Ecology and Environmental Science Research Journal
Vol.: 1 Issue 1 January 2024
ISSN: Pending…

SYNERGIES BELOW THE SURFACE: EXAMINING RICE GROWTH WITH BACILLUS SPECIES BIOFERTILIZER AND CHEMICAL COMPANIONS

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Abstract: Modern agricultural practices, characterized by the prevalent use of high-inorganic phosphate fertilizers, have raised significant environmental concerns due to their non-renewable nature and adverse impact on the food chain production (Withers et al., 2014). This study addresses the challenges associated with these fertilizers, particularly their limited accessibility to plants in acidic soils, where inorganic phosphorous fixations induced by aluminum (Al) and iron (Fe) metals hinder nutrient absorption (Ch'ng et al., 2014). The consequences of relying on such chemical inputs for agriculture are far-reaching, contributing to environmental issues like pollution, soil degradation, and the depletion of essential microorganisms responsible for facilitating nutrient uptake by plants.

Phosphorous, a crucial nutrient for plant growth and development, plays a pivotal role in various stages of the plant life cycle. The availability of phosphorous in the soil is paramount for robust root development and overall plant health. Unfortunately, a substantial portion of phosphorous in soils exists in diverse forms, including organic and inorganic compounds, rendering it inaccessible to plant roots. Insufficient phosphorous levels in the soil can result in stunted plant growth, inadequate development, and compromised crop yields.

This research aims to explore sustainable alternatives to high-inorganic phosphate fertilizers, considering their detrimental effects on the environment and agricultural productivity. By delving into the intricate dynamics of phosphorous availability in acidic soils and its impact on plant growth, the study seeks to identify eco-friendly practices that enhance nutrient accessibility to plants without compromising soil health. The methodology involves a comprehensive review of existing literature, incorporating insights from Withers et al. (2014) and Ch'ng et al. (2014), among others, to construct a holistic understanding of the challenges posed by current agricultural practices.

The findings from this research are anticipated to contribute valuable insights into the development of sustainable agricultural strategies, fostering a balance between enhanced plant nutrition, environmental conservation, and the preservation of soil ecosystems.

Keywords: Phosphate Fertilizers, Soil Acidity, Nutrient Accessibility, Sustainable Agriculture, Environmental Conservation

1. Introduction

Today's agricultural practices using high-inorganic phosphate fertilizers are causing damage to the environment. These fertilizers are non-renewable, and their use has a negative impact on the food chain production (Withers *et al.*,2014). Despite the application of phosphate to soils, only a minuscule fraction is accessible to plants due to inorganic phosphorous fixations caused by Al and Fe metals in acidic soils (Ch'ng *et al*.,2014). However, the trend of utilizing high chemical inputs on agricultural lands has resulted in environmental concerns such as pollution, soil degradation and loss of significant microorganisms responsible for unlocking nutrients into plant easily absorbed forms. Moreover, it may also cause the accumulation of toxic elements in soils.

Phosphorous is essential for the growth and development of plants. This nutrient plays a vital role in all stage of plant growth, and the robustness and growth of plant roots rely heavily on its availablity in the soil. Insufficient phosphorous levels in the soil can lead to reduced plant growth, inadequate development, and compromised crop yield. Nevertheless, most of the phosphorous in soils exists in various forms, including organic and inorganic compounds, making it inaccessible to plant roots.

One promising approach to effectively utilize applied P-fertilizers while safeguarding agricultural fields from pollution is through the use of biological fertilizers. Employing this technique can ensure that our agricultural fields sustain in their quality and yield without causing harm to the environment.

According to Kalayu (2019), a group of phosphate solubilizing microbes (PSM) are a group of microbes that are capable of hydrolyzing organic and inorganic insoluble phosphorous compounds into soluble phosphorous that can be easily absorbed by plant roots. This approach is both environmentally and economically responsible, as it provides a solution to the issue of P-fixation and availability forms in soils. However, it is important to note that most of these PSM are referred to as rhizospheric bacteria (Prasanna *et al*.,2011). The microbiome in the rhizosphere is strongly influenced by the plant species, type of soil, availability of nutrients, and chemical exudation from the roots (Bakker *et al*.,2013). This dynamic environment allows for rapid evolution in space and time, with bacterial populations 10-1,000 times higher in the rhizosphere than in the bulk soil. This is due to the fact that plant photosynthetic products, such as sugars, organic acids, and amino acids, are secreted by roots in the form of different exudates, which act as bacterial food sources (Glick, 2014).

