based on their nature and function (**Table 1**).

Table 1: Types of Biofertilizers.

S. No.	Groups	Examples
N2 fixing Biofertilizers		
1.	Free-living	*Azotobacter, Beijerinkia, Clostridium, Klebsiella, Anabaena, Nostoc,*
2.	Symbiotic	*Rhizobium, Frankia, Anabaena azollae*
3.	Associative Symbiotic	*Azospirillum*
P Solubilizing Biofertilizers		
1.	Bacteria	*Bacillus megaterium* var. *phosphaticum, Bacillus subtilis Bacillus circulans, Pseudomonas striata*
2.	Fungi	*Penicillium sp, Aspergillus awamori*
P Mobilizing Biofertilizers		
1.	Arbuscular mycorrhiza	*Glomus sp.,Gigaspora sp.,Acaulospora sp., Scutellospora sp. & Sclerocystis sp.*
2.	Ectomycorrhiza	*Laccaria sp., Pisolithus sp., Boletus sp., Amanita sp.*
3.	Ericoid mycorrhizae	*Pezizella ericae*
4.	Orchid mycorrhiza	*Rhizoctonia solani*
Biofertilizers for Micro nutrients		
1.	Silicate and Zinc solubilizers	*Bacillus* sp.
Plant Growth Promoting Rhizobacteria		
1.	Pseudomonas	*Pseudomonas fluorescens*

In the present study, different fruits are used as bio fertilizers to check the efficiency in improving plant growth. The microorganisms present in various fertilizers which benefit the plant growth have also been studied.

MATERIALS AND METHODS

Collection of Samples

Agro – wastes (rotten fruits) were collected from the fruit market near CMBT. The five different fruits used for the present study are watermelon, papaya, pine apple, custard apple and guava (Figure 2). Fruits were cut into small pieces and smashed. They were used for Solid-State Fermentation (SSF). The soil samples were collected from Kolathur (Figure 1).

Figure 1: Kolathur.

Figure 2: Collection of fruits wastes.

Preparation for fermentation process

Two batch of fermentation process were carried out - BATCH – I & II.

Materials Required

1. Polyethene bottle
2. Fruit wastes (rotten)
3. Distilled water

Batch – I

Five hundred grams of water melon wastes was placed in a polythene bottle which has a capacity of 2.5 L. Hundred milliliters of water was added to it. The bottle was kept undisturbed for 30 -40 days until the soluble product was formed. This soluble product was filtered with a fabricated filter. The fermented solution is the first batch water melon biofertilizer.

Batch – II

Hundred milliliters of this filtered solution was used as inoculum precursor to the next SSF process. 500 g of new water melon wastes were placed in a polythene bottle. The precursor increases the rate of fermentation and minimizes the duration of SSF process. The bottle was kept undisturbed for 20-30 days at room temperature until the soluble product was formed. This soluble product was filtered with a fabricated filter. This filtered solution is called second batch water melon biofertilizer. Agro-wastes from pine apple, papaya, and custard apple were also used to produce first and second batches of biofertilizer.

Soil Fertility Analysis

Soil Fertility Analysis was carried out by estimating the Soil pH, Electric Conductivity, Calcium, Magnesium, Sulphate, Chloride, Phosphorous, Total Organic Carbon, Nitrogen, Sodium, Potassium, Iron, Zinc, Manganese and Copper.

Atomic Absorption Spectrophotometer

Standard solutions were prepared in the range 0, 1,2,5,10 µg/ml of the trace metal. The standard solutions were diluted with DTPA solution. The working condition of the instrument is optimized and the readings were taken for standard as well as soil samples.

Calculation

µg Te/g soil= µg Te/ml_{sample} * 20ml/5 g soil.

Isolation of Microorganisms from Sample

10 gm of the soil sample was added to 90 ml of sterilized water and was mixed with a magnetic blender for 30 minutes to separate the microorganisms from the soil completely. After being deposited for 20 minutes, 1ml of suspension was added to the broth and was incubated at 37°C for 24 hours.

