Combination of bacteria-fungi in five formulations of carrier and its effectiveness on composting of corn stalk waste

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Abstract. Bacteria and Fungi combination as decomposer in a few formulation and stored for several months after production. Research aim to study viability and effectivity of bacteria-fungi combining decomposer for maize waste composting. Material that using there were five carrier composition formula ie. BAGM (rice flour, charcoal flour, peat soil, and molasses), AAM (charcoal flour, jelly, and molasses), SAM (husk, jelly, and molasses), BKM (rice flour, coconut water, and molasses), SGM (husk, peat soil, and molasses), and commercial biodecomposer Promi and EM4 as the check treatments. Isolate combination were using ie. bacteria+fungi BO(B7.1+O5) and EP(E7.7+P7). Results indicated that commonly carrier composition formula where tested was effective better than Promi and EM4 in maize waste composting. Carrier formula plus bio decomposer that consistently produce the better of compost after stored 12 and 24 WAP (weeks after production) i.e. combination of SAMEP, BAGMBO, SGMBEP and AAMEP and have opportunity to mass production for expansion.

1. Introduction

Previous research has conducted isolation of several fungi and bacteria originating from the soil in various types of plantations in South Sulawesi. A total of 119 bacterial isolates were tested, 23 cellulolytic isolates were obtained [6]. Subsequently, 23 effectiveness of fungal isolates were taken to test the effectiveness of 13 fungi isolates that had the most potential to decompose corn plant waste [7]. Furthermore [8] have tested the combination of bacterial isolates and decomposer fungus in vitro. The microbial viability test was continued on three types of storage media and tested its effectiveness in leaf waste and corn stalk in vitro [9]. Fungi represented by 12 genera generally produce extra cellular enzymes that can degrade plant cell walls [1]. Intermediate decomposition products of fungi are utilized by bacteria and total bacterial activity is low without the presence of fungi [16]

Furthermore it is said that bacteria and fungi contribute to essential ecological functions, such as recycling organic carbon material trapped in cellulose and lignin. White and Brown rot fungi contribute greatly to the mitigation of environmental pollution [17]. The success of organic waste decomposition depends on several factors including: type of raw material, nutritional composition, humidity, temperature, acidity, salinity and aeration [3].

As a quick composting starter it is better to use sawdust for cellulolytic fungi inoculum [11]. BFC8 bacterial isolates have high efficiency to degrade carboxymetil cellulose because it is used as composting inoculants [12]. To optimize the activity of carboxymethyl bacteria and fungi in decomposing cereal waste, the combined formulations of these isolates were tested using five types of carrier formulations.

This research aims to get the best carrier formulation in supporting the viability of microbial bio decomposers for the manufacture of organic fertilizer.
2. Materials and Methods

The study was conducted in May to August 2017 at the Pest and Disease Laboratory and Green House Cereals Plant Research Institute, Maros, South Sulawesi, Indonesia. The experimental design used was a randomized group with three replications. The materials used are five carrier compositions namely BAGM formulations (rice flour, charcoal flour, peat, and molasses), AAM (charcoal flour, jelly, and molasses), SAM (husk, jelly, and molasses), BKM (rice flour, coconut water, and molasses), SGM (husk, peat soil, and molasses), plus 2 commercial bio decomposers Promi and EM4 as a comparison. This isolate was the result of selection from the decomposition of the trunk, leaf and corn waste from the researchers’ collection. To test the effectiveness of formulation of decomposer carriers against the stems of the leaf and stems, 11 treatments were tested (Table 1).

2.1. Preparation of Carrier and Inoculant Formulations

Preparation of carrier material, each carrier was mixed in accordance with each treatment in Table 1, then the powder carrier was put into heat-resistant plastic and closed using a clip while the liquid carrier was put into an erlenmeyer, then sterilized using an autoclave with temperature 121°C for 30 minutes.

Decomposer bacterial inoculant production, a single colony of bacterial isolates was scratched on Nutrient Agar (NA) medium and incubated 2x24 hours. After incubation, it is then dissolved into 50 ml of Nutrient Broth (NB) media and then incubated in a shaker for 1x24 hours (pre culture). Then 1 ml of pre culture was taken mixed with 200 ml of coconut water, then incubated for 3x24 hours in a shaker with a speed of 200 rpm (main culture).

