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STUDY ON THE BACTERIOLOGICAL EXAMINATION OF PREPARED EM SOLUTIONS AND ORGANIC FERTILIZERS USING VEGETABLE WASTES, SOIL, BAT DUNG AND RICE STRAW

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ABSTRACT

In this research, the microorganisms that contained in prepared EM (effective microorganism) solutions and organic fertilizers were studied. EM solutions were prepared from different conditions such as vegetables waste only, vegetables with soil, vegetables with soil and bat dung, vegetables with bat dung by an aerobic digestion. During the digestion, biogas evolved. The two organic fertilizers were prepared by aerobic and anaerobic methods. The microorganisms were isolated from prepared EM solutions and prepared organic fertilizers according to cultural and microscopic morphology at the Department of Biotechnology, Mandalay Technological University, Myanmar. The microorganisms that contained in prepared EM solutions and prepared organic fertilizers were found to be Bacillaceae, Staphylococcaceae and Leptotrichiaceae and Enterobactriaceae. Moreover, physicochemical properties such as pH, moisture, available nitrogen, available phosphorus, available potassium, organic carbon, carbon-nitrogen ratios of these fertilizers were determined revealing the values of pH 7.07 and 7.16, 7.83% and 9.92% moisture, 1.69% and 1.66% available nitrogen, 1.59% and 1.63% available phosphorus, 1.67% and 1.46% available potassium, 13.03% and 17.48% organic carbon, 7.71 and 10.53 carbon- nitrogen ratio which were based on aerobic and anaerobic conditions. The yield percent of these fertilizers were found to be 50.56% and 46.21%. The elemental contents were examined by using EDXRF Spectrometer. The minerals present in two prepared organic fertilizers were Si 10.71% and 8.70%, Ca 2.12% and 1.76%, Al 2.12% and 1.76%, Fe 1.78% and 1.38%, K 1.51% and 2.05%, P 0.93% and 0.72%, S 0.17% and 0.22% respectively.

KEYWORDS: EM solutions, prepared organic fertilizers, microorganisms, aerobic and anaerobic conditions, NPK

1. INTRODUCTION

Generally, both organic and inorganic fertilizers are used for the cultivation of plants. With the escalating interest in organic vegetable cultivation due to its health and environmental benefits both locally and globally, intensified research on all aspects of organic farming is timely and urgent. High-quality compost is one of the essential organic matter inputs, along with green manures, used to manage soil health in organic farming and gardening systems (Miles, 2015).

One of the main goals of every organic farmer is to build long-term soil fertility by feeding the soil with a variety of natural amendments. The regular addition of compost is one of the best ways to enhance soil organic and humic content, which helps to build a fertile soil structure. Such a soil structure makes better use of water and nutrients. It is easier to till and, overall, is better able to achieve optimum yields on a long-term (Baldwin and Jackie, 2006).

Compost, as a product of recycling processes, can be a very appropriate input material for organic farming, provided the composting process is well-managed, the input materials are free of contaminants, and the resulting product is applied according to the system's ecological needs (Streminska and Raviv, 2016). EM solution rich in plant nutrients and has excellent fertilizer qualities and has great potential worldwide as a sustainable alternative to mineral fertilizers. EM consists of mixed cultures of beneficial and naturally according microorganisms that can be applied as inoculants to increase the microbial diversity of soil and plant. The inoculation of EM cultures to the soil/ plant ecosystem can improve soil quality, soil health and the growth, yield and quality of crops (Higa *et al.,* 1994).

EM contains selected species of microorganisms including predominant populations of lactic acid bacteria and yeasts and smaller numbers of photosynthesis bacteria, actinomycetes and other types of organisms. All of these are mutually compatible with one another and coexist in liquid culture (Higa *et al.* 1994).The organic fertilizer production line is commonly used to process different fermented organic substance into bio-organic fertilizer. Animal manure and agricultural waste are recycled as the main raw materials, thus manure and dung waste is not only creating economic benefits for the interprise , but also making a great contribution to environment projects for mankind.

Therefore, the present works explores the preparation of EM solution form four different conditions, the production of biogas from these four different conditions and the preparation of two organic fertilizers and isolation of microorganisms.

Aim

The main aim of the research is to prepare EM solutions and organic fertilizer, and analyze the isolated microorganism form EM solutions and prepared organic fertilizers.

