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Inoculation Effect of N_2-Fixer and P-Solubilizer into a Mixture of Fresh Manure and Phosphate Rock Formulated as Organonitrofos Fertilizer on Bacterial and Fungal Populations

Sutopo Ghani Nugroho[1*], Dermiyati[1], Jamalam Lumbanraja[1], Sugeng Triyono[2], Hanung Ismono[3], Missy Kurnia Ningsih[1] and Fitri Yani Saputri[1]

[1]Soil Science Division, [2]Department of Agriculture Engineering, and [3]Department of Agribussiness Faculty of Agriculture, University of Lampung, Jl. Sumantri Brojonegoro No. 1, Bandarlampung 35145, Indonesia. Tel/fax: +62-721-781822, [*]e-mail: sutopo@unila.ac.id.

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ABSTRACT

Microbial N_2-fixer and P-solubilizer were innoculated in a mixture of fresh manure and phosphate rock formulated as an Organonitrophos fertilizer. The population dynamics of bacteria and fungi growing during the composting process were observed. The inoculation treatments consisted of: K = mixture of 20% phosphate rock and 80% of fresh manure + decomposers (control), N = mixture of 20% phosphate rock and 80% of fresh manure + decomposers + N_2-fixer (*Azotobacter* and *Azospirillum* sp.) , P = mixture of 20% phosphate rock and 80% of fresh manure + decomposers + P-solubilizer (*A. niger* and *P. fluorescens*), and NP = mixture of 20% phosphate rock and 80% of fresh manure + decomposers + N_2-fixer + P-solubilizer. The results showed that inoculation of microbial N_2-fixer and combination inoculation of N_2-fixer and P-solubilizer increased the total bacterial population compared to that of the control as well as the only inoculation of microbial P-solubilizer on the 14th day of observation in which the bacteria reached the highest population. On all the observation days, the population of fungi in the inoculation of microbial P-solubilizer treatment increased significantly compared to that of the control. However, there was no difference between the populations of fungi in the inoculation of N_2-fixer and combination inoculation of N_2-fixer and P-solubilizer. The genus of fungy identified in the compost of the mixture of fresh manure and phosphate rock were *Chytridium* sp., *Aspergillus* sp., *Rhizopus* sp., and *Fusarium* sp.

Keywords: Bacterial and fungal populations, composting, microbial N_2-fixer and P-solubilizer inoculations, organonitrophos fertilizer

INTRODUCTION

The main problems of national fertilizer in Indonesia are the expensive price of chemical/ inorganic fertilizers and their uneven distribution in some areas (districts). The price of chemical fertilizers is continuously increasing because most of the raw materials needed, such as fossil fuels and others, for manufacturing the chemical fertilizers are still imported. The high price of fertilizer will generally not be affordable by the farmers, unless it is subsidized by the government. On the other hand, the National Budget (*APBN*) for fertilizer subsidy increases significantly every year (Kementerian Koordinator Perekonomian Republik Indonesia 2009).

Farmers are generally very dependent on the chemical/inorganic fertilizers for their intensive cultivation practices and have a tendency of ignoring the use of organic fertilizer. They do not realize that the impact of the application of chemical/inorganic fertilizers continuously in the long term will result in a serious decrease of soil quality (Parnes 1986). Lately, the farmers realize those phenomena and starting to re-use the organic fertilizers. Actually, since 1990, FAO has promoted the program and it called as go organic (Dalzell *et al*. 1987). Unfortunately, however, the organic fertilizers available in the market are generally poor in quality. Although the common organic fertilizers can provide some essential nutrients for plants, but the nutrient contents are not adequate, particularly N and P nutrients.

Nugroho *et al*. (2012) formulated a new type organic fertilizer by mixing the raw materials of organic matter in the form of fresh manure and mineral-rich P element in the form of phosphate rock, that was composted using controlled composting technique. In addition, the mixture of raw materials was inoculated with N_2-fixer and P-solubilizer microbes, intending to function in increasing the nutrient content of N and solubilizing P from phosphate rock in the compost produced. The compost product is formulated as an organomineral NP fertilizer with relatively enough content of N as well as high soluble-P. The formulated fertilizer is called as Organonitrophos fertilizer. Both sources of the raw materials of the formulated fertilizer are available abundant in Lampung Province, so it is expected that the price of the new formulated organic fertilizer will be appropriately low to be afforded by the farmers.

