

**THE ROLE OF NITROGEN-FIXING ENDOPHYTIC BACTERIA IN REDUCING NITROGEN FERTILIZER APPLICATION RATES AND ENHANCING MAIZE GROWTH ON KENTRONG ULTISOLS**

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**ABSTRACT**

Maize cultivation on Ultisols is constrained by low nitrogen availability. One strategy to improve nitrogen availability is the application of biofertilizers containing effective nitrogen-fixing bacteria. This study aimed to determine the best combination of nitrogen fertilizer application rate and biofertilizer application rate to enhance maize growth on Ultisols from Kentrong. The experiment was conducted in a greenhouse using a randomized block design consisting of 16 treatments with three replications. Treatments consisted of four urea fertilizer application rates (200, 160, 120, and 80 kg ha<sup>-1</sup>) combined with different biofertilizer application rates and methods, namely 1.2 kg ha<sup>-1</sup> as seed coating, 2.4 kg ha<sup>-1</sup> as soil treatment, and 3.6 kg ha<sup>-1</sup> as a combination of seed coating and soil treatment. The results showed that the combination of urea fertilizer and biofertilizer application rates significantly affected endophytic bacterial populations in roots and leaves, plant fresh weight, root dry weight, leaf dry weight, plant height, stem diameter, nitrogen uptake, and nitrogen content, but did not significantly affect leaf number. The application of 80 kg ha<sup>-1</sup> urea fertilizer combined with 3.6 kg ha<sup>-1</sup> biofertilizer was identified as the optimal treatment for increasing maize dry biomass while reducing urea fertilizer application rate.

**Keywords:** Nitrogen-fixing Bacteria, Biofertilizer, Ultisols, Maize.

**1. INTRODUCTION**

Maize (*Zea mays* L.) is an important cereal crop widely cultivated in Indonesia due to its role as a staple food, animal feed, and industrial raw material. Increasing maize production on marginal soils such as Ultisols remains a major challenge. Ultisols are characterized by low soil fertility, acidic reaction, low organic matter content, and limited availability of essential nutrients, particularly nitrogen (N) (Prasetyo and Suriadikarta, 2006).

Nitrogen is a primary macronutrient required for plant growth and development, as it is a major component of chlorophyll, amino acids, proteins, and nucleic acids (Marschner, 2012). In maize cultivation, nitrogen deficiency leads to reduced vegetative growth, chlorosis, and decreased yield (Tamba et al., 2016). Farmers generally rely on high rates of inorganic nitrogen fertilizers to overcome nitrogen limitations in Ultisols. However, excessive nitrogen fertilizer application

can reduce fertilizer use efficiency and cause environmental problems such as nitrate leaching and greenhouse gas emissions (Rasyid et al., 2010; Wallace and May, 2018).

The use of biofertilizers containing nitrogen-fixing bacteria offers a sustainable alternative to reduce dependence on inorganic nitrogen fertilizers. Nitrogen-fixing bacteria are capable of converting atmospheric nitrogen ( $N_2$ ) into ammonium that can be utilized by plants (Vessey, 2003). Among these beneficial microorganisms, endophytic nitrogen-fixing bacteria are of particular interest because they colonize internal plant tissues without causing disease and directly contribute nitrogen to the host plant (Sturz and Nowak, 2000; Hardoim et al., 2015).

Endophytic bacteria can enter plants through root hairs, lateral root emergence sites, and natural openings, and subsequently colonize intercellular spaces or vascular tissues (Mercado-Blanco and Prieto, 2012; Putri et al., 2018). In addition to nitrogen fixation, endophytic bacteria promote plant growth through the production of phytohormones such as indole-3-acetic acid (IAA), solubilization of nutrients, and enhancement of stress tolerance (Matsumura et al., 2015).

Several studies have reported that the application of nitrogen-fixing endophytic bacteria can reduce inorganic nitrogen fertilizer requirements while maintaining or improving crop growth and yield (Rasyid et al., 2010; Wallace and May, 2018). However, the effectiveness of biofertilizers depends on the compatibility between bacterial strains, application methods, fertilizer rates, and soil conditions. Information regarding the optimal combination of nitrogen fertilizer application rate and endophytic biofertilizer application rate for maize grown on Ultisols from Kentrong remains limited.