Serial Dilution

The incubated tubes were taken for serial dilution. 9 ml of saline was added to 10 sterilized test tubes. 1 ml from the incubated test tubes was added to the first test tube that gives 1:10 dilution. The tube was mixed well and 1 ml from the first test tube was transferred to the second tube. This was continued till the eighth tube. And 1 ml from the eighth tube was discarded. Dilutions such as 10^4, 10^5, 10^6 and 10^7 were chosen for plating.

Spread Plate Technique

Once the plates solidified, 0.1 ml from 10^4 dilution was taken and added to the petri plate, L-rod was flame sterilized using ethanol and it was used to spread the sample on agar surface. The same procedure was repeated for 10^5, 10^6 and 10^7 dilutions. 1 plate was used as control and the plates were incubated at 37°C for 24 hrs. After the incubation period (24 - 48hrs) the plates were observed for growth on the media.

Selective Media were prepared for Bacillus, Yeast and Rhizobium Species

Biochemical Characterization

Biochemical screening was done by performing tests such as Indole, Methyl Red Test, Citrate Utilization Test, Triple Sugar Iron Test, Urease Test, Oxidase Test and Catalase Test.

Applicability of the biofertilizer in vegetable plantation

The biofertilizers were applied on the various seed samples of 2 weeks of age in order to determine the effectiveness of the biofertilizer. Each batch of the biofertilizers were applied on 100 plant samples. At the same time, another 100 samples were planted in the absence of any fertilizer.

Experimental Design – Pot Culture

250 g of soil was taken in empty box which has a capacity of 500gm. 50 g of Cumbu seeds were taken. 5 ml of watermelon fertilizer and 5 ml of water were mixed and applied to the soil. The procedure was followed for the rest of the fruits as well. At regular intervals, the fertilizer was sprinkled on the soil.

RESULTS AND DISCUSSION

Solid State Fermenation

The fermented solution from Batch II was used to check the efficiency of vegetation plantation (Figure 3).

BATCH – I **BATCH -II**

Figure 3: Batch 1 and II Fermentation Process.

Pot Culture (Soil Sample – (Kolathur)

500g of soil sample from Kolathur was weighed and taken in a tray. 50 g of seeds (Cumbu) was taken. 5ml of the biofertilizer (watermelon) and 5ml of water is taken and mixed well. The fertilizer is applied daily to the soil. The following method is carried out for other fertilizers. The growth of the plants was observed periodically and the height was noted (Figure 4).