The inoculation of N_2-fixer bacteria into the mixture of raw materials of the formulated fertilizer is expected to increase the total population of bacteria, especially the population of N_2-fixer bacteria. By increasing the population of N2-fixing bacteria, it is expected to increase the N fixation significantly, which in turn it will contribute to the increase of the N content in the compost product (organic fertilizer). Similarly, the inoculation of microbial P-solubilizer (bacteria and fungi) into the mixture of raw materials of formulated fertilizer is expected to increase the populations of bacteria and fungi. The growing population of P-solubilizers will act synergistically with the H^+ of organic acid produced during the decomposition proscess of fresh manure to solubilize P from the phosphate rock (Traina *et al*., 1986; Kumari *et al*. 2008). It will be possible when the organic materials such as fresh manure and the phosphate rock were mixed and composted, as it has already been reported by Asea *et al*. (1988); Soeleman (2008); Noor (2008); Taiwo and Ogundiya (2008); Nugroho *et al*. (2012).

This research was aimed to study the inoculation effects of microbial N_2-fixers (*Azotobacter* sp. and *Azospirillum* sp.) and microbial P-solubilizers (*A. niger* and *P. fluorescens*) in a mixture of fresh manure and phosphate rock formulated as an organonitrophos fertilizer on the bacterial and fungal populations during the composting process with the purpose to increase the quality of organic fertilizer.

MATERIALS AND METHODS

Research Implementation

The materials used were fresh manure obtained from PT. Juang Jaya Abdi Alam, Sidomulyo, South Lampung and phosphate rock obtained from the community mining in the Selagai Lingga, Central Lampung. Prior to mixing, the phosphate rock was exposed to air-dry and grainded then sieved with a 3 mm diameter sieve. The manure was taken in a fresh condition. Phosphate rock amounted of 100 kg and fresh manure amounted of 400 kg (corresponding to a mixing percentage of 20% : 80%) based on finding by Nugroho *et al.* (2012) were mixed and then was placed in a wooden constructed box with size (180 × 80 × 50) cm^3. In the treatment of inoculation of microbial N_2-fixer, the bacterial inoculants of *Azotobacter* sp. and *Azospirilum* sp. were added with population density as much as 2.97 x 10^8 CFU g^{-1} (application per gram material = 1.3 × 10^6 CFU) and 2.29 × 10^8 CFU g^{-1} (application per gram material = 8 x 10^5 CFU), respectively. The bacterial inoculants of both bacterial species were developed from the pure isolates which had been mixed into the molasses with concentration of 40%. Similarly, in the microbial P-solubilizer treatment, bacteria *Pseudomonas flourescens* and fungi *Aspergillus niger* were added with the population density as much as 2.62 × 10^8 CFU g^{-1} (application per gram material = 1 × 10^6 CFU) and 1.21 × 10^6 CFU g^{-1} (applications per gram material = 4.2 × 10^3 CFU), respectively. The two inoculants were also developed from the pure isolates which had been mixed into the molasses with concentration of 40%. In the control treatment, the mixture of the raw materials was only added by molasses with a concentration of 40%. The mixture was then composted aerobically for two months. The composting technique used was a simple batch system in static pile (Diaz *et al*. 1993; Haug 1993; Misra 2003). During composting process, the box was sealed with plastic to prevent evaporation.

Research Methods

The study was conducted using a randomized block design with four replications. The inoculation treatments consisted of: K = mixture of 20% phosphate rock and 80% of fresh manure + decomposers (control), N = mixture of 20% phosphate rock and 80% of fresh manure + decomposers + N_2-fixer (*Azotobacter* sp. and *Azospirillum* sp.), P = mixture of 20% phosphate rock and 80% of fresh manure + decomposers + P-solubilizer (*A. niger* and *P. fluorescens*), and NP = mixture of 20% phosphate rock and 80% of fresh manure + decomposers + N_2-fixer + P-solubilizer.

