The Effect of Biofertilizer Combined with Organic or Inorganic Fertilizer on Growth of *Caesalpinia pulcherrima* and Bacterial Population in Soil

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**Abstract.** The greenhouse experiment was carried out to study the effect of biofertilizer combined with organic or inorganic fertilizer on the growth of *Caesalpinia pulcherrima* and bacterial population in the soil. Biofertilizers including *Azotobacter* sp. (mixture of 5 isolates) *Azospirillum* sp. (mixture of 5 isolates) and *Rhizobium* sp. (mixture of 5 isolates), were applied with organic fertilizer (Palm Oil Mill Effluent/POME) or inorganic fertilizer (Muller Solution/MS) were laid out in Randomized Complete Block Design (RCBD) with three replicates. The result showed that plants which were inoculated with biofertilizer (*Azotobacter, Azospirillum*, and *Rhizobium*) showed better growth compared than control treatment. Plants treated with a combination of biofertilizers with POME or MS had higher growth than plants treated with organic or inorganic fertilizers individually. The application of an organic fertilizer on plant showed more excellent plants’ growth than the usage of organic fertilizer. However, there were no significant differences in plant growth between POME and MS combined with biofertilizer. Biofertilizer combined with organic fertilizer combination produced higher bacterial population than inorganic fertilizer combination.

1. **Introduction**

Fertilizer is among the most utilized substance in agriculture. Based on the production process, fertilizer is categorized into three types; chemical fertilizer (inorganic), organic and biofertilizer. In modern agriculture, inorganic fertilizers especially nitrogen and phosphorus are widely used in soil management plan. This phenomenon resulted in many detrimental implications to the environment. The use of chemical fertilizer contributes to the contamination of soil, water, and air and is also harmful to soil microorganisms and fertility [1-3]. Organic fertilizer and biofertilizer can be alternative solutions to reduce the usage of chemical fertilizer with the purpose of maintaining and improving the quality of the soil.

Biofertilizers are a nutritious, renewable and eco-friendly substance which contain living microorganisms that are able to colonized rhizosphere and internal tissue of plants [4-6]. *Rhizobium, Azotobacter, Azospirillum* are some examples of biofertilizer that has been used for a good period of time to provide nutrients through nitrogen fixation and induce plant’s growth through growth hormone synthesis and dissolve phosphate [7-10].
Organic fertilizers have an important role in maintaining soil sustainability and improve both soil physicochemical characteristics and microorganisms activity [11,12]. One sample of widely utilized organic fertilizer is wastewater of palm oil industry. Palm Oil Mill Effluent (POME) is the waste of oil winning process in palm oil industry which contains a high level of organic matter and plant nutrients especially potassium (K), nitrogen (N), magnesium (Mg) and calcium (Ca) but relatively low phosphorus (P) [13]. Wu et al. reported that POME has been extensively applied in irrigation and liquid organic fertilizer in palm plantations [14]. The application of organic and biological nutrients is important for plant growth and shows positive interaction with chemical fertilizer, thus increase fertilization efficiency.

*Caesalpinia pulcherrima* is flowering species that frequently planted as ornament and shades trees. Plant component such as flower, leaves, bark and root is recognized contain various types of a bioactive compound and have been used for medicinal purposes. The application of fertilizer is recommended for excellent plant growth. The objectives of the research was to investigate the impact of organic, inorganic, biofertilizer and its combinations’ application on *C. pulcherrima* growth and soil microbial population.

2. Materials and Methods

2.1. Bacteria

The bacteria used consisted of *Rhizobium* (a mixture of 5 isolates), *Azospirillum* sp. (5 isolates) and *Azotobacter* sp. (5 isolates). They were isolated from soil of former tin mine in Bangka, Batu Belubang village, Pangkalan Baru, Bangka (S. 02° 11' 409' and E. 106° 11' 265’), Bangka Belitung province. The bacteria’s ability to solubilize phosphate and produce IAA has been tested. Phosphate solubilization by bacteria was tested using [15]. Solubilization of inorganic phosphate was qualitatively performed by inoculating culture of bacteria isolates on pikovskaya agar medium containing 0.5% Ca₃(PO₄)₂ as a source of P, 0.5 g (NH₄)₂SO₄; 0.2 g NaCl; 0.1 g MgSO₄, 7H₂O; 0.2 g KCl; 10 g Glucose; 0.5 g yeast extract; 20 g agar; MnSO₄ and a few FeSO₄, distilled water 1000 ml [16]. Media in the petri dish was inoculated with 10 μl (10⁶ cfu/ml) of bacterial culture using drop plate method and incubated for 7 days at room temperature. The bacterial colonies capable to solubilize tricalcium phosphate were characterized by halo zone around the colonies. The obtained bacterial isolates were then tested quantitatively on liquid Pikovskaya media. The amount of soluble phosphate was measured on 72 h. Indole Acetic Acid production by bacterial culture was analyzed by Pattern and Glick method. It was performed in a liquid medium of TSB (20 ml) containing L Tryptophan (200 μg/ml) at 72 h based on [17].

