Utilization of bio organic fertilizers for increasing upland rice production

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Abstract. Productivity of upland rice plants is lower than lowland. Water availability and soil fertility are determined by plant growth. The aim of this study was to investigate the effectiveness of bio organic fertilizers (BOF) in upland rice production. The genetic material used was Inpago LIPI Go 1 upland rice. Seeds were planted in plots 16 m² with plant spacing of 20 x 20 cm. Four treatments consisted of P1 (50% inorganic fertilizer), P2 (100% inorganic fertilizer), P3 (50% inorganic + BOF), P4 (100% BOF) were applied to a randomized block design with four replications. The results showed that the application of BOF single or combination did not significantly effect on yield component of upland rice. The application of BOF + 50% inorganic fertilizer increased fibrous root formation and the rate of mycorrhizal infection in the roots. The highest colonization of mycorrhiza in upland rice roots was shown by P3 treatment of 34%.

1. Introduction
Organic fertilizer was composed from living things material and had been claimed for its efficacy for supporting the growth of rice plant [16] [15]. The application of organic fertilizer was considered to be effective in increasing the soil quality and improving plant growth [9] [20]. Nowadays, organic farming is very popular as an effective way to obtain healthy foods without causing harmful impact to the environment. Furthermore, the value of organic farming products is higher than conventional farming. Therefore farmers are expected to produce rice by application the organic farming concept when working their field including the utilization of organic fertilizer.

The development of upland rice is an effort to provide solution to food security issue in Indonesia. Utilization of marginal land for upland rice production is very possible. This is because Indonesia has very large marginal land. Sub-optimal land in Indonesia is in the form of dry land: ultisol and oxisol and also wetlands such as: peatland, acid soil, tidal swamps [23]. Millions of hectares of sub-optimal or marginal land are spread across several islands in Indonesia. Dry land area and potential for agriculture in Indonesia are reported at 12.9 million hectares [7].

Problems encountered in upland rice production is limited water availability. Nutritional balance for the growth of upland rice plants must be considered. The availability of sufficient nutrients will guarantee the yield. This availability must be maintained, starting from the beginning of the plant growth until harvest. The right fertilizer composition, type, and dosage will determine the success of rice production. The application of organic fertilizer as a source of plant nutrition has been widely carried out. Bacteria-based organic fertilizer was reported to reduce the usage of conventional fertilizer up to 50% on the soil of paddy rice and upland rice [1] while application of different biostimulants and its combination gives various response depend on the upland rice varieties[21].

Soil microorganisms, such as arbuscular mycorrhizal fungus are important component in the plant or soil system. Symbiosis between arbuscular mycorrhizal fungus and most plant can improve plant tolerance to abiotic stress and improve plant nutrition and protection against the oxidative damage produced by the water stress [18]. Mycorrhizal colonized plants can also interact with several soil
microorganisms including plant growth promoting rhizobacteria (PGPR) that also able to make the plant more tolerant to these stressed conditions [8]. The interaction between mycorrhizal and PGPR might be useful for the development of re-vegetation in soils having water and nutrition limited.

2. Methods

2.1. Field experimental design

The research was conducted from August to December 2018 at field trial station, Research Center for Biotechnology LIPI. Experiment genetic material used upland rice LIPI Go 1. Amount of 2-3 seeds (per hole) were planted on the 4 m x 4 m plot with plant spacing of 20 x 20 cm. There were four treatments applied consisted of P1 (50% organic fertilizer), P2 (100% inorganic fertilizer), P3 (50% organic fertilizer + 50% inorganic fertilizer) and P4 (100% organic fertilizer). Organic fertilizers used in this study were contained mycorrhiza fugal such as Acalauspore and Scutellospore which infection root biomass form and BioPlus (contained Azospirillum brasilense dan Azotobacter sp. bacteria’s), StarTmi (contained bacteria consortia), and microalgae biomass. A mixture of 5 kg/ha of BioVam and 50 mL of BioPlus were applied prior to plantation by soaking the seeds in the mixture solution. StarTmi fertilizer was applied by spraying directly onto rice plants on the 10th, 30th and 45th day after planting. As much as 0,5 g/m² of microalgae biomass were mixed into soil on each plot in the beginning of plantation. Treatments were applied to randomized block design with four replications.

Root characteristics and yield component parameters such as: plant height, total amount of productive tillers, flowering time, harvesting time, panicle length, number of filled and empty grain per panicle, and weight of 100 harvested grains were observed. All acquired data were analyzed using Minitab v.16 and SPSS v.16 software.

