Population Dynamics of *Azotobacter sp., Azospirillum sp., Bacillus subtilis, Talaromyces pinophilus, Trichoderma asperellum,* and *Syncephalastrum racemosum* in the Medium with Various Earthworm Applications

Nur Ulina Warnisyah Sebayang[1], Tengku Sabrina[1], Abdul Rauf[1], Mariani Sembiring[1], Asmarlaili Sahar[1], and Erman Munir[2]

[1]*Department of Agriculture, Universitas Sumatera Utara Padang BulanMedan, Indonesia 20155*

[2]*Department of Math and Science, Universitas Sumatera Utara Padang BulanMedan, Indonesia 20155*

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Abstract: The quality of the biological fertilizer was based on the population of inoculated microorganism. The important efforts made to increase the population and maintain the population of microorganisms during storage was using the best carrier. The aim of this study was to measure the population dynamics of some microbes due to the application of various types of earthworms in producing biofertilizer. The study was conducted in Biology and Biotechnology Soil Laboratory, Department of Agriculture, Universitas Sumatera Utara. The design used was a Factorial Randomized Block Design consisting of 2 factors. The first factor was a type of earthworm with 4 treatments viz; without earthworm, anecic earthworms, endogeic earthworms, and epigeic earthworms. The second factor was a type of microorganism inoculants with 6treatments, viz; *Azotobacter sp.*, *Azospirillum sp.*, *Bacillussubtilis*, *Trichodermapinophilus*, *Talaromyces asperellum*, and *Syncephalastrum racemosum*. The results showed that there was a significant interaction between the types of earthworms in influencing the population of each microorganisms in the medium. The highest alteration of microbial population was found in *Azotobacter sp.* and *Trichodermaasperellum*. The best type of earthworm that could be used on the inoculum medium was the epigeic earthworm.

1 INTRODUCTION

The use of organic fertilizers has been used to reduce the use of chemical fertilizers. Development of biofertilizer technology is being actively conducted to resolve the limitations of biofertilizer that is a relatively short storability thus inhibiting its commercialization. Many studies have been done to get the best carrier. Fungus phosphate-solvent growth of $2.0x10^7$ after 7 days of storage can be maintained from wheat skin mixed with 20% (w / w) perlite when compared to peat carrier, corn cobs + 20% (weight / weight) perlite and cow manure compost with 20% (weight / weight) perlite(Wang, 2015).Increased proportion of carrier material vermicast in improving the survival of microbes. The results also showed that vermicasts can be used as alternative carriers for *Azotobacter chroococcum*, *Bacillus megaterium* and *Rhizobium leguminosarum* (Sekar and Karmegam, 2015).

Earthworms have the ability as a vector of microorganisms and the vermicast have highavailability nutrient and population ofmicroorganisms. Earthworm is an organismcapable of selecting and increasing thepopulation of microorganisms through peptidesderived from its excretion. Digestive fluid of earthworms released amino acids, sugars and organic molecules from organic residues. Cellulotic bacteria are present in the digestive canal and casts of earthworms indicating that they live in the digestive canal of earthworms and actively degrade food containing cellulose (Suhartanti, 2013).

By using the role of the earthworm, a new innovation can be found in biofertilizerproduction (enriched product of vermicompost and some microbial inoculant). The biovermi to be produced must be investigated in advance to identified the quality through the parameters of microbial population observed for some time.

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The observed microbial population dynamics indicate that the quality of the biofertilizer to be developed in terms of both variation of earthworm and microorganism and to know the fertilizer's storage capacity. Therefore, it is necessary to know the effect of calibrationvariation of earthworms and microorganisms in organic compost carrier to know the dynamics of the microbial population in order to produce qualified biofertilizers that can be utilized as an innovation and environmentally technology.

2 MATERIALS AND METHODS

2.1 Experimental Design

The experiment was conducted in Laboratory of Biology and Biotechnology Soil, AgriculturalFaculty, Universitas Sumatera Utara, Medan, Indonesia. The experiment used Factorial Random Block Design with 2 factors with 2 replications, so this study gets 48 treatments. The first factor is type of earthworms with 4 stage of treatments that are; without earthworm (C_0), anecic earthworm (C_1), endogeic earthworm (C_2), and epigeic earthworm (C_3). The second factor is type of microorganismwith 6 stage of treatments that are; *Azotobactersp.* (M_1), *Azospirillumsp*. (M_2), *Bacillus subtilis*(M_3), *Talaromycespinophilus*(M_4), *Trichodermaasperellum*strain G (M_5), and *Syncephalastrumracemosum*isolate VPCI 1857/11 (M_6). Data wereanalyzed statistically by using Analysis ofVariance (ANOVA) and then following by Duncan Multiple Range Test (DMRT) at 5 % level.