Therefore, this study aimed to evaluate the effects of combined application of urea fertilizer and endophytic biofertilizer on endophytic bacterial populations, growth, biomass production, nitrogen uptake, and nitrogen content of maize grown on Ultisols from Kentrong, and to identify the optimal combination that can reduce nitrogen fertilizer application rate.

## **2. MATERIALS AND METHODS**

### **Isolation and Selection of Nitrogen-Fixing Endophytic Bacteria**

The study began with the isolation and selection of nitrogen-fixing endophytic bacteria conducted at the Soil Biology Laboratory, Department of Soil Science, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, Indonesia. Endophytic bacteria were isolated from roots, stems, and leaves of plants growing on Ultisols from Kentrong, including rice, maize, peanut, sweet potato, and *Imperata cylindrica*. Isolation of nitrogen-fixing endophytic bacteria was performed using selective JNFB (James Nitrogen-Free Bromothymol Blue) medium.

Selected isolates were screened for their ability to promote maize seedling growth using the rolled paper method and ranked using the Simple Rank Method for treatment selection. Parameters observed included germination percentage, germination rate, root length, shoot length, seedling vigor, and vigor index. The most effective isolation was subsequently formulated into a solid biofertilizer using an organic carrier material.

### **Application of Biofertilizer and Urea Fertilizer**

Biofertilizer application as soil treatment was carried out by placing solid biofertilizer at a rate of  $2.4 \text{ kg ha}^{-1}$  into planting holes. For seed coating treatment, maize seeds were coated with arabic gum solution (4 g per 100 seeds) followed by application of solid biofertilizer at a rate of  $1.2 \text{ kg ha}^{-1}$ . Coated seeds were incubated for 30 minutes prior to planting. The biofertilizer application rate of  $3.6 \text{ kg ha}^{-1}$  represented a combination of seed coating and soil treatment.

Urea fertilizer was applied in two equal splits (50% each) at 7 and 30 days after planting. Phosphorus (SP-36) and potassium (KCl) fertilizers were applied uniformly to all treatments at planting, at rates of 100 kg ha<sup>-1</sup> and 120 kg ha<sup>-1</sup>, respectively.

### **Experimental Design and Statistical Analysis**

The greenhouse experiment employed a randomized block design with 16 treatment combinations and three replications, resulting in 48 experimental units. Treatments consisted of combinations of four urea fertilizer application rates (200, 160, 120, and 80 kg ha<sup>-1</sup>) and three biofertilizer application rates and methods. Data were analyzed using SPSS software. Normality of data distribution was assessed using the Shapiro–Wilk test. Analysis of variance (ANOVA) was performed at a 5% significance level, and least significant difference (LSD) tests at 5% were conducted when treatment effects were significant.

## **3. RESULTS AND DISCUSSION**

### **Endophytic Bacterial Population in Roots and Leaves**

The combined application of urea fertilizer and biofertilizer significantly affected the population of endophytic bacteria in maize roots but did not significantly affect endophytic bacterial populations in maize leaves (Table 1). Endophytic bacterial populations were generally higher in roots than in leaves, as roots represent the primary entry point of endophytic bacteria from the soil environment. Bacteria from the rhizosphere can penetrate root tissues and colonize internal plant tissues.

According to Mercado-Blanco and Prieto (2012), endophytic bacteria enter plants through root hairs and subsequently spread within plant tissues, with some migrating to aerial parts through the xylem (Hardoim et al., 2015). The highest root endophytic bacterial population ( $81.33 \times 10^5$  CFU g<sup>-1</sup> root) was observed in treatment G (120 kg ha<sup>-1</sup> urea fertilizer combined with 1.2 kg ha<sup>-1</sup> biofertilizer applied as seed coating). Some endophytic bacteria remain localized in root tissues, while a small proportion migrate to aboveground plant parts. Sturz and Nowak (2000) reported that endophytes located in the root cortex can spread throughout the plant via the apoplastic pathway. This explains why endophytic bacteria detected in maize leaves originate primarily from root colonization.

