

Enrichment of Mushroom Compost Heap Quality Using *Azotobacter* and *Pseudomonas* as a Renewable Ameliorant

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Abstract – *Azotobacter* and *Pseudomonas* were able to enrich the quality of mushroom compost heap to become a new ameliorant and able to improve the performance of cauliflower (*Brassica oleracea*). A different levels of combination materials of Phase-1 study has resulting the best of combination formula that is 10% *Azotobacter* + 10% *Pseudomonas* and had given the highest point in total population bacteria of *Azotobacter* and *Pseudomonas* throughout composting time. The highest population bacteria of *Azotobacter* found on day 21th at total of 51,000 x 10⁵ CFU/g soil; whilst that of *Pseudomonas* emerges on day 14th at total of 197.17 x 10⁵ CFU/g soil. Similar treatment gives the fastest reducing ratio of CN as well. In terms of chemical matters, the combination of 10% *Azotobacter* + 10% *Pseudomonas* had also resulting the highest new ameliorant quality. Its portion combination of ameliorant were implemented in the Phase-2 study and were applied to the selected composition ratio of one to two of compost ameliorant and soil formulation and were applied as a planting medium of a cauliflower in the nursery time. Result of the Phase-2 study showed that this composition formulation had gave the highest performing in height as well as in dry weight of the cauliflower.

Keywords – Ameliorant, *Azotobacter*, Mushroom Compost Heap, *Pseudomonas*.

I. INTRODUCTION

Quality improvement of compost using biological agent aims to meet the nutrient that is quite low in this type of fertilizer. Hamastuti *et al.*, (2012) explain that the organism can be used to enrich the quality of nutrients that are best applied as a biological fertilizer in soil or attached to compost which is then used in the cultivation process. The use of some types of functional microbial which metabolite outcomes can improve various types of compost nutrients will have an impact on improving the quality of the compost obtained. According Widawati *et al.*, (2003) that in order to produce high quality compost, it is necessary to add bio-activator containing nitrogen and phosphorus that can be inoculant. *Azotobacter* and *Pseudomonas* are examples of a functional microbe that have been widely used as a biological fertilizer in order to improve the quality of the compost in the form of N and P.

It has been widely known that *Azotobacter* has the ability to fixing N so that its presence in the soil be able to meet the needs of N for plants. Razie and Iswandi (2005) reported that *Azotobacter* obtained from rhizosphere of rice variety IR 64 in peat lands can improve nutrient absorb of nitrogen by rice plants up to 188%. *Azotobacter* able to convert nitrogen (N₂) in the atmosphere into

ammonia (NH₄⁺) through the process of nitrogen fixation, where the ammonia produced is converted into protein needed by plants (Saribay & Fidan, 2003). The addition of *Azotobacter* in compost beside increasing the availability of N content in compost, it can also shorten the composting time because it can increase the amount of N during the composting so that the rate of decline in C / N ratio run faster. Hamastuti *et al.*, (2012) suggests that adding *Azotobacter* is able to increase nitrogen levels up to 500% in sewage sludge compost dairy processing industry.

Pseudomonas sp is one of functional microbe that has ability to dissolve P. Ginting and Soul (2006) explained that *Pseudomonas fluorescens* is able to increase the solubility of P from natural phosphate at 16.4 ppm to 59.9 ppm, increasing the solubility of AlPO₄ from 28.5 ppm to 30.6 ppm and increasing P available on the soil from 17.7 ppm to 34.8 ppm. P in the soil quite a lot but low availability to plants. Enrichment of organic matter during composting *Pseudomonas* were able to increase the population of *Pseudomonas* so that when the compost is inserted into the planting media it can simplify plant in absorbing nutrients P in soil. Application of inoculant *Pseudomonas* compost in large amounts can increase the population in soil so that the competition between microorganisms in the soil does not affect the solubility of P in the soil (Ginting & Soul, 2006).

