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The Synergy Test of Solubilizing Phospate Highland Bacteria and *Azotobacter vinelandii* Lowland Bacteria on FLUFF Tea Compost as Solid Carrier Base

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ABSTRACT: Laboratory-scale experiments have been conducted to test the effect of FLUFF tea compost and mix it with zeolit as carrier base for Solubilizing Phosphate Highland Bacteria (BPF) and *Azotobacter vinelandii* as Nitrogen fixing lowland bacteria (BPN). The experiment was using a randomized block design, two factors, three repeated. The first factor is the carrier base (100% FLUFF Tea Compost; 90% FLUFF Tea Compost + 10% Zeolite; and 75% FLUFF Tea Compost + 25% Zeolite) and the second factor is the kind of inoculant (100% BPF; 100% BPN; and mix of BPF 50% + BPN 50%). The water content from FLUFF Tea Compost was 59,8% with pH after 2 days incubation was 6,3-6,7. The respons were total bacteria and temperature on 4, 8, and 12 days after incubation. The results showed that the total bacteria from the carrier base and kind of inoculants have significant on 4 days after incubation, but not significant on 8 and 12 days after incubation. The A3B3 (75% FLUFF Tea Compost + Zeo25%) & (BPF 50%+azoto50%) was the best treatment, but if compare from before, A3B1 (75% FLUFF Tea Compost + Zeolite 25%) & (BPF 100%) have the highest average percentage during observation. The temperature was stable, it means that the decomposition proccess was not occur. From the data, we can tell that the BPF highland more adaptable than BPN lowland on FLUFF tea compost. The BPF and BPN have synergy growth on each carrier base treatment.

KEYWORDS: *Azotobacter vinelandii*, BPF, FLUFF tea compost, Incubation, Zeolite.

1 INTRODUCTION

Biofertilizers is the living microorganism that provide into the soil as inoculant to facilitated or supply a particular nutrient for plants [1]. One of them is Solubilizing Phosphat Bacteria, that can increasing the phosphat solubilising by fosphatase and organic acid as secondary metabolite. Phosphat is the secondary essensial nutrient to the plant. Availability of phosphat around 0,01% from the total of phosphat on soil. Most of fosphate bounded by soil colloidal and chelated by Fe, Al, or Ca ion. [2] Only 15 – 20% fosphat could be absorb by plant from anorganic fosphate fertilizer [3].

Azotobacter sp is the rhizobacteria that can fixation the nitrogen from the air. Generally, it can use to increasing the nitrogen on the soil and also as PGPR by produce fithohormon [4], [5]. This bacteria also have a potential to excrate some eksopolisacarida (EPS) and organic acid [6]. *Azotobacter vinelandii* particulary can produced biosurfactant [6], sitokinin [7], nitrogen fixation superior bacteria, root growth stimulant [8], and contribute on soil fertility.

A carier should be need as a carbon sources to produce biofertilizer. One of the important thing to choice a material to carier is the capability to kept population of inoculant. Some of the material can be used a carrier base are compost, zeolit, bentronite, vermiculite, and peat. The FLUFF tea compost was offgrade from tea processing. It can use a organic fertilizer on tea plantation by composting first [9]. The mixture from compost and zeolit (9:1) on 28 ^{O}C was a better carier than peat [2], [10], [11].

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2 MATERIALS AND METHODS

2.1 MULTIPLIED INOCULANT

This experiment used BPF from Research Institute for Tea and Cinchona (RITC)'s collection and *Azotobacter vinelandii* (BPN) was Padjadjaran University (UNPAD)'s collection. The inoculant source of BPF from tea rhizosphere on RITC's experimental garden with elevation 1350 – 1600 m above sea level and BPN from agricultural food plants with elevation 600 – 800 m above sea level. Each of bacteria multiplied on each liquid medium, that was Pikovskaya and Ashby's medium. After two days incubation (TPC-0), the population of BPF was 10 x 10^7 population/ml and BPN was 185 x 10^7 population/ml.

2.2 EXPERIMENTAL DESIGN

This experiment used Randomized Blok Design Factorial with two factors and three repeated. The statistic analyxe used was Analyze of Variants (ANOVA) with Duncan different test. 100% BPF meaning that 10 ml BPF on 100 g carrier. 50% BPF + 50% BPN meaning that 5 ml BPF + 5 ml BPN on mixture of 75 g FLUFF Tea Compost and 25 g Zeolite as the carrier. Thw water content of FLUFF Tea Compost was 59,8%. The arrangement treatment were :

A. Carier Factor, the level was :
    1. 100% FLUFF tea compost
    2. 90% FLUFF tea compost + 10% Zeolite
    3. 75% FLUFF tea compost + 25% Zeolite
B. Inoculant Factor, the level was :
    1. 100% BPF
    2. 100% BPN
    3. 50% BPF + 50% BPN

The respons are :

a. Bacteria Population with Total Plate Count methode
b. The temperature of compost with thermometer

3 RESULTS AND DISCUSSION

3.1 TOTAL BACTERIA POPULATION

TPC-0 was the total bacterial population before treatment, after 2 days incubation that was 195 x 10^7 population/g. After each 4, 8, and 12 days after incubation the total bacterial population was counted. The total bacterial of population on TPC-1 (after 4 days incubation), TPC-2 (after 8 days incubation), and TPC-3 (after 12 days incubation) shown on Table 1 until 3.

