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Effectiveness of *Dyella japonica* and *Enterobacter cloacae* as biofertilizers to increase maize (*Zea mays*) production on andisol soil

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Abstract. *Sembiring M, Sabrina T. 2022. Effectiveness of* Dyella japonica *and* Enterobacter cloacae *as biofertilizers to increase maize (*Zea mays*) production on andisol soil. Biodiversitas 23: 3338-3343.* Nitrogen is a very important nutrient in plant growth because it is one of the constituents of plant cells. The availability of nitrogen nutrients in the soil is very low, and this is because nitrogen is easily leached and easy to evaporate. Application of N-fixing microbes is one way to increase the availability of nitrogen in the soil. This study aims to utilize nitrogen-fixing microbes to increase nutrient uptake, production, and efficiency of urea fertilization. The study used a factorial randomized block design (RBD) consisting of Factor I, namely biofertilizer consisting of 4 treatments, namely without the application of bacteria (N0), *Dyella japonica* 5g (N1), *Enterobacter cloacae* 5g (N2) and *D. japonica* 2.5g + *E. cloacae* 2.5g (N3). Factor II is urea fertilizer with 5 levels U0 = No urea fertilizer, U1 = 1.25 g, U2 = 2.50 g, U3 = 3.75g and U4 = 5g. Parameters observed were plant height observed in weeks I-VIII, plant nitrogen (N) uptake (mg/plant), phosphorus (P) uptake (mg/plant), potassium (K) uptake (mg/plant), and plant production (g). Sampling of plants for analysis of nutrient uptake was carried out at the end of the vegetative period, namely the 8th week, while for plant production parameters, plant samples were taken at the 12th week. The results showed that the applications of nitrogen-fixing bacteria could increase N uptake up to 89.35%, P uptake up to 79.57%, K uptake up to 48.81%, and crop production increase up to 49.76%. The applications of nitrogen-fixing microbes can streamline the use of urea fertilizer by up to 50%. In general, the best treatment is the applications of *D. japonica* and *E. cloacae* plus 2.5g urea (N3U2).

Keywords: Biofertilizer, fertilization efficiency, nitrogen nutrient, nitrogen-fixing microbes

INTRODUCTION

Nitrogen (N) is one of the main nutrients that play an important role in stimulating growth and giving green color to leaves. The lack of nitrogen in the soil causes the growth and development of plants to be disrupted and crop yields decrease because the formation of chlorophyll, which is very important for the photosynthesis process is disrupted. Soil is a dynamic and most diverse ecosystem for microorganisms to live. Microorganisms play a very important role in the nitrogen cycle in ecosystems (Wang et al. 2015). Microorganisms can carried out nitrogen fixation (Levy-Booth et al. 2014; Suliasih and Widawati 2015). Nitrogen-fixing bacteria (NFB) are able to increase N nutrients in the soil by fixing N from the atmosphere so that it can be absorbed by plants (Alfaro-Espinoza and Ullrich 2015).

A fertilizer is a source of artificial nutrients needed to overcome nutritional deficiencies, especially nitrogen elements. The availability of nitrogen is often a problem in agricultural land because nitrogen fertilizers that are applied to the soil are often washed and evaporated, especially in areas that have high rainfall and high temperatures. The most common nitrogen fertilizer used by the community is urea, but long-term use of urea fertilizer will cause soil acidity. The continuous use of chemical fertilizers pollutes groundwater and can adversely affect soil organisms (Solomon et al. 2012), and also has a negative effect on biogeochemical cycles (Savci Serpil 2012; Amundson et al. 2015; Steffen et al. 2015). The use of chemical fertilizers can cause leaching and runoff of nutrients, especially N and P hich can have a polluting impact on the environment (Omidire et al. 2015; Leontidou et al. 2020). The efficiency of the use of urea fertilizer is very low ranging from 30-40%, even in some cases even less. The low efficiency of urea fertilization is due to the loss of part of the applied urea fertilizer, either through abstersion or evaporation in the form of ammonia. In the application of nitrogen fertilizers, soil moisture is very influential because the presence of groundwater greatly affects the solubility of nitrogen fertilizers that can be absorbed by plants and fertilization efficiency can be increased (Usman et al. 2015; Carrijo et al. 2017; Hamoud et al. 2019), The presence of nitrogen in the soil greatly affects the growth and production of plants (Paungfoo-Lonhienne et al. 2019; Amanullah et al. 2020).