2.2 Experimental Pot Preparation

The experimental pot used was made of plastic with size 19cm x 13cm x 9cm as figure 1.

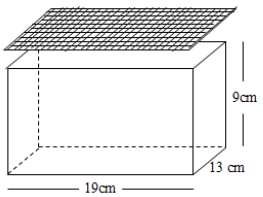

Figure 1 : The Sketch of Experimental Pot.

2.3 Earthworm Preparation

Identification of earthworm was based on Blakmore identification (Blakmore, 2002). The same weight of each type earthworms was chosen for further experiment. All selected earthworms soaked in sterile water in order to remove its sewage so the microbes on the digestive canal. Then they were acclimatized in the laboratory with culture media compost that has been sterilized.

2.4 Preparation of Inoculant Microbial

Azotobacter sp. and *Azospirillum sp.* were obtained from the AgriculturalFaculty, Universitas Sumatera Utara, Medan, Universitas Padjadjaran, Bandung. *Bacillus subtilis*was obtained from the Laboratory of Institut Pertanian Bogor Culture Collection, Bogor, Indonesia. *Talaromycespinophilus* was obtained from the collection of Soil Biology Laboratory of AgriculturalFaculty, Universitas Sumatera Utara, Medanfrom potato plant (Sembiring, 2015). *T. Asperellum* strain strain G and *Syncephalastrumracemosum* isolates VPCI 1857/11 were obtained from a previous study (Sabrina, 2017) derived from oil palm trunkchips and have been identified. Multiplication of each isolate was carried out using specific media such as Jensen medium for *Azotobacter sp.,* Okonmedium for *Azospirillumsp.,*Pikovskayamedium to *Talaromycespinophilus*and *Bacillus subtilis.*Potato Dextrose Broth medium for *Trichodermaasperellum*and *Syncephalastrumracemosum*. After each isolate was inoculated then incubated for 3-5 days, then microbial population was calculated using colony counter with the Pour Platedilution technique.

2.5 Preparation of Culture Media

Pot experiment of earthworm culture medium filled with compost sterile as much as 400 gram and covered with cloth net. Culture media regulated moisture by adding water using hand sprayer.

2.6 Application of Microbes and Earthworm

Each microbial was applied to a sterile culture medium (compost sterile) of 10 mL and mixed evenly. After 1 week of microbial application, the earthworm application was performed with the same individual weights selected and fed into the experimental pot in accordance with the treatment and then covered with a net cloth.

Treatments	*Azotobacter*	*Azospirillum*	*Bacillus subtilis*	*Talaromycespinophilus*	*Trichodermaasperellum*	*Syncephalastrumracemosum*	Mean
	10^{-8} CFU/mL						
Without Earthworm	12,1cdefg	11,5defg	12,4cdef	0,4defg	0,9defg	0,4efg	6,3
Anecic Earthworm	23,7b	26,0a	22,5bc	0,3fg	0,2g	0,2g	12,1
Endogeic Earthworm	22,5cd	27,5a	21,4cd	0,3fg	0,7defg	1,1defg	12,2
Epigeic Earthworm	20,2cd	10,7defg	12,7cde	0,3fg	4,9defg	0,5defg	8,2
Mean	19,6	18,9	17,2	0,3	1,7	0,6	

Table 1: The average of microbialpopulation in differenttypes of earthwormapplication on the second observation.

2.7 Maintenance and Observation of Microorganism Population

Maintenance of various types of earthworms by the addition of sufficient water using hand sprayer into each culture medium. Observations were made on the number of microorganisms in the culture medium. Observations and sampling were performed at the start of the study, a week after application the microbial and a week after application the earthworm (it means that 2 weeks after microbial application). Ten (10) g of media using a spatula and dissolved in 90 mL of sterile water and shaker until homogeneous (dilution 10^{-1}).