2.2. Effect of fertilizer on growth of *C. pulcherrima* on pot in greenhouse

The experiment was carried out using completely randomized design (CRD), consisted of Plant without fertilizer nor inoculant as control (C), *Azotobacter* inoculant (At), *Azospirillum* inoculant (Ap), *Rhizobium* inoculant (R), Organic fertilizer (POME/P), Inorganic fertilizer (Muller solution/M), *Azotobacter* inoculant + POME (At + P), *Azotobacter* inoculant + Muller solution (At + M), *Azospirillum* inoculant + POME (Ap + P), *Azospirillum* inoculant + Muller solution (Ap + M), *Rhizobium* inoculant + Muller solution (R+ P), *Rhizobium* inoculant + Muller solution (R+M). Three replications were prepared for each treatment.

The experiment was carried out in the greenhouse of Microbiology department, Research center for Biology, Cibinong. Growing medium used for the experiment was sterilized sand. The sand was sterilized in an autoclave for an hour. As much as 300 g of the sand was filled into the pot. Seeds of *Caesalpinia pulcherrima* was sterilized by being soaked in a solution of 1% sodium hypochlorite for 30 seconds and rinsed with aquadest three times. The seeds then were germinated in wet (using 10 mL of sterile aquadest) and sterile filter paper on a petri dish. The seed germination was observed every day. Three seedlings were placed on a pot after seven days germination. The application of organic (POME) and inorganic fertilizer (Muller solution) was performed by pouring 10 mL fertilizer every day. The concentration of POME was 5 %. Muller solution was consisted of mixture of A solution.
(CuSO$_4$ 2H$_2$O 5g; Na; MgSO$_4$ 7H$_2$O 5g; distilled water /DW 20L) + 10 mL B solution (Ferric citrate 30g; DW 1L) + 100 mL C solution (K$_2$HPO$_4$ 34g; KOH 1.96g; DW 1L) + 10 mL D solution (MnSO$_4$ H$_2$O 1g; Na; ZnSO$_4$ 7H$_2$O 0.25g; CuSO$_4$ 5H$_2$O 0.25g; Na$_2$MoO$_4$ 2H$_2$O 0.06g; H$_3$BO$_3$ 0.50g; CaCl$_2$ 6H$_2$O 0.05g. Azotobacter (a mixture of At1, At2, At3, At4, At5), Azospirillum (a mixture of Ap1, Ap2, Ap3, Ap4, Ap5) and Rhizobium (a mixture of R1, R2, R3, R4, R5) isolates which used as biofertilizer. The shoot length, the number of branches and fresh weight were measured when the plants were 30 and 60 days old. Rhizosphere soil sample from each treatment was analyzed to obtain bacterial population (CFU/g soil).

2.3. Effect of fertilizer plant on bacterial population in soil

Bacterial population count was performed by solving 1 gram of soil in an Erlenmeyer filled with 90 ml of 0.8% sterile NaCl. The Erlenmeyer was then put in a 120 rpm shaker for 30 minutes and was diluted into $10^1$-$10^7$ dilution. As much as 0.2 ml of each dilution was dropped in a sterile petri dish then respective media was poured over. The media used for Azotobacter, Azospirillum, and Rhizobium consecutively were Mannitol Ashby [18], Caceres medium [19] and YEMA agar [18]. The number of viable cells was measured by CFU (colony forming unit/ soil (g) after seven days incubation.

The acquired data were analyzed using SPSS 16.0 software, and significant differences of treatments were determined by LSD (P<0.05).