Production of decomposer fungus inoculants, fungus isolates were propagated on Potato Dextrose Agar (PDA) medium and incubated for 3x24 hours. After incubation, it was then dissolved into 50 ml of Potato Dextrose Broth (PDB) medium and then incubated in a shaker for 1x24 hours (pre culture). Then take 10 ml of pre culture mixed with 200 ml of coconut water, then incubated for 3x24 hours in a shaker with a speed of 200 rpm (main culture).

Formulation of decomposer microbial isolates, each combination of 100 g carrier material mixed with 5% sucrose, 0.5% Yeast Extract, and 0.5% molasses and then sterilized for 15 minutes at 121°C. After that, 20 ml of microbe/100 g suspension is added. Furthermore, the carrier material is flattened until it is homogeneous, stored in sterile plastic bottles, and labeled according to the name of the carrier and the type of inoculant. The bottles are stored at room temperature (30 °C).

2.2. Preparation of Bacteria and Fungi Bio decomposer Isolates for Application

The viability test of the formulation is carried out by inserting 1 g of the carrier formula into 9 ml of physiological solution), then shaking it so that the solution becomes homogeneous and after that makes a series of dilutions. Each inoculant was grown on NA media for bacteria and PDA for fungi. Furthermore, the observations made were counting the number of bacterial colonies and fungi that grew. Observations are made periodically at 4, 8, 12, 16, 20 and 24 weeks after production. Colonies of bacteria and mold growing spores were observed and the total population was calculated using the agar plate method (Plate counting) [14], using the formula:

\[
\text{Bacterial population (Cfu g}^{-1}) = \frac{A}{V \cdot \text{df}}
\]

Information:
Cfu: Colony forming unit
A: Average number of colonies/petri
df: Dilution factor
V: Volume of suspended culture is spread
Table 1. Types of combined treatment of decomposer carriers and microbes, Maros 2017.

| Treatments     | Carrier combination                      | Microbe Combination |
|----------------|------------------------------------------|---------------------|
| BAGM+EP (liquid) | rice flour + charcoal flour + peat + molasses 350 g + 500 g + 900 g + 1500 ml | E7.7 + P7          |
| BAGM+BO (liquid) | rice flour + charcoal flour + peat + molasses 350 g + 500 g + 900 g + 1500 ml | B7.1 + O5          |
| AAM+EP (powder)  | charcoal + jelly + molasses 1000 g + 35 g + 5% | E7.7 + P7          |
| AAM+BO (powder)  | charcoal + jelly + molasses 1000 g + 35 g + 5% | B7.1 + O5          |
| SAM+EP (powder)  | husk + jelly + molasses 750 g + 35 g + 5% | E7.7 + P7          |
| SAM+BO (powder)  | husk + jelly + molasses 750 g + 35 g + 5% | B7.1 + O5          |
| BKM+EP (liquid)  | rice flour + coconut water + molasses 100 g + 800 ml + 5% | E7.7 + P7          |
| BKM+BO (liquid)  | rice flour + coconut water + molasses 100 g + 800 ml + 5% | B7.1 + O5          |
| SGM+BEP (powder) | husk + peat + molasses 500 g + 1000 g + 5% | B7.1 + E7.7 + P7    |
| EM 4 (liquid)    | -                                        | -                   |
| Promi (powder)   | -                                        | -                   |

2.3. Testing the Effectiveness of Bio decomposers on composting of corn leaf and stalk waste.

The effectiveness test of bio decomposers with various carrier formulations is carried out in a greenhouse. Using corn stalk as compost material with the following procedures: The testing activity of a combination of bacteria and fungi was effective to decompose corn stalk waste. The bacteria-fungus combination decomposers used were effective isolates from the 2016 study. Using a randomized block design, three replications, treatment consisted of 3 kinds of decomposer combinations with the best carrier or carrier as a result of the 2016 research. The isolate combinations were: E7.7 + P7, E7.7 + O5, B7.1 + E7.7 + P7 use various carrier formulations with EM4 and Promi as a comparison, so we get 11 treatment combinations.

