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Utilization of agro waste to produce biofertilizer and its efficacy on tomato plant

Atchaya A, Kannahi M
Department of Microbiology, STET Women's College (Autonomous), Sundarakkottai, Mannargudi, Thiruvarur, Tamil Nadu, India

Abstract
A Biofertilizer 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. The objective of the study was to develop a simple and cost- effective method to produce biofertilizer using agro waste. There were five types of agro wastes being used in this study they were wastes from water melon, pineapple, citrus orange, guava and banana. Solid state fermentation method was used to produce biofertilizer, which was then applied into vegetable plantation. Physical property tests were done on the plant samples of 5 weeks of age in order to determine the effectiveness of the biofertilizer. The results of the experiment showed that the plant samples treated with biofertilizer from watermelon, papaya, pineapple, citrus orange and banana wastes had promising physical characteristics. Other tests such as analyses of p^H values and potassium content in the biofertilizer were also done in this research.

Keywords: biofertilizer, agro waste, solid state fermentation

Introduction
Biofertilizers are defined as preparation of living cells or efficient microorganisms which helps to uptake the nutrients for the growth of plants [1]. Biofertilizers are used in 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 [4]. 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 [8].
Green waste composting has also been linked to suppression of soil borne disease such as dumping off and root rots that affect large agricultural ventures like greenhouses and large-scale forms [11]. This disease suppressive quality has positive implication for lesser developed nations that do not have the technology or resources to purchase expensive fertilizer. Addition of composts that contain residues and particle of heavy metals to soil can raise the soil's heavy metal content and increase the probability of these metals to transfer into crop plants. When biological, or green waste is added to these soil samples, plant uptake of heavy metal has been shown to decrease crop uptake of metals compared to other types of compost composed of things such as sewage sludge. This can product consumers and the environment from biomagnification caused by long- term accumulation of heavy metal particles with the soil and plant life of an area [14].
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 [7]. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a from easily assimilated by Plants [8]. Very often microorganisms are not efficient in Natural surrounding 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 [10]. 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 [3]. Several microorganisms and their association with crop plants are being exploited in the production of fertilizers [13]. They can be grouped in different ways based on their nature and function.
Biofertilizer is commonly referred to as the fertilizer that contains living microorganisms and it is expected that their activities will influence the soil ecosystem and produce supplementary substances for the plants [12]. The extensive use of chemical fertilizer and pesticide according to this strategy caused numbers of deaths and illnesses to the farmers. The poor form management technique and improper use of agrochemicals has also resulted in both soil quality and environmental degradation. Their common objective is to provide socioeconomic and ecological benefits. Among these benefits, improvement of soil quality is one of the interesting aspects since it contributes to a broad attributes including food quality and safety, human and animal health, and also environmental quality.
The application of effective microorganism aims to function as inoculum of microorganisms to the soil in which it will help to establish or re- establish soil ecosystems. EM is commercially available in concentrated from that needs to be processed before the application [2]. According to the preparation suggested by EM manufacture. The concentrated EM can be used directly by mixing with molasses and water. However, the common method is to use EM as a starter to ferment the raw materials and produce either liquid or solid biofertilizer. The common raw

materials include left- over plant are animal materials in the farms.

The fermentation period was suggested to be at least seven days and the product is recommended to be used within three months. Nowadays, the production of ready- to- use liquid biofertilizer from EM is becoming available in the market due to the convenience for small- scale farming or domestic application in which the users do not have space and raw materials available for fermentation. Efficiency of biofertilizer depends on the components available in raw materials as well as contribution from living microorganisms in them. However, as far as the authors known there is no previous published documentation of tests done to analyze the diversity of the microorganisms during the storage.

Agro- waste is defined as waste which is produced from various agriculture activities. These agro- wastes include manures, bedding, plant stalks, hulls, leaves and vegetable matter. Agro- waste is usually produced through farming activities. In farming situation, the agro- waste is often useless and will be discarded [6]. The accumulation of agro-waste may cause health, safety, environmental, and esthetic concern. Thus, this represents a problem which requires safe disposal. Agro-waste contain insoluble chemical constituents (e.g., cellulose and lignin) and soluble constituents (e.g., sugar, amino acids and organic acids). Other constituents are fats, oil waxes, resins, pigment, protein and mineral. The agro-wastes such as decaying part of plants are the primary source of organic matter in soil. Therefore, agro-waste are the cheapest source that can be used by farmers to improve the fertility of soil.

Plant growth and development depends on nutrients derived from the soil or air, or supplemented through fertilizer. There are eighteen essential elements for plant nutrition, each with their own functions in the plant, levels of requirements, and characteristics. Nutrient requirements generally increase with the growth of plants, and deficiencies or excesses of nutrients can damage plants by slowing or inhibiting growth and reducing yield. Many deficiencies can be recognized by observing plant leaves.