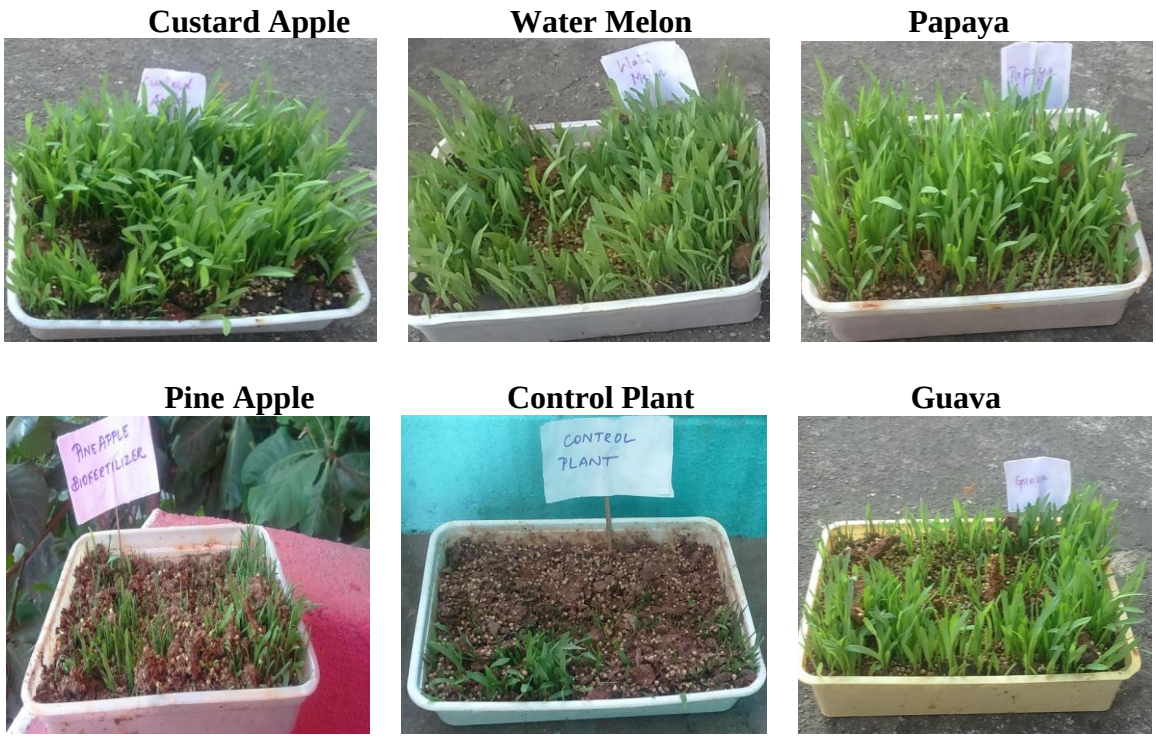


Figure 4: Growth of Plants tested in Soil sample - Kolathur.

Measurement of Plants - (Soil Sample - Kolathur)

Observation of plant growth was noted in Soil sample from Kolathur and the measurement of plant height was taken at 3 week age of the plant. Root elongation, shoot length and number of leaves germinated were also recorded (Figure 5).

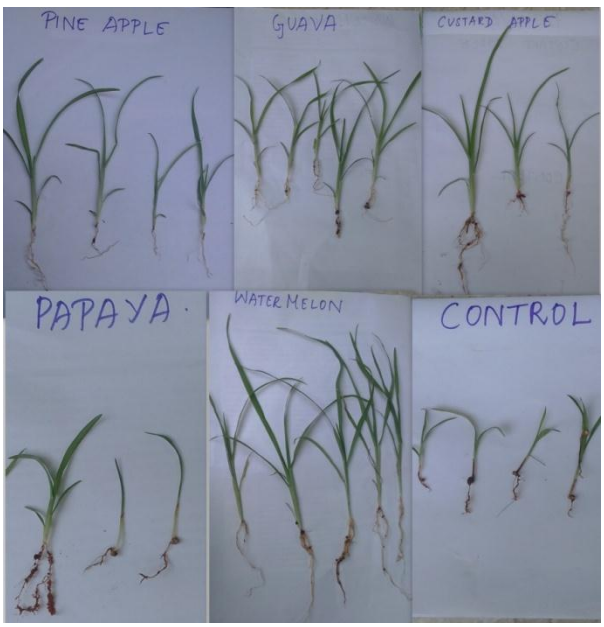


Figure 5: Measurement of Plant Growth in Soil sample – Kolathur.

Quantitative analysis of soil sample - Kolathur

Each fruit is unique in its nutritional elements which make the plant growth differ in their morphological characters such as length of root, shoot and height of plant and seed germination. Custard apple, watermelon and guava showed better growth in plant rate with reference to the height of plant, length of root, shoot and seeds germinated in soil sample. The soil sample taken from Kolathur showed better seed germination (Table 2).

Table 2: Quantitative analysis of plant growth -Soil sample.

S.no	Agro – waste (rotten fruits)	Total height of plant	Root length	Shoot length	No. of seeds germinated
1	Control Plant	5 cm	2cm	3cm	20 - 45%
2	Custard Apple	10 – 25 cm	7 – 22 cm	5 – 20 cm	75 - 80 %
3	Water Melon	20 – 35 cm	5 – 20cm	10 – 15 cm	80 - 85%
4	Guava	15 – 30 cm	10 – 25 cm	10 -20 cm	75 - 85%
5	Pine Apple	7-10 cm	1 – 3cm	4-6 cm	60 - 65%
6	Papaya	5 – 10 cm	4 – 7 cm	3 – 6 cm	40 - 60%

Quantification of Trace Elements in Agro - Wastes

The trace elements were present in the fermented Agro-waste. In water melon, custard apple and guava the level of Potassium (K) and Phosphorus (P) were high. Hence the plants can utilize the amount of K and P present in the fertilizer as well as in the soil. The potassium helps the plants in growth, whereas phosphorous helps in the growth of plants and also in the ripening of fruits (Table 3).

Table 3: Quantification of Trace Elements in Agro – wastes.