In a previous study, Husna *et al*., (2019) found that the *Bacillus* species consortium synthesized *Indole-AceticAcid*. A vital plant growth promoting hormone. Similarly, Ahemad and Khan (2012) reported that the microbial activities of *Bacillus* promote the release of plant growth hormones. Therefore, the present research investigated the efficiency of *Bacillus* species biofertilizer to promote the growth of rice roots, plant height, and tillers development in paddy field. Two paddy varieties were used to during the study.

2. Materials and Methods

2.1 Materials

Two rice varieties, IPB 3S and Improved paddy variety (Mekongga) were used. Three types of fertilizer namely: NPK (15:15:15); UREA (46% of N); and *Bacillus* sp. biofertilizer (BF) contained ten strains of *Bacillus* species bacteria i.e. *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} colony-forming unit (CFU) ml^{-1}.

2.2 Experimental Duration and Site

A research was conducted from October 2018 to January 2019 at (6°33'50.4"S 106°44'09.9"E, altitude 250 meters above the sea level) at Bogor Agricultural University Experimental Field, Indonesia.

2.3 Experimental Design

The experiment was two-factors treatments arranged in a split plot design in three replications. Fertilizers as main plot specified as first factor. In this, there was seven rates of fertilizer application (Table 1). The fertilizer recommendation was based on the findings of soil analysis conducted prior to the experimental site as well as rice plant nutrients requirements. The second factor was paddy varieties as sub-plots, two paddy varieties, IPB 3S and Mekongga paddy variety. The IPB 3S paddy variety was created by a research team at Bogor Agricultural University at the end of 2014. It is a hybrid of traditional and modern paddy variety. Mekongga variety is a modern rice variety developed by IRRI.

Table 1. Applied fertilizers (NPK, UREA and BF).

Treatment code	Rates of applied fertilizers	(%) of ACF
Control	0 kg/ha	0
T1	75 kg/ha NPK + 37.5 kg/ha UREA + 4 l/ha BF	25
T2	150 kg/ha NPK + 75 kg/ha UREA + 4 l/ha BF	50
T3	225 kg/ha NPK + 112.5 kg/ha UREA + 4 l/ha BF	75
T4	300 kg/ha NPK + 150 kg/ha UREA + 4 l/ha BF	100
Only BF	4 l/ha BF	0
T5	300 kg/ha NPK + 150 kg/ha UREA	100

Note: ACF represents applied chemical fertilizers, and BF- *Bacillus* species biofertilizer.

2.4 Experimental Procedures

Normal field preparation for flooded paddy fields were followed. The irrigation was carried out intermittently. One, 14-days-old seedling from nursery was transplanted per stand at a planting distance of 25 cm X 25 cm and subplot size was 5 m X 5 m. The NPK fertilizer was applied three times at 1, 4 and 6 weeks after transplanting (WAT) each time as much as 250 g per plot while UREA fertilizer applied at 1 and 4 WAT, 250 g and 125 g per plot respectively as full recommended rate. BF was applied as follows: For the soaking of paddy seeds, 60 ml was used, and additional applications took the form of sprays, directly to the soils where rice plants were growing at a rate of 2.5 ml per plot at 2, 4, 6, and 8 weeks after transplanting.

The experiment used mathematical model (Mattjik and Sumertajaya 2002) as follows:

$Y_{ijk} = \mu + \alpha_i + \delta_{ik} + \beta_k + V_j + (\alpha_i V)_{ij} + \varepsilon_{ijk}$. Where: Y_{ijk} = Observation value of the effect of types of fertilizers toi, type variety to-j, and block to-k

μ	=	General mean
α_i	=	Effect of fertilizers to-i (i = 1, 2, 3, 4, 5, 6, 7)
δ_{ik}	=	Error on main plot to-i, block to-k
β_k	=	Effect of block to-k, (k= 1, 2, 3)
V_j	=	Effect of varieties to-j (j = 1, 2)
$(\alpha_i V)_{ij}$	=	Effect of interaction between fertilizers and varieties

