The application of biofilm biofertilizer-based organic fertilizer to increase available soil nutrients and spinach yield on dry land (a study case in Lithosol soil type)

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Abstract. Climate change has an adverse effect on dryland by decreasing soil organic matter content and hence improving soil fertility through innovative research is urgent. The research aims to investigate the optimum doses of organic fertilizer composted with liquid biofilm biofertilizer to increase soil nutrients availability and on dryland Lithosols. The experiment was conducted on Lithosols at Geneng Duwur village, Sragen, Central Java, Indonesia, with the elevation 112 m above sea level. The experimental was arranged in the randomized complete block design with a single factor, namely doses of organic fertilizer which was composted by liquid biofilm biofertilizer (0, 3, 6, 9, 12, 15, 18 and 21 tons ha⁻¹) with three replications. Each treatment occupied a 2 x 3 m² size plot, then each plot was prepared with holes to plant spinach with plants spacing of 15 cm x 20 cm. Three seeds of spinach were planted at each hole, and they were harvested at the maximum vegetative stage. The observed variables including plants parameters (plant height, stove fresh and dry weight) and soil parameters (organic matter content, pH, cation exchange capacity (CEC), total nitrogen (N), available phosphorus (P) and exchangeable potassium (K)). Data were analyzed using the F test at α=0.05 and followed by Duncan's multiple range test (DMRT). The results showed the application of organic fertilizer composted with liquid biofilm biofertilizer on dryland of Lithosols increased soil nutrients availability (total-N, available-P, exchangeable-K and soil organic matter) and spinach yields. Composting organic fertilizers with biofilm biofertilizer can support integrated farming systems because it provides microbes as the main agents in biogeochemistry reaction process in soil.

1. Introduction

Food security is an issue that is very important to be achieved in Indonesia. Many factors affect food security, including the scarcity of fertile agricultural and, caused of it decrease due to land conversion to non-agricultural sectors. Therefore the use of marginal land to replace the fertile one should be improved. Marginal land is land that has low quality due to several limiting factors when used for specific purposes [1], but the potential for agricultural land when is managed appropriately. Rainfed cultivated land [2], as well as dry land with shallow solum, steep slope, high erosion rates and a lot of rocks on the surface [3], can be included as marginal. According to Center for Agriculture Data and...
Information System, Indonesian Ministry of Agriculture [4] there are more than 11 million hectares of the dry field and more than 3 million hectares of rainfed paddy rice in Indonesia. Marginal land spread in almost all parts of Indonesia areas.

Global climatic change has an adverse effect on soil fertility, especially on dry land. This because of the increase soil temperature will fasten soil organic matter decomposition process and it will decrease soil organic matter content. Yuwono [1] stated that marginal land island which has a level of fertility at low margins. Increased utilization of marginal land can be through various means, including the application of biological fertilizer technology in integrated farming systems to develop vegetable production and increase the soil fertility status. Biofilm biofertilizer is a biological fertilizer technology that forms a thin layer of microbes when applied. Biofilms biofertilizer and or biopesticides have been developed to improve fertilizer efficiency, increase crop yields, conservation of natural resources and food safety [5][6]. According to Seneviratne et al. [7] the biofilm is a complex community of various species of microbes attached to or located close to the plant roots. Biofertilizer biofilms are more resistant to environmental stress, predators and antagonists and have the ability to increase crop yields were higher than conventional biofertilizer. Many types research has been suggesting the advantages of biofertilizer which microbial form biofilms than not form biofilms, both in improving nutrients availability, produce plant growth promoting substances (e.g. IAA) and inhibiting plant diseases [8][6][7].