In Andisol soils, the availability of nitrogen nutrients can be a limiting factor for plant growth because soil organic matter always accumulates in large quantities. The mineralization of N in the soil is strongly influenced by the presence of microbes in the soil. The number and activity of microbes are strongly influenced by environmental conditions. As investigated by Qadaryanty et al. (2020) and Zebua et al. (2019), soil pH and plant vegetation greatly affect the population of organisms in the soil. Application of both symbiotic and non-symbiotic nitrogen-fixing bacteria can increase the N content in the soil. The application of Nitrogen-fixing microbes can increase the availability of N nutrients in the soil so that nutrient uptake will increase which can increase plant growth and production (Widawati and Suliasih 2019). Soil N nutrient content increased up to 15.6% with the application of Nitrogen-fixing microbes (Sembiring et al. 2021). *Enterobacter* sp is one of the soil microbes included in plant growth-promoting rhizobacteria (PGPR) which is able to produce indole-3-acetic acid (IAA) and stimulate plant growth (Ghosh et al. 2015; Li et al. 2016). One approach to save the use of inorganic fertilizers is to use microbes that can increase the availability of nutrients in the soil that can be absorbed by plants. According to Sembiring and Sabrina (2021), application of Nitrogen-fixing bacteria can increase soil N nutrient content. Application of *Enterobacter cloacae* can increase soil N up to 111.16% and *Dyella japonica* bacteria can increase soil N up to 41.17% the increase in nutrient N levels in the soil with the application of these microbes shows that both bacteria have the ability to convert the atmospheric nitrogen to ammonia so that the availability of N in the soil increases. Utilization of environment-specific Nitrogen-fixing microbes is an alternative to increase the availability of N in the soil which plays a role in increasing plant growth and production and can increase fertilization efficiency.

MATERIALS AND METHODS

The experiments were conducted in Kuta Rayat Village, Karo Regency. The indicator plant used was maize. The material used is Andisol soil with the characteristics of C - organic 6.55% (Walkey and black titration method), available P 137.54 ppm (Bray II method), soil N 0.62% (Kjedahl-Titrimetry method), cation exchange capacity (CEC) 21.87 me/kg and pH 5.43. The biological fertilizers used were *D. japonica* (8×109 CFU/g), and *E. cloacae* (8×10^9 CFU/g). The experiment design used a factorial randomized block design (RBD) consisting of: Factor I: Biofertilizer, N0 = Without application, N1 = *D. japonica* 5 g/plant, N2 = *E. cloacae* 5 g/plant, N3 = D. *japonica* 2.5 g + *E. cloacae* 2.5 g. Factor II urea fertilizer, U0 = No urea fertilizer, U1 = 1.25 g/plant, U2 = 2.50 g/plant, U3 = 3.75 g/plant and U4 = 5 g/plant. The land used as research area was cleared of grass and other plants first by digging and then leveling it again. Maize was planted in polybags containing 10 kg of soil. The fertilizers used were urea (application according to treatment), SP36 2 g/plant, and KCl 1 g/plant. Fertilizer application was carried out 2 days before the plants were planted. The microbial application was carried out 1 week after planting soil and plant samples were taken for analysis purposes after 8 weeks of microbial application.

Parameters observed were: plant height at I-VIII weeks, plant N uptake (mg/plant), P uptake (mg/plant), K uptake (mg/plant), and plant production (g). Plant sampling for nutrient uptake analysis was carried out at the end of the vegetative period, namely the 8th week, while for plant production parameters, plant samples were taken at the 12th week.

Statistical analysis

In general, the effect of treatment was carried out by the F test at the 5% level and followed by the Duncan Multiple Range Test (DMRT) test at the 5% level.

