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Impact of Biofertilizer and Inorganic Fertilizers on Soil Available Phosphorus, Productive Tillers, and Rice (*Oryza sativa* L.) Yield

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ABSTRACT

The use of Bacillus bacteria as biofertilizers (BF) increases since the detrimental effects of inorganic fertilizers on the agricultural environment spread out. The objective of this research was to study the influence of bio fertilizer and in combination with inorganic fertilizer on the productivity of rice fields. The experiment was a two-factor treatment arranged in a split-plot design with three replications using a randomized complete block design (RCBD). The first factor was fertilizers, which were used as the main plot. The second factor was paddy varieties as a subplot. The results showed that the combination of inorganic and bio fertilizer application of (150 kg NPK ha^{-1} + 75 kg Urea ha^{-1} + 4 L BF ha^{-1}) and (300 kg NPK ha^{-1} + 150 kg Urea ha^{-1} + 4 L BF ha^{-1}), improved soil available phosphorous (P) of 12.04%, 40.69%, and 44.05%, respectively compared to control treatment, the inorganic fertilizer application (300 kg NPK ha^{-1} + 150 kg Urea ha^{-1}), and the bio fertilizer application (4 L BF ha^{-1}). In addition, the application of (300 kg NPK ha^{-1} + 150 kg Urea ha^{-1} + 4 L BF ha^{-1}) increased productive tillers per plant as 28.13% and 16.48% compared to the control and the inorganic application, respectively. Moreover, by using Mekongga variety, applying the inorganic and bio fertilizer at rates of (300 kg NPK ha^{-1} + 150 kg Urea ha^{-1} + 4 L BF ha^{-1}) increased rice yield of 64.08% and 30.33% compared to the control and the inorganic fertilizer application; while, by using IPB 3S variety, the rice yield increased by 85.80% and 10.34%, respectively. These findings are significant as they demonstrate the potential of biofertilizers to enhance soil fertility and rice productivity, thereby advancing sustainable farming practices.

Keywords: *Bacillus*, inorganic fertilizer and phosphate solubilization

INTRODUCTION

Widely grown rice (*Oryza sativa* L.) is a staple food in East, Southeast, and South Asia (Zheng et al., 2019). The demand for rice and the population are rising significantly. In order to boost food production, this has led to the application of high-inorganic phosphate fertilizers to the majority of agricultural fields in use today (Withers et al., 2014). Most importantly, because phosphorus (P) is essential for transferring energy through adenosine triphosphate, plants depend on it to ensure energy transfer during photosynthesis (Carstensen et al., 2018).

However, only a minute of applied P to soils is left available for plant uptake when soil pH is acidic due to inorganic P fixations in soil by Al^{3+} and Fe^{3+} (Ch'ng et al., 2014). However, rice plant production also depends on high nitrogen (N) fertilizer applications because N deficiency exists in most rice fields globally (Bashan and de-Bashan, 2010). On the other hand, weak and sparse root formation, combined with N and P nutrient deficits, leads to poor nutrient and water absorption from the soil, further impeding plant growth, eventually, and low rice yields.

Plant growth is also influenced by phytohormones such as indole-3-acetic acid (IAA). A variety of beneficial bacteria, including *Bacillus* sp., can produce this hormone (Spaepen, 2015). According to Ahemad and Khan (2012) and Dazzo and Yanni (2006), the molecule IAA plays a crucial role in the processes that promote and facilitate plant growth. Furthermore, Husna *et al*. (2019) examined a group of *Bacillus* species in a lab setting and discovered that the bacteria could dissolve phosphate, fix N_2, and synthesize IAA, thereby promoting plant growth and health.

Therefore, this study assessed the suitability of the *Bacillus* bacteria consortium for the rice field. Our investigation aimed to determine the effects of *Bacillus* biofertilizer on soil available phosphorous (P), productive rice tillers, and yields of two paddy varieties, either in isolation or combined with inorganic fertilizers (NPK and Urea).

MATERIALS AND METHODS

Experimental Duration and Site

The experiment was carried out at the experimental station of Bogor Agricultural University (IPB), Sawah Baru, a location known for its significant contributions to agricultural research and practice. This site, situated at (6°33'50.4"S 106°44'09.9"E, altitude 250 m above sea level), was chosen for its diverse soil characteristics and its representative nature of many rice-growing regions. The study was conducted in the dry season when the monthly average air temperature was 25.6°C. The experimental site's pre-transplanting soil characteristics included available phosphorous (P) of 3.0 mg kg^{-1}, available potassium (K) of 177.6 mg kg^{-1}, organic carbon of 2.4%, organic matter of 4.2%, and N-total of 0.39%.