3 RESULT

3.1 Microbial Population (CFU/ml)

The results showed thatapplication oftypes of earthworms, various microbes and theinteractions of both in compost have a significantly effect onmicrobial population. The average population of microbes after a week earthworm application available in table 1.

The population of *Azospirillum sp.* on boxtreated with endogeic earthworms was the (27.5 x 10^{-8} CFU/mL), however was not significant different with its population in treatment anecic earthworms (26 x 10^{-8} CFU/mL). The lowest microbial population was at treatment anecic earthworms and *Trichodermaasperellum*(0.2 x 10^{-8} CFU/mL) which is a fungal decomposers and has a value of the average population of microbes similar to earthworms anecic treatment with the microbial *Syncephalastrumracemosum* (table 1).

3.2 The Microbial Population Dynamics

The dynamics of microbial populations was determined from the difference between early microbial population (a week after the application of microbial) with microbial populations after application of earthworm (figure 2).

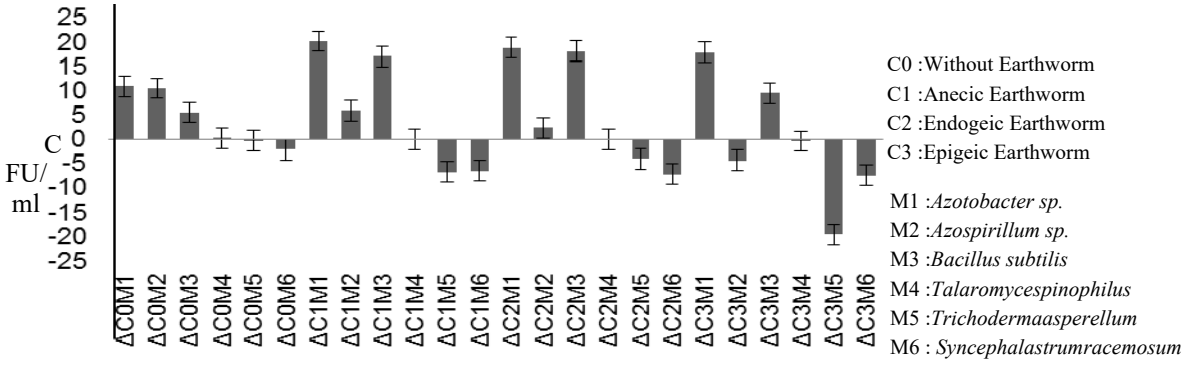

Figure 2 : Dynamics of Microbial Population

The dynamics of microbial populations in biovermi compost suistainthe increased and decreased. The bacterial population increased from the first observation to the second observation, but the average of fungus population decreased from the first observation to the second observation (figure 2). However, the treatment without earthworms was the lowest population in the first observation, whereas in the second observation the lowest population was treated with anecic earthworms. In this study, the application of earthworms in the second week after the application of earthworms had the percentage of live earthworms continued to decline, indicating that earthworms are not able to survive on the medium that used in this study. On the second day after the application of earthworms, the percentage of live anecic earthworms dropped dramatically, so that all anecic earthworm were died. Anecic earthworms did not survive on carrier media, it may be due to anecic type earthworms usually live in the soil and not live in waste habitats as decomposer worms. The anecics earthworm (length 13-17 cm; diameter 10-15 mm) is larger than other types and usually move vertically in soil. The same thing happens withendogeic earthworms prefer live on the soil with medium clay content for their habitat. While the comparison of culture media used may not be comparable, so that this endogeic cannot survive. In this case the endogeic worm used is *Pontocolexcorethrurus.* However, it is inversely proportional to the epigeic earthworm that used *Eisenia Andrei* that survive from application to completion of observation. This happens because the epigeic earthworm is an active earthworm as a decomposer of organic matter in soil. Furthermore, epigeic earthworms are also more relative tolerant to pH and can tolerate pH levels and are relatively tolerant of environmental conditions of organic waste (Dominguez and Edwards, 2011), (Neuhauser, 1988).

4 CONCLUSION

The interaction between the types of earthworms and microorganisms influenced the population of microorganisms. The type of earthworm that could be used on the medium was the epigeic earthworm (*Eisenia andrei*). The highest microbe population were *Azospirillum sp.* (bactery) and *Trichoderma asperellum* (fungus).

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