RESULT AND DISCUSSION

The acceleration (Δ) of plant height growth in the treatment of *D. japonica* and *E. cloacae* added with 5g urea fertilizer (N3U4) at week 1 observation showed slower growth when compared to other treatments and growth increased with increasing time, while without nitrogen-fixing microbes, plant growth increased at week 1-V increased then decreased until observation VIII (Table 1). The application of nitrogen-fixing bacteria significantly affected the uptake of N, P, K, and crop production (Table 2). The application of *D. japonica* and *E. cloacae* (N3) can increase the N uptake of plants up to 301.18 mg/plant. This indicates that the treatment of N3 can increase the N uptake of plants up to 32.88% higher than the control. P uptake of plants increased from 28.47 mg/plant (N0) to 42.58 mg/plant (N3) which indicated an increase in P uptake of plants by 49.56%, uptake of K increased from 367.52 mg/plant to 435.17 mg/plant which indicated that K uptake of plants increased up to 18.4% and the highest plant production was 532.94 g/plant which showed an increase of up to 18.58% when compared to the control. The application of bacteria *E. cloacae* which was able to increase the N nutrient content of the soil up to 111.16% and *D. japonica* increased the nutrient N content of the soil up to 41.17% when compared to without the application of microbes, this indicates that these microbes have the ability to fix nitrogen from the atmosphere (Sembiring and Sabrina 2021). This shows that nitrogen-fixing microbes used can increase plant nutrient levels so that nutrient uptake by plants will increase and plant production will increase. Increased nitrogen fixation by bacteria so that plant growth will also increase (Paul and Lade 2014; Shrivastava and Kumar 2015). *Enterobacter cloacae* is a microbe that can stimulate growth (Ramesh et al. 2014; Khalifa et al. 2016; Li et al. 2016). The ability of microbes to increase the availability of nutrients in the soil varies depending on their ability to adapt to their environment such as temperature, humidity and others. One of the environmental factors that greatly affects the growth and activity of nitrogen fixation by microbes from the air is the optimum pH (Xie and Yokota 2005; Navarro et al. 2012; Agnolucci et al. 2019).

Table 1. The acceleration (Δ) Plant Height Increase (cm) observations at 1-8 weeks

Treatment	Week							
	I	II	III	IV	V	VI	VII	VIII
N0U0	20.83	17.77	20.10	20.67	21.67	15.60	9.10	8.43
N0U1	16.23	19.00	32.20	23.17	25.67	19.77	13.43	24.30
N0U2	20.13	18.73	31.20	17.43	32.03	22.10	22.67	11.57
N0U3	12.40	19.53	29.73	28.63	31.13	26.10	19.47	30.63
N0U4	16.17	19.23	26.73	25.33	32.10	27.43	13.50	22.23
N1U0	15.47	19.50	20.47	13.33	22.27	32.27	26.20	27.43
N1U1	18.37	20.20	23.00	33.67	23.77	20.17	16.80	20.57
N1U2	17.30	14.20	38.33	27.07	27.83	22.33	12.47	29.83
N1U3	12.20	18.23	27.03	25.43	33.70	26.87	16.90	23.00
N1U4	14.27	20.43	26.07	22.33	32.80	23.17	14.07	40.93
N2U0	16.57	19.53	26.90	19.23	21.53	18.27	16.07	21.60
N2U1	16.43	20.03	31.17	22.17	30.97	16.70	14.30	25.50
N2U2	16.00	17.87	27.30	24.03	23.77	27.50	12.20	22.70
N2U3	12.70	18.80	28.70	30.80	25.03	20.73	9.93	15.03
N2U4	14.90	18.37	27.37	23.70	28.20	18.90	19.37	19.90
N3U0	16.90	20.73	22.50	28.43	3.40	14.50	16.20	33.60
N3U1	20.30	18.37	30.13	19.23	34.83	23.93	10.97	16.13
N3U2	17.73	15.53	31.20	22.87	29.67	22.80	12.73	23.70
N3U3	15.27	20.57	28.33	21.47	27.10	21.53	10.57	15.00
N3U4	14.63	19.67	26.03	24.10	27.97	31.63	20.53	25.50

