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Study of biofertilizers production and isolation of microorganism from fruits waste

Bhimrao N. Jadhav[1], Kailas B. Temkar, Somwanshi B. V., Jyoti Galande, Rutuja Kanade,

Department of Biotechnology, Vinayakrao Patil Mahavidyalaya Vaijapur, Dist. Aurangabad Maharashtra State India 423701

Email: bhimaarjun1982@gmail.com

Abstract:-

Food wastes have tremendous potential to enhance the production of bio-fertilizers because these wastes are present in bio-degradable forms and may efficiently accelerate the activity of the microbial metabolic. Thus, the present review summarizes an overview of the production strategy of bio-fertilizers using the combination of food wastes and microorganisms

For ever increasing demand of agricultural products chemical fertilizers are traditionally used on large scale to obtained more yield. But now a day, we realized the over use of chemical fertilizers effects on soil fertility causes soil pollution and water pollution also harmful to the human beings. Hence, to get better and sustainable agricultural production, it important to used renewable nutritional sources and ecofriendly substances, which is produced from biological compounds that contain nutrients which useful for the soil fertility and does not contain any toxic effects on environment.

The aim of present study is to produce bio-fertilizers from fruit wastes and its efficiency on crop yield. In our investigation, the growth of seedling of wheat is more in Pineapple fertilizer (7-9 cm), followed by Banana fertilizer (7-8 cm), Papaya fertilizer (6-7 cm), Watermelon fertilizer (5-7 cm) and control (4-5 cm). Where as in case of *Panicum miliaceum L (Proso millet)* the growth of seedling is more in Watermelon fertilizer (4-5 cm) followed by Banana fertilizer (3-4 cm), Pineapple fertilizer (2-3 cm), Papaya fertilizer (2 cm), and control (1-2 cm).

Key Words: Bio-fertilizers, Microorganisms, Soil fertility, Solid state fermentation.

Introduction:

After green revolution over use of chemical fertilizer damage the soil fertility and increase the soil pollution. Massive utilizations of chemical fertilizer in agriculture sector to improve the farming productivity had created increasing possibility of environmental damages. Severe human health issues, global warming, poor fertility and high cost of soil maintenance are the major side effects of the

utilizations of inorganic fertilizers and needs immediate attention. To overcome these issues, agriculture farming has been shifted towards the development of organic fertilizers using natural bio-resources. Organic fertilizers have several advantages like low-cost, being produced from the renewable resources and are highly efficient to improve the productivity of soil and agriculture product, rapidly. Additionally, bio-fertilizers not only increase the production, nutrients and organic matter but also neutralize the harmful impacts caused by the chemical fertilizers due to the potential combination of the microorganisms with organic wastes.[2]

Biofertilizers have now emerged as a highly potent alternative to inorganic fertilizers and offer an ecologically sound and economically attractive route for augmenting nutrient supply and increasing crop production. These include live cells of diverse genera of microorganisms and have the potential to fix atmospheric nitrogen and solubilize and mobilize plant nutrients from insoluble form through microbiological process. It has also the potential to diminish the gap between nutrient supply through fertilizers and nutrient removal by crops. Hence, biofertilizers can be a feasible option to the farmers to increase crop productivity and should find greater acceptance from the extension workers and commercial biofertilizer manufacturers.[3]

Moving away from synthetic and apply only naturally derived product, it is the aim of organic farming. Biofrtilizer is the best solution because combining naturally occurring microorganisms with organically derived, nutrient-rich fertilizers, provides the plants and soils with a healthy growing environment that is sustainable for future growing seasons. Biofertilizers also enhance a plant's resistance against pests and abiotic stressors such as drought, excess water, and extreme temperature changes. Providing plants with natural protection against external threats and restrictive conditions is necessary for successful plant growth and development, and reduces the need for traditional, inorganic fertilizers and pesticides.

