The application of Plant Growth Promoting Rhizobacteria and Organic Matters from Black Soldier Fly Larva Culture, Calliandra, Cocopeat in the Pre-nursery Oil Palm Seedling

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Abstract. Plant growth promoting rhizobacteria (PGPR) are some of the bacteria that live in the rhizosphere. This research was aimed to evaluated the effect of the application of PGPR and various organic matter from black soldier fly larvae (BSF) tehnology, calliandra humus and cocopeat on the growth of pre-nursery oil palm. The research was conducted in Maguwoharjo, Depok, Sleman, Yogyakarta in April to July 2020. The research used factorial experimental method arranged in a completely randomized design (CRD) which consisted of two factors. The first factor is the concentration of PGPR which consists of four levels namely control (without PGPR), PGPR 10 mL polybag^{-1}, 20 mL polybag^{-1}, and 30 mL polybag^{-1}. The second factor is various of organic matter consisting of four levels namely: control (without organic matter), organic matter from BSF larvae technology, calliandra humus and cocopeat. The research data were analyzed using analysis of varians (ANOVA) and follow up test with DMRT. The results showed that the various of organic matter affect the pre-nursery oil palm growth, the best is Calliandra humus. The PGPR application was able to increase the growth of pre nursery oil palm seedlings, the best dose is 10 mL polybag^{-1}.

Keywords: Bio fertilizer**,** *Calliandra haematocephala, Cocos nucifera, Elaeis guineensis, Hermetia illucens***,** Enviromentally-friendly.

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1 Introduction

Plant growth promoting rhizobacteria (PGPR) are some beneficial bacteria that are lived in the soil around and on the root surfaces, that are directly or indirectly have positive effect in plant growth and development through the secretion of various organic component in the rhizosphere [1–3]. Rhizosphere is a soil zone around plant roots which is an area that is very important for plant with microbial populations. A large number of microorganisms coexist in the rhizosphere. This zone is rich in nutrients from plant exudates, such as amino acids and sugars which provide a source of energy and nutrients for bacteria [4–6]. Free living and beneficial soil bacteria that inhabit the rhizosphere are called PGPR [4, 7, 8]. Bacteria that have been identified as PGPR include symbiont nitrogen-fixing bacteria, among others *Rhizobium* which is an obligate symbiont in Leguminosae, *Frankia* which is symbiont nitrogen-fixing bacteria in non leguminous plant and *Cyanobacteria*. Non-symbiotic nitrogen-fixing bacteria among others *Azotobacter* sp., *Acetobacter*, *Bacillus*, and *Azospirillum* [5]. In addition, there are *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Bacillus* sp. [3, 5, 9, 10]. PGPR helps plant growth indirectly by controlling phytopathogens [11, 12] This can occur with the production of antagonistics agents or by the induction of resistance to pathogens. Research on PGPR shows improved plant health and productivity under normal and stressfull conditions. PGPR has the potential for bioremediation and detoxification of heavy metal pollutans and pesticides, and exhibits antifungal activity [1, 3, 4, 13]. The direct effect of PGPR in increasing soil fertility through increased availability of nutrients in the soil, symbiotic and non-symbiotic nitrogen fixation [3–5, 9, 10, 13] and increased phosphate solubilization [3, 10, 13, 14]. In addition PGPR produced siderophore, which is a low molecular mass iron binding protein and has a high affinity for Fe^{+3} to become a siderophore-Fe^{+3} complex that can be absorbed by plants [13, 15]. Under aerobic conditions, reduced iron is unstable and easily oxidized to form of Fe^{+3}, which is not available for biological systems [3, 4, 15]. PGPR performs the synthesis of hydrolytic enzymes, such as chitinase, glucanase, proteinase and lipase, which can destroy pathogenic fungal [2, 4]. PGPR secretes auxins, cytokinins and gibberellins and decreases plant ethylene. Rhizobacteria involved in the synthesis of these phytohormones include *Bacillus licheniformis, Bacillus pumilus* and *Pseudomonas fluorescens* [3, 9, 10, 13, 15]. Because of the increased soil pollution, climate change, pathogens and overuse of land, the soil becomes infertile and unproductive, PGPR can reduce the use of chemical fertilizers and pesticides that have a negative impact on agriculture. The PGPR application supports the development of biosafety-based agriculture to the development of environmentally friendly sustainable agriculture [1–4, 13, 14, 16]. PGPR which can be classified as biological fertilizers, biostimulants and biopesticides is expected to contribute to overcoming food insecurity, environmental sustainability and reducing public health risks [3, 9, 13, 17, 18]. Research on PGPR applications containing *Pseudomonas fluorescens* and *Bacillus subtilis* are able to increase the growth of PS 882 sugarcane bud chip. Application of 20 mL of PGPR with a combination of 10 t ha^{-1} of rabbit manure can increase corn yield. The application of 20 mL of PGPR and 30 t ha^{-1} green manure was able to increase maize yields [19–21].