Testing the effectiveness of decomposer formulations using 10 kg of corn stalk each treatment as a single material for composting through the following work processes: Waste corn stalks cut into pieces 2-3 cm, then dried in the sun for 3 days, so the water content of about 10%. The stems of corn leaf stems are weighed 10 kg and put into each black plastic bag according to the combination treatment of the isolates used. Corn stalk waste material in a plastic bag mixed with decomposer bacterial isolates that have been aged 36 hours shaken for 2 hours until evenly mixed, diluted as much as 25 g/250 ml of distilled water and diluted to a volume of 600 ml for three replications. Potential decomposer fungus isolates that have been propagated in the media of husk + glucose concentration of 3% which have been sterile and incubated for 7 days are mixed evenly with corn leaf stem waste according to the treatment combination. Added distilled water (50 ml) to reach about 60% humidity, marked by squeezing out no water. Compost material that has been mixed evenly is put back into a plastic bag container pressed/compacted and then closed bound and stored in a greenhouse for fermentation. All 33 units were arranged neatly in the order of treatment and repetition. Reversal/stirring once a week to spread the temperature, humidity and air, while making several observations.

Observations include: Initial weight of corn stalk waste compost material, measurement of temperature and pH of corn stalk waste once every week, period (weeks) needed to decompose completely, weigh the final weight of corn stalk waste when fully decomposed, C: N ratio, and analysis nutrient content of N, P, K compost after mature.
3. Results and Discussion

3.1. Biodecomposer Viability Test in Carrier Formulations.

Viability of bacteria and decomposer fungi in each sterilized carrier until the storage period of 4 weeks after production at room temperature (30 °C) showed an increase in total (Tabel 2).

| Treatments  | Bacteria population (x10^8 cfu g^-1) 4 weeks after production (wap) | Total microbial (x10^8 cfu g^-1) |
|-------------|-----------------------------------------------------------------------|---------------------------------|
| BAGMBO      | 1,3                                                                   | 2.5                             | 3.8  |
| BGMO        | 2.6                                                                   | 24.1                            | 26.7 |
| AAMEP       | 22.0                                                                  | 2.6                             | 2.6  |
| AAMBO       | 33.0                                                                  | 1.8                             | 34.8 |
| SAMEP       | 11.1                                                                  | 1.9                             | 13.0 |
| SAMBO       | 4.1                                                                   | 2.7                             | 6.8  |
| BKMEP       | 10.8                                                                  | 3.2                             | 14.0 |
| BKMO        | 31.1                                                                  | 0.7                             | 31.8 |
| SGMBEP      | 30.7                                                                  | 1.2                             | 31.9 |
| EM4         | 25.2                                                                  | 1.6                             | 26.8 |

Using various carrier materials composition as decomposer combine bacteria-fungi do not give significant difference with control of EM4 and Promi. Untill 35 days after inoculation to accelerate in maize stover decomposition upon which compost at 12 weeks after production (wap). But if seen at degradation of weight compost there were some carrier formula giving degradation toward initial weigh is better than EM4 by in series SGMBEP, SAMEP, AAMEP, BKMEP, SAMBO (Table 3).

| Treatments | Days after inoculation (dai) | Weight decrease (kg) |
|------------|------------------------------|----------------------|
|            | 0 dai                        | 7 dai                | 14 dai               | 21 dai               | 28 dai               | 35 dai               |
| BAGMBO     | 15,65 b                      | 15,51 t              | 15,46 t              | 15,28 t              | 15,12 t              | 15,05 t              | 0,53 t               |
| AAMEP      | 16,30 ab                     | 15,91                | 15,83                | 15,63                | 15,42                | 15,20                | 0,88                 |
| AAMBO      | 15,60 b                      | 15,44                | 15,36                | 15,18                | 15,17                | 15,04                | 0,43                 |
| SGMBEP     | 16,27 ab                     | 15,71                | 15,67                | 15,42                | 15,14                | 14,71                | 1,13                 |
| SAMEP      | 16,93 a                      | 16,32                | 16,24                | 16,14                | 15,99                | 15,48                | 0,94                 |
| BAGMEP     | 15,68 b                      | 15,53                | 15,39                | 15,24                | 15,13                | 14,81                | 0,55                 |
| BKMEP      | 16,24 ab                     | 15,86                | 15,66                | 15,56                | 15,49                | 15,14                | 0,75                 |
| BKMO       | 16,14 ab                     | 16,03                | 15,92                | 15,76                | 15,65                | 15,44                | 0,49                 |
| SAMBO      | 16,29 ab                     | 16,11                | 16,02                | 15,94                | 15,55                | 15,65                | 0,74                 |
| EM4        | 16,49 ab                     | 16,28                | 16,18                | 16,12                | 15,86                | 15,54                | 0,63                 |
| PROMI      | 16,38 ab                     | 16,16                | 16,12                | 16,03                | 15,93                | 15,69                | 0,45                 |