2.2. Infection of mycorrhiza analysis

The percentage of mycorrhiza root infection was estimated by visual observation of fungal colonization after clearing washed roots in 10% KOH and staining with 0.05% Trypan blue in lactic acid (v/v), according to Phillips and Hayman (1970). Quantification of the root colonization was performed according to grid-line intersect method (Giovannetti and Mosse, 1980). Five replicates per treatment were used. Root colonization is measured by the following formula Deguchi et al., (2017):

\[
\text{Root colonization (\%) = } \frac{\text{Number of infected roots}}{\text{Number of observed roots}} \times 100\%
\]

3. Result and Discussion

The effect of different applications of fertilizer on growth and yield of upland rice is presented in Table 1-4 and Figure 1-2. There was no significant effect of bio fertilizer application to increase upland rice production based on some parameters: yield components, root characteristics, and the rate of mycorrhizal infection in the root. However, the root volume (Figure 1) and the rate of mycorrhizal infection (Table 1) were increased.

Mycorrhizal fungi infect rice roots in the treatment P3 and P4 compare to control. The highest percentage of colonization (34%) was observed in plant treated with BOF + 50% inorganic (P3), while the lowest colonization at P1 (11%). This result suggested that the addition of BOF improves health and fertility in the root area. Previous research showed the application of bio-ameliorant combined with consortia for biofertilizer has increased induced systemic resistance and enhanced the rice productivity significantly [20]. Similar research found that there was highly root infection by mycorrhiza in 10 Indonesian upland rice varieties under drought condition [14]. An other study showed that mycorrhizal inoculation increase shoot and root growth at five lowland rice varieties in Southwestern Nigeria [115]. Arbuscular mycorrhizae fungi has high root colonization at soil pH 5.9 but upland rice plant growth is not responded to root colonization [22]. The rice plants that are grown under aerobic conditions respond strongly to mycorrhizal infection [13]. The effectiveness of mycorrhizal fungi to colonize plant roots is determined by combination of mycorrhizal fungi and host plant. The different type of mycorrhizal fungi will cause differences ability to colonize plant roots.
Colonization of mycorrhizal fungi on plant roots depend on mechanisms of nutrition exchange between mycorrhizal fungi and host plant, mycorrhizal survival and host sensitivity [2]. The ability of mycorrhizal fungi to adapt and tolerate is influenced by its presence on the roots of plant [11].

Table 1. The mycorrhizal infection at upland rice root after treatments.

| Treatments                          | Colonization of mycorrhiza (%) |
|-------------------------------------|-------------------------------|
| P1 (Inorganic 50%)                  | 11                            |
| P2 (Inorganic 100%)                 | 14                            |
| P3 (BOF+50% Inorganic)              | 34                            |
| P4 (BOF 100%)                       | 32                            |

*Source: The Institute of Mychorrizal Research and Development, USDA; very low (0 – 5%), low (6 – 25%), medium (26 – 50%), high (51 - 75%), very high (˃ 75%).

The rice root weights after fertilizer treatment was presented in Table 2. There was no significant difference between root weights after being treated. However, the root volume showed different appearance (Figure 1). Although statistically not significant, the treatment of BOF single and combination were produced a thicker root volume than inorganic treatment. The P3 treatment showed a lot fibrous root obtained, as the result not significant on root weight. This is supported by the previous study [16] that the effect of biofertilizer on aerial biomass dry weight is not significant, otherwise on dry weight of root biomass at rice.

Table 2. Root weight after treatments

| Treatments                          | Root Weight (g) |
|-------------------------------------|-----------------|
| P1 (Inorganic 50%)                  | 16.35           |
| P2 (Inorganic 100%)                 | 14.24           |
| P3 (BOF+50% Inorganic)              | 17.71           |
| P4 (BOF 100%)                       | 16.13           |

Figure 1. Root conditions of upland rice after fertilizing treatments

F-Test result showed no significant differences between the treatments (Table 3). A small coefficient of variation indicates that the treatment has been applied properly. However, Table 3 also showed that the application of 100% BOF (P4) produced the lowest number of productive tillers about
9 and 7 tillers compared to inorganic (P1). While, 50% BOF + 50% inorganic (P3) treatment had an equal number of tillers to P1 and P2. The treatment 50% inorganic fertilization treatment have the highest percentage of empty grains (33.2%). Otherwise, the application 100% inorganic (P2) produced the highest filled grain (115 grains). These results are in line with [21] that the 6 types of biofertilizer applications combined with 50% NPK had no significant effect on plant height and number of tillers at upland rice. The application of biofertilizer had no significant effect on yield components nor on grain yield of three soybean varieties [17]. Another study suggested that biofertilizer would have been more effective and helpful in the rainy environment with limited inorganic fertilizer input in irrigated rice production [4].

Some factor inhibited the plant growth during the experiment such us brown plant hopper (BPH) attacked. The highest population of BPH at generative affected at seed filling and decreasing the yield. Another factor is poor nutrient content in the soil of experiment site due to removal of topsoil several years earlier. For this reason, other research needs to be done about BOF fertilizer dosages specifically in the land of the experiment. The population of BPH is positively correlated to temperature and relative humidity, negative correlation to rainfall [16] [5]. The Brown plant hopper attacked at 40-50 days after transplanting (last week of August), and explosion of BPH at third week of October, than decreased at the harvesting stage [5]. The biofertilizer usage should be evaluated under abiotic stress conditions such as low soil fertility, soil acidity, and drought [4].