The composition of planting media has much influence to the cauliflowers. The composition of planting media will support the vegetative and generative phase of cauliflower plants. Organic fertilizer can increase the number of P and K in the planting media (Berry *et al.*, 2003). While Khai (2007) added that organic fertilizers can improve the balance of N in the soil. The availability of nutrients during the seedling phase would be a good starter and the plant will not stress when transferred to cultivate. The use of compost enriched as seedling media providing improved quality of cauliflower plants because apart from adequate nutrients can also stimulate root growth better because compost has the ability to improve the physical, chemical and biological that unable to do by inorganic fertilizers. (Subardja *et al.*, 2016).

The purpose of this study was to get the best dose of *Azotobacter sp* and *Pseudomonas sp* during composting and enriched its compost with the best total population of *Azotobacter sp* and *Pseudomonas sp* in order to improve the quality of the produced compost chemical properties as an new ameliorant. Compost with the best inoculation dose is then applied as a planting medium of cauliflower.

II. MATERIALS AND METHODS

The study was conducted in a Laboratory and Greenhouse of the Faculty from May to July 2016. Research using mushroom compost heap from the nearest farmers that consists of composition of rice straw, cotton, and lime. Compost enrichment was using *Azotobacter sp* and *Pseudomonas sp* with a density of 10^6 CFU / ml carrier material. Analysis of the total population of *Azotobacter sp* was using NFM media while *Pseudomonas sp* was using PDA. Calculation of total colonies was using a colony counter while the chemical quality compost was using spectro-photometry and C / N ratio using the C / N analyzer.

The study conducted in two stages using a single randomized block design. The Phase-1 study was to determine the dose inoculation *Azotobacter sp* and *Pseudomonas sp* with treatment A = without inoculation, B = 5% *Azotobacter sp* + 5% *Pseudomonas sp*, C = 5% *Azotomonas sp* + 10% *Pseudomonas sp* and D = 10% *Azotobacter sp* + 10 % *Pseudomonas*. The whole treatments was repeated 4 times to obtain 16 experimental units. Microbial inoculation performed on 250 g of straw mushroom compost heaps that had been sterilized and composted for 28 days. Observations were made every 7 days on 7, 14, 21 and 28 days after inoculation with the observation that the total population parameter of *Azotobacter* and *Pseudomonas* and C / N ratio. At the end of the composting, the chemical properties that includes nutrient C, total N, total P and total K, pH and water content was measured.

The Phase two of this study conducted by using the best results of the test compost obtained in the preceding Phase for planting media of cauliflower. The treatment consists of 5 different planting media compositions that are K_0 = soil, K_1 = not enriched compost, K_2 = 1 compost + 1 soil, K_3 = 1 compost + 2 soils and K_4 = 1 compost + 3 soils. The whole treatment was repeated 4 times to obtain 20 experimental units. Planting media was inserted into seedling and sowing seeds of cauliflower for 7 days, at day 7 the whole growing seedling was transferred to cultivate into a polybag and piled. The study then continued until 14 days after sowing. Observations were made at 7 and 14 days after sowing in terms of height and dry weight.

III. RESULTS AND DISCUSSION

Stage 1: Enrichment of Mushroom Compost Heap with *Azotobacter* and *Pseudomonas*.

The Total Population of *Azotobacter* and *Pseudomonas*

Based on the analysis results, there were influence of inoculation *Azotobacter* and *Pseudomonas* to the total population of *Azotobacter* at various times of observations. The use of dose inoculation 10% *Azotobacter* + 10% *Pseudomonas* on composting media of mushroom compost heap provided a highest total population of *Azotobacter* at 7, 14, 21 and 28 days after inoculation and significantly different in the treatment of

5% *Azotobacter* + 10 % *Pseudomonas* and treatment without enrichment.