Notes: BAGMBO=rice flour+charcoal+peat soil+Molase+B7.1+O5; AAMEP=Charcoal+Jelly+Molase+E7+P7; AMBO=Charcoal+Jelly+Mol+B7.1+O5; SGMBEP=chaff+Peat+Mol+E7+P7; SAMEP=chaff+Jelly+Molase+E7.1+P7; BAGMEP=rice flour+Charcoal+Peat+Molase+E7+P7; BKMEP=rice flour+Coconut water+Mol+E7.1+P7; BKMO=rice flour+Coconut water+Mol+E7.1+O5; SAMBO=chaff husk+Jelly+Molase+B7.1+O5.

To see the viability and effectiveness of the fungus bacterial decomposer at 24 dap (days after production), the effectiveness of composting on corn leaf stem waste was tested and the results showed that there was a significant difference in compost weight from the carrier formulation tested at 7, 14 and 21 dai (days after inoculation). But there is no difference in 28 dai and 35dai (Table 4). This shows that microbial activity increased at 7, 14 and 21 dai. The decrease in compost weight to initial
weight is higher than EM4. Consecutively from highest to lowest in the formulation: BAGMBO, AAMEP, BAGMEP, SAMBO, and SGMBEP (Table 3). And when seen in decreasing the weight of compost, the combination of decomposer fungus bacteria at 24 wap with SAMEP, BAGMBO, AAMEP, BAGMEP and SAMBO carrier formulations has the opportunity to be developed through mass propagation.

**Table 4.** Weight of compost corn waste in a combination of bacteria and fungi with various carrier formulations at 24 wap, Maros 2017

| Treatment  | Days after inoculation (dai) | Weight reduction (kg) |
|------------|-----------------------------|----------------------|
|            | 0   | 7   | 14  | 21  | 28  | 35  |            |
| BAGMBO     | 16.46 tn | 15.23 ab | 14.93 bc | 14.45 ab | 13.22 tn | 12.94tn | 3.24 |
| AAMEP      | 15.35 | 13.87 b | 13.57 c | 13.33 b | 13.16 | 13.09 | 2.19 |
| AAMBO      | 15.49 | 14.96 ab | 14.52 abc | 14.16 ab | 13.72 | 13.53 | 1.77 |
| SGMBEP     | 15.83 | 15.13 ab | 14.77 abc | 14.21 ab | 13.96 | 13.85 | 1.87 |
| SAMEP      | 16.10 | 14.20 ab | 13.56 c | 13.41 ab | 13.20 | 12.64 | 3.46 |
| BAGMEP     | 16.22 | 15.79 ab | 15.44 a | 14.78 ab | 14.28 | 13.82 | 1.94 |
| BKMEP      | 15.99 | 15.21 ab | 15.06 ab | 14.88 ab | 14.76 | 14.18 | 1.23 |
| BKMBO      | 16.72 | 16.20 a | 15.65 a | 15.18 ab | 14.99 | 13.97 | 1.73 |
| SAMBO      | 16.47 | 16.09 ab | 15.49 a | 15.03 ab | 14.34 | 14.01 | 1.89 |
| EM4        | 16.38 | 15.44 b | 14.85 abc | 15.02 ab | 14.58 | 14.30 | 1.80 |
| PROMI      | 16.36 | 16.11 ab | 15.82 a | 15.52 a | 15.14 | 14.44 | 1.22 |
| KK         | 3.26 | 4.47 | 4.60 | 4.46 | 4.89 | 5.54 | 4.35 |

Note: BAGMBO = Rice + Charcoal + Peat + Molasses + B7.1 + O5; AAMEP = Charcoal + Jelly + Molassel + E7 + P7; AAMBO = Charcoal + Jelly + Molasses + B7.1 + O5; SGMBEP = Husk + Peat + Molasses + E7 + P7; SAMEP = Husk + Jelly + Molasses + E7.1 + P7; BAGMEP = Rice + Charcoal + Peat + Molasses + E7.1 + P7; BKMEP = Rice + Coconut Water + Molasses + E7.1 + P7; BKMBO = Rice + Coconut Water + Molasses + B7.1 + O5; SAMBO = Husk + Jelly + Molasses + B7.1 + O5.