Lithosols is an order of soil that is still relatively young. The solum of Lithosol soil is generally shallow (<10 cm) and located above the parent rock material [9]. According to Sarief [10], the productivity of Lithosols is classified as low because of the naturally bad physical, chemical and biological soil characteristics. Lithosols coarse soil texture tends to make the soil porous and have low water holding capacity. Its organic matter content is very low even nothing. Organic matter is one of plant nutrients sources, so that the low organic matter content of the soil makes the nutrient-poor soil, besides caused by weathering of parent rock that has not been further. Low levels of organic matter also cause soil cation exchange capacity (CEC) low [11]. Lithosols generally spread in upland and are the elements of dry land. The soil in this order actually has the potential to be a fertile one when it is developed as a commercial crop cultivation lands, with intensive use of organic fertilizers. Implementation of integrated farming systems is one way of proper management on marginal land with emphasis on recycling organic matter and other low input technology such as biofertilizer. This research aims to study the effect of the application of biofilm biofertilizer-based organic fertilizer on nutrient available and spinach yield on Lithosols. Biofilm biofertilizer is new technology in exploiting the microbes in providing plant nutrients. Biofilm biofertilizer has higher effectivity than the conventional one. The microbial consortia of biofilm biofertilizer can make microbial biofilm which in this form, their activity, as well as their tolerance to the environmental stress, is higher than if they do not form a biofilm. The use of organic fertilizer which was decomposed with biofilm biofertilizer may have higher quality because it contains available nutrients and functional microbial can form a microbial biofilm.

2. Material and methods

Isolates of nutrient provider and biological agents microbes used as consortia of biofilm biofertilizers is obtained from Sudadi et al. [12][13]. Phosphate solubilizer microbes used were isolates of A. niger (YD17), bacteria isolates of PBH 17 and TBH 18. For sulfur-oxidizing microbes, a fungal isolate of P. nalgiovense (UGM1) and bacteria isolates of NBH 12 and TBK 3 were used. Potassium solubilizer microbes used were isolates of JH 7, JK 7 and JK 17. For non-symbiotic nitrogen-fixing bacteria used azotobacter isolate AG 17.

The materials used to make organic fertilizer composted with biofilms biofertilizer were phosphate rock, feldspar rock, sulfur, quail dung and rice husk. Auxiliaries include molasses, lime and water. Phosphate rock, feldspar rock and sulfur were finely grounded to passes 100 mesh sieve. The composition (per 100 kg of organic fertilizer to be made) is 10 kg of rock phosphate, 5 kg of feldspar, 1 kg of sulfur, and 5 kg of lime, 3 liters molasses, 5 kg rice husk ash and 70 kg of quail dung). All
materials evenly mixed. Then added inoculum of liquid biofilm biofertilizer evenly as much as 5 liters. The water content of the dough of organic fertilizer made up about 60% by adding clean water. After the dough, an organic fertilizer made a pile as high as 0.5 m and covered with a tarpaulin and was incubated for two weeks. The pile mixed one for a week.

The research focused on testing the dose of organic fertilizer composted with liquid biofilm biofertilizers for the cultivation of spinach and improvement of soil fertility of Lithosol. The experiments were conducted in the field with soil order Lithosol in the sub-district of Gemolong, Sragen district, Central Java, Indonesia at an altitude of about 112 m above sea level. Tillage is done manually with a hoe, to loosen the soil, grass cleaned and made beds with a size of 2 x 3 m² for each treatment. Experiments using completely randomized block design with single factor ie the dose of organic fertilizer (0; 3; 6; 9; 12; 15; 18 and 21 tons ha⁻¹) with three replications. Spacing was 15 cm x 20 cm with each hole was planted 3 seeds. Organic fertilizer evenly mixed with soil up to the tillage layer as prescribed treatment, then watered and incubated for three days before planting.

Variables observed were planted in height, the fresh and dry weight of stover and variable soil fertility i.e organic matter content, soil pH, cation exchange capacity (CEC), total-N, available-P and exchangeable-K. Data were analyzed using the F test at the level confidence of 95%, followed by Duncan's multiple range test (DMRT) when there is a significant influence.

3. Results and discussion

According to Rosmarkam et al. [14], Lithosol is a type of soil that is still very young and therefore has not demonstrated the progress of diagnostic horizon. Solum of Lithosol is still very shallow so often the parent material appears on the surface as a solid rock. Land with this soil order is generally not much undergo a process of pseudogenes such as the influence of climate, volcanic eruptions and too sloping topography. So to be used as cropland, Lithosols require treatment that can accelerate the process of soil formation. Lithosol included in the order of Entisols the USDA system. According to Fanning and Fanning [15] generally, the order of this soil has a lot of obstacles to cultivation. Lithosolson infertile dry land included in the category of marginal (sub-optimal) land. Marginal land is the least fertile land due to the properties of the chemical, physical, morphological and minerals, including soil type of red-yellow podzolic, rainfed [2] as well as dry land with shallow solum, steep slope, high erosion rates and a lot of rocks on the surface is also a marginal lands [3]. Land used for the study had a chemical fertility as follows:

| Variable          | Value | Level      |
|-------------------|-------|------------|
| pH                | 7.47  | slightly alkaline |
| C-organic,%       | 1.23  | very low   |
| Total-N,%         | 0.30  | low        |
| Available-P, ppm  | 3.65  | very low   |
| Exchangeable-K, me% | 0.19  | high       |
| CEC, me%          | 11.44 | fair       |

Note: Classification according to Cottene, et al. (1982) [16]

Table 1 shows the status of soil nutrients and Cation Exchange Capacity (CEC), pH and organic matter content of the study site ranged from low to moderate. The main obstacle to the cultivation of annual crops, especially vegetables is the content of soil organic matter that very low which will affect the availability of N and P were low anyway. However, the problem can be solved by fertilization with organic matter adequately. Cation exchange capacity will be increased with organic matter that will enhance the soil's capability to store and exchange of nutrients provided in the form of fertilizer.
3.1. Spinach yield and soil nutrient status

Spinach is a vegetable that is commonly consumed by people. Spinach is easily cultivated and has a relatively short harvest time. The use of organic matter will help to improve the growth of spinach and vegetables in general. Spinach yield was presented in Table 2.

Table 2. The influence of a dose of organic fertilizer composted with biofilm biofertilizer to spinach yield on dry land Lithosol

| Dose of organic fertilizer, ton ha⁻¹ | Plant height, cm | Stover fresh Weight, kg per plot | Stover dry weight, g per plant |
|--------------------------------------|------------------|---------------------------------|-------------------------------|
| D0 (0 ton ha⁻¹)                      | 41.33 c          | 3.800 c                         | 9.367 d                      |
| D1 (3 ton ha⁻¹)                      | 45.53 bc         | 4.733 bc                        | 11.57 c                      |
| D2 (6 ton ha⁻¹)                      | 47.07 bc         | 5.267 ab                        | 9.900 d                      |
| D3 (9 ton ha⁻¹)                      | 48.90 bc         | 5.367 ab                        | 12.200 c                     |
| D4 (12 ton ha⁻¹)                     | 51.60 ab         | 5.533 ab                        | 12.000 c                     |
| D5 (15 ton ha⁻¹)                     | 58.70 a          | 5.600 ab                        | 11.970 c                     |
| D6 (18 ton ha⁻¹)                     | 60.33 a          | 5.850 ab                        | 16.670 a                     |
| D7 (21 ton ha⁻¹)                     | 61.47 a          | 6.133 a                         | 15.070 b                     |
| CV (%)                               | 10.10            | 11.990                          | 6.400                         |

Description: number followed by the same letter in the same column showed no significant difference in Duncan's multiple range test at α=0.05.

The highest spinach yield was obtained from the application of 21 tons ha⁻¹ organic fertilizers composted with biofilm biofertilizer treatment (D7). There was a tendency that increases doses of organic fertilizers will increase spinach yields. The optimum dose did not achieve maybe cause of very low levels of soil organic matter. That is, the dose of organic fertilizer used still needs to be raised to gain maximum spinach yield.

Table 3. Influence of the dose of organic fertilizer composted with biofilm biofertilizer on soil nutrient availability