S.no	Parameters	Units	Custard apple	Pine apple	Papaya	Water melon	Guava
1.	pH	-	4.5	3.42	4.13	3.010	3.640
2.	Phosphorous	mg/kg	21.6	128.2	71	169	11.40
3.	Potassium	mg/kg	382.3	8.6	362	535.3	396.23
4.	Sodium	mg/kg	4.1	0.89	4	15.3	2.3
5.	Calcium	mg/kg	107	241	290	113	82.18
6.	Magnesium	mg/kg	39.2	356	135	543.13	174.23
7.	Iron	%	0.7	0.74	0.1	0.40	0.26
8.	Copper	mg/kg	2.6	2.6	2.2	2.47	2.50
9.	Manganese	mg/kg	0.1	12.36	0.1	0.10	0.15
10.	Zinc	mg/kg	0.3	0.53	0.1	0.20	0.23
11.	Selenium	mg/kg	0.1	0.23	8.1	0.60	0.60

Soil Fertility Analysis

Soil fertility analysis was carried out at Tamil Nadu Test House (Table 4).

Table 4: Comparison of Soil Fertility Analysis.

S.no	Parameters	Units	Soil – 1	Soil – 2	Normal range
1.	pH	-	7.24	7.1	6 – 8
2.	Electrical conductivity	µs/cm	1489	735	-
3.	Phosphorous	mg/kg	131	117	0.2%
4.	Potassium	mg/kg	470	420	1.0%
5.	Total organic carbon	%	0.50	0.60	-
6.	Sodium	mg/kg	650	520	100 – 500mg/kg
7.	Calcium	mg/kg	348	204	0.5 %
8.	Magnesium	mg/kg	98.11	75.00	0.2 %
9.	Sulphate	mg/kg	643.30	149.68	0.1 %
10.	Chloride	mg/kg	154.91	169.60	100 mg / kg
11.	Nitrogen	%	2.90	2.10	1.5%
12.	Iron	%	1.20	0.98	2 – 5%
13.	Copper	mg/kg	65.21	68.16	70 – 100 mg/kg
14.	Manganese	mg/kg	55.63	58.00	70 – 100 mg/kg
15.	Zinc	mg/kg	82.13	86.12	70 – 100mg/kg
16.	Molybdenum	mg/kg	0.001	0.001	0.1%
17.	Boron	mg/kg	0.001	0.001	0.1%
18.	Nickel	mg/kg	0.001	0.001	0.1%

Isolation of soil organism

Soil was collected from Kolathur. Soil fertility analysis was done for the soil. The organisms were isolated from the soil and also from the fermented fruits. The organism present in the fermented solution helps to enhance the growth of the plants (Figure 6).

Rhizobium spp. ***Bacillus spp.***

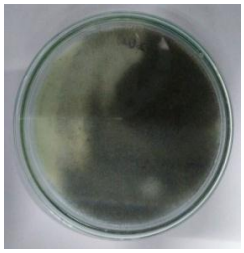
Figure 6: Microorganisms isolated from Soil Sample from Kolathur.

Isolation of organism in fermented solution

The following microorganisms were isolated from the fermented solution (Figures 7 a- c & 8 – 10 and Table 5).

Custard Apple and Papaya

Aspergillus spp.



Guava and Watermelon

Bacillus spp.

Figure 7: Isolation of microbes from the fermented solution.

PINE APPLE

Bacillus spp.

Figure 8: Isolation of Microorganisms in Fermented Solution.

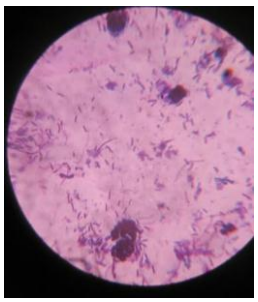


Figure 9: *Bacillus spp*. (Gram positive rods)