Table 2. Nitrogen, phosphrus, potassium uptake and maize plant production

Treatment	N uptake (mg/plant)	P uptake (mg/plant)	K uptake (mg/plant)	Plant production (g/plant)
Nitrogenf fixing bacteria				
No microbial application (N0)	226.65±60.46a	28.47±5.19a	367.52±48.30a	466.28±40.08a
Dyella japonica (N1)	275.11±53.48ab	35.15±9.74bc	412.32±43.97bc	505.61±77.93a
E. cloacae (N2)	242.84±20.58a	33.43±7.81b	374.61±57.46b	445.22±68.35a
D. japonica and *E. cloacae* (N3)	301.18±31.08bc	42.58±4.11d	435.17±38.94cd	532.94±64.72b
Urea Fertilizer Dosage (g)				
0.00 (U0)	240.78±44.65a	33.66±4.84a	362.75±33.02a	432.43±91.28a
1.25 (U1)	233.26±68.00a	29.86±7.40a	370.22±75.78a	477.85±67.96a
2.50 (U2)	261.53±43.95ab	34.65±12.27ab	418.93±63.91ab	509.44±66.43ab
3.75 (U3)	295.72±24.40bcd	34.09±6.79a	427.15±18.09bc	517.29±44.41bc
5.00 (U4)	176.05±58.78bc	42.29±7.25bc	407.97±37.91a	500.55±64.94a
N	*	*	*	*
U	*	*	*	*
NxU	*	*	*	*
Cv	18.03	16.37	13.77	15.55

Note: *significant p ≤0.05. Means in a column followed by a common letter are not significantly different at the 0.05 lavel by DMRT.

The results showed that the application of urea fertilizer could increase the uptake of N, P, K, and plant production. The application of a single urea fertilizer can increase the N uptake of plants. Nutrient N uptake of control plants was 240.78 mg/plant while the application of 3.75g urea fertilizer could increase N uptake to 295.72 mg/plant, which means an increase in plant N uptake up to 22.81%, P plant nutrient uptake increased from 33.66 mg/plant to 42.29 mg/plant (U4), which means that plant P uptake increased by 27.9% when compared to no fertilizer application (U0), K uptake up to 17.76%, and increase plant production up to 27.98% when compared to control. The increase in nutrient uptake by plants with the application of urea fertilizer indicates that plant growth is getting better so that plant production increases from 424.1 g to 542.78 g/plant. K nutrient uptake increased from 362.75 (U0) mg/plant to 427.15 mg/plant (U3), increased nutrient uptake up to 17.76% and increased plant production to 27.98% when compared to control. The increase in nutrient uptake by plants with the application of urea fertilizer showed that plant growth was getting better so that plant production increased from 424.1 g to 542.78 g/plant. Urea fertilizer application as much as 3.75g (U3) is the best dose in increasing plant nutrient uptake and increasing plant growth. Nutrient uptake of N, P, K of plants decreased with the increase in the amount of fertilizer applied, namely 5g (U4). Nitrogen plays a role in plant cell division so that if the nitrogen needs of plants are met then nutrient uptake, growth, and plant production will increase (Liu et al. 2014; Hamoud et al. 2019). The availability of nitrogen in the soil greatly affects the growth of plant roots so that nutrient uptake by plants will increase and can affect plant production (Paungfoo-Lonhienne et al. 2019; Amanullah et al. 2020).

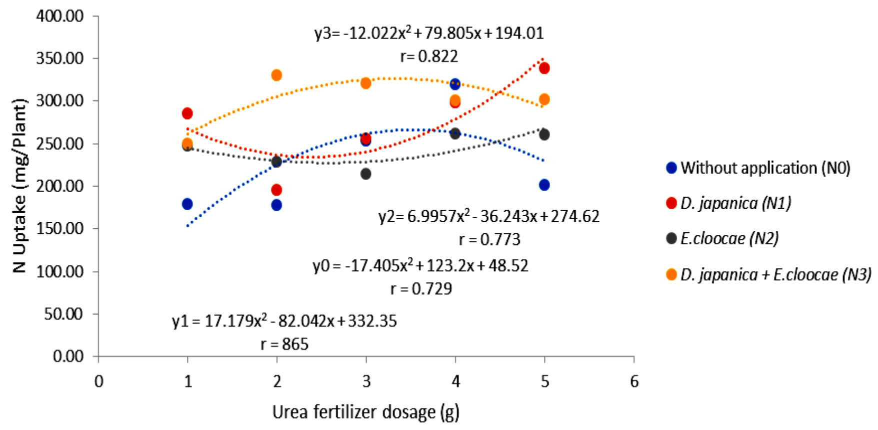

Figure 1. The effect of interaction between biofertilizer and urea fertilizer on N uptake (mg/plant)

