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PRODUCTION OF BIOFERTILIZER FROM FRUIT WASTES

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ABSTRACT

India, an agriculture based country has a wide variety of flora and fauna. Agriculture along with fisheries and forestry is one of the largest contributors of Gross Domestic Product (GDP).India's agriculture is composed of many crops besides wheat and rice such as pulses, potatoes, sugarcane, coffee, oil seeds and jute. Currently, the total agricultural output is lost due to inefficiencies in harvesting, transport and storage of government subsidized crops. 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 problems faced by the farmers are high cost of inorganic fertilizers which are required for plant growth. The chemical fertilizers used pollute the air, soil and water ecosystems. To overcome this, Researchers have found "**Bio fertilizer**" as an excellent alternate to Chemical fertilizers. Bio fertilizers or Organic fertilizers supply 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. The present study is aimed at producing Biofertilizers from Fruit wastes using Solid State Fermentation.

KEYWORDS: India, Agriculture, GDP, Chemical Fertilizers and Bio fertilizers.

INTRODUCTION

Biofertilizers are defined as preparation of living cells or efficient microorganism which helps to uptake the nutrients for the growth of plants **(Abdullahi *et. al.*, 2012).** Biofertilizers are used to improve the soil fertility using the biological wastes. The biological waste don't contain toxic materials, hence the living microorganism present in the soil are able to enrich the fertility of the land **(Dumitrescu *et. al.*, 2009).** Thus Biofertilizers can increase the output and improve the quality of the soil. It is well known that the continued use and overuse of petrochemical based fertilizers and toxic pesticides have caused a detrimental effect to the soils, water supplies, foods, animals and even people **(Laditi M. A *et. al.*, 2012).** Biofertilizer can be grouped in different ways based on their nature and function **(Table 1).**

Table 1: Types of Biofertilizers.

S.no	Groups of Biofertilizer	Organisms
1	Nitrogen fixing Bio fertilizer	*Rhizobium, Azotobacter, Azospirillum, Bradyrhizobium.*
2	Phosphorous solubilizing Bio fertilizer	*Bacillus, Aspergillus and Pseudomonas*
3	Phosphate mobilizing Bio fertilizer	*Mycorrhiza*
4	Plant growth promoting Bio fertilizer	*Pseudomonas sp.*

In the past century, the farmers were eager in the usage of chemical fertilizer as it yielded great number of crops. But eventually, they realized that chemical fertilizer affects the soil fertility and kills the beneficial microbes which enhance the growth of crops. The major issue they faced using chemical fertilizers were affecting not only the soil but also human beings who eat these farm products **(Shah Alam and Rajendra Kumar Seth, 2012).**

To overcome the problems faced by farmers using chemical fertilizers, Biofertilizer came as the solution. As the term Biofertilizer implies, it is eco-friendly to environment and farmers. The Biofertilizer and biological waste are used to replace the usage of chemical fertilizers as it does not contain any toxic substance and makes the soil enriched. Use of such natural products like biofertilizers in crop cultivation will help in safeguarding the soil health and also the quality of crop products **(Ajay Kumar Singh *et. al.*, 2013).**

AGRO – WASTE

Agro wastes are defined as waste which is formed from various agricultural activities. The agro-wastes are fruits,

vegetables, weeds and organic manure. The accumulation of agro-waste may cause some environmental issues. In order to avoid this proper and safe disposal methods are undertaken. The agro-wastes can be used in the production of Biofertilizer using fermentation method such as: Solid State Fermentation (SSF) which is a simple and cost effective method **(Soh-Fong Lim and Sylvester Usan Matu, 2015).**

SOLID STATE FERMENTATION

Solid State Fermentation has been defined as a fermentation process which is used in cultivation of microorganisms under controlled conditions in the absence or near absence of free water. It is a potential technology that is used in the production of microbial products such as feed, fuel, food, chemical and pharmaceutical products. Solid substrate generally provides a good environment to the microbial flora containing bacteria, fungi and yeast (**Muhammad Yasin *et. al*., 2012).**