Table 1. Total Bacteria on TPC-1

Treatment	Total Population (10^7 cfu/g)
A1B1 (100% FLUFF Tea Compost) & (BPF 100%)	151.67 a
A2B1 (90% FLUFF Tea Compost + Zeolite 10%) & (BPF 100%)	295 ab
A1B2 (100% FLUFF Tea Compost) & (Azoto 100%)	475 ab
A2B2 (90% FLUFF Tea Compost + Zeolite 10%) & (Azoto 100%)	706.67 abc
A1B3 (100% FLUFF Tea Compost) & (BPF 50% + Azoto 50%)	883 abc
A3B1 (75% FLUFF Tea Compost + Zeolite 25%) & (BPF 100%)	928 abc
A2B3 (90% FLUFF Tea Compost + Zeo10%) & (BPF 50%+Azoto50%)	1055 abc
A3B2 (75% FLUFF Tea Compost + Zeo 25%) & (azoto 100%)	1206.67 bc
A3B3 (75% FLUFF Tea Compost + Zeo25%) & (BPF 50%+azoto50%)	1633 c

Note : The ANOVA's analysis was significant on 5% level's. The figures followed by the same letter does not significantly on 5% level's of Duncan test

Table 2. Total Bacteria on TPC-2

Treatment	Total Population (10^7 cfu/g)
A1B1 (100% FLUFF Tea Compost) & (BPF 100%)	330
A2B1 (90% FLUFF Tea Compost + Zeolite 10%) & (BPF 100%)	1176,67
A1B2 (100% FLUFF Tea Compost) & (Azoto 100%)	523,33
A2B2 (90% FLUFF Tea Compost + Zeolite 10%) & (Azoto 100%)	686,67
A1B3 (100% FLUFF Tea Compost) & (BPF 50% + Azoto 50%)	1501,67
A3B1 (75% FLUFF Tea Compost + Zeolite 25%) & (BPF 100%)	636,67
A2B3 (90% FLUFF Tea Compost + Zeo10%) & (BPF 50%+Azoto50%)	1335
A3B2 (75% FLUFF Tea Compost + Zeo 25%) & (azoto 100%)	600
A3B3 (75% FLUFF Tea Compost + Zeo25%) & (BPF 50%+azoto50%)	1150

Note : The ANOVA's analysis was not significant on 5% level's

Table 3. Total Bacteria on TPC-3

Treatment	Total Population (10^7 cfu/g)
A1B1 (100% FLUFF Tea Compost) & (BPF 100%)	348,33
A2B1 (90% FLUFF Tea Compost + Zeolite 10%) & (BPF 100%)	425
A1B2 (100% FLUFF Tea Compost) & (Azoto 100%)	1513, 33
A2B2 (90% FLUFF Tea Compost + Zeolite 10%) & (Azoto 100%)	760
A1B3 (100% FLUFF Tea Compost) & (BPF 50% + Azoto 50%)	1066,67
A3B1 (75% FLUFF Tea Compost + Zeolite 25%) & (BPF 100%)	1386 ,67
A2B3 (90% FLUFF Tea Compost + Zeo10%) & (BPF 50%+Azoto50%)	1405
A3B2 (75% FLUFF Tea Compost + Zeo 25%) & (azoto 100%)	1093,33
A3B3 (75% FLUFF Tea Compost + Zeo25%) & (BPF 50%+azoto50%)	1301, 67

Note : The ANOVA's analysis was not significant on 5% level's

The A3B3 was the best treatment on TPC-1. After 8 and 12 days incubation, all treatment was not significant on ANOVA's analysis. With compare the present TPC with TPC before (TPC-1 with TPC-0. TPC-2 with TPC-1, and so), the trend of population bacteria each treatment shown on Table 4.

Table 4. Trend of Population Bacteria

Treatment		Percent of Compare Total Population (%)			
		TPC-0	TPC-1	TPC-2	TPC-3
100% BPF	100% FLUFF Tea Compost	0,00	1416,70	117,58	5,55
	(90% FLUFF Tea Compost + Zeolite 10%)	0,00	2850,00	298,87	***-63,88***
	(75% FLUFF Tea Compost + Zeolite 25%)	0,00	9180,00	***-31,39***	117,80
100% BPN	100% FLUFF Tea Compost	0,00	156,76	10,17	189,17
	(90% FLUFF Tea Compost + Zeolite 10%)	0,00	281,98	***-2,83***	10,68
	(75% FLUFF Tea Compost + Zeolite 25%)	0,00	552,25	***-50,28***	82,22
50% BPF + 50% BPN	100% FLUFF Tea Compost	0,00	352,82	70,06	***-28,97***
	(90% FLUFF Tea Compost + Zeolite 10%)	0,00	441,03	26,54	5,24
	(75% FLUFF Tea Compost + Zeolite 25%)	0,00	737,44	***-29,58***	13,19