Laboratory analysis of corn leaf stalk mature compost at 4 weeks after inoculation. Using bacterial-fungus combination bio decomposers after being stored for 24 wap (weeks after production) at room temperature of 30°C, showed that the N, P and K nutrient content of compost was higher than that of corn stover without inoculants. While the content of C-organic and C: N there was a decrease in compost material. The pH is somewhat alkaline and the water content is quite good because all of them are below 50% (Table 5). The decomposition rate of compost material with the main indicator is C:N [17]. Some other indicators to assess the rate of composition of agricultural waste such as increased CO2 release, loss of crude fiber, reduction of glucose, and reduction in fat and protein [4]. There are several bacteria-fungi combination bio decomposers giving lower C: N compared to EM4 and Promi sequentially, namely: SAMEP (19.74), BAGMBO (20.88), AAMEP (21.08), SAMBO (21.23), BKMEP (21.50) and SGMBEP (21.93). If C: N > 20, the microbes use N for the process of metabolism, conversely if C: N < 20 then N can be available to plants [5]. In general, plant residues have C: N around 50-150 [19]. While [14] reported that the best C:N compost for use was in the range of 20-40. As for carrier formulations which consistently show good compost results when storing 12 and 24 MSP are a combination of SAMEP, BAGMBO, SGMBEP, SAMBO and AAMEP.
Table 5. Chemical analysis of corn waste mature compost using a combination of fungal bacteria various carrier formulations at 24 wap, Maros 2017.

| Treatment  | N (%) | P (%) | K (%) | Organic-C (%) | pH  | C:N  | Water content (%) |
|------------|-------|-------|-------|---------------|-----|------|-------------------|
| BAGMBO     | 1.50  | 0.17  | 4.76  | 31.32         | 8.02| 20.88 | 40.1              |
| AAMEP      | 1.48  | 0.17  | 4.93  | 31.20         | 8.10| 21.08 | 38.8              |
| AAMBO      | 1.44  | 0.15  | 5.04  | 32.86         | 7.80| 22.82 | 43.5              |
| SGMBEP     | 1.46  | 0.16  | 4.95  | 32.02         | 8.10| 21.93 | 38.7              |
| SAMEP      | 1.52  | 0.20  | 5.03  | 31.40         | 7.70| 19.74 | 29.5              |
| BAGMEP     | 1.45  | 0.15  | 4.72  | 32.39         | 8.01| 22.34 | 40.3              |
| BKMEP      | 1.47  | 0.21  | 4.85  | 31.61         | 7.80| 21.50 | 31.7              |
| BKMBO      | 1.31  | 0.15  | 4.76  | 32.92         | 8.00| 25.12 | 36.6              |
| SAMBO      | 1.52  | 0.14  | 4.90  | 32.27         | 7.74| 21.23 | 34.1              |
| EM4        | 1.35  | 0.15  | 4.66  | 35.58         | 7.75| 26.36 | 38.5              |
| PROMI      | 1.44  | 0.14  | 4.72  | 32.90         | 7.90| 22.84 | 25.7              |
| Maize stalk | 0.92 | 0.12  | 2.36  | 37.29         | 7.63| 40.53 | 12.3              |

Note: BAGMBO = Rice + Charcoal + Peat + Molasses + B7.1 + O5; AAMEP = Charcoal + Agar + Molasses + E7 + P7; AAMBO = Charcoal + Agar + Molasses + B7.1 + O5; SGMBEP = Husk + Peat + Molasses + E7 + P7; SAMEP = Husk + Agar + Mol + E7.1 + P7; BAGMEP = Rice + Charcoal + Peat + Molasses + E7.1 + P7; BKMEP = Rice + Coconut Water + Molasses + E7.1 + P7; BKMBO = Rice + Coconut Water + Molasses + B7.1 + O5; SAMBO = Husk + Agar + Molasses + B7.1 + O5.

4. Conclusions
In general, all carrier formulations tested showed better effectiveness than EM4 and Promi. Carrier formulations consistently showed good compost results when 4 wai used 12 and 24 wap stored formulations were a combination of SAMEP, BAGMBO, SGMBEP, SAMBO and AAMEP that were likely to be mass-propagated for development.

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