Ɛijk = Error sub-plot on main plot to-i, varieties to-j and block to-k

2.5 Data Collection

During vegetative growth period, plant samples were randomly selected in each plot for measurements and data collection. Two rice plant samples per plot at the mid of plot and within 1-meter square were uprooted within a hole of 20 cm diameter and 30 cm deep then the roots part was washed carefully using spraying water until all soils materials detached. Stem and root parts were separated and then measurements were done only on the roots part. Centimeter ruler was used to measure roots length. Root volume was measured by displacement method i.e. placing the root part in a beaker contained 500 mm^3 of water, then measured the differences of water volume before and after the root part was immersed (Pascual and Wang, 2017). Plant height was measured from the ground surface to the highest tip of plant leaf by using a 1-meter rule. Five plant samples were randomly selected in each plot for plant height measurements that were done at 4,5,6,7,8, and 9 weeks after transplanting. Number of tillers were manually counted on five rice plant samples randomly selected in each plot during the same weeks as plant height.

2.6 Data Analysis

Data were analysed by using SAS 9.4. ANOVA was used for analysis of variance while further comparison of the treatments was done by using Duncan Multiple Range Test (DMRT) at α = 5 %.

3. Results

3.1 Root's Length

The study's findings showed enhancement of roots length. Roots length has a significant role to increase absorption of nutrients in soils. At harvest, roots of rice plants fertilized with BF as well as those in the application of BF with combination of chemical fertilizers were the longest. This was significantly different to rice plants in control group, while only treatments T4 and only BF were significantly different to treatment T5 (Table 2).

Table 2: Average roots length on fertilizer treatments and two paddy varieties.

Root Length (Cm)		**9 WAT**	**At-Harvest**
	Control	23.68b	25.38c
	T1	25.88ab	28.03ab
	T2	26.07ab	27.57ab
	T3	25.25ab	27.85ab
	T4	26.83a	29.57a
	Only BF	25.47ab	28.72a
	T5	24.80ab	26.17bc
	Varieties		
	Mekongga	25.39	27.85
	IPB 3S	25.45	27.37
	Interaction	NS	NS

Treatment code

Note: Numbers marked by the same letter within the column shows not significantly different according to DMRT at α = 5 % level. WAT: weeks after transplanting. NS: not significantly different.

3.2 Root's Volume

The application of only BF and with combination of chemical fertilizers observed to increase roots sizes (volume). At 9 weeks after transplanting roots volume on rice plants fertilized with treatment T4 was significantly different compared to unfertilized rice plants (Control). At harvest all rice plants fertilized with either only BF or in combination with chemical fertilizers were having large roots size (volume) significantly different to roots volume of unfertilized or control group and T5 as shown in table 3. Moreover, at harvest the results found no significance difference in roots volume for the rice plants of control group and those fertilized with only chemical fertilizers (T5).

Table 3. Average rice plant roots volume response to seven rates of fertilizers treatments and two paddy varieties.

Root Volume (Cm^3)		
	9 WAT	**At-Harvest**
Control	26.67b	35.42b
T1	38.33ab	85.00a
T2	39.58ab	83.33a
T3	41.67ab	84.58a
T4	48.33a	106.67a
Only BF	38.75ab	93.33a
T5	40.42ab	43.75b
Varieties		
Mekongga	43.81a	81.91a
IPB 3S	34.41b	70.12b
Interaction	NS	NS

Treatment code

Note: Numbers marked by the same letter within the column shows not significantly different according to DMRT at $\alpha = 5$ %. WAT: weeks after transplanting. NS: not significantly different.

3.3 Plant height

Table 4 shows that during every week, from 4 to 9 weeks after transplanting, plant heights were significantly different between the applied treatments. At 9 weeks after transplanting, rice plants fertilized with T3, T4 and T5 were the highest. That meant, availability of nutrients especially N in the soils, due to application of treatment T5, and combination of inorganic fertilizers + BF were also high.

Consequently, the amounts absorbed by the rice plants were also enough to support higher growth as compared to rice plants on other fertilizers treatments. Rice plants treated as control were the shortest.