2. EXPERIMENT

Sample Collection and Preparation

The natural waste materials such as vegetable wastes, bat dung, soil and rice straw were collected for the production of biogas and organic fertilizers. Vegetable waste was collected from local market, Chanmyatharsi Township, Mandalay Region. Bat dung was collected from Ballon Kyune Village, Nyaung Oo Township, Mandalay Region. Rice straw and soil were collected from Tamokesoe Village, Amarapura Township, Mandalay Region. Vegetable waste samples were cut into small pieces and then used throughout experiment. Bat dung samples were powdered and sieved with 60 seive mesh size to get the size of powder. This condition was ready to use. Soil samples were powderedd and sieved with 60 seive mesh size to get powder sample. Rice straw samples were cut into small pieces.

Vegetable wastes | Bat dung Powder | Soil Powder | Rice Straw

Preparation of Effective Microorganism (EM) solution

Effective microorganism (EM) solution was prepared from vegetable wastes in four different conditions by anaerobic digestion. These conditions are (1) vegetable wastes only (2) vegetable waste and soil, (3) vegetable waste and bat dung, (4) vegetable waste, bat dung and soil. The fermentation time took about one month to obtain effective microorganism (EM) solution.

Production of Biogas

The biogas was also produced from these four different conditions. In the first condition, small pieces of vegetable waste only (6kg) were put into the anaerobic digester. The neck of the digester was entwined with Teflon. It was also tightly sealed with the lid connecting delivery tube (pipe). While the preparation of effective microorganism solution, the biogas was evolved. The biogas was collected by downward displacement of water. After the digester was kept for the five days, the gas production was checked. The amount of liberated biogas was recorded as day by day. The prepared digester was shown in fig (1). Similarly, according to the above procedure, biogas was collected from the condition 2, 3 and 4.

Fig 1 Preparation of effective microorganism solution and production of biogas

Preparation of Organic Fertilizer

The organic fertilizers were prepared from vegetable waste, soil, bat dung, straw and EM solution by two different conditions such as aerobic condition and anaerobic condition.

The following successive layers are piled on top to this.

1. A layer of 0.25 kg of straw
2. A layer of 0.175 kg bat dung
3. A layer of 0.75 kg soil
4. A layer of 0.25 kg vegetable waste
5. A layer of 0.8 kg EM solution
6. A layer of 0.25 kg straw
7. A layer of 0.175 kg bat dung
8. A layer of 0.75 kg soil
9. A layer of 0.25 kg vegetable waste
10. A layer of 0.8 kg EM solution
11. A layer of 0.25 kg straw
12. A layer of 0.175 kg bat dung
13. A layer of 0.75 kg Soil
14. A layer of 0.25 kg vegetable waste
15. A layer of 0.8 kg EM solution
16. A layer of 0.25 kg Straw

In this way, the container was composed of sixteen layers. The layers were arranged in order of increasingly easier to decompose from the bottom to the top.

Fig 2 The preparation of organic fertilizer in aerobic condition

Turning Over for the Sample under Aerobic Condition

During decomposition the layers were turned over regularly, in order that it remains well aerated and all the materials were converted into compost. The first turning over was done after two weeks. The second turning over took place after two weeks. Then, each turning over was done after one week. During the process, water was sprinkled over the container, if necessary. After two months, decomposition was complete because the plant materials were changed into an unrecognizable crumbly dark mass. However, some stalks do not decompose completely and can still be seen.

Fig 3 The preparation of organic fertilizer in anaerobic condition

Determination of Yield Percent of Prepared Organic Fertilizer (POF)

The prepared organic fertilizers were dried and the yield percent of these fertilizers were determined based upon the total weight of selected materials used.

POF – 1
(Aerobic condition)

POF – 2
(Anaerobic condition)

Table(1) Determination of Physicochemical Properties of Organic Fertilizer

No.	Analytical items	Analytical methods / Instruments
1.	Elemental contents	EDXRF Spectrophotometer
2.	pH	pH meter
3.	Moisture content	Oven drying method
4.	Available N	Alkaline Permanganate method
5.	Available P	Olsen's method
6.	Available K	Atomic absorption spectrophotometer
7.	Organic carbon	Walky and Black's method

Determination of Elemental Analysis of Prepared Organic Fertilizer

Elemental Analysis of prepared organic fertilizers was performed at Department of Physics, University of Mandalay, by applying EDXRF (Energy Dispersive X- Ray Fluorescence) Spectrometer.

Isolation of Microorganism from EM solutions and Two Prepared Organic Fertilizers

Isolation of microorganism from four conditions of EM solutions and two prepared organic fertilizers were done at Department of Biotechnology, Mandalay Technological University, Myanmar. The microscopic morphologies of microorganisms were also studied.