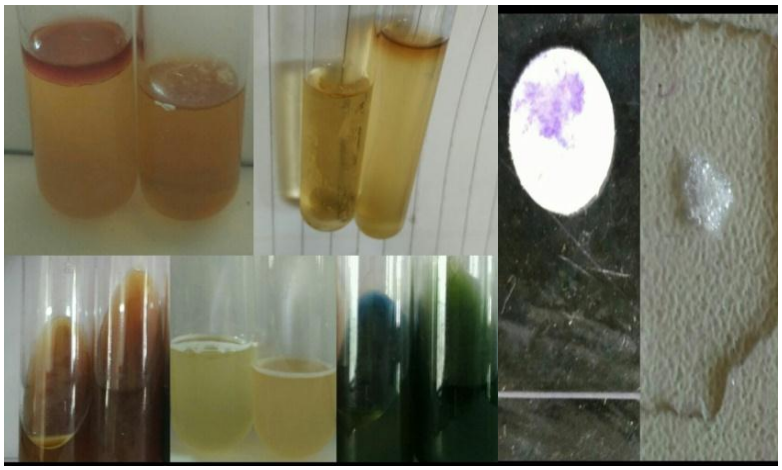


Figure 10: Biochemical tests.

Table 5: Biochemical Tests.

S.no.	Biochemical test	Result
1.	Indole test	Negative
2.	Methyl red test	Negative
3.	VogesProskauer test	Positive
4.	Citrate utilization test	Positive
5.	Triple sugar iron agar test	A/K, no gas production
6.	Oxidase test	Positive
7.	Catalase test	Positive

Identification of organism

1) The selective media MYP agar was used to confirm the presence of *Bacillus spp.* Three *Bacillus spp*. was isolated whose colony morphology are as follows:

a) ***Bacillus spp*.** 1- Red Colour Colony- Lecithinase Activity –Present (Positive Opaque Zone around the colony)

b) ***Bacillus spp***. 2 - Yellow Colour colony-Lecithinase activity – Absent

c) ***Bacillus spp***. 3 - Red Color colony.

2) The selective media YEMA agar was used to confirm the presence of ***Rhizobium spp.***

3) The selective media SDA agar was used to confirm the presence of ***Aspergillus spp.***

a) ***Aspergillus spp.* 1**- Yellow-green, powdery and pale yellowish.

b) ***Aspergillus spp.* 2**- The initial growth is white and becomes black later on giving "salt and pepper appearance" which results from darkly pigmented conidia borne in large numbers on conidiophores.

c) ***Aspergillus spp.* 3**- Blue – green, powdery and pale yellow.

d) ***Aspergillus spp.* 4** - Greenish-blue with whitish edge, yellow to brownish colour.

Advantages of Microorganism

1*) Bacillus spp.*

Bacillus spp. is a plant growth promoting bacteria which helps to enhance the availability of nutrient in the soil. *Bacillus spp.* produces plant hormones and solubilizes the insoluble form of phosphates. *Bacillus spp.* like *Bacillus subtilis*, *Bacillus megaterium* and *Bacillus pumillus* are the beneficial microorganism to the soil for improving the growth of plants. *Bacillus spp.* is called as a Synergistic plant promoter because both the soil and the plant get benefited. Thus *Bacillus spp.* helps the plant to absorb the phosphate by solubilizing the phosphates and produces the auxin which is used to stimulate cell elongation and delays fruit ripening.

2) Aspergillus spp.

Aspergillus spp. is one of the most important filamentous fungal genera. *Aspergillus* species are used in the fermentation industry. *Aspergillus spp.* is a saprophytic fungus that plays an essential role in recycling environmental carbon and nitrogen. It is very effective in removing the exchangeable, carbonate, and Fe/ Mn oxide fractions of Cu, Cd, Pb and Zn.

CONCLUSION

Biofertilizers are defined as carrier based materials which improves soil fertility. The collected Agro-wastes were subjected to Solid State Fermentation process to produce soluble fermented solution. The Agro – wastes used were water melon, guava, papaya, custard apple and pine apple. Solid state fermentation aided in the formation of soluble product and helped to produce the microorganism such as bacteria, fungi and yeast. The fermented solution was applied to vegetation to check the efficiency of the Biofertilizer. The soil collected from Kolathur showed better seed germination due to the presence of *Aspergillus spp*. It was also able to prevent root diseases. Cumbu (*Pennisetum glaucum*) seeds were tested using the biofertilizer. The elongation of root, shoot and germination of seeds were compared. Watermelon, Custard Apple and Guava fertilizer showed the best efficiency in comparison to others.

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