Endophytic bacterial populations in maize leaves were lower than those in roots because colonization of aerial tissues requires time, even though bacteria can enter xylem vessels within the vascular system (Hardoim et al., 2015). The highest endophytic bacterial population in leaves ( $96.00 \times 10^5$  CFU g<sup>-1</sup> leaf) was observed in treatment H (80 kg ha<sup>-1</sup> urea fertilizer combined with 1.2 kg ha<sup>-1</sup> biofertilizer). This may be attributed to the high phenolic compound content in leaves, which can serve as an energy source for endophytic bacteria (Putri et al., 2018). Endophytic bacteria may enter plant tissues through lateral root emergence points, radicle growth zones, lenticels, natural wounds, stomata, and damaged trichomes (Putri et al., 2018).

**Table 1. Effect of Combined Application of Urea Fertilizer and Biofertilizer on Endophytic Bacterial Populations**

Treatments	Treatments Combination		Endophytic of Root x 10 <sup>5</sup> (CFU g <sup>-1</sup> )	Endophytic of Leaf x 10 <sup>5</sup> (CFU g <sup>-1</sup> )
	Urea (kg ha <sup>-1</sup> )	Biofertilizer (kg ha <sup>-1</sup> )		
A	200	0	55,00 cde	18,00 a
B	160	0	47,67 abcd	4,67 a
C	120	0	21,67 ab	14,33 a
D	80	0	18,50 a	3,67 a
E	200	1,2	20,00 a	11,33 a
F	160	1,2	31,33 abcd	5,00 a
G	120	1,2	81,33 e	9,00 a
H	80	1,2	45,33 abcd	96,00 b
I	200	2,4	20,00 a	15,00 a
J	160	2,4	58,00 de	10,33 a
K	120	2,4	36,00 abcd	5,50 a
L	80	2,4	46,33 abcd	8,67 a
M	200	3,6	37,67 abcd	3,00 a
N	160	3,6	52,67 bcde	5,00 a
O	120	3,6	30,67 abcd	3,33 a
P	80	3,6	24,00 abc	13,67 a

Low urea fertilizer application rate (80 kg ha<sup>-1</sup>) combined with seed coating biofertilizer application promoted the migration of endophytic bacteria colonizing maize seeds toward aerial plant parts, leading to increased bacterial populations in leaves. In leaf tissues, endophytic bacteria can fix sufficient nitrogen to support maize growth, thereby reducing competition between plants and bacteria for nutrients.

In contrast, high urea fertilizer application rate (200 kg ha<sup>-1</sup>) combined with biofertilizer generally reduced endophytic bacterial populations in roots, while no significant effect was observed in leaves. Conversely, high urea fertilizer application rate without biofertilizer increased root endophytic bacterial populations. This phenomenon is likely due to the availability of nitrogen supporting the growth of indigenous endophytic bacteria from the soil environment, enabling their colonization of maize root tissues.

### Plant Height, Stem Diameter, and Leaf Number

The combined application of urea fertilizer and biofertilizer resulted in significantly improved maize plant height, stem diameter, and leaf number compared with treatments without biofertilizer (Table 2). These results indicate that nitrogen-fixing endophytic bacteria can promote plant growth through nitrogen contribution to the host plant.

Treatment P (80 kg ha<sup>-1</sup> urea fertilizer combined with 3.6 kg ha<sup>-1</sup> biofertilizer) produced the greatest maize plant height compared with treatments without biofertilizer. Differences in plant height may be attributed to reduced inorganic nitrogen input combined with increased biofertilizer application, which enhances biological nitrogen fixation and phytohormone (IAA) production. Nitrogen is a critical nutrient supporting vegetative plant growth (Tamba et al., 2016).

Stem diameter was also influenced by nitrogen availability derived from both urea fertilizer and biofertilizer. Treatment O (120 kg ha<sup>-1</sup> urea fertilizer combined with 3.6 kg ha<sup>-1</sup> biofertilizer) produced the largest stem diameter (1.37 cm), exceeding treatments receiving high urea fertilizer rates with or without biofertilizer. The combined seed coating and soil treatment ensured sufficient nitrogen availability to support stem development at reduced urea fertilizer rates. According to Gao et al. (2017), nutrients absorbed during early growth stages primarily affect plant height, whereas nutrients absorbed during later vegetative stages contribute more to stem diameter development. Larger stem diameter enhances nutrient transport capacity due to increased vascular tissue area (Zao et al., 2024).