PLANT NUTRITION

Usually plant requires 80 – 90% of water. Essential elements required for the plant growth are classified into **Macronutrients** and **Micronutrients**. Macronutrients can be broken into two or more groups of primary and secondary nutrients. The primary nutrients present in the soil are **Nitrogen (N), Phosphorous (P)** and **Potassium (K)**. These major nutrients are usually lacking in the soil because plants use large amounts of them for their growth and survival (**Bákonyi *et. al*., 2013).** The secondary nutrients present in the soil are **Calcium (Ca), Magnesium (Mg)** and **Sulphur (S).** Also, large amounts of Calcium and Magnesium are added when lime is applied to acidic soils (**Moola Ram1 *et. al*., 2014)**. Sulphur is usually found in sufficient amounts from the slow decomposition of soil organic matter. Micronutrients are those elements essential for plant growth which are need in only very small quantities. These elements are sometimes called minor or trace elements. The micronutrients are **Boron (B), Copper (Cu), Iron (Fe), Chloride (Cl), Manganese (Mn), Molybdenum (Mb)** and **Zinc (Zn) (Aher *et. al*., 2015).** A fertile soil should possess all the macro and micronutrients as these minerals promote Plant Nutrition (**Tables 2 -5**).

Table 2: General elements present in the soil.

Element	Symbol	mg / Kg	Percent
Nitrogen	N	15,000	1.5
Potassium	K	10,000	1.0
Calcium	Ca	5,000	0.5
Magnesium	Mg	2,000	0.2
Phosphorous	P	2,000	0.2
Sulphur	S	1,000	0.1
Chlorine	Cl	100	-
Iron	Fe	100	-
Boron	B	20	-
Manganese	Mn	50	-
Zinc	Zn	20	-
Copper	Cu	6	-
Molybdenum	Mo	0.1	-
Nickel	Ni	0.1	-

Table 3: Major elements of plant nutrient.

Element	Uses	Deficiencies
Carbon	Allcarbohydrates, proteins and fatsare composed of a backbone of carbon atoms.	Plants can use up enough carbon dioxide to slow photosynthesis and reduce growth.
Hydrogen	Component of Carbohydrates and fats.	Usually not a problem
Oxygen	Component of carbohydrates, proteins and fats; necessary for respiration	Can be a problem within the root zone root rot, and plant death.

Table 4: Macronutrients.

Element	Uses	Deficiencies
Nitrogen	To stimulate the growth of leaf and stem. Also gives dark green color to leaves.	Lack of nitrogen is often indicated by a yellowish color of leaves and short growth of the stalk.
Phosphorous	It is responsible for growth of plant and play an important role in ripening of fruits.	Poor root/plant growth and flowering, "purplish" under leaves.
Potassium	Acts as a catalyst or activator of enzymes promotes overall growth, critical for stomata turgor.	Poor growth, leaf choruses/necrosis (death), slowed gas exchange
Calcium	Involved in acid/base regulation during metabolism and as an enzyme activator	Poor growth of meristems (growing tip), blossom end rot.
Magnesium	The "heart" of chlorophyll, and an activator for ATP/ADP metabolism, Photosynthesis, respiration & DNA/RNA formation.	Interveinal chlorosis/necrosis of lower Mature leaves.
Sulphur	Forms sulphur bridges to establish and maintain protein structure.	Reduced growth in mid/young leaves, thin brittle stems.

Table 5: Other Micronutrients.

Element	Uses	Deficiencies
Iron	Acts as a catalyst for enzymes involved in chlorophyll production, protein synthesis, respiration and other reactions.	Interveinal chlorosis of young leaves.
Manganese	Involved in enzyme activation during carbohydrate reduction, chlorophyll and RNA/DNA synthesis and other reactions.	Interveinal chlorosis of young leaves, necrotic spots, leaf shed.
Boron	The metabolism of calcium and potassium	Poor growth, blackening then dies back of roots/shoots
Zinc	Acts as an enzyme activator in protein	General stunting especially of young growth.
Copper	Involved in chlorophyll synthesis.	Stunting, tip death, new leaf twist, blue-green leaves, necrosis, loss of turgor.
Molybdenum	Involvedin nitrogen and Carbohydrate metabolism.	Interveinal chlorosis, mottling and marginal scorching or inward cupping of older leaves.
Chlorine	Involved in respiration, regulation of cell turgor	Older leaves chlorotic then Necrotic, wilt, overall Stunting.

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 Kanchipuram (**Figure 1).**

Figure 1: Kanchipuram.

Figure 2: Collection of fruit wastes.