Experimental design

The experiment was a two-factor treatment arranged in a split-plot design with three replicates using a randomized complete block design (RCBD). The first factor was fertilizers applied in seven combination rates, as the main plot consisted of: 0 (NPK + Urea) + 0 BF, 75 kg (NPK) ha^{-1} + 37.5 kg (Urea) ha^{-1}+ 4 L BF ha^{-1}, 150 kg (NPK) ha^{-1} + 75 kg (Urea) ha^{-1} + 4 L BF ha^{-1}, 225 kg (NPK) ha^{-1} + 112.5 kg (Urea) ha^{-1} + 4 L BF ha^{-1}, 300 kg (NPK) ha^{-1} + 150 kg (Urea) ha^{-1} of + 4 L BF ha^{-1}, 0 kg (NPK + Urea) ha^{-1}+ 4 L BF ha^{-1}, and 300 kg ha^{-1} of (NPK) + 150 kg ha^{-1} of (Urea). The second factor was the rice varieties that consisted of Mekongga and IPB 3S and were assigned as sub-plots. Materials used in the experiment are rice seeds of Mekongga and IPB 3S; fertilizer: NPK (15:15:15); and Urea (46% N). *Bacillus* species biofertilizer (BF) contained ten strains of *Bacillus* species (*B. catenulatus, B. cereus, B. drentensis, B. firmus, B. flexus, B. megaterium, B. niacin, B. subtilis, B. tequilensis,* and *B. thuringiensis*) with a total population of 7.6 x 10^{11} CFU mL^{1}. As with other flooded paddy fields, the field was prepared. It was done occasionally with irrigation. With a planting distance of 25 cm by 25 cm and a sub-plot size of 5 m by 5 m, one 14-day-old seedling from the nursery was transplanted per hole. To reduce contamination from fertilizers, a 50-cm-wide ridge was used to divide the subplots. Three times, at one, four, and six weeks after transplanting (WAT), 250 g of NPK fertilizer were applied per plot; at one and four WAT, 250 g and 125 g of Urea fertilizer were applied per plot, respectively. The following is how the *Bacillus* sp. biofertilizer was applied: 60 mL were used to soak the seeds, and at 2, 4, 6, and 8 week after seedlings transplanting, up to 2.5 mL of direct soil spraying were applied to each plot.

Crop management (weeds and pests control)

Weeds were controlled manually. Pests were controlled by using a pesticide (Dimehipo 410 g L^{-1}. This pesticide was applied to rice plants fertilized under the control group using only inorganic fertilizers. Pest attacks in rice plants fertilized with inorganic fertilizers in combination with BF were controlled by sprays of *Bacillus species* on the plant canopy. *Bacillus thuringiensis* is also known to paralyze and destroy the pest's digestive systems and guts, eventually leading to death as the pests eat the leaves and ingest the bacteria. Birds attacks were controlled by the installation of a bird's net.

Data collection

Samples of soil were taken using composite soil sampling techniques before planting. After harvest, each plot's soil was sampled, and the Bray and Kurtz Method was used to analyze the soil's available P content in each plot. Five randomly chosen plant samples were marked in each plot to be observed and collected data. The number of productive tillers was counted for five chosen plant samples. Grain yields per ha were calculated after harvest by taking grain samples per plot (at 12% moisture content) within 2.5 m × 2.5 m, then multiplied by 1600.

Data analysis

The data were analyzed through ANOVA using SAS version 9.4. The treatments were further compared using the Duncan Multiple Range Test (DMRT) at α 5%.

RESULTS AND DISCUSSION

Soil-available phosphorous (P)

Table 1 shows that soil-available P improved from deficient status at pre-planting to medium-high status at post-harvest. Soils fertilized with 150 kg

Table 1. Soil-available P at pre-planting and post-harvest.

Treatments			Soil available P (mg kg^{-1})	
NPK (kg ha^{-1})	UREA (kg ha^{-1})	BF (L ha^{-1})	Pre-planting	Post-harvest
0	0	0		10.8^{M}
75	37.5	4		9.8^{M}
150	75	4		12.1^{MH}
225	112.5	4	3.0^{VL}	11.2^{MH}
300	150	4		12.1^{MH}
0	0	4		8.4^{M}
300	150	0		8.6^{M}

Composite soil sampling procedures were applied at pre-planting. According to the Bray-P1 phosphorous level classification in soil, VL is Very Low, M is Medium, and MH is Medium-High.

