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Research Article
Nitrogen Fertilization and Corn Growth (*Zea mays*) Effect in Andisols by Using Non-Symbiotic Nitrogen-Fixing Bacteria

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Abstract
Background and Objective: The availability of Nitrogen (N) is very limited in the soil because this nutrient is easily washed and evaporates so that it cannot be absorbed by plants. Efforts that can be made to increase the availability of N in the soil is to utilize non-symbiotic N-fixing bacteria. The purpose of this study was to obtain superior microbes that could increase the efficiency of N fertilization and the growth of corn in Andisol soil. **Materials and Methods:** The materials used in this study were Andisol soil, *Dyella japonica*, *Enterobacter cloacae*. The study applies a factorial randomized block design consisting of 2 factors. The factor I is nitrogen-fixing bacteria consisting of 4 treatments, no bacteria (N_0), *D. japonica* 5 g/plant (N_1), *E. cloacae* 5 g/plant (N_2) and *D. japonica* 2.5 g+*E. cloacae* 2.5 g (N_3). Factor II is urea fertilizer with 5 treatments, U_0 = No urea fertilizer, U_1 = 1.25 g/plant, U_2 = 2.50 g/plant, U_3 = 3.75 g/plant and U_4 = 5 g/plant. Plant height in week I-VIII, soil pH, N nutrient content in the soil, microbial population, plant dry weight and plant moisture content. **Results:** The results showed that the treatment of *D. japonica*+*E. cloacae* (N_3) with 1.25 g urea fertilizer per plant (N_3U_1) increases the N nutrient content in s dry weight of the plant and moisture content of the plant, which means it can increase the growth of corn on Andisol soil. **Conclusion:** The treatment of *D. japonica* + *E. cloacae* (N_3) + urea fertilizer 1.25 g/plant (N_3U_1) can increase the efficiency of urea fertilization by 75%.

Key words: Andisol, corn, fertilization efficiency, nitrogen, nitrogen-fixing bacteria, urea fertilizer

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plant growth highly needs nutrients. During the growth period of plants, they need nitrogen in large quantities as the main nutrient[1]. Nitrogen is absorbed by plants in the form of NO_3^- and NH_4^+. The amount of nitrogen in most types of soil (including Andisol) is often limited for plants. The nitrogen element in Andisol soil is an essential nutrient of which content is higher than other soil. However, the problem is the content of nitrogen in the soil is easily lost due to leaching and evaporation. Based on the data, the total nitrogen in Andisol soil in some places ranged from 0.11-0.76%. According to Sembiring and Sabrina[2], the nitrogen content in Andisols around Mount Sinabung ranges from 0.11-0.65%. The total N content in the soil affected by Mount Sinabung volcanic ash is 0.56-0.61%[3]. The total N content in Andisol soil is largely determined by the soil organic-c content[4]. With less carbon available, the bacterial population is reduced and the absorbed free soil nitrogen is reduced as well[5]. Sarah *et al.*[6] state that the activity of microorganisms is strongly influenced by the pH and soil organic-c content. The results of the research by Pakolo *et al.*[7] states that the lower the soil pH, the lower the microbial population in the soil.

The availability of nitrogen in the soil is very low, including in Andisol soil so that it can be a limiting factor for plant growth. The accumulation of organic matter in large quantities in Andisol soil always contains organic nitrogen. Therefore, Andisol soil can supply large amounts of N minerals for plants. It shows that organic N in volcanic ash soil is highly resistant to microbial decomposition[8]. Applying nitrogen-fixing bacteria, both symbiotic and non-symbiotic can increase the soil nitrogen content. Vitousek *et al.*[9] state that non-symbiotic nitrogen-fixing bacteria can increase the total N content in the soil by 3.2%. In the Andisol soil around Mount Sinabung, the high sulfur and Al-dd content from volcanic ash affect the total number of microorganisms in the soil. The higher the levels of Sulfur and Al-dd in the soil, the less the number of microorganisms. Low soil pH can affect the number of microorganisms in the soil[10]. According to scientists[11-14], the soil pH of Andisol in Sinabung ranged from 4.4-6.5. According to Massignam *et al.*[1], the quantity and activity of soil microbes are affected by soil pH, soil type, plant growth, soil treatment, cultivation and macro/microclimate of each location. Qadaryanty *et al.*[15] found that soil pH and plant vegetation is highly influential on the population of organisms in the soil.