In general, high urea fertilizer application rate (200 kg ha<sup>-1</sup>) resulted in lower leaf number compared with lower application rates, particularly in the absence of biofertilizer. Treatment P produced the highest leaf number, whereas treatment A (200 kg ha<sup>-1</sup> urea fertilizer without biofertilizer) produced the lowest leaf number. Leaf number is commonly used as an indicator of nitrogen availability in soil (Wu et al., 2024). Increased leaf number under biofertilizer application demonstrates the importance of endophytic nitrogen-fixing bacteria in supporting maize growth.

**Table 2. Effect of Combined Application of Urea Fertilizer and Biofertilizer on Plant Height, Stem Diameter, and Leaf Number**

Treatments	Treatments Combination		Plant Height (cm)	Stem Diameter (cm)	Leaf Number
	Urea (kg ha <sup>-1</sup> )	Biofertilizer (kg ha <sup>-1</sup> )			
A	200	0	29,81 a	1,22 a	4,1 a
B	160	0	42,95 b	1,29 bc	4,9 abc
C	120	0	43,79 bc	1,31 bcde	5,1 bc
D	80	0	43,12 b	1,32 bcde	4,9 abc
E	200	1,2	45,16 bc	1,27 ab	5,0 bc
F	160	1,2	49,02 bcd	1,31 bcde	5,2 bc
G	120	1,2	43,19 b	1,31 bcde	4,8 abc
H	80	1,2	45,45 bc	1,33 cde	5,2 bc
I	200	2,4	49,95 bcd	1,31 bcde	5,1 bc
J	160	2,4	50,24 cd	1,34 cde	5,3 bc
K	120	2,4	45,55 bc	1,36 de	5,0 bc
L	80	2,4	46,25 bc	1,30 bcd	5,1 bc
M	200	3,6	44,62 bc	1,29 bc	4,6 ab
N	160	3,6	49,97 bcd	1,34 cde	5,3 bc
O	120	3,6	48,87 bcd	1,37 e	5,3 bc
P	80	3,6	53,38 d	1,36 de	5,5 c

**Plant Fresh Weight, Root Dry Weight, and Leaf Dry Weight**

The combined application of urea fertilizer and biofertilizer significantly affected maize biomass production (Table 3). The experiment was conducted until the end of the vegetative growth stage, as visual differences among treatments were already evident.

Treatment P produced the highest plant fresh weight, root dry weight, and leaf dry weight among all treatments. This indicates that nitrogen supplied by endophytic bacteria enhanced maize

biomass production. Nutrient deficiency can disrupt root activity in nutrient and water uptake, thereby limiting biomass synthesis (Tamba et al., 2016).

Treatment A exhibited low biomass production despite receiving a high urea fertilizer application rate. This suggests that maize plants relied solely on nitrogen derived from urea mineralization, which is prone to leaching and volatilization losses. Effective nitrogen accumulation is essential for optimal plant growth, and endophytic nitrogen-fixing biofertilizers can compensate for nitrogen losses. Similar findings were reported by Rasyid et al. (2010), who observed that high nitrogen application rates do not necessarily increase plant biomass.

Root and leaf dry weights followed similar trends to plant fresh weight. Approximately half of plant fresh weight was lost during drying, indicating high water content in plant tissues. Plant dry biomass represents accumulated photosynthates translocated and stored within plant organs (Chen et al., 2018).