The use of black soldier fly (BSF) larvae (*Hermetia illucens* Linnaeus, 1758) to process organic material is growing rapidly. BSF larvae culture is the problem solving of sanitation by using insects to develop biological, as an alternative food source for livestock and fisheries as well as for reuse of organic waste. The use of BSF larvae is a potential alternative for recycling biological waste [22]. BSF larvae (maggot) are able to degrade organic waste, both waste originating from animals and plants better than other insects [23–25]. BSF larvae have a high protein content, ranging from 28.2 % to 42.5 % [26, 27].

Organic fertilizer derived from maggot has a pH of 7.78 and elemental N content reaches 3.36 % [28].

Calliandra humus is humus produced from the biomass of *Calliandra calothyrsus* Meisn.as an organic fertilizer provides nitrogen as the main nutrient and adds soil organic matter for plants. In Calliandra compost, nitrogen content is obtained in the form of 3.23 mg kg^{-1} nitrate and 6.11 mg kg^{1} ammonium, more high than the control with nitrate content of 1.26 mg kg^{-1} and ammonium of 5.33 mg kg^{-1} [29]. In many studies show that Calliandra is better than other Leguminosae. Research on Calliandra compost shows that Calliandra is one of the plants that has the ability to fix nitrogen better than other legum plants [30]. Research in maize shows that Calliandra humus is able to increase maize yields [31].

Cocopeat is a planting medium derived from coconut (*Cocos nucifera* L.) fiber waste. The use of cocopeat media is better than topsoil media for chrysolite (*Magnolia elegans* (Blume) H. Keng) growth [32]. Cocopeat is an organic material that is environmentally friendly for use as a composite material [33]. The nutrient content in cocopeat is Nitrogen 0.39 %, Phosphorus 0.41 %, Potassium 2.39 %, Calcium 0.18 %, and Magnesium 0.11 % [34]. In tea nurseries, soil medium + cocopeat showed the same results as soil medium + baglog (mushroom media) [35]. Research on potato plantlet shows that cocopeat media gives the same results as husk charcoal media and humus media, and better than chicken manure media [36]. Research on Merbau seedling shows that 25 % and 50 % cocopeat media were better than 100 % soil media and 75 % cocopeat media [37]. Nurseries are the first step in the entire series of oil palm cultivation activities. Good oil palm seedlings have the strength and appearance of optimal growth and the ability to deal with environmental stress conditions during transplanting. To obtain good oil palm seedlings, special treatment is required of the planting media and fertilizers used during the nursery process [38]. Good and healthy seedlings can be achieved, among others, by improving the planting medium. Improvement of planting media can be done by mixing organic materials into the media, including PGPR combined with calliandra, cocopeat and BSF larvae compost. This study aims to examine the PGPR application combined with organic matters from cocopeat, calliandra and BSF larvae (maggot) for improving planting media.

2 Research and method

2.1 Time and place

The research was conducted at the Institut Pertanian Stiper's Educational and Research Garden, Krodan in Maguwoharjo, Depok District, Sleman, Yogyakarta (coordinate 7º45'37"S 110º25'25"E). The altitude of the research site is 156 m above sea level.