Nitrogen is an element required to form essential compounds in cells, including proteins, DNA and RNA. Plants need to extract their nitrogen requirements from the soil. Sources of nitrogen contained in the soil are being insufficient to meet the needs of plants, so it is necessary to supply synthetic fertilizer as a source of nitrogen to increase production. The demand to increase crop production to match food supply results in large amounts of fertilizer in demand. One approach to efficiently use inorganic fertilizers is to increase the efficiency of the available N in the soil through N-fixing, either directly or by interaction with nitrogen-fixing bacteria. According to Sembiring and Sabrina[2], the treatment of nitrogen-fixing bacteria *E. cloacae* and *D. japonica* can increase soil N nutrient levels up to 111.16 and 41.17%, respectively. It shows that these two bacteria have the nitrogen fixation ability to increase the availability of N in the soil. The utilization of N-fixing bacteria can increase the efficiency of N fertilization. The use of non-symbiotic nitrogen-fixing bacteria is an alternative to increase the availability of N in the soil which plays a role in increasing plant growth and production. The purpose of this study was to obtain superior microbes that were able to increase the efficiency of N fertilization and the growth of jarung plants in Andisol soil.

MATERIALS AND METHODS

Study area: The research is conducted in Desa Kuta Rayat, Karo regency. The indicator crop is corn. The research is carried out for 3 months. This research was conducted from May-August, 2021.

Material: The material used in this study is Andisol soil with 6.55% organic (Walkley and Black titration method), 137.54 ppm available P (Bray II method), 0.62% N soil (Kjeldahl-Titrimetry method), 21.87 me kg^{-1} CEC and soil pH 5.43. *Dyella japonica* (8×10^9 CFU g^{-1}) is used as biological fertilizer, *Enterobacter cloacae* (8×10^9 CFU g^{-1}), The research applies a factorial randomized block design consisting of 2 factors.

Factor I: Biological fertilizer:

- N_0 = Treatment
- N_1 = *Dyella japonica* 5 g/plant
- N_2 = *Enterobacter cloacae* 5 g/plant
- N_3 = *D. japonica* 2.5 g+*E. cloacae* 2.5 g

Factor II: Urea fertilizer:

- U_0 = No urea fertilizer
- U_1 = 1.25 g/plant
- U_2 = 2.50 g/plant
- U_3 = 3.75 g/plant
- U_4 = 5 g/plant

Observed parameters: Plant height week I-VIII, soil pH, soil N nutrient content, microbial population, plant dry weight and plant moisture content.

Research implementation: The land used as the research area was 1st cleaned of weeds and other vegetation by using a hoe and then doing the levelling. Corn is 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 treatment is carried out 2 days before the plants are planted. Microbial treatment is carried out 1 week after planting, soil and plant sampling for analysis purposes are carried out on 8 weeks of plant age after microbial treatment.

Statistical analysis

Applied statistical analysis: To observe the effect of treatment in general, the F-test was carried out at the 5% level and followed by the Least Significance Different (LSD) test at the 5% level[16].

RESULTS AND DISCUSSION

The observation and statistical analysis showing the treatment of a combination of microbes and urea fertilizer towards all observed parameters can be seen in Table 1 and 2.

Statistically, the treatment of nitrogen-fixing bacteria does not significantly affect plant height in observations 1-8 (Table 1). The treatment of *D. japonica* (N_1) can increase plant height by 2.22% when compared with no microbial treatment. The treatment of urea fertilizer has a significant effect on plant height on observations 1, 4, 5 and 6. The treatment of urea fertilizer as much as 5 g/plant could increase plant height by 6.26% when compared to no treatment. The interaction of nitrogen-fixing bacteria and urea fertilizer has a significant

Table 1: Plant height observations on week I-VIII after the treatment of nitrogen-fixing bacteria and urea