Measurement of Bacterial and Fungal Populations

Observation of bacterial and fungal populations were performed on days 1, 7, 14, 28 and 56 after incubation. Measurement the populations of bacteria and fungi were carried using the plate method. The medium culture for the bacteria was Nutrient Agar (NA) while for the fungi was Potato Dextrose Agar (PDA). To counting the microbial populations in the plate (petri dish), Quebec Colony Counter (QCC) was used. The total population of the sample (CFU) was calculated by multiplying the average number of colonies by the dilution factor (Poincelot 1982).

RESULTS AND DISCUSSION

The population dynamics of the bacteria during incubation (composting process) of the mixture of fresh manure and phosphate rock are shown in Figure 1. The total bacterial population increased from day 1 to day 14 and decreased thereafter. The increasing pattern of the bacterial population is considered normal, because most of bacteria are grouped as mesophilic type of microbes which initiate the composting process. During the initial stage of decomposition, readily available substrates (proteins, sugars, and starch) are rapidly oxidized. Metabolism of the simpler compounds by mesophiles causes physical and chemical changes in the decomposition environment in the compost pile that eventually selects for those microbes better able to cope with the new substrates and with the increasing heat (thermophilic stage) generated by the mesophiles (Zibilske 2005). The phenomena of increase (in the mesophilic stage) and decrease (in the thermophilic stage) of the numbers of bacteria shown in Figure 1 was in line with the results reported by Poincelot (1982). His report showed that the numbers of bacteria (determined by plate counting) could increase by two log units, to reach the figure up to 10^8 g^{-1} by the end of the mesophilic stage and then decrease to a level of 10^6 g^{-1} during the thermophilic stage of the composting process.

The dominant mesophiles bacteria in the compost pile were usually *Pseudomonas* spp., *Achromobacter* spp., *Bacillus* spp., *Flavobacterium* spp., *Clostridium* spp., and *Streptomyces* spp.; whereas the thermophiles bacteria generally consisted of *Bacillus* spp.; *Streptomyces* spp.; *Thermoactinomycetes* spp., *Thermus* spp.; *Thermomonospora* spp.; and *Microployspora* spp. (Strom 1985).

On the day 14, when the bacterial population reached the highest level, in both inoculation treatments (either inoculation with only N_2-fixer or inoculation combination with N_2-fixer and P-solubilizer), the bacterial populations were significantly higher than that of the control (without inoculation). On the other hand, in the inoculation with only P-solubilizer, the bacterial population was not significantly differ to that of the control (without inoculation). It suggested that, besides obtaining N nutrient from metabolizing the available organic substrates, the growing population of N2-fixers will eventually obtain significant amount of N nutrient through atmospheric N2 fixation; both mechanisms contributed to the increase of growth (biomasses) and populations of the bacteria.