Nutrient mobility various among the essential elements, and represents an important consideration when planning fertilizer applications. For increase, NO_3^- nitrogen is very mobile in the soil, and will leach easily. Excessive or improper application increases the risk of water contamination. Meanwhile, phosphorus is relatively immobile in the soil, and is thus less likely to runoff. At the same time, it is also less available to plants, as it cannot "migrate" easily through the soil profile. Thus, P is often banded close to seeds to make sure it can be reached by starting roots. Nutrients also have variable degrees of mobility in the plant, which influences where deficiency symptoms appear.

Meterials and Methods

Sample Collection

Agrowaste (rotten fruits) were collected from the fruit market. The five different fruits used for the present study were Watermelon, Banana, Citrus Orange, Pine apple and Guava.

Fruits were cut into small pieces and smashed. They were used for Solid – State Fermentation (SSF). The soil sample were collected from Paddy Field, STET Women's College, Mannargudi, Thiruvarur District, Tamilnadu

Preparation for Fermentation Process

Batch–I

Five hundred grams of fruit waste was placed in a polythene bottle which has a capacity of 2.5 L. Hundred millilitres 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 of Biofertilizer.

Batch–II

Hundred milliliters of the filtered solution was used as an inoculum precursor to the next SSF process. 500 g of new watermelon wastes were placed in a polythene bottle. The Precursor increases the rate of fermentation and minimizes the duration of Solid State Fermentation 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 watermelon Biofertilizer.

Agro – wastes from Banana, Citrus Orange, Pine apple and Guava were also used to produce first and second batches of Biofertilizer.

Agro- wastes from Watermelon, Banana, Citrus Orange, Pine apple and Guava were also used to produce first and second batches of Biofertilizer. The procedure is the same as above.

Isolation of Microorganisms from sample

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

Spread Plate Technique

The plates solidified, 0.1 ml from 10^4 dilution was taken and added to the petriplate, 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. One plate was used as controland the plates were incubated at 37ºC for 24 hrs. After the incubation period (24 -28 hrs), the plates were observed for growth on the media.

Pot Culture method (Grime *et al*., 1987)

250 g of soil was taken in empty box which has a capacity of 500gm.

50g of Tomato seeds were taken.

5ml of Liquid Biofertilizer and 5ml 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.

Plant Analysis

Growth parameter studies

After 30 days of growth, plants per pot were removed from all samples and studied for the following morphological growth parameter.

1. Height of plant (in cm)
2. Number of leaves (per plant)
3. Number of root (per plants)
4. Shoot length (in cm)
5. Root length (in cm)

Phytochemical Analysis

To studied for the following Phytochemical analysis
(Table-3**)**

1. Chlorophyll
2. Carbohydrate
3. Carotenoids
4. Protein

Statistical Analysis (Gupta, 2004)

All the experiments were repeated as triplicates. The result Obtained in the present study were subjected to statistical analysis like mean $\bar{X}$ and standard deviation (SD).
Mean (x) = Sum of all values of the variable
N = Number of observation.
Where, add together all values of variable X and obtained $\bar{X}$. Divide the total by the number of observation. The standard deviation (SD) was calculated by the formula.
The formula of calculating standard deviation = S.D = $\frac{\sqrt{\Sigma(x-\bar{x})^2}}{n-1}$ Where,

$\bar{X}$ = Arithmatic mean
X = Number of values
N = Total number of observation
Find out the deviation of each value from the mean
(X-$\bar{X}$) square the deviation and take the total of square deviation. Divide the total by number of observation.

Result

The effect of fertilizer treatment on vegetative growth of Tomato plant was significantly higher in single inoculation than control plants. A significant variation in plants height and number of leaves due to application of fertilizers was found. Statistically significant increase in plant height, number of leaves, shoot length, root length and number of roots was observed.

Isolation of Organism in Fermented Solution and Biochemical Analysis

Serial dilution technique ware used to isolate the Bacteria. Gram staining, Motility test and Biochemical tests, Indole, MR-VP test, Citrate Utilization test, Oxidase Gram Positive, Non-motile, showing positive result for Indole, MR, Triple Sugar iron test and negative result for VP, Oxidase, Catalase, Urease, Citrate test. The Organism was identified as *Lactobacillus acidophilus*.
Gram Positive, Motile, Showing Positive result for MR, Oxidase, Urease, Citrate and negative result for Indole, VP, Catalase, Triple Sugar Iron test. The Organism was Identified as *Bacillus subtilis.*
Gram negative, Non-motile, showing positive result for VP, Catalase, Urease, Citrate, Triple Sugar Iron Test and negative result for Indole, MR, Oxidase test. The Organism was Identified as *Klebsiella pneumoniae.*
Gram positive, non motile showing positive result for Triple Sugar Iron test and negative result for Indole, MR, VP, Oxidase, Catalase, Urease, Citrate Test. The organism was identified as *Lactobacillus bulgaricus.*
Gram Positive, Motile showing positive result for MR, VP, Oxidase, Catalase, Citrate, Triple Sugar Iron test and negative result for Indole, Urease test. The Organism was identified as *Bacillus cereus.*
Based on the results obtained above the organisms were confirmed using Bergey's Manual of Determinative Bacteriology