Biofertilizers are living microbes that enhance plant nutrition by either by mobilizing or increasing nutrient availability in soil. [1] Bio-fertilizers help the soil to gain its nutrients back and make the soil available for cultivation. It don't make any kind of pollution so, it is not a threat to use them instead of the chemical fertilizers which causes lot of soil pollution. The plant nutrients are important to the production of crops and healthy food for the human being. Also the soil fertility are dependent on fertilizers. Bio-fertilizers has been identified as an alternative for increasing soil fertility and crop production in sustainable farming

Thus, the exploitation of beneficial microbes as bio-fertilizers has important in the agricultural sector. The micro- organisms are commonly used as biofertilizers component including, nitrogen fixers, potassium and phosphorus solublizers, Growth Promoting Rhizobacteria (PGPR), Endo and Ectomycorrhizal fungi, Cynobacteria and Other useful microscopic organisms

Thus, biofertilizers which are produced from biological wastes are used to replace of chemical fertilizers. Biofertilizers are biological preparation of efficient micro- organisms that promote plant growth by improving nutrient acquisition. They enhance soil productivity by fixing atmospheric nitrogen, solubilizing soil phosphorus, and stimulating plant growth.

Here, solid state fermentation was used to produce biofertilizers. Solid state fermentation is the process for the cultivation of micro-organisms in a controlled environment. The solid substrate

(fruits waste) provide good environment to micro- organisms containing bacteria, fungi and yeast.

Material and Methods:

Present study has been conducted in January 2022 to June 2022 under the UGC STRIDE Scheme Component-1, in Vinayakrao Patil Mahavidyalaya Vaijapur, district Aurangabad. The material for the study was collected from the fruit market of the Vaijapur and other fruit selling stalls onside of highway. The four different fruits waste used for the study i.e. Banana, Papaya, Watermelon and Pineapple. Collected fruit waste was cut into small pieces and fills it in to the polythene bottles for the solid state fermentation. Two seed samples used to check efficiency of biofertilizers is *Triticum* (Wheat) and *Panicum miliaceum L* (Proso millet). [1]

Results and Discussions:

Fermentation Procedure:-

Two batch of fermentation process was carry out

Batch I and Batch II

Batch I: Solid state fermentation: -

Collected fruit waste was cut into small pieces and fills it in to the polythene bottles for the solid state fermentation. Add 100 ml of distilled water into polythene bottle and keep the entire bottle undisturbed i.e. Banana and papaya keep undisturbed for 20-30 days and watermelon and pine-apple keep undisturbed for 30-40 days at room temperature. Then soluble product of each bottle was filtered. This filtered solution use as an inoculum for second batch fermentation.

	
1. Banana and Papaya kept undisturbed for 20-30 days 2. Watermelon and Pine-apple kept undisturbed for 30-40 days at room temperature	**Filtered solutions of** Batch I fermentation process

Batch – II: Solid state fermentation:

100 ml filtered solution of each samples was used as inoculum to the next solid state fermentation process. Mix fresh 500 gm. new fruit wastes of Pineapple, papaya, watermelon and Banana in each inoculum respectively. They were placed in a polythene bottle. Added

inoculum precursor increases the rate of fermentation and minimized the duration. Keep the all bottle undisturbed i.e. Banana and Papaya keep undisturbed for 10-20days and Pineapple and Watermelon keep undisturbed for 20-30 days at room temperature. Lastly, the soluble product of each bottle was filtered and this filtrate is called 'biofertilizer'.

Batch II solid state Fermentation process

Filtered solutions of Batch II solid state Fermentation process

Experimental observations:

Soluble product of each bottle was filtered and these filtrates were applied on various seeds samples, to determine the effectiveness of the biofertilizers. Each solution of biofertilizers applied on approximately on 100 plant samples (Experimental) and another sample of approximately 100 plants taken as a control.

Experimental procedure: [1]

Two seed samples used to check efficiency of biofertilizers is *Triticum* (Wheat) and *Panicum miliaceum L* (Proso millet).

1. **Pot culture [Seed sample - *Triticum (Wheat)*]**

Take 500 gm. Of soil sample in a plastic pot and add 50 gm. seed of *Triticum* (Wheat). Then add 5ml of biofertilizers (Filtrate of Batch II solid state Fermentation process) and 5ml of water was mix well. One pot sample taken as a control; on each day (up to one week) 5 ml filtrate of batch II solid state fermentation process was applied on the soil.