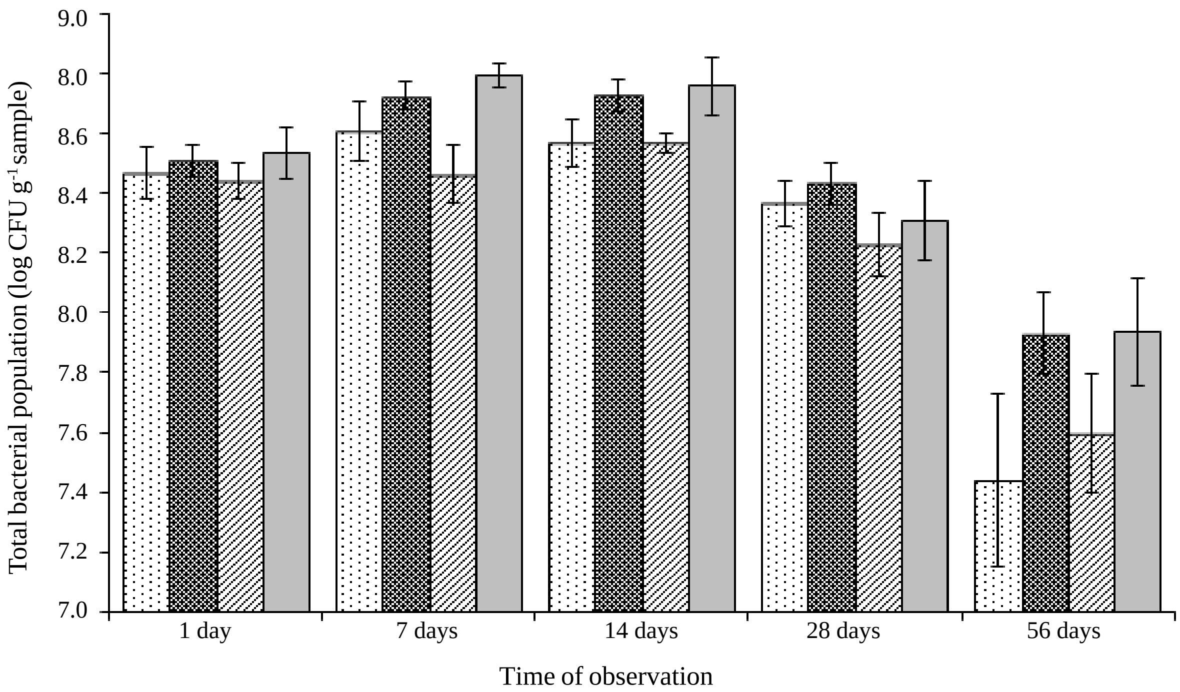

Figure 1. The population dynamics of bacteria in the mixture of fresh manure and phosphate rock which was inoculated with the microbial N_2-fixer and P-solubilizer. = control, = inoculated with the N_2-fixers, = inoculated with the P-solubilizer, and = inoculated with the N_2-fixers and P-solubilizer.