Table 3. The result of yield component observation of upland rice to fertilizing treatments.

| Treatments   | Plant height | Flowering Days | Number of tiller | Panicle | Number of grains |
|--------------|--------------|----------------|------------------|---------|------------------|
|              | (cm)         | Prior 50%      | Total (straw)    | Length (cm) | Weight (g)     | Filled (grain) | Empty (%) |
| P1 (Inorganic 50%) | 116,6        | 77,8           | 89,3             | 12       | 10               | 23,3           | 2,4       | 102       | 33,2 |
| P2 (Inorganic 100%)   | 113,6        | 81,0           | 92,5             | 10       | 10               | 23,1           | 2,4       | 115       | 27,7 |
| P3 (BOF+50%) Inorganic | 113,1        | 78,8           | 92,8             | 11       | 10               | 23,4           | 2,3       | 107       | 28,7 |
| P4 (BOF 100%)        | 113,5        | 78,5           | 90,5             | 9        | 7                | 23,0           | 2,3       | 101       | 30,0 |

Coefficient of Varian Significations

*ns=non-significant based on F test

Pearson correlation value showed the applied fertilizer correlated to each variable (Table 4). The analysis showed close and positive correlation on several yield component parameters. Plant height was positively correlated with weight and length panicle, and also number of grain. The number of productive tillers correlated closely with the total number of tillers. Otherwise, the panicle length and panicle weight were highly positive correlated with the amount of grain. The longer panicle produced more grain.

The relationship between plant parameters on fertilizer treatment was presented in Figure 2. The results of the Biplot analysis indicated that 50% inorganic BOF (P2) and 100% inorganic (P3) treatment had a similar effect to the growth plant. While, single BOF treatment (P4) resulted in a far different effect to the growth. A very wide vector angle value explains there is a high negative correlation between flowering age and number of filled grains with plant height, panicle weight and percentage of empty grain. The application of BOF + 50% inorganic (P3) increased the number of tillers (productive and total) and the panicles length, while the 100% inorganic treatment affects the age of flowering and the filled tillers. The 50% inorganic + BOF (P3) showed comparable result to
100% inorganic treatment on rice plant growth. Bakrie et al (2010) found the similar result that the fertilizer treatment with 50% inorganic + 200 kg biofertilizer gave the same yield with 100% anorganic fertilizer using System of Rice Intensification (SRI) cultivation. Previous experiment was reported that three-kind application of biofertilizers (Probio, Glicompost and StarTmik) combined with 50% NPK rice produced the same yield with single NPK [21]. Lowland inoculated rice plants with biofertilizers were not significantly taller and higher in number than biofertilizers, but significant at number of leave [15].

Table 4. Correlation between observed parameters after treatments

| Pearson Correlations Value | 50% Flowering day | Plant Height | Number of Total Tiller | Number of Productive Tiller | Panicle Length | Panicle Weight | Number of Filled Grain | Percentage of Empty Grain |
|----------------------------|-------------------|--------------|-----------------------|----------------------------|----------------|---------------|------------------------|--------------------------|
| 50% Flowering day          | 1                 | -.232        | -.340                 | -.458                      | -.082          | -.012         | -.363                  | .345                     |
| Plant Height               | -.232             | 1            | .380                  | .337                       | .596**         | .641**         | .690**                 | .129                     |
| Number of Total Tiller     | -.340             | .380         | 1                     | .882**                     | .346           | .251          | .403                   | -.284                    |
| Number of Productive Tiller| -.458             | .337         | .882**                | 1                          | .240           | .285          | .430                   | -.338                    |
| Panicle Length             | -.082             | .596**       | .346                  | .240                       | 1              | .643**        | .778**                 | .131                     |
| Panicle Weight             | -.012             | .641**       | .251                  | .285                       | .643**         | 1             | .841**                 | -.232                    |
| Number of Filled Grain     | -.363             | .690**       | .403                  | .430                       | .778**         | .841**        | 1                      | -.311                    |
| Percentage of Empty Grain  | .345              | .129         | -.284                 | -.338                      | .131           | -.232         | -.311                  | 1                        |

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).
Figure 2. The biplot analysis fertilizers effect of fertilizer treatments to yield component.

4. Conclusion
The application of BOF both (single or combination) with inorganic fertilizer increased the percentage of mycorrhizal colonization in upland rice roots, but had no effect on root weight and yield components. The application of BOF in combination with 50% inorganic fertilizer had a similar effect to 100% inorganic treatment on the growth of rice plants while single BOF treatment resulted in highest significant different effect to the growth of rice plant from that of inorganic single and inorganic combination with BOF treatment.

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