PREPARATION FOR FERMENTATION PROCESS

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

MATERIALS REQUIRED

1. Polythene 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

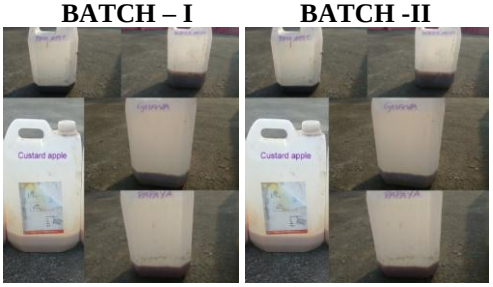
250 g of soil was taken in empty box which has a capacity of 500gm. 50 g of Cumbu seeds were taken. 5 ml of watermelon fertilizer and 5 ml of water were mixed and applied to the soil. The procedure was followed for the rest of the fruits as well. At regular intervals, the fertilizer was sprinkled on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

RESULTS AND DISCUSSION

SOLID STATE FERMENATION

The fermented solution from Batch II is used to check the efficiency of vegetation plantation (**Figures 3 - 4**).

BATCH – I **BATCH -II**



Figures 3 – 4: Batch 1& II Fermentation Process.

POT CULTURE (SOIL SAMPLE – (Kanchipuram)

500g of soil sample from Kanchipuram 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 5).**

Figure 5: Growth of Plant in Soil sample – (Kanchipuram).

MEASUREMENT OF PLANT (SOIL SAMPLE - KANCHIPURAM)

Observation of plant growth was noted in Soil sample from Kanchipuram 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 6).**

Figure 6: Measurement of Plant Growth in Soil sample – Kanchipuram.

QUANTITAIVE ANALYSIS OF PLANT GROWTH – SOIL SAMPLE (Kanchipuram)

Each fruits 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** shows 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 Kanchipuram showed better seed germination **(Table 6).**

Table 6: Quantitative Analysis of Plant growth (Soil sample – Kanchipuram).

S.no	Agro – waste (rotten fruits)	Total height of plant	Root length	Shoot length	No. of seeds germinated
1	**Control plant**	2 - 4 cm	2cm	3cm	20 - 45%
2	**custard apple**	5 – 10 cm	2 – 5 cm	3-7 cm	75 - 85 %
3	**water melon**	10 – 15 cm	5 – 10 cm	3 – 10 cm	85 - 90%
4	**guava**	15 – 20 cm	10 – 15 cm	5 - 20 cm	80- 85%
5	**pine apple**	7- 9 cm	1 – 3cm	4-7 cm	60 - 65%
6	**papaya**	5 – 10 cm	4 – 7 cm	3 – 6 cm	40 - 60%

QUANTIFICATION OF TRACE ELEMENTS IN AGRO - WASTES

Trace elements were present in the fermented Agro-waste. In water melon, custard apple and guava the level of Potassium (K) and Phosphorus (P) are rich and shows various parameters of agro-wastes. Hence the plants can utilize the amount of K and P present in the fertilizer as well as in the soil. The potassium helps in the plants in growth, whereas phosphorous helps in the growth of plants and role in the ripening of fruits **(Table 7).**

Table 7: 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 8).**

Table 8: 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 Kanchipuram. 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 7).**

Aspergillus spp

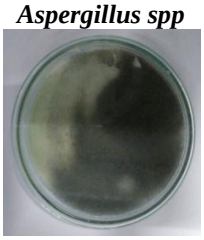
Figure 7: Microorganism isolated from Soil Sample of Kanchipuram.

ISOLATION OF ORGANISM IN FERMENTED SOLUTION AND BIOCHEMICAL ANALYSIS

The following microorganisms were isolated from the fermented solution (**Figures 8 a- c & 9 and Table - 9**).

CUSTARD APPLE AND PAPAYA

Aspergillus spp



GUAVA AND WATERMELON

Bacillus spp

PINE APPLE

Bacillus spp

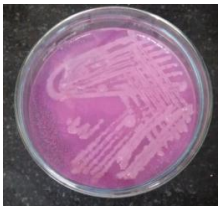


Figure 8: Isolation of microbes from the fermented solution.

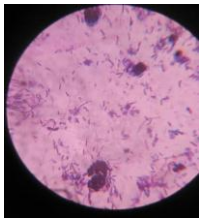


Figure 9: *Bacillus spp*.(Gram positive rods).

Figure 10: Biochemical tests for *Bacillus spp*.

Table 9: Biochemical tests for *Bacillus spp*.

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 Kanchipuram 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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