Analysis of Physicochemical Parameter

The physicochemical parameters such as pH (6.4), Carbon (3), Potassium (36ppm), Phosphorus (53.5ppm), Biomass (76), Temperature (9.2°C), Nitrogen (68.7ppm), and Moisture(49) was observed in before and after inoculation and the above said parameters were increased after fertilizer inoculation treatments. The effect of fertilizer on the growth of *Lycopersicum esculentum* was studied and compared with control.

Quantitative Analysis of Trace Elements in Agro – Waste

The trace elements were present in the fermented Agro Waste.
In Banana, Watermelon, Citrus Orange, Pine Apple, 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 1).

Table 1: Quantification of Trace Elements in Agro-waste

S.No	Parameters	Units	Banana	Watermelon	Citrus Orange	Pine Apple	Guava
1	p^H	-	3.640	4.13	3.42	4.5	3.010
2	Phosphorous	Mg/kg	11.40	71	128.2	21.6	169
3	Potassium	Mg/kg	392.23	362	8.6	382.3	535.3
4	Sodium	Mg/kg	2.3	4	0.89	4.1	15.3
5	Calcium	Mg/kg	82.18	290	241	107	113
6	Magnesium	Mg/kg	174.23	135	356	39.2	543.13
7	Iron	%	0.26	0.1	0.74	0.7	0.40
8	Copper	Mg/kg	2.50	2.2	2.6	2.6	2.47
9	Manganese	Mg/kg	0.15	0.1	12.36	0.1	0.10
10	Zinc	Mg/kg	0.23	0.1	0.53	0.3	0.20
11	Selenium	Mg/kg	0.60	8.1	0.23	0.1	0.60

Test, Catalase test, Triple Sugar Iron test, Urease test were used to identify bacterial Species.

Pot Culture

The seed inoculation with all fertilizer were planned as (T1) – Control, (T2) – Banana Biofertilizer, (T3) –Watermelon Biofertilizer , (T4) – Citrus Orange Biofertilizer, (T5) –Pine Apple Biofertilizer, (T6) – Guava Biofertilizer. The treatment were significantly increased in plant growth and yield of *Lycopersicum esculentum.* After treatment the number of leaves, plant height, leaves length, number of nodules is increased compared the types of biofertilizer the plant height, number of leaves, number of nodules, leaves length was increased. **(**Table-2, Figure- 1**)**

Table 2: Effect of various fertilizer on morphological parameters of *Lycopersicum esculentum* (45th day)

Treatment	Height of the plant (cm)	Number of leaves/ plant	Shoot length (cm)	Root length (cm)	Number of roots / plant
T1	8±0.09	10±1.0	7.5±0.4	4.05±2.1	10.01±11.2
T2	12±4.4	15.56±1.23	10.3±7.12	8.44±.2.02	13.02±12.3
T3	14.4±1.82	18.7±2.24	11.6±6.72	7.66±3.3	15.05±5.5
T4	11±1.52	14.4±1.11	9.3±5.3	6.22±2.9	12.01±12.1
T5	15.9±2.32	20.3±1.14	13.5±03.2	10.11±2.0	19.09±4.5
T6	10.2± 1.37	12.02± 1.45	8.7±4.3	5.05± 2.4	11.03±11