**Table 3. Effect of Combined Application of Urea Fertilizer and Biofertilizer on Plant Fresh Weight, Root Dry Weight, and Leaf Dry Weight**

Treatments	Treatments Combination		Plant Fresh Weight (g)	Root Dry Weight (g)	Leaf Dry Weight (g)
	Urea (kg ha <sup>-1</sup> )	Biofertilizer (kg ha <sup>-1</sup> )			
A	200	0	9,88 a	0,40 a	0,48 a
B	160	0	11,67 ab	1,03 ab	1,64 b
C	120	0	12,05 abc	1,38 abc	1,67 b
D	80	0	12,08 abc	1,14 abc	1,94 b
E	200	1,2	12,76 abcd	1,58 abcd	2,19 bc
F	160	1,2	14,65 cd	3,44 de	2,21 bc
G	120	1,2	11,67 ab	1,15 abc	1,52 b
H	80	1,2	12,63 abc	1,67 abcd	1,96 b
I	200	2,4	13,28 bcd	2,13 abcd	2,15 b
J	160	2,4	14,86 cd	3,04 cde	1,92 b
K	120	2,4	12,88 bcd	1,68 abcd	2,19 bc
L	80	2,4	12,36 abc	1,34 abc	2,02 b
M	200	3,6	12,55 abc	1,26 abc	1,62 b
N	160	3,6	14,88 cd	2,72 bcde	3,16 cd
O	120	3,6	13,14 bcd	1,88 abcd	1,54 b
P	80	3,6	15,65 d	4,19 e	3,46 d

### Nitrogen Uptake and Nitrogen Content

The combined application of urea fertilizer and biofertilizer significantly affected nitrogen uptake and nitrogen content of maize plants (Table 4). The highest nitrogen uptake was observed in treatment P, indicating that endophytic bacteria effectively supplied nitrogen to maize plants through biological nitrogen fixation. The lowest nitrogen uptake was recorded in treatment A, where maize plants depended solely on nitrogen from urea fertilizer mineralization.

Nitrogen uptake increased with increasing biofertilizer application rate. Endophytic bacteria enhance plant nitrogen nutrition through diazotrophic activity (Matsumura et al., 2015). However, endophytic effects on plant nutrition are not always accompanied by visible

morphological changes because endophytes do not directly interact with soil nutrient pools (Wallace and May, 2018).

Nitrogen content increased with biofertilizer application at 1.2 kg ha<sup>-1</sup>, whereas higher biofertilizer application rates did not consistently increase nitrogen content. This suggests that seed coating application plays a more prominent role in influencing tissue nitrogen content. Higher nitrogen uptake under combined seed coating and soil treatment was primarily associated with increased biomass accumulation rather than increased tissue nitrogen concentration. Similar observations were reported by Rasyid et al. (2010), who found that nitrogen application rate influences plant nitrogen content but does not necessarily increase biomass production.

**Table 4. Effect of Combined Application of Urea Fertilizer and Biofertilizer on Nitrogen Uptake and Nitrogen Content**

Treatments	Treatments Combination		Nitrogen Uptake (mg)	Nitrogen Content (%)
	Urea (kg ha <sup>-1</sup> )	Biofertilizer (kg ha <sup>-1</sup> )		
A	200	0	13,05 a	0,96 abc
B	160	0	25,21 ab	0,79 abc
C	120	0	34,02 b	0,97 abc
D	80	0	33,41 b	0,85 abc
E	200	1,2	34,62 b	1,67 d
F	160	1,2	43,21 bc	0,71 ab
G	120	1,2	25,56 ab	1,11 bc
H	80	1,2	29,59 ab	1,11 bc
I	200	2,4	31,59 ab	0,73 ab
J	160	2,4	25,28 ab	0,77 abc
K	120	2,4	25,81 ab	0,67 a
L	80	2,4	25,93 ab	0,77 ab
M	200	3,6	25,53 ab	0,95 bc
N	160	3,6	43,90 bc	0,80 abc
O	120	3,6	31,08 ab	0,77 ab
P	80	3,6	55,77 c	0,75 ab

**4. CONCLUSION**

The combined application of urea fertilizer and endophytic biofertilizer significantly affected endophytic bacterial populations in roots and leaves, plant growth parameters, biomass production, nitrogen uptake, and nitrogen content of maize grown on Ultisols from Kentrong. The application of 80 kg ha<sup>-1</sup> urea fertilizer combined with 3.6 kg ha<sup>-1</sup> biofertilizer applied through seed coating and soil treatment was identified as the optimal treatment for increasing maize dry biomass while reducing the required urea fertilizer application rate.

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**Conflict of interest statement**

The authors declare that there is no conflict of interest associated with this study.

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