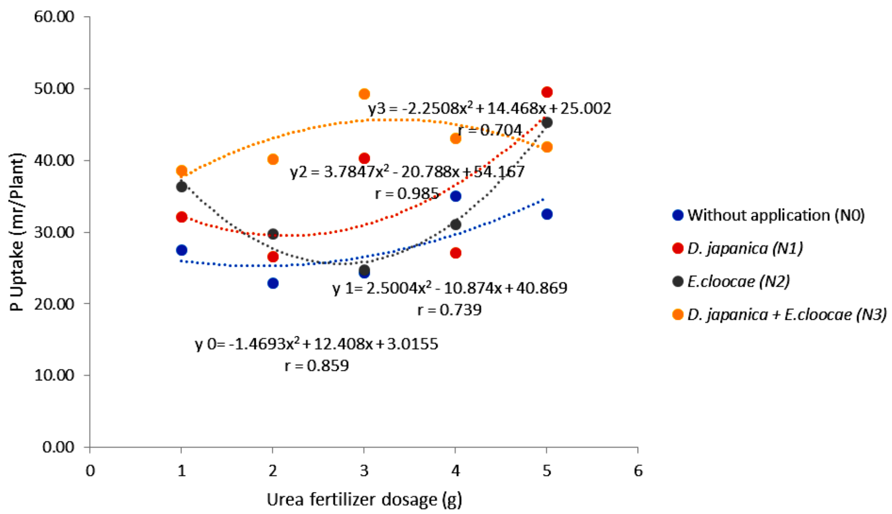

Figure 2. The effect of interaction between biofertilizer and urea fertilizer on P uptake (mg/plant)

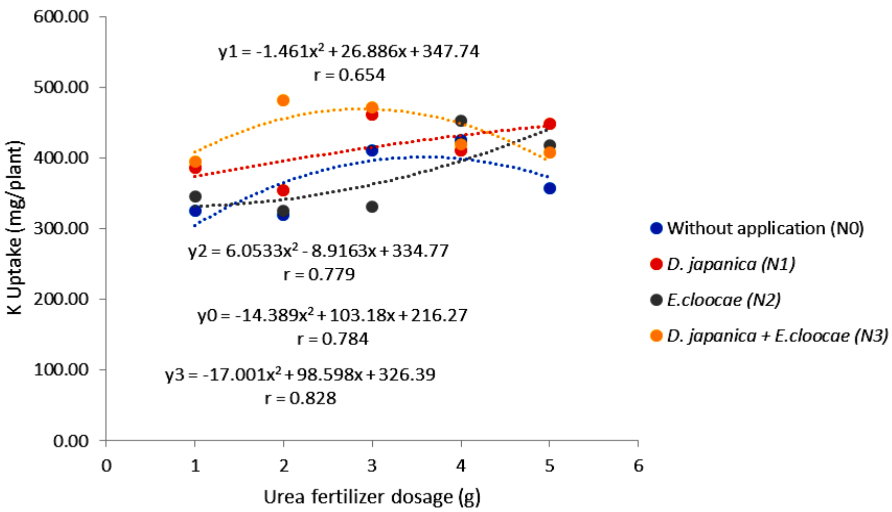

Figure 3. The effect of interaction between biofertilizer and urea fertilizer on K uptake (mg/Plant)