3. Results and Discussion

Table (2) Results of Evolved Biogas

No.	Sample	Biogas for one day(mL)	Biogas for five days(mL)
1.	Vegetable waste only (6 kg)	5400	13000
2.	Vegetable waste(3kg)+ soil(3kg)	3600	5600
3.	Vegetable waste(3kg)+ bat dung(3kg)	2225	6600
4.	Vegetable waste(2kg)+ bat dung(2kg) +soil(2kg)	2000	2700

According to experimental data, production of biogas from vegetable waste only gave rise to the highest amount of biogas.

Table(3) Yield Percent of Prepared Organic Fertilizer

No	Prepared Organic Fertilizer	Total weight of adding materials (g)	Dried weight of Prepared Organic Fertilizers (g)	Yield percent (%)
1.	POF -1	6925	3500	50.56
2.	POF -2	6925	3200	46.21

POF-1 = prepared organic fertilizer in aerobic condition
POF-2= prepared organic fertilizer in anaerobic condition

According to this table, the yield percent of prepared organic fertilizer-1 in aerobic condition was found to be higher than that of prepared organic fertilizer-2 in anaerobic condition.

Table(4) pH Values of Prepared Organic Fertilizer and Commercial Organic Fertilizer

No.	Organic Fertilizer	pH
1.	POF - 1	7.07
2.	POF - 2	7.16
3.	Commercial	7.67

This table showed that the pH of two prepared organic fertilizer is less than that of commercial organic fertilizer. It was observed that the two prepared organic fertilizers were nearly neutral.

Table (5) Moisture Content of Prepared Organic Fertilizer and Commercial Organic Fertilizer

No.	Organic Fertilizers	Moisture content (%)
1.	POF -1	7.83
2.	POF -2	9.92
3.	commercial	15.23

According to these results, it can be seen that the two prepared organic fertilizers were less moist than the commercial organic fertilizer.

Table (6) Available N, P, K Values of Prepared Organic Fertilizer and Commercial Organic Fertilizer

No.	Organic Fertilizer	Available Nitrogen (%)	Available Phosphorus (%)	Available Potassium (%)
1.	POF-1	1.69	1.59	1.67
2.	POF-2	1.66	1.63	1.46
3.	Commercial	0.86	0.55	0.46

From N, P, K determination, it can be observed that available nitrogen, phosphorus and potassium values of two prepared fertilizers are higher than that of commercial organic fertilizer.

Table (7) Organic Carbon Value and Carbon-Nitrogen ratio of Prepared Organic Fertilizer and Commercial Organic Fertilizer

No.	Organic Fertilizer	Organic Carbon(%)	C:N Ratio
1.	POF-1	13.03	7.71
2.	POF-2	17.48	10.53
3.	Commercial	4.88	5.67

According to this table, carbon – nitrogen ratios of both two prepared organic fertilizers were observed to be higher than that of commercial organic fertilizer.

Table (8) Elemental Analysis of POF-1 and POF-2

No	Element	POF-1	POF-2
1.	Si	10.71%	8.70 %
2.	Ca	2.12 %	1.76 %
3.	Al	1.88 %	1.10 %
4.	Fe	1.78 %	1.38 %
5.	K	1.51 %	2.05 %
6.	P	0.93 %	0.72 %
7.	Ti	0.18%	0.12 %
8.	S	0.17 %	0.22%
9.	Mn	0.08 %	0.07 %
10.	Ba	0.04 %	0.04 %
11.	Zn	0.02 %	0.02 %

According to elemental analysis, it can be seen that silicon was highest value in both prepared organic fertilizers. Phosphorus was found to be relatively low amount in both POF-1 and POF-2 .Because of the high amount of silicon, both prepared organic fertilizers may resist in many plants to disease and pests.

Microscopic Morphology and Family of Bacteria in EM Solution and Two Prepared Organic Fertilizers

The bacteria observed in all conditions were Gram positive. All of bacteria in EM solution were family of Bacillaceae, Staphylococcaceae and Leptotrichiaceae. Family of bacteria for aerobic prepared organic fertilizer was Bacillaceae and for anaerobic prepared fertilizer was Entero bacteriaceae. The shapes and sizes of bacteria were different.