NPK ha^{-1} + 75 kg Urea ha^{-1}+ 4 L BF ha^{-1} and 300 kg NPK ha^{-1} + 150 kg Urea ha^{-1} of + 4 L BF ha^{-1} had 40.69% more soil available P compared to the soil applied with 300 kg NPK ha^{-1} + 150 kg Urea ha^{-1}. Comparable treatments resulted in an increase in soil-available P of 12.04%, 23.47%, and 44.05% in comparison with control, 75 kg NPK ha^{-1} + 37.5 kg Urea ha^{-1} + 4 L BF ha^{-1}, and only 4 L BF ha^{-1}, respectively. Additionally, the application of 225 kg NPK ha^{-1} + 112.5 kg Urea ha^{-1} + 4 L BF ha^{-1} led to an increase in soil available P of 3.70%, 14.29%, 30.23%, and 33.33% compared to soil under control treatment, 75 kg NPK ha^{-1} + 37.5 kg Urea ha^{-1} + 4 L BF ha^{-1}, 300 kg NPK ha^{-1} + 150 kg Urea ha^{-1}, and only 4 L BF ha^{-1}, respectively. Medium-high amounts of soil-available P at post-harvest implied the success of the applied Bacillus bacteria in phosphate solubilization. However, research by Prasanna et al. (2011) also revealed that the use of phosphate-solubilizing bacteria (PSB) increased the amount of soil-available P in paddy soils.

Productive Tillers

Bacillus sp. must be combined with inorganic fertilizers to produce plenty of productive tillers (Table 2). However, after harvest, we found more lateral roots in the rice plants treated with BF (Figure 1). The result was attributed to the release of the exogenous auxin hormone by *Bacillus* sp. (Amara et al., 2015; Tolboys et al., 2014). Additionally, the results regarding the amount of P in the soil indicate that the soils treated with BF showed elevated levels of soil-available P. Further reveals *Bacillus*'s ability to do phosphate solubilization.

Furthermore, we observed that compared to the control group (Figure 4), the rice plants treated with BF were greenish (Figures 2, 3, and 5). The

Table 2. The average number of productive tillers per plant in fertilizers treatments and rice varieties.

Treatments			Productive Tillers
NPK (kg ha^{-1})	UREA (kg ha^{-1})	BF (L ha^{-1})	(number)
0	0	0	16.00^{c}
75	37.5	4	18.97^{ab}
150	75	4	19.03^{ab}
225	112.5	4	19.63^{ab}
300	150	4	20.50^{a}
0	0	4	17.00^{bc}
300	150	0	17.60^{bc}
Varieties			
Mekongga			19.94^{a}
IPB 3S			16.89^{b}
Interaction			NS

Means marked by the same letter within the column show no significant difference at á 5% according to DMRT. ns = not significant

Figure 1. Roots of rice plants after harvest. M1 represents roots under fertilization of 300 kg NPK ha^{-1} + 150 kg Urea ha^{-1} + 4 L BF ha^{-1}. M5 represents 300 kg NPK ha^{-1} + 150 kg Urea ha^{-1}. BSB represents only 4 L BF ha^{-1}. M2 represents 150 kg NPK ha^{-1} + 75 kg Urea ha^{-1} + 4 L BF ha^{-1}. M4 represents 225 kg NPK ha^{-1} + 112.5 kg Urea ha^{-1} + 4 L BF ha^{-1}. Co represents Control group or unfertilized rice roots. M3 represents 75 kg NPK ha^{-1} + 37.5 kg Urea ha^{-1} + 4 L BF ha^{-1}.

condition was affected by the N nutrients from fertilizers that were applied, as well as the ability of *Bacillus* sp. to fix N_2 in soils. Nitrogen is an essential part of chlorophyll, the green pigment used in photosynthetic processes, primarily to enhance the absorption of solar light energy. Moreover, by raising crop yields, the nutrient N plays a crucial role in agriculture (Leghari et al., 2016). Consequently, the overall effects of BF and inorganic fertilizer were positive in the sense that the roots' absorption of nutrients from the soil led to highly productive tillers. Danuwikarsa and Robana (2017) have also reported improved productive tillers due to biofertilizer applications for rice plant growth. The varieties' respective levels of productive tillers varied considerably. Regarding productive tillers, the Mekongga variety had an 18.4% advantage over IPB 3S as the descriptions of the varieties.