Comparison of growth of *Triticum* (Wheat) crops in experimental and controlled pot were observed through no. of seeds germinates growth of roots, growth of shoot, and total length of plants.

Experimental set up of Pot culture [Seed sample -*Triticum (Wheat)*]

2. **Pot culture [Seed sample - *Panicum miliaceum L (Proso millet)*]**

Take 500 gm. of soil sample in a plastic pot and add 50 gm. seed of *Panicum miliaceum L* (Proso millet). Then add 5ml of biofertilizers (Filtrate of batch II solid state fermentation process) and 5ml of water was mix well. One pot sample taken as a control; on each day (up to one week) 5 ml filtrate of batch II solid state fermentation process was applied on the soil.

Comparison of growth of *Panicum miliaceum L* (Proso millet) crops in experimental and controlled pot were observed through no. of seeds germinates growth of roots, growth of shoot, and total length of plants.

Experimental set up of pot culture [Seed sample - *Panicum miliaceum L (Proso millet)*]

Experimental observations:

Experimental observations of above experiments were recorded after one week as follows:

1. **Observations of pot culture [Seed sample - *Triticum (Wheat)*]**

Different morphological characteristics of *Triticum* (Wheat) crops such as root, shoot and no. of seed germination was recorded after the 1 week.

Biofertilizers	No. of seed germination	Total length of seedling in Centimeter	Root length of seedling in Centimeter	Shoot length Centimeter
Watermelon	90-95	5-7	3-4	3-4
Papaya	50-65	6-7	2-3	1-2
Pineapple	75 -85	7-9	2	2
Banana	80-90	7-8	3	2-3
Control	30-45	4-5	1-2	1

Table 1: - Showing the growth of seedling after applying various biofertilizers

In our study, we observe, the growth of seedling of wheat is more in Pineapple fertilizer (7-9 cm), followed by Banana fertilizer (7-8 cm), Papaya fertilizer (6-7 cm), Watermelon fertilizer (5-7 cm) and control (4-5 cm). Seed germination percentage is 90-95 % in Watermelon fertilizer followed by 80-90 % in Banana fertilizer,70-85 % in Pineapple fertilizer, and 50-65% in Papaya fertilizer. Whereas, the normal control soil showed 30-45%.

2. **Observations of pot culture [Seed sample - *Panicum miliaceum L (Proso millet)*]**

Biofertilizers	No. of seed germination	Total length of seedling in Centimeter	Root length of seedling in Centimeter	Shoot length Centimeter
Watermelon	90-98	04-05	3	3-4
Papaya	60-75	2	2-3	1-2
Pineapple	70-89	2-3	2	2-3
Banana	80-90	3-4	1-2	2
Control	30-35	1-2	1	1-2

Table 1: - Showing the growth of seedling after applying various biofertilizers

In our study, we observe the growth of seedling of *Panicum miliaceum L (Proso millet)* is more in Watermelon fertilizer (4-5 cm) followed by Banana fertilizer (3-4 cm), Pineapple fertilizer (2-3 cm), Papaya fertilizer (2 cm), and control (1-2 cm). [2]

Seed germination percentage is 90-98% in Watermelon fertilizer followed by 80-90 % in Banana fertilizer, 70-89% in Pineapple fertilizer, and 60-75% in Papaya fertilizer. Whereas, the normal control soil showed 30-35%.

Isolation of Micro-Organisms from soil and fermented solution:-

For the isolation of micro-organisms, two different agar sample was used which is Yeast Mannitol Agar and Mannitol Egg Yolk Polymyx in Agar to detect the presence of micro-organism. Soil and fermented sample was streak on the agar plates.

Isolation of micro-organisms from soil:-

Weight 10 gm. soil sample add 90 ml of sterilized water and mix with the magnetic blender for 30 min, 1 ml suspension add to the 100 ml of broth and incubated at 37^0Cfor 24 hours.