Table (9) Colony and Microscopic Morphologies of Microorganisim in EM Solution and Prepared Organic Fertilizers

Sample	Gram strain	Shape	Size	family	Microorganism
Condition-1 liquid	+	Rod chain	1 µm × 2-3 µm	Bacillaceae	2
	+	Single rod	1 µm × 2-3 µm	Bacillaceae	2
Condition-1 residue	+	Rod	2 µm × 2.5-3 µm	Bacillaceae	2
	+	Rod	3 µm × 4.5-5 µm	Bacillaceae	2
Condition-2 liquid	+	Rod cluster	1 µm × 2-3 µm	Bacillaceae	2
	+	Cocci cluster	1 µm	Staphylococcaceae	2
Condition-2 residue	+	Rod	1.5 µm × 3 µm	Bacillaceae	2
	+	Rod	1 µm × 2 µm	Bacillaceae	2
Condition-3 liquid	+	Rod chain	1 µm × 2-3 µm	Bacillaceae	2
	+	Cocci cluster	1 µm	Staphylococcaceae	2
Condition-3 residue	+	Rod	1 µm × 2 µm	Bacillaceae	2
	+	Rod chain	2 µm × 4 µm	Leptotrichiaceae	2
Condition-4 liquid	+	Rod chain	1 µm × 2-3 µm	Bacillaceae	2
	+	Rod chain	1 µm × 3-5 µm	Bacillaceae	2
Conditi	+	Rod	1.5 µm ×	Bacillacea	2

on-4 residue			2-3 µm	e	
	+	Rod chain	1.5 µm ×4-5 µm	Leptotrichiaceae	2
POF-1	+	Rod	1 µm × 2.5-3 µm	Bacillaceae	2
	+	Rod	1µm × 3.4 µm	Bacillaceae	2
POF-2	+	Rod	0.5 µm × 1 µm	Bacillaceae	2
	+	Spiral cluster	0.5 µm × 1.2 µm	Enterobacteriaceae	2

Condition -1 =Vegetable waste only

Condition -2 =Vegetable waste and soil

Condition -3 =Vegetable waste and bat dung

Condition -4 =Vegetable waste, bat dung and soil

According to this table, Condition-1(liquid and residue) contains microorganisms of Bacillaceae. Condition-2 (liquid) contains microorganisms of Bacillaceae and Staphylococcaceae. Condition-2 (residue) contains microorganisms of Bacillaceae. Condition- 3 (liquid) contains microorganisms of Bacillaceae and Staphylococcaceae. Condition-3 (residue) contains microorganisms of Bacillaceae and Leptotri chiaceae. Condition- 4 (liquid) contains microorganisms of Bacillaceae. Condition- 4 (residue) contains microorganisms of Bacillaceae and Leptotrichiaceae. POF-1 contains microorganisms of Bacillaceae. POF-2 contains microorganisms of Bacillaceae and Enterobacteriaceae.

Condition - 1 (Vegetable Waste Only)

Condition-1 (liquid and residue), the two aerobic bacterial were observed by the following figures.

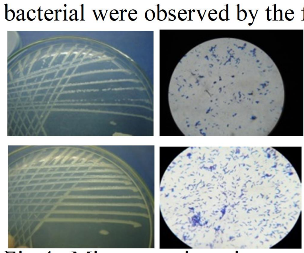

Fig 4 Microorganisms in Condition- 1 (liquid)

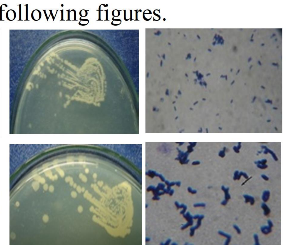

Fig 5 Microorganisms in Condition- 1 (residue)

Condition - 2 (Vegetable Waste and Soil)

Condition-2 (liquid and residue), the two aerobic bacterial were observed by the following figures.

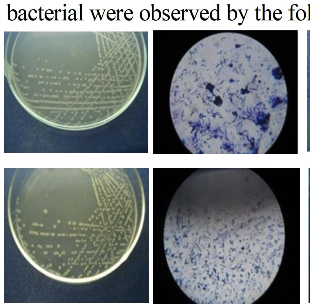

Fig 6 Microorganisms in Condition- 2 (liquid)

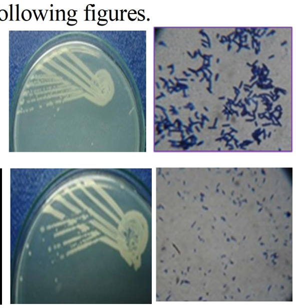

Fig 7 Microorganisms in Condition- 2 (residue)

Condition - 3 (Vegetable Waste and Bat dung)

Condition-3 (liquid and residue), the two aerobic bacterial were observed by the following figures.