Although A3B3 was the best treatment on TPC-1, but if compere with TPC-0 the highest percentage was A3B1 and A3B1 have the highest average percentage for three times observation, that was 30,89 times multiplied population from TPC-0. From the data, we can tell that the BPF highland more adaptable than BPN lowland on FLUFF tea compost as a solid carrier base. The BPF and BPN have synergy growth on each carrier base treatment, although BPN more lately growth than BPF. The 75% FLUFF Tea Compost + Zeolite 25% tratement influence the growth of both bacteria after 8 days incubation and reduce the bacteria population. Azotobacter was the nitrogen fixing microorganism that can growth on huge range of pH [12].

3.2 TEMPERATURE OF COMPOST

The average temperature of carrier before treatment was 21 ^{O}C. Temperature was measurable at the same times with population of bacteria counted. The each temperature of TPC-1, TPC-2, and TPC-3 was shown on Tabel 5 until 7.

Table 5. Temperature on TPC-1

Treatment	*Repeated 1 (°C)*	*Repeated 2 (°C)*	*Repeated 3 (°C)*	*Average (°C)*
A1B1	19.80	19.90	20.20	19.97
A1B2	20,00	20,00	20,00	20.00
A1B3	20,00	20.20	20.10	20.10
A2B1	20,00	19.90	20.20	20.03
A2B2	20,00	20,00	20.10	20.03
A2B3	20,00	19.80	20,00	19.93
A3B1	19.90	20,00	20,00	19.97
A3B2	19.90	20,00	20.10	20.00
A3B3	20,00	19.90	20.50	20.13

Table 6. Temperature on TPC-2

Treatment	*Repeated 1 (°C)*	*Repeated 2 (°C)*	*Repeated 3 (°C)*	*Average (°C)*
A1B1	21.25	21.15	21.00	21.13
A1B2	21.50	21.05	21.50	21.35
A1B3	21.10	21.10	21.10	21.10
A2B1	21.50	21.20	21,00	21.23
A2B2	21.40	22,00	21,00	21.47
A2B3	21.75	21.75	20.85	21.45
A3B1	21.30	21.40	21.55	21.42
A3B2	20.95	21.20	21.05	21.07
A3B3	20.80	21.05	21.40	21.08

Table 7. Temperature on TPC-3

Treatment	*Repeated 1 (°C)*	*Repeated 2 (°C)*	*Repeated 3 (°C)*	*Average (°C)*
A1B1	21,00	22,00	22.40	21.80
A1B2	21.40	21.60	21.60	21.53
A1B3	20,60	22.10	22.50	21.73
A2B1	21,00	20.40	22,00	21.13
A2B2	20.80	22.20	22.40	21.80
A2B3	21.60	21.40	22.70	21.90
A3B1	20.80	21.60	23,00	21.80
A3B2	21,00	22,00	22,00	21.67
A3B3	21,00	21.80	22.20	21.67

The table shown that the temperature was stable, it means that the decomposition proccess was not occur during the observation. The increasing of temperature on decomposition proccess as an indicator on metabolism of microorganism [13]. Beside of that, the range pH of refined compost was 6 – 7. Table 8 shown the pH after 12 days incubation (TPC-3).

Table 8. The pH on TPC-3

Treatment		Repetition			Average
		I	II	II	
100% BPF	100% FLUFF Tea Compost	6.6	6.7	6.7	6.7
	(90% FLUFF Tea Compost + Zeolite 10%)	6.6	6.6	6.6	6.6
	(75% FLUFF Tea Compost + Zeolite 25%)	6.3	6.4	6.4	6.4
100% BPN	100% FLUFF Tea Compost	6.7	6.7	6.8	6.7
	(90% FLUFF Tea Compost + Zeolite 10%)	6.6	6.6	6.6	6.6
	(75% FLUFF Tea Compost + Zeolite 25%)	6.3	6.3	6.4	6.3
50% BPF + 50% BPN	100% FLUFF Tea Compost	6.7	6.8	6.8	6.8
	(90% FLUFF Tea Compost + Zeolite 10%)	6.7	6.5	6.6	6.6
	(75% FLUFF Tea Compost + Zeolite 25%)	6.3	6.3	6.5	6.4

4 CONCLUSION

Biofertilizers is the living microorganism that provide into the soil as inoculant to facilitated or supply a particular nutrient for plants. For the best growth of biofertilizer, we can used a solid carrier base. To inoculation more than one microbe as a biofertilizer, we should do the synergy test of those microbes. The solubilizing phosphate (BPF) highland bacteria more adaptable than *Azotobacter vinelandii* (BPN) lowland bacteria on FLUFF tea compost as a solid carrier base. The BPF and BPN have synergy growth on each carrier base treatment, although BPN more lately growth than BPF.

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