| Dose of biofilms biofertilizer | Org-C (%) | Total-N,% | Available-P, ppm | Exch-K, me% | CEC, me% | pH |
|-------------------------------|-----------|-----------|------------------|-------------|----------|----|
| D0 (0 ton ha⁻¹)               | 1.20 b    | 0.29 b    | 4.8 d            | 0.22        | 12.92 e  | 7.57 |
| D1 (3 ton ha⁻¹)               | 1.20 b    | 0.30 b    | 5.3 d            | 0.30        | 14.94 d  | 7.57 |
| D2 (6 ton ha⁻¹)               | 1.52 ab   | 0.32 ab   | 7.2 c            | 0.26        | 15.65 d  | 7.30 |
| D3 (9 ton ha⁻¹)               | 1.58 ab   | 0.32 ab   | 7.5 c            | 0.25        | 17.03 e  | 7.40 |
| D4 (12 ton ha⁻¹)              | 1.64 ab   | 0.31 ab   | 7.4 c            | 0.24        | 17.85 bc | 7.40 |
| D5 (15 ton ha⁻¹)              | 1.63 ab   | 0.37 a    | 8.7 b            | 0.34        | 18.69 b  | 7.43 |
| D6 (18 ton ha⁻¹)              | 1.74 a    | 0.33 ab   | 10.0 a           | 0.32        | 18.85 b  | 7.43 |
| D7 (21 ton ha⁻¹)              | 1.93 a    | 0.37 a    | 10.0 a           | 0.35        | 20.01 a  | 7.57 |
| CV (%)                        | 15.39     | 7.83      | 8.43             | 22.40       | 3.79     | 0.95 |

Note: a number followed by the same letter in the column showed no significant difference in Duncan's multiple range test at the 95% confidence level.

Table 3 shows fertilizing with organic fertilizer improved soil organic matter content, total-N, available-P, exchangeable-K and soil CEC. That is because organic fertilizer from the manure of quail has a high content of N, P and K in addition to organic matter. Soil reaction (pH) was not affected significantly because organic fertilizer had lower pH than Lithosol soil. Lithosol soil reaction should
even be lowered slightly so that it becomes neutral. The use of organic fertilizer composted with biofertilizer biofilms on Lithosol is very appropriate for this type of soil generally have very low levels of organic matter. The use of biofilms biofertilizer will also encourage the development of soil microbial populations [17] which is very important to encourage the pedogenesis process, although the influence of the treatment on microbial population is not significant. Sudadi et al. [13] showed that the use of biofilms biofertilizer was able to increase the availability and uptake of P as well as the growth and yield of onion on a Vertisol soil. Hadiwiyono et al. [18] also suggested the use of biofilms biofertilizer increase the availability of P and suppress basal rot disease incidence of red onion on Alfisols, Entisols and Vertisols.

4. Conclusion
The use of organic fertilizer composted with biofilms biofertilizer on Lithosols increased soil organic matter content and available nutrients (N, P and K). The dose of organic fertilizer applied did not support spinach growth and yield optimally, allegedly due to very low initial soil organic matter levels, thus further study with higher doses is necessary.

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References
[1] Yuwono N W 2009 Developing Soil Fertility of Marginal Land Journal of Soil Science and Environment 9 137-41
[2] Notohadiprawiro T 1988 Reviews on Dry Land Position in the Food Agricultural Development Nasional Seminar at Faculty of Agriculture UNISRI Surakarta, Indonesia
[3] Suwardjo, Nurida N L and Irawan 1995 The Step to Develop Conservation farming Business in Critical Hills Area. Prosiding of Exposure of Conservation Farming Systems Technology and Machine Tools Research & Development. BPPP.Yogyakarta.17-19 January 1995.
[4] Center for Agriculture Data and Information System 2017 Statistics of Agricultural Land 2012 - 2016 (Jakarta: Secretariat General – Ministry of Agriculture)
[5] Yokohama T and Ohyama T 2007 Current status and future direction of commercial production and use of biofertilizers in Japan FFTC Ext. Bull. 600 pp 1-9
Chien S Y, Young C C and Wang C L 2007 Current status of Bio-fertilizers, development, farmers’ acceptance, utilization and future perspective in Taiwan FFTC Ext. Bull. 603 1-7
Bandara W M M S, Seneviratne G and Kulasingoory S A 2006 Interactions among endophytic bacteria and fungi, effects and potentials J. Biosci. 31 645–50
Javier P A and Brown M B 2007 Bio-fertilizer and bio-pesticides research and development at UPLB FFTC Ext. Bull. 02 1-22
Jee H J 2007 Current status of bio-fertilizers and bio-pesticides development, farmers’ acceptance and their utilization in Korea FFTC Ext. Bull. 601 1-8
Kao S S 2007 Current status of Bio-pesticides development, farmers’ acceptance, utilization and future perspective in Taiwan FFTC Ext. Bull. 599 1-20
Seneviratne G, Kecskés M L and Kennedy I R 2007 Biofilm biofertilisers: Novel inoculants for efficient nutrient use in plants Efficient nutrient use in rice production in Vietnam achieved using inoculant biofertilizers ed. Kennedy I R, Choudhury A T M A, Kecskés M L and Rose M T Proceedings of a project (SMCN/2002/073) workshop held in Hanoi, Vietnam pp 126 - 30
Seneviratne G, Thilakaratne R M M S, Jayasekara A P D A, Seneviratne K A C N, Padmathilage K R E and De Silva M S D L 2009 Developing Beneficial Microbial Biofilms on Roots of Nonlegumes: A Novel Biofertilizing Technique Microbial Strategies for Crop
Improvement Ed. Khan M S, Zaidi A and Musarrat J (New York: Springer-Verlag) pp 51-80.