The research was conducted from April to July 2020. Nutrient content of regosol soil: Nitrogen 0.021 %, Phosphorus 0.049 %, Potassium 0.02 %, Magnesium 0.01 %, C organic 0.99 %, and pH 6.4. All nutrient data and organic C content are categorized as very low.

2.2 Research method

Oil palm (*Elaeis guineensis* Jacq.) sprouts from the Medan Oil Palm Research Center were used in this research. The research used a factorial pattern experimental method consisting of two factors and arranged in a completely randomized design. These factors were: Factor I, the dosage of PGPR containing *Azospirillum* which consists of four levels, namely: Control (without PGPR), PGPR 10 mL $polybag^{-1}$, PGPR 20 mL $polybag^{-1}$, PGPR 30 mL $polybag^{-1}$. Factor II, types of organic matter consisting of four levels, namely:

Control (100 % Regosol soil), Regosol soil + Calliandra compost, Regosol soil + cocopeat, Regosol soil + BSF larvae compost. Each treatment combination with three replications. The parameters observed were seedling height, stem diameter, number of leaves, shoot and leaf fresh weight, root fresh weight, shoot and leaf dry weight, root dry weight, root length, leaf area, and number of roots. The data analysis used ANOVA at the 5 % real level. Further test using the Duncan Multiple Range Test at the 5 % real level [39, 40].

3 Result and discussion

Table 1. The application of PGPR on the pre-nursery oil palm seedlings.

Parameters	PGPR (mL polybag^{-1})			
	0 (control)	**10**	**20**	**30**
Seedling height (cm)	19.94 ± 3.87p	20.40 ± 4.07p	18.44 ± 3.41p	18.71 ± 2.83p
Stem diameter (cm)	0.55 ± 0.12p	0.55 ± 0.10p	0.55 ± 0.13p	0.59 ± 0.09p
Number of leaves	3.75 ± 0.25p	4.08 ± 0.27p	3.83 ± 0.37p	4.00 ± 0.36p
Shoot and leaf fresh weigth (g)	2.60 ± 1.10q	2.84 ± 1.23p	2.87 ± 1.07p	2.96 ± 0.98p
Root fresh weigth (g)	1.42 ± 0.51q	1.60 ± 0.48p	1.53 ± 0.73p	1.55 ± 0.60p
Shoot and leaf dry weigth (g)	0.67 ± 0.40q	0.78 ± 0.35p	0.86 ± 0.30p	0.78 ± 0.27p
Root dry weigth (g)	0.50 ± 0.12q	0.55 ± 0.14q	0.55 ± 0.13q	0.70 ± 0.37p
Root length (cm)	23.58 ± 6.51p	22.70 ± 6.99p	22.75 ± 6.83p	26.25 ± 8.75p
Leaf area (cm^2)	70.83 ± 26.25p	69.07 ± 30.3p	63.66 ± 20.77p	71.53 ± 20.8p
Number of roots	3.58 ± 0.51pq	3.75 ± 0.62p	3.66 ± 0.77p	3.00 ± 0.73q

Note: The average number followed by the same letter in the same row shows no significant difference according to the DMRT at the 5 % real level.

Table 2. The application of various organic matters on the pre-nursery oil palm seedlings.

Parameters	Organic matters			
	Control (Regosol)	**Calliandra compost**	**Cocopeat**	**Compost from BSF larvae**
Seedling height (cm)	21.50 ± 2.29a	21.79 ± 4.30a	17.19 ± 2.24b	17.02 ± 1.68b
Stem diameter (cm)	0.62 ± 0.06a	0.63 ± 0.11a	0.52 ± 0.06b	0.52 ± 0.06b
Number of leaves	4.08 ± 0.28ab	4.16 ± 0.71a	3.50 ± 0.67p	3.91 ± 0.79ab
Shoot and leaf fresh weight (g)	3.31 ± 0.77a	3.64 ± 1.36a	2.20 ± 0.52p	2.12 ± 0.55b
Root fresh weight (g)	1.83 ± 0.56a	1.84 ± 0.73a	1.38 ± 0.25p	1.07 ± 0.21b
Shoot and leaf dry weight (g)	0.93 ± 0.23a	1.00 ± 0.41a	0.57 ± 0.15p	0.58 ± 0.23b
Root dry weight (g)	0.56 ± 0.17a	0.60 ± 0.42a	0.56 ± 0.16q	0.58 ± 0.23a
Root length (cm)	23.45 ± 4.74bc	24.04 ± 8.37b	29.58 ± 6.75p	18.20 ± 3.88c
Leaf area (cm^2)	72.90 ± 14.07ab	87.77 ± 33.47a	55.43 ± 16.52p	58.93 ± 14.67b
Number of roots	3.83 ± 0.57a	3.66 ± 0.88a	3.08 ± 0.66p	3.41 ± 0.51ab