3. Results and Discussion

3.1. The potential of bacteria

Table 1 represents the ability of Azotobacter, Azospirillum, and Rhizobium to solubilize phosphate and produce IAA growth hormone. The activity of phosphate solubilization was varied in the range of 9.61–11.56 µg/ml, while IAA yield ranged from 0.3 – 6.82 µg/ml. Some species of Azotobacter, Azospirillum, and Rhizobium were reported to show the ability to solubilize phosphate and produce IAA [20-22].

| Isolates | P Solubilization | IAA (µg/ml) |
|----------|------------------|-------------|
| Solid medium | Liquid medium (µg/ml) |
| At1 | + | 9.61 | 4.04 |
| At2 | + | 10.01 | 3.21 |
| At3 | + | 10.45 | 0.30 |
| At4 | + | 10.0 | 0.30 |
| At5 | + | 10.65 | 6.82 |
| Ap1 | + | 11.01 | 1.56 |
| Ap2 | + | 10.21 | 2.81 |
| Ap3 | + | 11.01 | 1.82 |
| Ap4 | + | 11.56 | 0.30 |
| Ap5 | + | 10.45 | 0.30 |
| R1 | + | 10.09 | 4.39 |
| R2 | + | 10.37 | 1.35 |
| R3 | + | 11.01 | 1.99 |
| R4 | + | 10.51 | 4.92 |
| R5 | + | 4.81 | 4.81 |

Note: += halozone

3.2. Effect of fertilizer on growth of Caesalpinia pulcherrima on pot in greenhouse

Table 2 presents the data of each fertilizer and combinations’ effect on plant growth (shoot length, the number of branches and fresh weight) 1 and 2 months after transplanting. Plants which were inoculated with biofertilizer (Azotobacter, Azospirillum, and Rhizobium) showed better growth compared than control treatment. It was supported by the rise observed. The shoot length was
increased 12-31%. While 16-30% rise in the number of branches and 9-75% increase in fresh weight were measured. Correspondingly to this result, [23] reported that biofertilizer could improve plant height of various species. Yasari and Patwardhan, Hungria et al., Qin et al.) reported that plant growth can be increased by the inoculation of Azotobacter, Azospirillum, and Rhizobium [24,25,10].

The utilization of nitrogen-fixing bacteria as biofertilizer showed a positive result. It was indicated by the solubilization of phosphate and production of IAA growth hormone. The research by [8,26,10,2] showed that Rhizobium (symbiotic nitrogen-fixing bacteria) and Azotobacter or Azospirillum (nonsymbiotic nitrogen-fixing bacteria) as inoculant were able to improve plant growth due to phosphate solubilization. Nitrogen-fixing bacteria capability to generate growth hormone (IAA) was reported by [7,27-29]. The presence of IAA increased the formation of lateral roots. This phenomenon raised the amount of roots exudate and the nutrients absorbed by the roots which in turn stimulate bacterial growth thus enhance inoculation effect [30].

The application of both POME (organic fertilizer) and Muller solution (inorganic fertilizer) increased plant growth compared to control treatment one month and two months after transplanting. In the first month, the growth of fertilized plant was better than control treatment although the difference was insignificant. Two months after transplanting, the growth of plant fertilized with POME showed a significant difference to that of control treatment for shoot length, the number of branches and fresh weight respectively; 26%, 27.9%, and 21%. A similar result was obtained from Muller solution treatment. There were; 31.7% rise in shoot length, 38.9% rise of the number of branches and 75% rise of the fresh weight. In accordance with [31], reported that POME and inorganic fertilizer (Nitrogen, Phosphorus, and Potassium) significantly increased the growth and yield of Amaranthus cruentus compared to control treatment. Notable impact of POME and inorganic fertilizer (Nitrogen, Phosphorus, and Potassium) on soybean was reported by [32].

Plants that were fertilized with inorganic fertilizer presented better plant growth than those fertilized with POME. Falodun et al. stated that inorganic fertilizer increased the growth and yield of soybean higher than POME [32]. Inorganic fertilizer could provide nutrients more rapidly than organic fertilizer. Organic fertilizer released nutrients gradually [33].