[8] Seneviratne G and Jayasingheearachchi H S 2003 Mycelial colonization by bradyrhizobia and azorhizobia. J. Biosci. 28 243–7
Jayasingheearachchi H S and Seneviratne G 2006 Fungal solubilization of rock phosphate is enhanced by forming fungal–rhizobial biofilms. Soil Biology and Biochemistry 38 405–8
Adesemoye A O and Klopper J W 2009 Plant–microbes interactions in enhanced fertilizer-use efficiency. Appl. Microbiol. Biotechnol. 85 1–12
Danhorn T and Fuqua C 2007 Biofilm Formation by Plant-Associated Bacteria Annual Review of Microbiology 61 401-22
Gaind S 2011 Biofilms, Their Role in Agriculture http://www.biotecharticles.com/Agriculture-Article/Biofilms
Rodriguez-Navarro D N, Dardanelli M S and Ruiz-Sainz J E 2007 Attachment of bacteria to the roots of higher plants. FEMS Microbiol Lett 272 127–36
[9] Darmawijaya M I 1990 Soil Classification, Principles Theory for Soil Researcher and Agriculture Practician in Indonesia (Yogyakarta: Gadjah Mada University Press)
Hardjowigeno S 2003 Science Soil (Jakarta: Akademiqa Presindo)
[10] Sarief S 1986 Agriculture Science Soil (Bandung: Pustaka Guara)
[11] Brady N C and Weil R R 2004. The Nature & Properties of Soils. Mac Millan Publ. Co., New York.
[12] Sudadi, Sumarno and Widada J 2009 Developing of Biosulfo Fertilizer for Horticulture Commodities I: The Effect of Biosulpho Composition on Available P and S in Acid, Neutral and Alkaline Soils. Agrivita 31 9 - 16
Sudadi, Sumarno and Widada J 2011 Biosulfo Fertilizer Development for Horticulture Crops II. The Effect of Phosphate Rock Content and Inoculum Ratio of Biosulfo on P and S Uptake and Yield of Red Onion in Acid and Alkaline Soils. Agrivita 33 265 -72
[13] Sudadi, Sumarno, Hadiwiyono and Zafifah A 2015 Development of Biofilmed Biofertilizer: The effect on P-available and onion on Vertisol soil. Nasional Seminar of PIT Permi 2015 Semarang.
[14] Rosmarkam A, Shidieq D and Atmojo S W 1988 Soil Classification. Lecture Handbook dictate (Surakarta: Faculty of Agriculture, UNS)
[15] Fanning D S and Fanning M C B 1989 Soil. Morphology, Genesis and Classification (New York: John Wiley & Sons)
[16] Cottenie A, Verloop M, Kiekens L, Velghe G and Camerlynck R 1982 Chemical Analysis of Plants and Soil (Belgium: Lab. of analytical and Agrochemistry, State University Ghent)
[17] Buddhika U V A, Athauda A R W P K, Seneviratne G, Kulasooryi S A and Abayasekara C L 2013 Emergence of Diverse Microbes on Application of Biofilmed Biofertilizers to a Maize Growing Soil. Ceylon Journal of Science (Bio. Sci.) 42 87-94
[18] Hadiwiyono, Sudadi and Jariyah R A 2015 Biofilmed Biofertilizers As Nutrients P Provider and Biocontrol of Basal Rot Disease of Shallots. Nasional seminar of FKPTPI at Banjarmasin, Indonesia 29 - 30 September