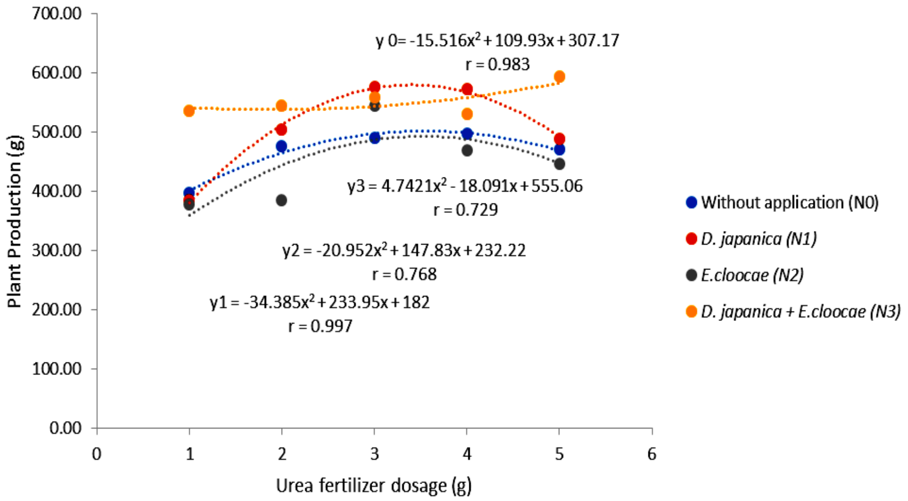

Figure 4. The effect of interaction between biofertilizer and urea fertilizer on plant production (g/plant)

The interaction of nitrogen-fixing microbes and urea fertilizer had a significant effect on increasing N, P, K nutrient uptake and crop production. The interaction application of *D. japonica* and *E. cloacae* coupled with the application of Urea 2.50 g/plant (N3U2) can increase the N uptake of plants up to 84.45%, and N uptake decreased with increasing doses of urea fertilizer applied (Figure 1), this indicates that the application of *D. japonica* and *E. cloacae* can reduce the use of Urea fertilizer doses up to 50%. While the treatment of *D. japonica* had a higher N uptake but could not reduce the use of urea fertilizer. Application of a mixture of bacteria *D. japonica* and *E. cloacae* plus urea 2.5g (N3U2) can increase P uptake up to 78.47% and decrease with increasing dose of P fertilizer applied, this indicates that the application of a mixture of bacteria *D. japonica* and *E. cloacae* can reduce the use of urea fertilizer 50% of the recommended dose (U4). Application of nitrogen-fixing bacteria can increase soil N and reduce the use of inorganic fertilizers (Amanullah et al. 2020). While the application of *D. japonica* alone has not been able to reduce the use of urea fertilizer. The application of *D. japonica* plus 5g of urea fertilizer (N1U4) had the highest P uptake up to 79.57% when compared to the control but could not reduce the dose of urea given. This shows that the application of *D. japonica* can increase plant P uptake with increasing doses of urea fertilizer applied (Figure 2). The application of *D. japonica* and *E. cloacae* added with 2.5g urea fertilizer (N3U1) can increase plant K nutrient uptake up to 481.54 mg/plant. The increase in plant K nutrient uptake by 48.01% when compared to control, 17.88% higher when compared to N3U4, this indicates that the application of *D. japonica* and *E. cloacae* can reduce the use of urea fertilizer, K uptake of plants decreases with increasing doses of urea fertilizer applied (Figure 3). The application of *D. japonica* and *E. cloacae* plus 3.75 g of urea (N3U3) fertilizer can increase crop production up to 530.83 g/plant. This has a higher production but this treatment can not reduce the use of urea fertilizer. While the N3U1 treatment could increase plant production up to 40.8% higher than the control (Figure 4). This shows that the application of N3 can streamline the use of urea fertilizer by up to 50%. From the results of this study, it was found that the application of nitrogen-fixing bacteria could increase plant growth and production. This is because the bacteria used have the ability to produce growth hormone IAA (Sembiring and Sabrina 2021), cytokinins, and gibberellins (Saini et al. 2015). *Enterobacter cloacae* can increase nitrogen fixation so that plant growth increases (Liu et al. 2017) and *D. japonica* (Xie and Yokota 2005). Nitrogen-fixing microbes can increase nitrogen fixation so that plant growth will increase (Paul and Lade 2014; Shrivastava and Kumar 2015; Nabti et al. 2015). The application of nitrogen-fixing bacteria can increase plant growth and increase soil fertility (Ramesh et al. 2014; Khalifa et al. 2016; Fu et al. 2021).

In conclusion, the application of non-symbiotic Nitrogen-fixing bacteria combined with urea fertilizer can increase plant N uptake up to 89.35%, plant P uptake up to 79.57%, K uptake up to 48.81%, and plant production up to 49.76%. The application of nitrogen-fixing microbes can streamline the use of urea fertilizer up to 50%. In general, the best treatment is the application of *D. japonica and E. cloacae* plus 2.5g urea (N3U2).

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