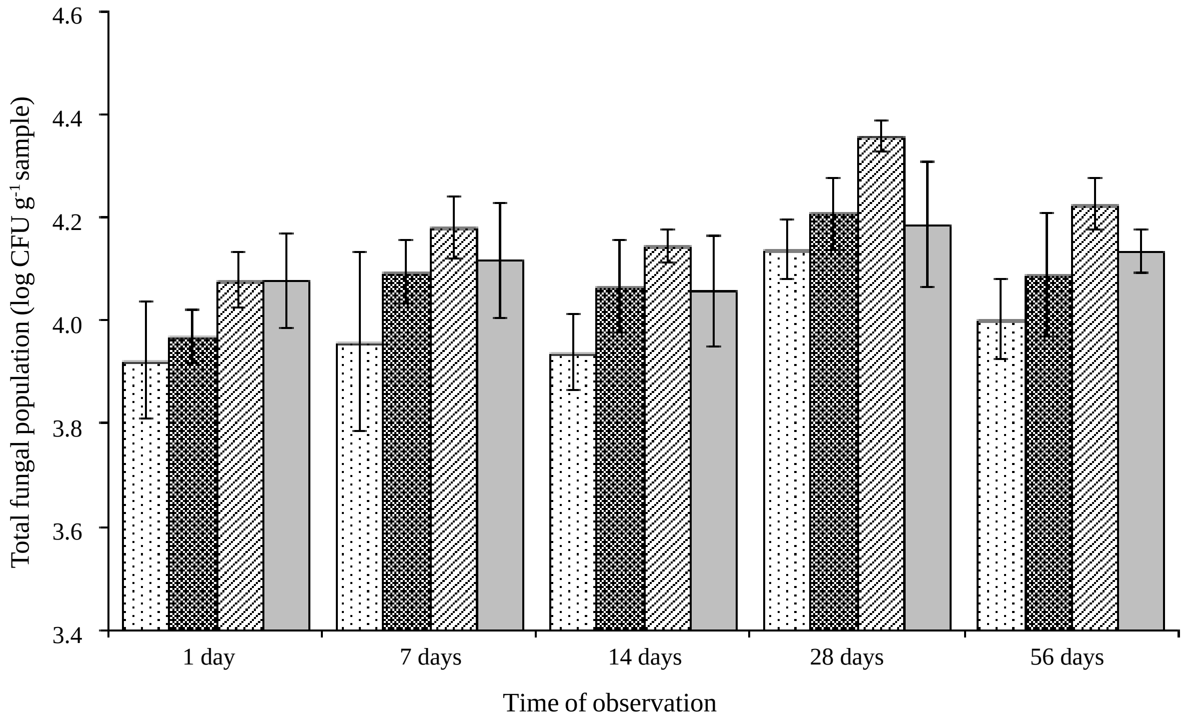

Figure 2. The population changes of fungi in the mixture of fresh manure and phosphate rock which was inoculated with the microbial N_2-fixer and P-solubilizer. □ = control, ▩ = inoculated with the N_2-fixers, ▨ = inoculated with the P-solubilizer, and ■ = inoculated with the N_2-fixers and P-solubilizer.

In the treatment of inoculation with only P-solubilizer, the bacterial population was not differ from that of the control (without inoculation); it also significantly lower compared to that of the inoculation treatment with only N_2-fixer or the combination inoculation with N_2-fixer and P-solubilizer. The N_2-fixer inoculation consisted of two bacteria strains, there were *Azospirillum* sp. and *Azotobacter* sp., whereas the P-solubilizer inoculation consisted of one strain of bacteria *Pseudomonas fluorescens* and one strain of fungy *Aspergillus niger*. It was likely that the P-solubilizer bacteria could not proportionally compete with the N_2-fixer bacteria in order to grow and to increase the population; so it is therefore, the P-solubilizer inoculation might not affect the total bacterial population (Figure 1).

The population changes of the fungi during incubation (composting process) of the mixture fresh manure and phosphate rock are shown in Figure 2. The fungal populations increased significantly from day 1 to day 28 and decreased thereafter. As it has already been explained that the mesophilic bacteria and fungy present at the start of the composting process begin to give way to thermophiles when the temperature reaches about 40 ^oC.

Temperature in the pile compost should rise beyond the mesophilic range within a few days, eliminating most of the mesophiles from the hottest parts of the pile, which reach about 60 ^oC. Generally, compost piles effectively restrict the free dissipation of heat generated during the decomposition of organic substrates, resulting in a continuous increase of pile temperature (Zibilske 2005). Thermophilic microbes (the bacteria, the yeast, the actinomycetes and the fungi) become more numerous; but the second succession occurs around 60 ^oC and the microbial participants increase, such as bacteria (principally *Bacillus* sp.) and fungi (*Chaetomium thermophile*, *Humicola lanuginosa*, *Thermoascus aurantiacus*, and *Aspergillus fumigatus*), and finally dominate the new hotter environment (Chang and Hudson 1967).

Figure 2 shows that the significant increase of the fungal population was in the inoculation treatment with P-solubilizer compared to those of the other inoculation treatments as well as the control. This was presumably due to the existing of the fungal strain (*A. Niger*) in the P-solubilizer inoculant, which contributed to the increasing of fungal population. However, the fungal population in the inoculation treatment with the mixture inoculants of P-solubilizer and N_2-fixer did not differ from that of the inoculation with the only inoculant of N_2-fixer. This was likely due to the strict competition between the P-solubilizer fungi (*A. niger*) to the P-solubilizer bacteria (*P. fluorescens*) and N_2-fixers bacteria