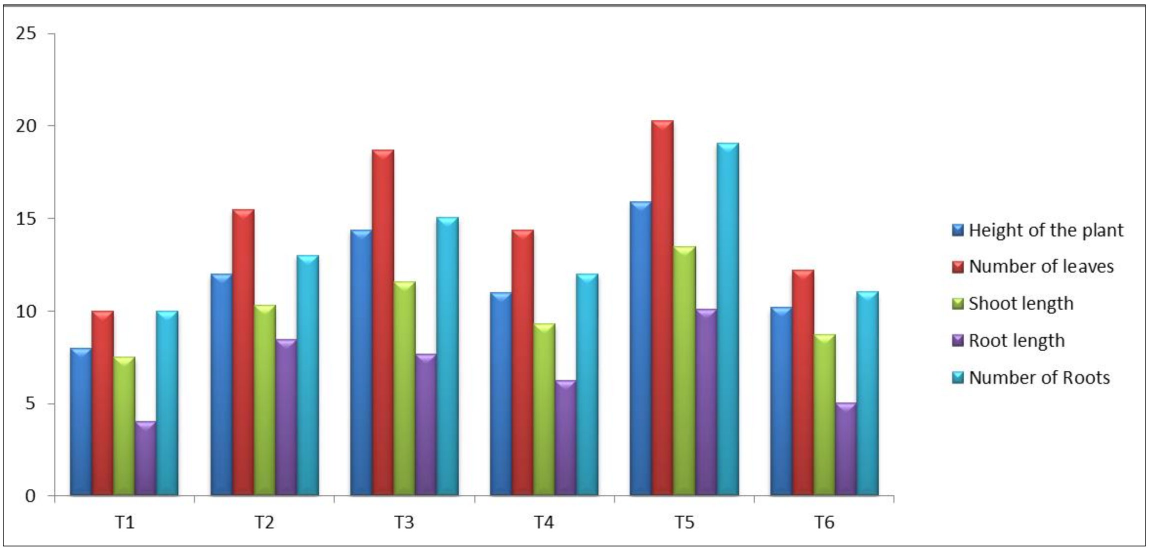

Fig 1: Effect of fertilizers on Height and Number of leaves in *Lycopersicum esculentum* (45th day)

Table 3: Phytochemical Analysis phytochemical constituent of (45th day) *Lycopersicum esculentum*

Treatment	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carbohydrate (mg)	Carotenoids (mg)	Protein (μg/g)
T1	0.33	0.114±1.0	0.112±0.10	1.312±0.11	0.155	2.163±0.53
T2	0.0345±0.34	0.299±0.85	0.355±0.45	4.69±.0.034	0.300	4.620±0.05
T3	0.453±0.43	0.0389±0.86	0.0452±0.12	6.397±0.45	0.389±0.80	4.367±0.040
T4	0.022±0.11	0.145±0.44	0.122±0.21	3.412±0.11	0.250	3.560±0.05
T5	12±4.4	0.0598±0.87	0.0678±0.87	6.643±0.56	0.598±0.67	4.620±050
T6	0.019± 0.07	0.136± 1.32	0.117±0.16	2.81± 0.11	0.186	3.250±0.4

Quality Assessment of Biofertilizer

The results of 60 days laboratory soil incubation study to compare soil aggregation in biofertilizers (Pineapple, Watermelon, Banana, Citrus Orange, Guava) developed from fruit and vegetable. Further increase in soil aggregate stability, was noted were the soil was amended with the biofertilizers respectively. The biofertilizer addition to soil enhanced it is stable aggregate upto 22 and 36% against the addition of raw waste material.

The water loss from soil treated by Pineapple Biofertilizer, Watermelon Biofertilizer, Banana Biofertilizer, Citrus Orange Biofertilizer, Guava Biofertilizer as compared to soil which received no amendment was determined in lab. The results of laboratory study are presented. Maximum water loss was observed in case of soil treated by Pineapple Biofertilizer, Watermelon Biofertilizer, Banana Biofertilizer, Citrus Orange Biofertilizer, Guava Biofertilizer containing Rhizobacterial stains. Average water loss during a period of 10 days was highest incase of unamended soil (50%) followed by soil treated with Citrus Orange Biofertilizer (45%), Guava Biofertilizer 9(42%), Banana Biofertilizer (41%), Watermelon Biofertilizer (39%), Pineapple Biofertilizer (35%).

Summary and Conclusion

Biofertilizers are defined as carried 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 Banana, Watermelon, Citrus Orange, Pine apple and Guava. Solid State Fermentation aided in the formation of soluble product an helped to produce the microorganisms such as bacteria , fungi and yeast. The fermented solution was applied to vegetation to check the efficiency of the Biofertilizer. The Biofertilizer environmental friendly and low cost. The Physiochemical parameters such as p^H, Temperature, Electrical conductivity, Nitrogen, Potassium, Phosphorus, Calcium and Magnesium were tested. The seedling of *Solanum lycopersicum* were transplanted in six pots of equal size, which were noted as (T1, T2, T3, T4, T5 and T6). The seedling of pots were treated with Control, Banana, Water melon, Citrus Orange, Pine apple and Guava (Biofertilizer).

Then the fertilizers could enhance the morphological parameters such as height of plants, number of leaves, number of roots, shoot length and root length were analyzed at 45th day.

Further, the study was needed for suitable combination of liquid biofertilizer with optimized field results is preferable for the sustainable production for various crops. Liquid biofertilizer is the only solution for restoration of soil health. Therefore liquid biofertilizer is believed to be the best alternative for the conventional carrier based biofertilizer witnessing the enhanced crop yields, regrinding soil health and sustainable global food production.

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