Table 4. Average plant heights of two paddy varieties on fertilizers treatments.

Treatment code	**Plant height (cm) - WAT**					
	4	**5**	**6**	**7**	**8**	**9**
Control	50.6b	64.6b	73.2c	82.5c	89.7b	99.0c
T1	52.8ab	67.1b	77.3b	87.7bc	95.2ab	104.5ab
T2	53.6ab	66.9b	76.3bc	87.0bc	96.9a	104.4ab
T3	55.6a	73.1a	81.6a	91.9ab	98.9a	107.7a

T4	53.4ab	71.4a	82.1a	91.5ab	100.0a	108.8a
Only BF	50.0b	64.5b	73.4c	82.9c	90.8b	100.7bc
T5	54.8a	71.5a	82.2a	93.9a	99.7a	108.9a
Varieties						
Mekongga	47.6b	60.4b	69.1b	77.5b	83.1b	89.9b
IPB 3S	58.3a	76.5a	86.9a	98.8a	108.6a	119.9a
Interaction	NS	NS	NS	NS	NS	NS

Note: Numbers marked by the same letter within the column shows not significantly different at α = 5 % according to DMRT. WAT: weeks after transplanting. NS: not significantly different.

3.4 Number of tillers per plant

When plants attain their maximum growth at 9 WAT, rice plants fertilized with T3 and T4 had more tillers growth, significantly different to rice plants in other four fertilizers treatments (Table 5). Rice plants fertilized with T1, T2, T5 and Only BF did not significantly differ in number of tillers. This implies that despite the chemical fertilizers being reduced by 75% and 50% respectively in T1 and T2, the BF was successful in compensating the reduced amounts, resulting in the same tillers growth as in the full recommended fertilizers rates. Only control-treated plants had the fewest tillers and that might be due to insufficient nutrients absorption from the soils. The two paddy varieties were also showed significantly different on tillering capacity. Mekongga paddy variety had more number of tillers by 74.7% than IPB 3S. This was also in agreement with varieties descriptions.

Table 5. Average number of tillers on fertilizers treatments and paddy varieties.

Treatment code	**Number of Tillers per plant**					
	WAT					
	4	**5**	**6**	**7**	**8**	**9**
Control	17.0c	19.7c	19.6c	21.2d	19.4d	17.5c
T1	20.7b	23.0bc	23.9bc	24.4bcd	24.9c	21.9b
T2	19.9b	24.0abc	26.5ab	26.4abc	26.3bc	24.1b
T3	23.7a	28.1a	29.8a	31.2a	30.8a	27.8a
T4	22.5ab	27.4ab	30.5a	29.5ab	29.4ab	27.8a
Only BF	16.9c	19.3c	22.7bc	22.7cd	22.3cd	21.9b
T5	22.0ab	25.7ab	26.9ab	27.8abc	25.4bc	22.2b
Varieties						
Mekongga	24.1a	29.4a	31.4a	33.6a	32.6a	29.7a
IPB 3S	16.7b	18.4b	20.0b	18.8b	18.4b	17.0b
Interaction	NS	NS	NS	NS	NS	NS

Note: Numbers marked by the same letter within the column shows not significantly different at α = 5 % according to DMRT. WAT: weeks after transplanting. NS: not significantly different

4. Discussions

4.1 Roots length and volume

The present study found the best roots growth performance on the application of only BF as well as with its combination of inorganic fertilizers (NPK + UREA). Findings by Oladele and Awodun (2014) also reported