The combination of biofertilizer with POME or inorganic fertilizer significantly increased plant growth. There was no significant difference in the plant growth between the plant fertilized with biofertilizer + POME and biofertilizer + inorganic fertilizer. As much as 9-60% rise was observed on the plant fertilized with the combination of biofertilizer + POME or inorganic fertilizer. It was not seen on plant fertilized with single fertilizer. A similar result was acquired by [34] which stated that the combination of biofertilizer and 50% inorganic fertilizer escalated lettuce yield as much as 25%. Whereas the combination of biofertilizer and 50% organic fertilizer generated 34% higher result compared to the application of single organic fertilizer. Shanmugam and Veeraputhan Nwangburuka et al. reported that biofertilizer and organic fertilizer were able to improve growth and yield of the plant [35][36].

### Table 2. Effect of fertilizer on growth of C. pulcherrima

| Treatment | Shoot length (cm) | No. of branches | Fresh weight (g) |
|-----------|-------------------|-----------------|-----------------|
|           | 1 MAT  | 2 MAT  | 1 MAT  | 2 MAT  | 1 MAT  | 2 MAT  | 1 MAT  | 2 MAT  |
| C         | 4.8±0.74 | 15.6±0.50 | 3.2±0.41 | 11.7±0.54 | 0.25±0.03 | 0.42±0.04 |
| At        | 7.6±0.22 | 20.6±1.10 | 4.8±0.29 | 15.2±0.67 | 0.41±0.07 | 0.80±0.09 |
| Ap        | 6.0±0.22 | 19.4±0.38 | 4.7±0.41 | 14.9±0.35 | 0.47±0.01 | 0.89±0.02 |
| R         | 4.7±0.07 | 17.8±1.88 | 4.0±0.12 | 13.5±1.42 | 0.33±0.03 | 0.56±0.15 |
| P         | 5.1±0.49 | 19.9±1.51 | 3.0±0.41 | 15.0±0.75 | 0.31±0.01 | 0.78±0.09 |
| M         | 5.1±0.49 | 20.8±0.92 | 3.7±0.41 | 16.3±0.91 | 0.32±0.05 | 0.51±0.27 |
| At+P      | 8.3±0.08 | 24.6±3.60 | 4.4±0.12 | 17.6±2.71 | 0.28±0.06 | 0.84±0.13 |
| At+M      | 7.4±0.41 | 20.4±0.43 | 4.3±0.41 | 15.2±0.62 | 0.27±0.01 | 0.50±0.07 |
| Ap+P      | 8.5±0.05 | 20.3±1.59 | 4.5±0.53 | 14.6±1.93 | 0.44±0.01 | 0.94±0.04 |
| Ap+M      | 8.2±0.42 | 20.0±0.41 | 5.0±0.01 | 16.4±0.82 | 0.42±0.03 | 1.01±0.04 |
R+P  5.9±0.57  19.27±1.03  4.3±0.01  14.3±0.61  0.30±0.01  0.66±0.06
R+M  6.1±0.83  22.1±0.84  5.0±0.01  16.3±0.84  0.37±0.02  0.86±0.26

Notes: MAT (a month after transplanting). ± SD is a value of 3 replications. Means in the same group followed by the same letter in the column are not significantly different (p≤0.05) as determined by the least significant difference (LSD) test.

3.3. Effect of fertilizer on bacterial population in soil harvesting (2MAT)
The application of biofertilizer and organic fertilizer significantly increase the soil bacterial population which was counted on harvesting (Figure 1). The highest number of population was seen on the soil inoculated with the combination of *Azospirillum* and POME (576x10⁵ CFU/g soil). There was no significant difference between the mentioned result with the number of population on the soil inoculated with *Azotobacter* + POME (573 x10⁵ CFU/g soil). This result was in accordance with the research of [37,38] which indicated that organic fertilizer impacted the microorganisms population at the rhizosphere positively. Muzaffar et al. reported that the application of biofertilizer and organic fertilizer increased the number of *Azospirillum* population [39]. According to [40], organic fertilizer could increase microorganism population since it provided adequate nutrients for soil microorganisms.

![Figure 1. Bacterial population in soil at 2MAT](image)

4. Conclusion
Plants which were inoculated with biofertilizer (*Azotobacter, Azospirillum,* and *Rhizobium*) showed better growth compared than control treatment. The application of inorganic fertilizer on plant showed more excellent plants’ growth than the usage of organic fertilizer. The application of biofertilizer and inorganic or organic fertilizer combination resulted in higher plants’ growth than single fertilizer usage. Biofertilizer and organic fertilizer combination produced higher bacterial population than inorganic fertilizer combination.

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