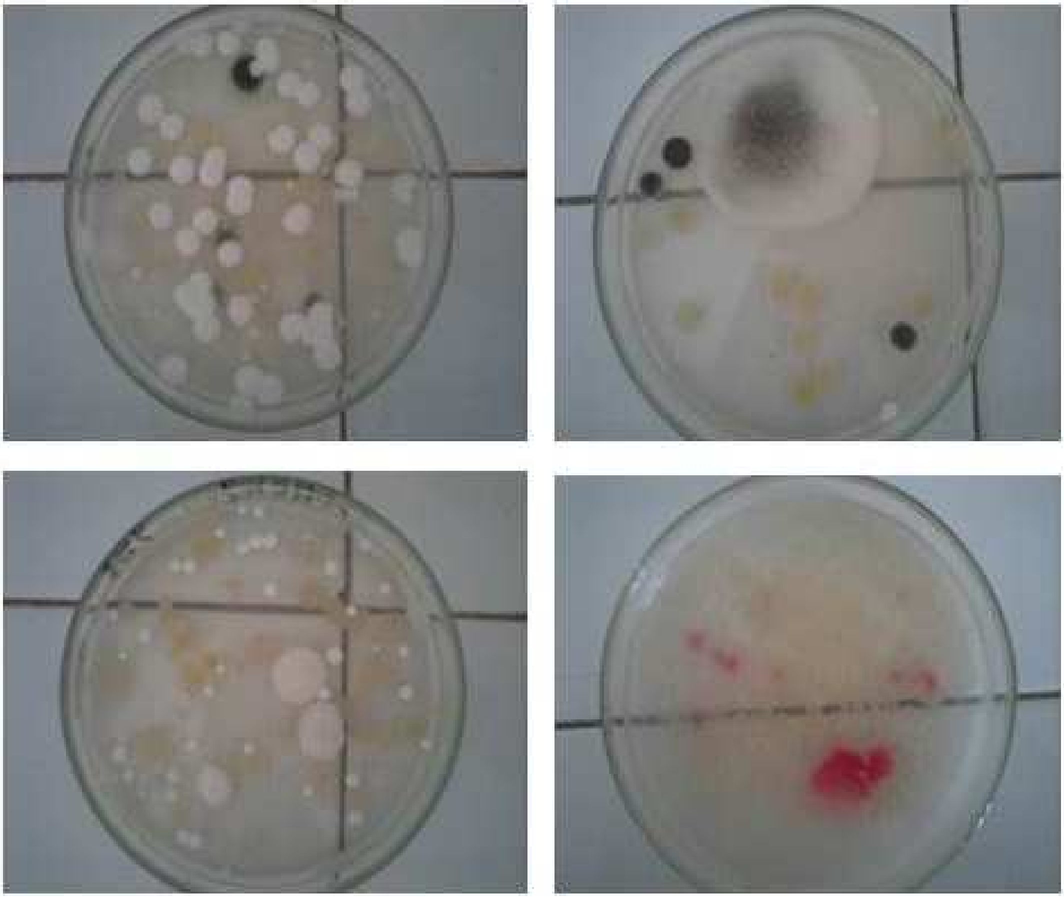

Figure 3. The fungi genus identified in the compost of the mixture of fresh manure and phosphate rock which were inoculated by microbial N_2-fixer and P-solubilizer. A = *Chytridium* sp. (white fibers, uneven edges), B = *Aspergillus* sp. (black, convex, filamentous), C = *Rhizopus* sp. (yellowish brown, uneven edges), and C = *Fusarium* sp. (pink, irregular edges).

(*Azospirillum* sp. and *Azotobacter* sp.) in all the stage of composting processes (mesophilic as well as thermophilic decomposition stages) (Zibilske 2005).

The fungal diversity in the compost of the mixture of fresh manure and phosphate rock was not different between all the inoculation treatments. This was likely due to the fungal population growing in the substrates were dominated by fungal strains originated from the decomposer inoculant which was applied into the all inoculant treatments; therefore, the similar fungal genus were indentified in all the treatments. There were 4 genus of fungy were identified, namely *Chytridium* sp., *Aspergillus* sp., *Rhizopus* sp., and *Fusarium* sp. (Figure 3).

CONCLUSIONS

The inoculation of microbial N_2-fixer and combination inoculation of N_2-fixer and P-solubilizer increased the total bacterial population compared to that of the control as well as the only inoculation of microbial P-solubilizer on the 14th day of observation in which the bacteria reached the highest population. On all the observation days, the population of fungi in the inoculation of microbial P-solubilizer treatment increased significantly compared to that of the control. However, there was no difference between the populations of fungi in the inoculation of N_2-fixer and combination inoculation of N_2-fixer and P-solubilizer. The genus of fungy identified in the compost of the mixture of fresh manure and phosphate rock were *Chytridium* sp., *Aspergillus* sp., *Rhizopus* sp., and *Fusarium* sp.

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