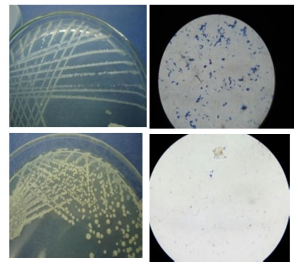

Fig 8 Microorganisms in Condition- 3 (liquid)

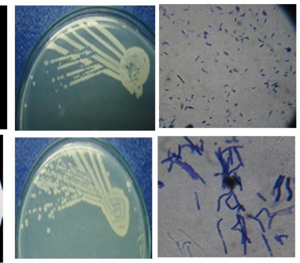

Fig 9 Microorganisms in Condition- 3 (residue)

Condition - 4 (Vegetable Waste, Bat dung and Soil)

Condition- 4 (liquid and residue), the two aerobic bacterial were observed by the following figures.

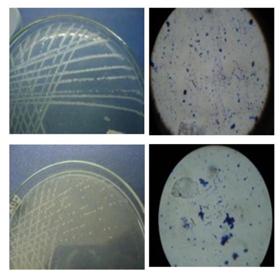

Fig 10 Microorganisms in Condition- 4 (liquid)

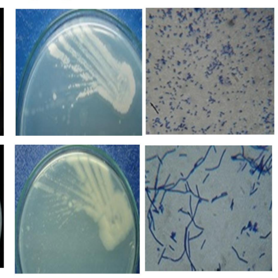

Fig 11 Microorganisms in Condition- 4 (residue)

Prepared Organic Fertilizer with Bat Dung (Anaerobic and aerobic Condition)

Two aerobic bacterial were observed in POF- I and POF-II by the following figures.

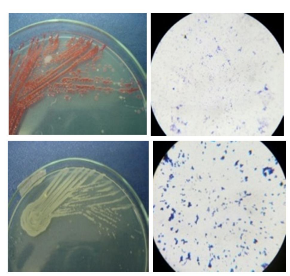

Fig 12 Microorganisms in Condition- 5 (liquid)

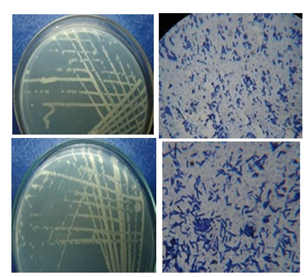

Fig 13 Microorganisms in Condition- 5 (residue)

4. Conclusion

In this research work, while EM solutions were prepared from vegetable wastes by primary fermentation, biogas was evolved. According to the experimental data, the evolved biogas from vegetable wastes only was found to be the highest amount. And then, organic fertilizers were prepared under the aerobic and anaerobic conditions by using vegetable wastes, bat dung, soil, rice straw and prepared EM solution. The yield percent of the prepared organic fertilizers were found to be 50.56% for aerobic condition and 46.21% anaerobic condition upon the total weight of used material. pH of the two prepared organic fertilizers was found to be nearly natural (7.07, 7.16). According to the moisture contents, the two prepared organic fertilizers (7.83%, 9.92%) were less moist than the commercial organic fertilizer.

From the isolation and microscopic morphology of the microorganisms, the effective microorganisms were observed in prepared EM solutions and prepared organic fertilizers. Bacillaceae, Enterobacteriaceae, Staphylococcaceae and Leptotrichiaceae species may be attractive biological control agents and good plant growth promoting bacteria for growth enhancement and plant disease control. From the comparison of N, P, K values, our prepared organic fertilizer can supply more N, P and K than that of commercial fertilizer. Carbon-nitrogen ratios of both fertilizers were higher than that of commercial organic fertilizer. Because of the organic matter present in organic fertilizer, soil structure is improved and as a result the soil's ability to hold onto water and nutrients increases.

According to the elemental analysis, silicon was the highest value in both prepared organic fertilizers. Since the silicon generates the resistance in many plants to disease and pests, it may contribute to reduce the rate of application of pesticides and fungicides. Therefore, organic fertilizer should be used widely in agriculture instead of mixed with chemical fertilizer because of their low cost, good fertility of the soil and supplying more trace elements.

Myanmar is an agricultural country. And so the findings of this research will be grate helpful for the people working on agriculture. Instead of using chemical fertilizer, using organic fertilizer can reduce the danger of the lives of the consumers and can give the consumers the benefit of the health.

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