Figure.1, showed an increment of the total population of *Azotobacter* from the observation at day 7 to day 21 and then decreased at day 28 in all treatments. The highest total population was on the observation of day 21 which treated by 10% *Azotobacter* + 10% *Pseudomonas* had gave the highest total population of *Azotobacter* that is $51,000 \times 10^5$ CFU g^{-1} soil and significantly different from the treatment of 5% *Azotobacter sp* + 10% *Pseudomonas sp* and without enrichment, i.e. a total population of $24,000 \text{ Azotobacter sp} \times 10^5$ CFU g^{-1} soil.

In contrast to the total population of *Azotobacter*, in Figure 2. the *Pseudomonas* total population during composting fluctuated on all treatments. The increment occurred in the observation at 14 days after inoculation and decreased at day 21 of observation, however, the population increased again at day 28 of observation, but not as much as the population at day 14 of observation

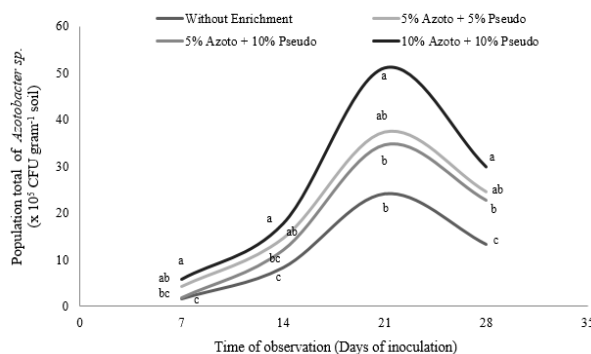


Fig. 1. Effect of dose enrichment *Azotobacter sp* and *Pseudomonas sp* to the total population of *Azotobacter* at various times of observation.

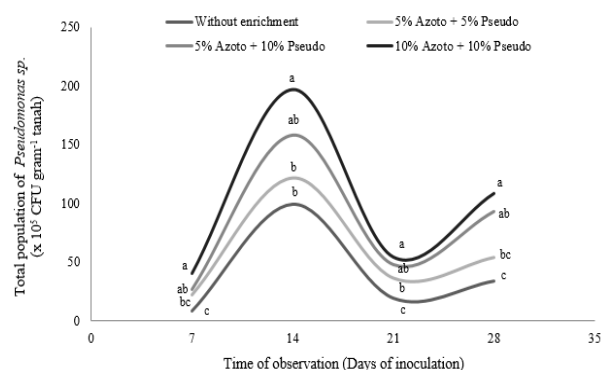


Fig. 2. Effect of dose enrichment of *Azotobacter sp* and *Pseudomonas sp* to sp total *Pseudomonas* population at various times of observation.

The total population of *Pseudomonas* (Figure 2) showed the highest point due to treatment of 10% *Azotobacter* + 10% *Pseudomonas* at day 14 of observation that was as much as 197.17×10^5 CFU g^{-1} soil, while the lowest was $99,170 \times 10^5$ CFU g^{-1} soil which is without enrichment. The decline in the total population of *Pseudomonas* occurred at 21 days after inoculation, where the lowest

population was up to $19,500 \times 10^5$ CFU g⁻¹ soil without enrichment, but increased at day 28 to $34,000 \times 10^5$ CFU g⁻¹ soil. In general, the highest total population of *Pseudomonas* was 10% *Azotobacter* + 10% *Pseudomonas* on the observation day 7, 14, 21 and 28 with respective total population of *Pseudomonas* was 40,000, 197 170, 54 000 and $108\ 330 \times 10^5$ CFU g⁻¹ soil.

Enrichment of a type of microbe in composting process will increase the diversity of microbes during composting. *Azotobacter* and *Pseudomonas* which were inoculated with the greater number of organic material were able to multiply more quickly so that the population during composting will become dominant, it was in line with the study results of Danapriatna (2012) which stated that an increase in the population of *Azotobacter* on compost enriched N fixing microbes in soil. Rao (2001) explained that the enrichment of *Azotobacter* and *Pseudomonas sp* on vermin-compost increased the total population of N-free fixing bacteria (free living Nitrification bacteria) and increased phosphate solubilizing microbes on organic materials composted for 3 weeks.