Note: The average number followed by the same letter in the same row shows no significant difference according to the DMRT at the 5 % real level.

The results in Table 1 show that the application of PGPR does not affect seed height, stem diameter, number of leaves, root length and leaf area, but it does affect shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and number of plant seed roots oil palm in the pre-nursery. This shows that PGPR has a role in improving the growth of oil palm seedlings. The shoot dry weight and root dry weight indicated an increase in the biomass of oil palm seedlings. Biomass is formed from photosynthate and respiration, which are the sources of the carbon skeleton that makes up organic compounds. The increased dry weight indicates an increased photosynthetic capacity with effective respiration, so that the organic matter making up the plant body increases. The increase in photosynthetic capacity in PGPR applications containing *Azospirillum* can occur because *Azospirillum* has the ability to fix nitrogen [5, 10, 15], available P dissolution, and IAA synthesis [9, 41], so there is an increase in nitrogen availability and phosphorus, as well as auxins in the rhizosphere which can be utilized by plants. In the plant body, nitrogen has a role as a constituent of amino acid compounds which are the building blocks of protein (both structural and functional), the constituent of chlorophyll, nucleic acids, purines, pyrimidines, coenzymes and many other organic compounds [42–44]. Phosphorus has a role as a constituent of ATP, NADP, DNA, RNA, cell membrane compounds [45]. Auxins have a role in regulating various metabolic processes and root growth [46, 47]. The optimal conditions formed in the PGPR application are able to increase the biomass of oil palm seedlings.

Table 2 shows that the type of organic matter has a significant effect on all parameters of oil palm seedling growth, except for the dry weight of the roots. In general, Calliandra compost was better than cocopeat and compost produced by BSF larvae technology. These results illustrate the increased growth of oil palm seedlings after application with Calliandra compost. The increased growth in the application of compost indicates an increase in biomass of the plant body, as well as an increase in seedling height and diameter and was supported by the addition of leaf area and the number of leaves. This is because Calliandra compost contains complete nutrients as plant remains in general, with a high nitrogen content. Calliandra compost contains 3.3 % N, 0.2 % P, 1.1 % K, 0.9.2 % Ca and 0.4 % Mg [48]. Compared to cocopeat which has an N content of 0.39 % [35] and compost made from BSF larvae made from vegetables and fruits, nitrogen content of Calliandra compost higher, especially because Calliandra has the ability to fix nitrogen [29]. As mentioned above, nitrogen has a role as a constituent of amino acid compounds which are the building blocks of protein, constituent of nucleic acids and chlorophyll [43]. Chlorophyll content can increase photosynthetic capacity [49]. The increase of functional protein content (enzymes) will increase metabolic capacity, while structural protein is part of the constituent of plant tissue, so that biomass increases and plant growth increases.

The findings in this research support the statements [50–52] and a previous study [53, 54, 8] that the application of PGPR must be accompanied by the application of organic fertilizers, especially in soil-like material of this research with deficient nutrient and C ingredient [55, 56, 7].

4 Conclusion

The conclusion from this research is that the PGPR containing *Azospirillum* is able to increase the growth of oil palm seedlings. In organic matter apllication, the mix of calliandra compost is best in increasing the growth of oil palm seedlings in the pre-nursery compared to cocopeat and compost from BSF larvae technology.

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