	Observations on week							
Treatments	I	II	III	IV	V	VI	VII	VIII
Microbial treatments								
Without microbe application (N_0)	34.02±2.85	52.87±2.49	80.87±5.90	103.91±6.20	132.43±9.78	154.63±13.91	170.27±18.26	189.70±25.00
D. japonica (N_1)	32.06±1.86	50.57±2.92	77.55±5.38	101.92±10.14	129.99±12.41	154.95±8.83	172.24±4.29	193.93±14.98
E. cloacae (N_2)	32.71±4.10	51.63±4.12	79.91±4.52	103.90±3.24	129.80±6.58	150.22±7.11	164.59±6.96	185.54±9.38
D. japanica+E. cloacae (N_3)	34.28±2.36	53.25±2.66	80.89±3.53	104.11±2.46	128.71±10.99	151.59±15.36	165.79±15.10	199.57±12.49
Urea fertilizer dosage (g)								
0.00 (U_0)	34.29±1.30	53.68±1.64	76.17±3.48	96.58±8.68^{a}	113.80±5.35^{a}	133.96±7.30^{a}	150.85±12.70	173.62±18.82
1.25 (U_1)	34.07±2.12	53.47±1.54	82.59±5.32	107.15±1.91^{a}	135.96±3.57^{bcde}	156.10±4.84^{a}	169.98±2.96	191.60±2.59
2.50 (U_2)	35.74±1.92	52.33±3.97	84.33±3.20	107.18±3.93^{ab}	135.51±4.97^{bcd}	159.19±2.97^{ab}	174.21±6.13	187.83±10.47
3.75 (U_3)	30.07±3.53	49.35±4.37	77.80±4.77	104.38±4.06^{a}	133.63±5.12^{bc}	157.43±7.73^{a}	171.65±12.39	192.57±19.81
5.00 (U_4)	32.17±1.70	51.59±1.73	78.14±1.85	102.01±1.31^{a}	132.28±3.09^{b}	157.56±4.98^{a}	174.43±4.57	201.57±10.11
N	Ns	Ns	Ns	Ns	Na	Ns	Ns	Ns
U	*	Ns	Ns	*	*	*	Ns	Ns
N×U	*	*	Ns	Ns	Ns	Ns	Ns	Ns
CV	9.01	8.04	9.64	9.08	9.24	11.79	12.71	13.01

N: Nitrogen, U: Urea, CV: Coefficient of variation, means in a column followed by a common letter are not significantly different at the level 0.05 level by LSD, *Significant at $p \le 0.05$ and NS: Not significance

Table 2: Soil pH, soil N nutrient content, microbial population, plant dry weight and plant moisture content after the treatment of nitrogen-fixing bacteria and urea

Treatments	Soil pH	Soil N content (%)	Microbe population (10^6 CFU g^{-1})	Plant dry weight (g)	Plant water content (g)
Microbial treatments					
Without microbe application (N_0)	4.88	0.661	24.00	113.47	389.53
D. japonica (N_1)	4.90	0.670	40.87	135.07	362.93
E. cloacae (N_2)	4.82	0.678	39.80	129.60	484.07
D. japanica+E. cloacae (N_3)	4.88	0.693	52.47	142.73	558.33
Urea fertilizer dosage (g)					
0.00 (U_0)	4.98	0.641	35.50	115.58	401.08
1.25 (U_1)	4.90	0.676	33.17	120.17	442.75
2.50 (U_2)	4.82	0.685	40.58	142.50	464.17
3.75 (U_3)	4.86	0.689	50.50	141.50	463.50
5.00 (U_4)	4.80	0.687	36.67	131.33	472.08
N	Ns	*	*	*	*
U	Ns	*	*	*	Ns
N×U	Ns	*	*	*	*
CV	3.31	2.57	26.52	13.08	21.23

N: Nitrogen, U: Urea, CV: Coefficient of variation, means in a column followed by a common letter are not significantly different at the level 0.05 level by LSD, *Significant at $p \le 0.05$ and NS: Not significance