Study results of Rajani (2001) explained that the increase of phosphate solubilizing microbes population was highest in vermin-compost on week 3 and returned to decrease in the next week. It was in line with the results of this study that an increase of *Azotobacter sp* population at 21 days after inoculation, the log phase of *Azotobacter sp* happened at days 14 to 21 of composting day and decreased at day 28, it might be caused by the population at initial time of composting still perform adaptation phase as the temperature fluctuations so that impact the fluctuation population of *Azotobacter sp*. Subardja and Sudjana (2015) explained that the fluctuation of total microbial population was caused by temperature fluctuations during composting process.

Decline Rate of the C/N ratio

Inoculation of *Azotobacter* and *Pseudomonas* on mushroom compost heap gave significant effect on the decline rate of C / N ratio. Inoculation of 10% *Azotobacter*+ 10% *Pseudomonas* provided the most rapid decline rate which significantly different when compared to other treatments.

The fastest decline rate of C / N ratio was in the treatment of 10% *Azotobacter* + 10% *Pseudomonas* where the decline rate of C / N ratio on observation day 7, 14, 21 and 28 respectively were 46,033; 35,533; 21,867 and 16,033 and significantly different with other treatments.

Treatment without enrichment provided the lowest decline rate of 49,200; 40,667; 25,767 and 18,500.

One indicator of compost maturity was the decrease of the C / N ratio. Organic materials undergone weathering will experience organic C decline due to degradation of microbe which produced N, so the value of C / N ratio decreased. The decline rate of C / N ratio on organic materials enriched 10% *Azotobacter* + 10% *Pseudomonas* was faster compared to other treatments, due to organic materials enriched N-fixing microbe and P solubilizing microbes could provide N and P for microbes thus decomposition of C by microbe more optimal.

According to the regulations of Minister of Agriculture of Republic Indonesia in 2009 the ideal C / N ratio of compost was 15-25. This study was in accordance with that regulation at day 28 after inoculation. The process of waste decomposition of mushroom waste medium enriched N fixing and P solubilizing microbes had occurred ideally. The slow decline rate of C / N ratio indicated that microbes growth was slow while in the rapid decomposition indicated by uncontrolled microbes growth that formed aerobic composting and produce volatile gases (odours). Suriadikarta and Setyorini (2006) explained that the use of compost with high C / N ratio would cause a negative impact for plants due to the deficiency of N.

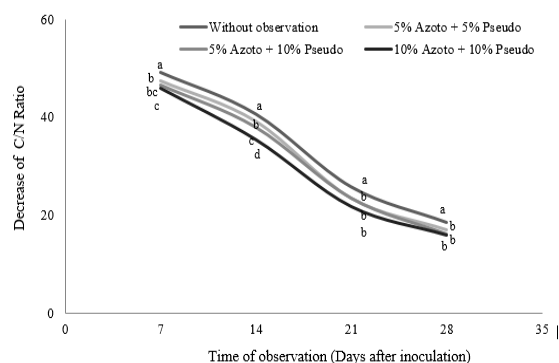


Fig. 3. Effect of *Azotobacter sp* and *Pseudomonas* spinoculation on the decline rate of C / N ratio at various time of observation

Chemical Properties of a New Ameliorant

Based on analysis results there were significant different of *Azotobacter* and *Pseudomonas* inoculation on the chemical properties of the compost ameliorant obtained. Table 1. showed the chemical properties of compost as a new ameliorant for each treatment.