Serial Dilution: -

Take 10 incubated test-tube for serial dilution. 9ml of saline was added to 10 sterilized test-tubes. Then 1ml from the incubated test tubes was added to the first test-tube.1 ml sample of first test-tube was transferred into second test-tube. Then 1ml of sample from second test-tube was added to third test-tube .Procedure follow till 9test tube. This sample of diluted test tube used for the plating [10^{-4}, 10^{-5},10^{-6},10^{-7}].

Identification of micro-organisms:

- **MYP** (Mannitol Egg Yolk Polymyxin Agar):-This is selective media was used to confirmation of *Bacilus Spp*. Here MYP agar containing *Bacilus Spp* shows red, powdery, faint red, yellowish colonies.
- **SDA** (Sabourad Dextrose Agar):-It is selective media for the conformation of *Aspergilus spp*. SDA agar containing *Aspergilus spp* shows yellow-green, greenish blue, powdery colonies.
- **YEMA** (Yeast Mannitol Agar):- It is used to confirm of presence of *Rhizobiums pp* YEMA agar containing *Rhizobiums pp* shows white colour or powdery colonies.

Spread Plate Technique: -

- Prepared **YEMA and MYP** agar containing plates.
- 1ml sample of diluted test tube (10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}) spread on the plates.
- For fermented solution of watermelon and Banana, 1ml of fermented sample was spread on both agar plates.
- 1 plate of both agars was taken as control.
- All the plates are incubated at 37°c for 24 - 48hrs.
- Observed the growth of media.

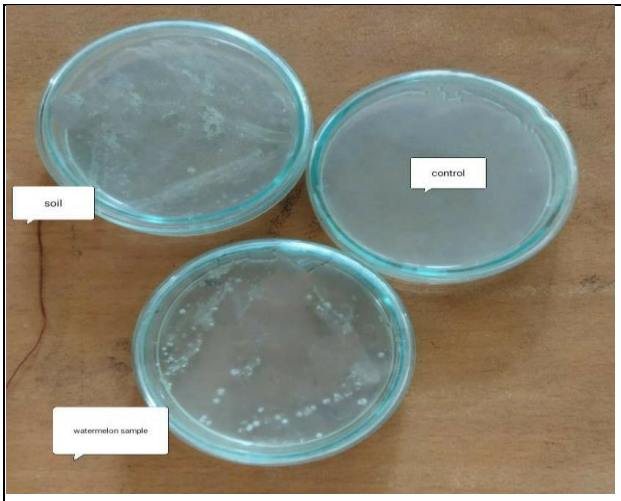

Figure 6 :-YEMA Agar Containing Plates of Watermelon

Figure 7 : - MYP Agar Containing Plates of Banana

Isolation of soil micro-organisms:-

After the 24 - 48 hr. incubation, micro-organisms from the soil were isolated. On YEMA media soil sample containing *Rhizobium spp* were isolated. And on the MYP media soil sample containing *Bacillus spp* were isolated. *Rhizobium spp* helps to fixing of nitrogen in the soil. *Bacillus spp* helps to enhance nutrients availability in the soil.

Isolation of Micro-Organisms: -

After the 24 -48 hr incubation, fermented sample containing micro-organisms were isolated. On YEMA media Watermelon sample was streak, *Rhizobium spp* was isolated from the sample. This procedure followed for the other samples of fruit wastes. On the MYP media banana sample were streaks. The *Bacillus spp* was isolated from this banana sample. Follow the procedure for other sample. Isolated *Rhizobium spp.* helps to plant for fixation of nitrogen whereas isolated *Bacillus spp*. produced hormones and solubilized insoluble phosphate.

Biochemical Characterization Rhizobium: -

Biochemical screening done by performing test such as - Congo red, Urease test, Citrate test, Gelatin test, Oxidase test, Keto lactose test, V.P. test, Catalase test and Triple sugar test.