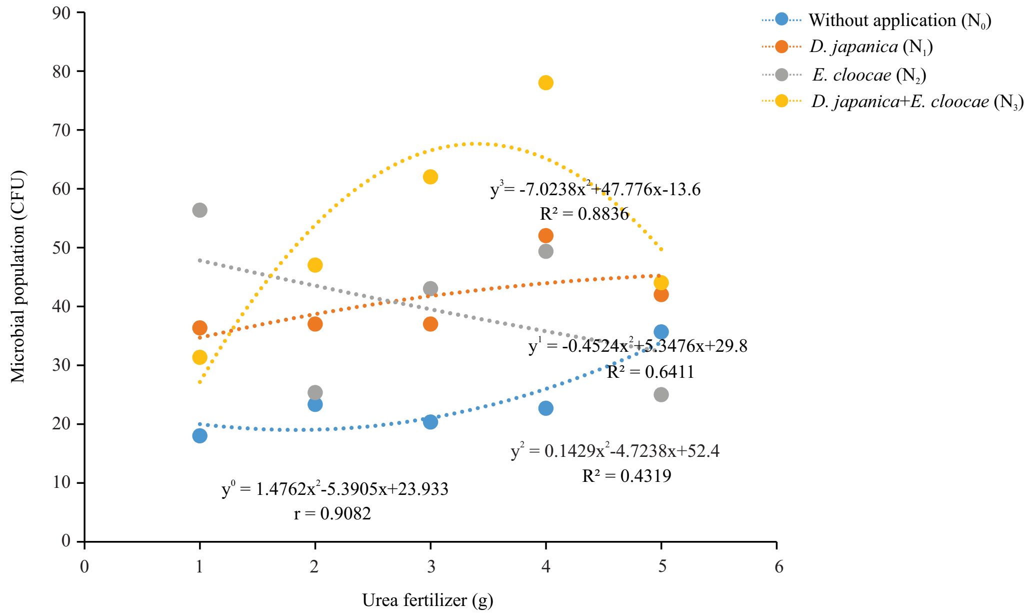

Fig. 1: Effect of nitrogen-fixing bacteria and urea fertilizer mixing on the microbial population in the soil (CFU)

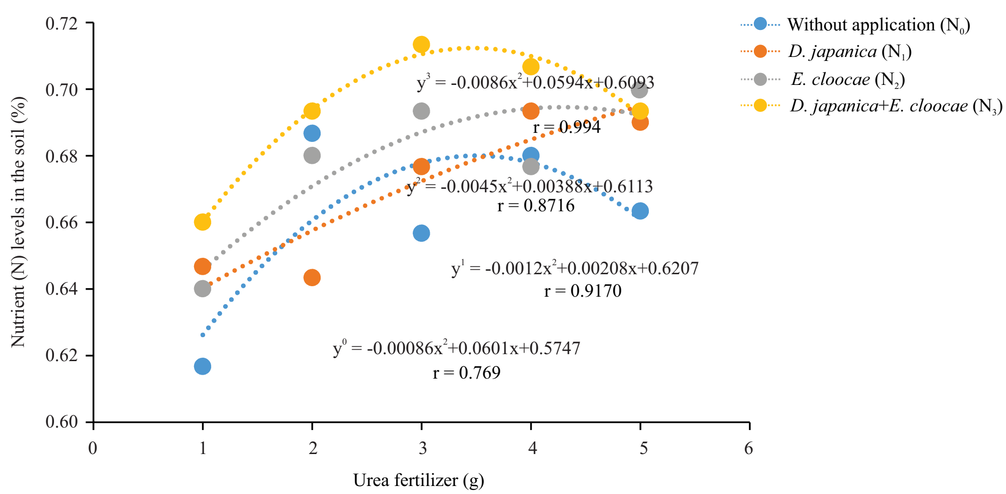

Fig. 2: Effect of nitrogen-fixing bacteria and urea fertilizer mixing on nutrient (N) levels in the soil (%)

effect on increasing plant height in observations 1 and 2 but have no significant effect on observation 3-8.

The treatment of nitrogen-fixing bacteria has no significant effect on soil pH. On the contrary, it has a significant effect on soil N nutrient levels and soil microbial population (Table 2). The treatment of *D. japonica+E. cloacae* (N_3) can increase soil N content up to 4.84% when compared to the treatment without bacteria (N_0). The results of the research by Sembiring and Sabrina[2] show that the treatment of *E. cloacae* bacteria on andisol soil could increase N nutrient levels by 111% with an incubation period of 30 days. The activity of N-fixing bacteria is strongly influenced by soil pH, the increase in nitrogen levels by microbes in this study has not been optimal since the soil pH is too low. Zebua *et al.*[14] found that the microbial population decreases in line with the decreasing soil pH. Urea fertilizer treatment can increase soil N nutrient content up to 7.49%, the best treatment is U_3. The interaction of nitrogen-fixing bacteria and urea fertilizer can increase the microbial population (Fig. 1) and nutrient N levels in the soil (Fig. 2). The treatment of *D. japonica+E. cloacae*+1.25 g urea (N_3U_1) and *D. japonica+E. cloacae*+2.50 g urea per plant (N_3U_2) can increase the N nutrient content in the soil up to 14.52% when compared to N_0U_0. Soil N nutrient content decreases in line with the