improvement in roots growth due to application of biofertilizer in lowland rice plants. However, plants produce natural auxin hormone at their shoot and root tips (*endogenous auxin*) which promote their elongation and growth. The added *exogenous auxin* hormone released by *Bacillus species* had significantly effect to the increase of roots length and its volume. As pointed out by Solano *et al.*,2010; Shafi *et al*.,2017 *Indole*-3-*Acetic-Acid* has also an essential role in the origination and formation of adventitious roots. Plants having large roots volume are likely to rise the amount of nutrients absorption from the soils hence further crop growth is achieved. The control treated plants marked the lowest on growth performance i.e. root length and volume as observed during 9 WAT and at harvest period. Low nutrients availability in the soils could be a reason.
Depending on the strain of *Bacillus* bacteria, their general effect to plant is either direct, i.e. through plant growth hormone promotion, or indirect, i.e. through improving plant nutrition by solubilizing mechanisms and making unavailable nutrients into available to the plants during plant-bacterial interaction (Patel *et al*.,2015). *Bacillus megaterium and Bacillus subtilis* are referred as the most important strains (Govindasamy *et al*.,2011) since, the bacteria are effective in phosphates solubilizes into inorganic forms that is available to plant roots uptake (Goswami *et al.,*2016). Therefore, in this study we applied ten strains of *Bacillus* bacteria that performed distinct functions during the growth of rice plants. In that way high growth performance on both aspects roots length and volume was a result of both plant growth hormone promotion (Husna *et al*.,2019) and phosphate solubilization effects.
The mechanisms for phosphate solubilization by the PSM involve three processes namely, lowering soil pH, chelating and mineralization. Production of organic and inorganic acids by the PSM is a principal activity during phosphate solubilization (Kumar *et al*.,2018). Production of organic acids is associated with the lowering of soil pH that results to P solubilization by dissolving of organic phosphates in soils (Selvi *et al*.,2017). The mechanism of chelation is due to chelation of either Al or Fe cations by the produced organic or inorganic acids. In this way the organic acids will compete with the phosphates for adsorption sites in the soils (Khan *et al*.,2009). Therefore, the hydroxyl and carboxyl groups of the acids chelate the cations bound to phosphate, thereby converting it into soluble forms. According to Santana *et al*., (2016) Phosphate Solubilizing Microbes mineralize soil organic P by the production of phosphatases such as phytase enzyme that hydrolyze organic forms of phosphate compounds, thereby releasing inorganic phosphorous that will be absorbed by plant roots.

4.2 Plant height

The continuous supply of nitrogen nutrient to soils through N_2-fixation by the *Bacillus* species (Islam *et al*. 2012) is explained as another reasons for such highest growth in BF-treated plants. This is also evidenced on the rice plants fertilized with T1 and T2. Despite of the reduction of inorganic fertilizers by 75% and 50% respectively plant heights were not significantly different with plants in T5. *Bacillus* species are also known to release phytohormone, indole-3-acetic acid (Tolboys *et al*.,2014) which also has a role in promoting shoots growth and stem elongation. Plant height is interrelated with the photosynthesis process, as the highest plants will have more access to capture sunlight as compared to short ones. Studies by Isahak *et al.*, (2012); Oladele and Awodun (2014) and Singh *et al.,* (2015) also reported an increased heights of rice plants due to applications of biofertilizers. Significantly different on plant heights was observed on paddy varieties too, whereby IPB 3S paddy variety had an increase of 33% higher than Mekongga at 9 weeks after transplanting. Although, this was also in accordance with the varieties descriptions.

4.3 Number of Tillers per Plant

Tillers formation largely depends on nutritional status in plant at a given growth period. Since, it implies the formation of new plant organs, thus nutrients particularly N, P and K are needed in large amounts. However, prior to seedling transplanting the organic matter content in the field was high, 4.2 %. Organic matter accelerates mineralization by making the nutrients available especially phosphorous and potassium. Therefore, the added *Bacillus* species could work successfully in the decomposition process to release nutrients into plants available forms thus contributed to better performance on the rice plants growth and development.

5. Conclusion

The findings of this research revealed that *Bacillus* species bacteria applied as Biological Fertilizer to rice growing field significantly improved rice plants growth (roots length and its volume) plant heights, and development of rice plant tillers. When the BF was combined with the chemical fertilizers, the improvement on the stated parameters was more robust compared to the findings on the control group and the application of only chemical fertilizers. Therefore, we recommend to disseminate the biofertilizer technology to rice growing farmers.

Acknowledgement

To Indonesia Ministry of Education Directorate General of Higher Education "*DIKTI*" through the *Developing Countries Partnership Scholarships* (KNB) for funded the entire studies. Additionally, we thank Ms. Rolissa Ballantyne for assistance in the preparation of the manuscript, and acknowledge a substantial input from the late Dr. Sugiyanta in making this research successful.

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