Table 1. Effect of *Azotobacter sp* and *Pseudomonas sp* inoculation to chemical properties of waste compost of straw mushroom

Treatment	Chemical Properties of Organic Fertilizer					
	C Organic	N Total	P Total	K Total	Moisture	pH
	-----%-----		--ppm--		-----%-----	
A (without enrichment)	39.40a	0.47c	32.25b	1.71d	68.04a	8.6a
B (5% Azoto + 5% Pseudo)	33.74b	0.51b	33.53b	2.05c	65.87ab	8.5a
C (5% Azoto + 10% Pseudo)	30.01b	0.53ab	35.73a	2.46b	63.83b	8.5ab
D (10% Azoto + 10% Pseudo)	29.00c	0.56a	36.67a	3.00a	63.74b	8.3b
CV (%)	2.99	2.34	2.08	6.02	2.32	2.9

Description: Different letters behind the numbers showed significantly different results. $\alpha = 0.05$.

The highest C-organic content was found in the treatment without enrichment of 39.4% and significantly different with other treatments, while the lowest content of C-organic presented in the treatment of 10% *Azotobacter sp* + 10% *Pseudomonas* of 29%. The highest content of N, P and K total was in the treatment of 10% *Azotobacter* + 10% *Pseudomonas* and lowest in the treatment without enrichment. The highest N total was 0.56% which significantly different with the treatment of 5% *Azotobacter* + 10% *Pseudomonas* without enrichment only 0.47%. the highest P total was 36.67 ppm and significantly different with the treatment of 5% *Azotobacter* + 10% *Pseudomonas* without enrichment. The significant different was found in K total, in which 10% *Azotobacter* + 10% *Pseudomonas* resulted 3:00% and the lowest in the treatment without enrichment of 1.71%.

The high of C-organic content of compost without enrichment might be caused by the low number of microbes populations contained in compost compared with compost enriched. The low of microbial population affected the high number of undegradable organic C. The highest content of N, P, and K was in the treatment of 10% *Azotobacter* + 10% *Pseudomonas* due to the compost had N-fixing and P solubilizing microbes which higher than other treatments. In line with Rao *et al.*, (2012) which obtained the highest availability of nutrients on compost vermi-compost and manure enrichment

Azotobacter and *Pseudomonas* during the composting period of these materials. Enrichment of compost with *Azotobacter*, *Pseudomonas*, and phosphate rock were able to increase the nutrient content of N and P of compost around 1.75% compared with without enrichment Kavita and Subramanian (2007).

The water content in the treatment without enrichment was 68.04%, highest compared to other treatments, but not significantly different with the treatment of 5% *Azotobacter* + 10% *Pseudomonas* and significantly different to the treatment of 10% *Azotobacter* + 10% *Pseudomonas* with water content of 63.75 %. The lowest pH 8.3 found in treatment of 10% *Azotobacter* + 10% *Pseudomonas* and showed significantly different to without enrichment that is pH 8.6.

The water content and pH are the chemical properties were much influenced by external factors during composting. Subardja *et al.*, (2015) explained that the use of *Aspergillus* with different doses during composting affect the microbes to produce compost therefore water content and pH were varied. Lower water content levels in compost-enriched indicated the use more water caused by higher microbial populations than the compost without enriched.

Stage 2: Biological Test of Enriched Compost as Ameliorant of Cauliflowers

Based on the results of the Phase 1. study, showed that compost enriched 10% *Azotobacter* + 10% *Pseudomonas* was the best, then composting conducted based on that treatment which furtherly used as an ameliorant for planting media on cauliflower seedling. Based on the observation of plant height and plant dry weight at 7 and 14 days after sowing so the data obtained as presented in

in Table 2.

Table 2. Effect of various media composition on plant height and plant dry weight of cauliflower

Planting media composition	Plant Height		Dry Weight	
	7 DAP	14 DAP	7 DAP	14 DAP
	-----cm-----		-----g-----	
Soil	2.437b	4.362b	0.035bc	0.262a
Compost without enrichment	1.250c	2.850c	0.010c	0.010b
1 ameliorant + 1 soil	3.170ab	5.082ab	0.117a	0.282a
1 ameliorant + 2 soil	3.300a	5.740a	0.075ab	0.342a
1 ameliorant + 3 soil	2.962ab	4.807ab	0.067b	0.240a
CV (%)	18.80	13.32	47.81	42.10