Paddy Yields

Table 3 shows that, the rice plants fertilized with 225 kg NPK ha^{-1} + 112.5 kg/ha^{-1} of Urea + 4 L of BF ha^{-1}, 300 kg ha^{-1} of NPK + 150 kg ha^{-1} of Urea + 4 L of BF ha^{-1}, and only 4 L of BF ha^{-1} resulted in more paddy yields. The results were noticeably different from the other four fertilizer treatments in the Mekongga variety. In the Mekongga variety, treatment with 300 kg NPK ha^{-1} + 150 kg Urea ha^{-1} + 4 L BF ha^{-1}, and 4 L BF ha^{-1} increased paddy yields by 10.46%, 30.33%, and 64.08%, respectively, when compared to only 4 L BF ha^{-1}, only inorganic fertilizers, and the control group, respectively. The same treatment increased yields by 14.29%, 10.34%, and 85.81% in the IPB 3S variety compared to rice plants treated with only BF, inorganic fertilizer, and the control group, respectively. The results further show

Figure 2. Rice plants fertilized with 300 Kg NPK ha^{-1} +150 kg Urea ha^{-1} + 4 L BF ha^{-1}.

Figure 3. Rice plants fertilized with 225 kg NPK ha^{-1} + 112.5 kg Urea ha^{-1} + 4 L BF ha^{-1}

Figure 4. Unfertilized rice plants (Control).

Figure 5. Rice plants applied with only Bacillus sp. biofertilizer.

that, in the Mekongga and IPB 3S varieties, the application of 225 kg NPK ha^{-1} + 112.5 kg Urea ha^{-1} + 4 L BF ha^{-1} increased paddy yield by 27.51% and 8.06% in comparison to the application of inorganic fertilizers only and only BF, respectively. The lowest paddy yield was produced by plants treated as the control group for both paddy varieties. Moreover, paddy yields resulted to the application of 300 kg NPK ha^{-1} + 150 kg Urea ha^{-1} did not statistically differ from those obtained in application of 75 kg NPK ha^{-1} + 37.5 kg Urea ha^{-1} + 4 L BF ha^{-1} and 150 kg NPK ha^{-1} + 75 kg Urea ha^{-1} + 4 L BF ha^{-1} for the Mekongga variety. Our findings regarding the increase in paddy yields brought about by the use of biofertilizer in rice fields are consistent with (Fitri and Gofar, 2018).

Despite reducing inorganic fertilizers by 75% and 50% in the latter two fertilizer treatments compared to application of 100% of the chemical fertilizer recommended rates, no significant difference in paddy yields was observed. It indicates that the *Bacillus* sp. biofertilizer added to improve nutrient availability through phosphate solubilization and N- fixation (Islam et al., 2012) was successful. However, pre-transplanting soil properties indicated a high level of organic matter, which meant that the added *Bacillus* bacteria could work successfully in mineralization (Ishak and Brown, 2019). It could be explained in the case of the application of 4 L BF only ha^{-1}, which yielded 17.2% more paddy yield compared to rice plants under treatment with 300 kg NPK ha^{-1} + 150 kg Urea ha^{-1} in the Mekongga region. As a result, rice plant roots could absorb a significant quantity of nutrients from the soil because of the extensive root volume observed in all BF treatments. It was due to the influence of the auxin hormone secreted by the *Bacillus* bacteria (Aeron et al., 2011; Husna et al., 2019).

Table 3. The effects of applied fertilizer treatments and rice varieties on paddy yield.

Treatments			Paddy Yields (Mg ha^{-1}) 12% MC	
NPK (kg ha^{-1})	UREA (kg ha^{-1})	BF (L ha^{-1})	Mekongga	IPB 3S
0	0	0	3.09^{cde}	1.55^{g}
75	37.5	4	3.31^{bcd}	2.29^{f}
150	75	4	3.73^{bc}	2.51^{ef}
225	112.5	4	4.96^{a}	2.83^{def}
300	150	4	5.07^{a}	2.88^{def}
0	0	4	4.59^{a}	2.52^{ef}
300	150	0	3.89^{b}	2.61^{ef}

Means marked by the same letter within the column show no significant difference at á 5% according to DMRT. MC. Moisture content.

only ha^{-1}, which yielded 17.2% more paddy yield compared to rice plants under treatment with 300 kg NPK ha^{-1} + 150 kg Urea ha^{-1} in the Mekongga region. As a result, rice plant roots could absorb a significant quantity of nutrients from the soil because of the extensive root volume observed in all BF treatments. It was due to the influence of the auxin hormone secreted by the *Bacillus* bacteria (Aeron et al., 2011; Husna et al., 2019).

CONCLUSIONS

Applying special-purpose, non-pathogenic bacteria (*Bacillus* sp.) in conjunction with inorganic fertilizers resulted in a notable increase in rice yields and yield-producing tillers. Additionally, it was discovered that the *Bacillus* bacteria improved soil-available P in rice fields. However, regarding paddy yield and rice plant growth, the Mekongga variety responded more favorably to BF and its combination of inorganic fertilizers. Therefore, we acknowledge increasing rice fields' productivity by incorporating *Bacillus* sp. with inorganic fertilizers.

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