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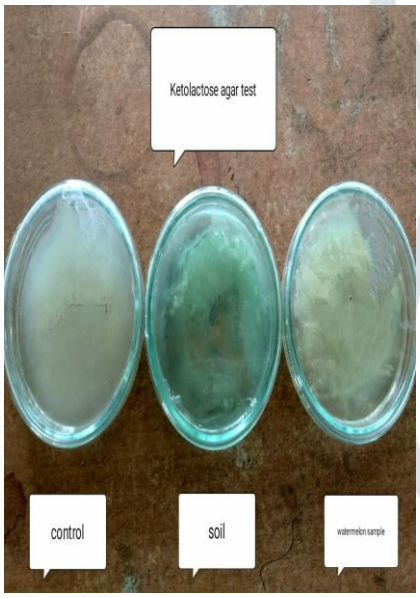
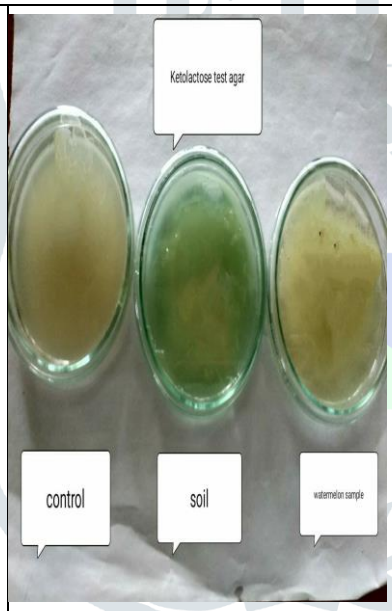
<td colspan="2"> 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<td colspan="2">Keto lactose test: Biochemical test for Rhizobium Spp</td>
<td>Congo Red Test: Biochemical test for Rhizobium Spp</td>
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Biochemical Tests	Result
Congo red test	Positive
Urease test	Positive
Citrate test	Negative
Gelatin test	Negative
V.P test	Negative
Oxidase test	Positive
Keto lactose test	Positive
Catalase test	Positive
Triple sugar glucose peptone agar test	Positive

Table:-Biochemical test for Rhizobium Spp.

1] Congo red test:

Prepared YEMA agar containing Congo red bacterial isolate streak on plates. The plates are incubated at 48hr on 28^0C. It shows, *Rhizobium* produce white colonies where as many other bacteria take up the dye strong.

2] Keto lactose test:

Prepare Keto lactose agar medium and bacterial isolates streak on plates. Incubate plates for 48hr at 28^0C. Then plates are flooded with Benedict reagent, incubate for 1hr at 25^0C. This test is used for differentiate *Rhizobium* from other contaminating bacteria. In Keto lactose test all the isolates showed no yellow colour formation on Keto lactose medium. After adding Benedict reagent isolates showed yellow colour formation. It indicates presence of *Rhizobium.*

3] Citrate utilization test:

In this medium citrate is the only carbon source available to the bacteria; however, *Rhizobium* cannot grow on the citrate. Prepare Simmon citrate agar streak loopful culture of *Rhizobium* and incubate 24hr at 37^0C. No change in colour, showed negative test. It indicates absence of *Rhizobium*

4] Triple sugar glucose peptone agar test:

Prepare triple sugar glucose peptone agar with Bromo –Thymol blue (indicator) is widely used for pure *Rhizobium* colonies. Then Streak the *Rhizobium* isolates on plates. The plates are then incubated at 28^0C for 48hr. No growth or very poor growth on glucose peptone agar medium showed the positive test.

5] Gelatin test:

This test performed to determine capability of microorganism to produce gelatinase enzyme and use gelatin as media source. Grown culture incubated in gelatin medium for 48hr. On low temperature at 4^0C for 30min. the culture which produces gelatinase liquefied, while other due to presence of gelatin becomes solid.

6] Catalase test:

This test was performed to study the presence of catalase enzyme in bacterial colonies. *Rhizobium* colonies where taken on glass slide and 1 drop of H_2O_2 (30%) was added appearance of gas bubble indicated the presence of catalase enzyme.

7] Urease test:

Christensen's urea agar was prepared .medium was pour into the sterile test tube and allow to solidify. The *Rhizobium* isolate where incubated separately into the test tubes and incubated at 30^0C for 4 days. Deep pink colour indicate positive test.