Description: Different letters behind the numbers showed significantly different results. $\alpha = 0.05$

Table 2. illustrated that the dose of compost ameliorant in planting media significantly effected on plant height and dry weight both at 7 and 14 days after sowing. Plant height at 7 days after seedling was highest in the treatment of 1 ameliorant+ 2 soil that was 3.300 cm but not significantly different with treatment of 1 ameliorant + 1 soil and treatment of 1 ameliorant+ 3 soil. The lowest plant height at 7days after sowing found in the treatment of compost without enrichment that was 1.250 cm. Similar results were obtained in the observations of 14 days after sowing, treatment 1 compost + 2 soil provided highest plant height of 5.740 cm and significantly different with the treatment of soil and compost without enrichment of plant height 2.850 cm. The combination of compost enriched *Azotobacter* and *Trichoderma* with soil as a planting medium was also used by Espiritu (2011) and provided the most excellent plant height on green bean compared to compost without enrichment+ soil.

Plant dry weight at 7 days after sowing was highest in the treatment of 1 ameliorant + 1 soil with a dry weight of 0.117 g and significantly different with the treatment of 1 ameliorant + 3 soil, soil and compost without enrichment with plant dry weight, respectively 0.067 g, 0.035 g and 0.010 g. At 14 days after sowing, plant dry weight was highest in treatment 1 ameliorant + 2 soil that was 0.342 g and only different with treatment without enrichment compost 0.010 g. The use of compost enriched *Azotobacter* and *Trichoderma* on green bean seedling media gave the higher biomass yield compared with the use of compost without enriched (Espiritu, 2011). Similar results were obtained in the study of Shazadet *et al.*, (2008) which stated that an increase of fresh biomass grown bean plants which planting with media compost enriched+ soil. The same study results also obtained from Abdelaziz *et al* (2007) which used a mixture of soil with compost enriched with a ratio of 1: 2 revealed the best high crop of other treatments.

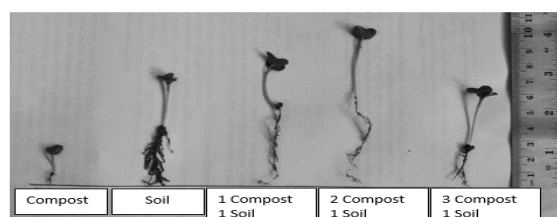


Fig. 4. Effect of various composition of planting media in Cauliflower seedling

The use of enriched compost with N fixing microbe and P solubilizing could provide a better plant performance compared with without compost or without enriched compost. The appearance of plants that grow in the mixed media of soil and compost enriched would be better, it due to compost enriched *Azotobacter* and *Pseudomonas* had enough nutrients as a planting media so that the nutrient needs at the initial of seeds imbibition already available. Beside the availability of more N and P, *Azotobacter* had the ability to produce in dole acetic acid (in dole Asetic Acid / IAA), which acted as a growth regulator and required when seeds were planted. IAA had a positive impact for plant growth, especially in early phases of seedling, IAA could be produced by several strains of *Azotobacter* isolated from soil (Egamber dieva et al, 2010). Yildirim et al (2009) reported that the use of the same inoculant (*Azotobacter* + *Pseudomonas*) on compost used as cauliflower growing medium provided high-yield crops and highest plant weight compared to that without enrichment or singly enrichment.

IV. CONCLUSION

Based on observations of Phase 1.study,the obtained inoculation formula of 10% *Azotobacter* + 10% *Pseudomonas* as the best dose for the total population of *Azotobacter* and *Pseudomonas*. The ameliorant compost produced at the dose has the fastest declining rate of C / N ratio compared to other doses and control. The bestperformingchemical properties of compost ameliorant is also contained in compost with in noculation formula of 10% *Azotobacter* + 10% *Pseudomonas*. The combination composition formula of planting mediawas shown by the1 ameliorant + 2 soil provides the best performing of plants height and plant dry weight compared to other planting media compositions.

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