8] Oxidase test:

Trypticase soya agar was prepared the *Rhizobium Spp.* isolate were streak on the plates and incubate all the plates at 30^0C for 4 days. After incubation period 2-3 drops of p- amino dimethylaniline oxalate were added to the surface of the plates and observed the colour. Purple colour indicates positive test.

9] Vogas Paskeur test (V.P):

MR-VP broth was prepared 5 ml of broth was poured into the sterile test tubes. The *Rhizobium* isolate were incubated separately into the test tubes and tubes are incubated at 30^0C for 2 days. After incubation period 5 ml Barritt's reagent A and B was added. Development of red colour indicates negative test.

Conclusion:

In our observations, the growth of seedling of wheat is more in Pineapple fertilizer (7-9 cm), followed by Banana fertilizer (7-8 cm), Papaya fertilizer (6-7 cm), Watermelon fertilizer (5-7 cm) and control (4-5 cm). Where as in case of *Panicum miliaceum L (Proso millet)* the growth of seedling is more in Watermelon fertilizer (4-5 cm) followed by Banana fertilizer (3-4 cm), Pineapple fertilizer (2-3 cm), Papaya fertilizer (2 cm), and control (1-2 cm). Seed germination percentage is 90-95 % in Watermelon fertilizer followed by 80-90 % in Banana fertilizer, 70-85 % in Pineapple fertilizer, and 50-65% in Papaya fertilizer. Whereas, the normal control soil showed 30-45%.

Seed germination percentage is 90-98% in Watermelon fertilizer followed by 80-90 % in Banana fertilizer, 70-89% in Pineapple fertilizer, and 60-75% in Papaya fertilizer. Whereas, the normal control soil showed 30-35%.

When fermented solution spread on the soil, it shows that better seed germination due to the development of microorganism. So, use of such biofertilizers is beneficial to the farmers. The elongation of root, shoot along with germination of seed shows best efficiency in comparison with the others.

The Watermelon fermented solution and Banana fermented solution both are spread on different agar plates i.e Watermelon sample spread on YEMA agar and Banana sample spread on MYP agar And also soil sample spread on both agar.

The result is the YEMA agar containing Watermelon and soil sample show presence of *Rhizobium spp* microorganism and the MYP agar containing Banana and soil sample shows the presence of *Bacillus spp* microorganism. Both micro-organisms are help to well growth of crops. Also they are enhancing the soil fertility.

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References:

1. Gowthami.S1 , and Neethu C.S2(2020) PRODUCTION OF BIO FERTILIZER FROM TUBER CROPS WASTE - AN REVIEW International Journal of Scientific & Engineering Research Volume 11, Issue 3, March-2020

2. (Mohammed Y. Areeshi (2022) Recent advances on organic biofertilizer production from anaerobic fermentation of food waste: Overview International Journal of Food Microbiology Volume 374, 2 August 2022)
3. Seyed Mahyar Mirmajlessi and Ramalingam Radhakrishnan (2019) Applications and Constraints of Plant Beneficial Microorganisms in Agriculture FROM THE EDITED VOLUME Biostimulants in Plant Science
4. Mustapha et al: 2021 Study on the Use of Banana and Pineapple Peel Waste as Biofertilizers: Enhancing soil fertility, Promoting Sustainable Agriculture and Environmental Sanitation.AZOJETE 17(4):569-574. ISSN 1596-2490; e-ISSN 2545-5818
5. Mamouni, FZ., Razouk, R., Kajji, A., Daoui, K., El Ouali, A. andBoukhlifi, F. 2016. Characterisation of Organic Waste used as Biofertilisers: Case of PatotoPeelings, Almond Hulls and Shrimp Shells. International Journal of Agriculture, Innovations and Research, 4(5): 993-998.
6. Hamid, HA., Qi, LP., Harun, H., Sunar, NM., Ahmad, FH., Muhamad, MS. and Hamidon, N. Development of Organic Fertilizer from FoodWaste by Composting in UTHM Campus Pagoh, Journal of Applied Chemistry and Natural Resources, 1(1): 1-6