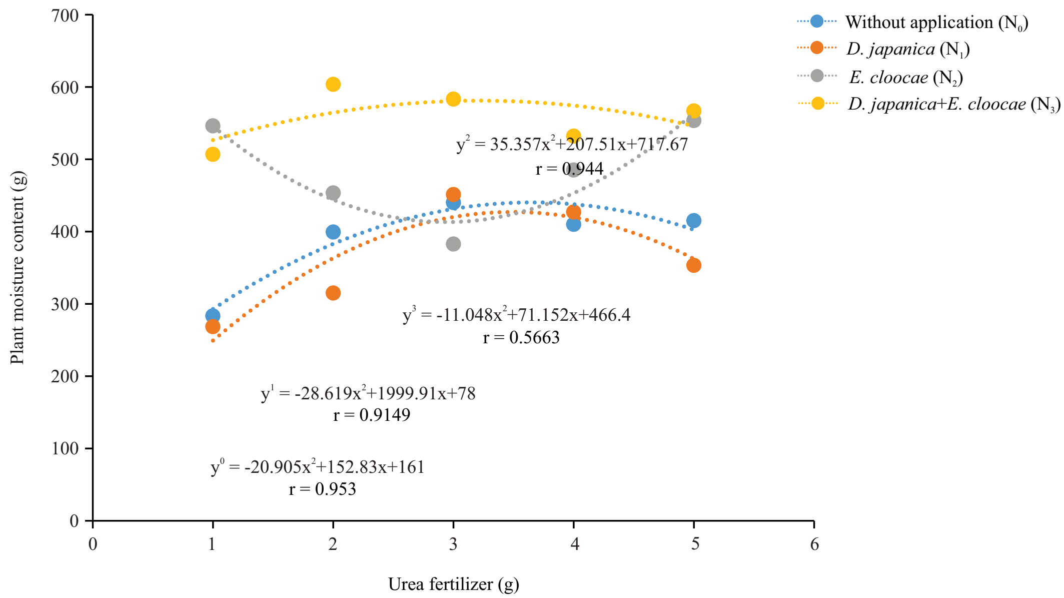

Fig. 3: Effect of nitrogen-fixing bacteria and urea fertilizer mixing on plant moisture content (g)

increasing treatment of urea fertilizer (U_3 and U_4). The mixed treatment of bacteria *D. japonica+E. cloacae* had better potential when compared to a single treatment. The finding by Sembiring and Sabrina[2] shows that *E. cloacae* can increase nutrient levels of N to 111.16% and *D. japonica* to 41.17%. It shows that these two bacteria have the ability of nitrogen-fixing. The ability of nitrogen-fixing bacteria for N-fixation varies depending on their ability to adapt to the environment where the soil pH is between 4.80-4.98 indicating a very acidic pH so that it restricts the growth and activity of bacteria in the soil. The result also shows that the microbial population stands between 25-52$\times 10^6$ CFU g^{-1}. According to previous authors[17,18] pH, 6.6-10 is the optimum pH for the growth and nitrogen-fixation process.

The treatment of *D. japonica+E. cloacae* (N_3) can increase the dry weight of the plant by 25.78%, higher than no microbial treatment (Table 2). The treatment of bacteria *D. japonica* and *E. cloacae* (N_3) can increase plant growth. With the mixed inoculant, the role of each bacteria can function properly so that plant growth increases compared to a single treatment. Nitrogen-fixing bacteria can produce IAA hormone so that it can increase plant growth[2,19-22]. According to Paul *et al.*[23], *E. cloacae* can increase nitrogen-fixation so that it increases plant growth. *D. japonica* is a bacterium capable of fixing N from the air[17]. The treatment of urea fertilizer can increase plant dry weight by 23.3% when compared to no treatment of urea fertilizer. Interaction of *D. japonica* (N_1) and urea fertilizer 2.5 g/plant can increase plant dry weight up to 23.3% when compared with no treatment. The Treatment of *D. japonica* and *E. cloacae* plus 1.25 g urea (N_3U_1) can increase plant dry weight up to 14.52%. Plant dry weight decreases in line with the increasing fertilizer dose (Fig. 3).

Plant moisture content generally increases with the treatment of nitrogen-fixing bacteria. The N_3 treatment can increase the moisture content of plants up to 43.33% when compared to no bacteria treatment. The N_3 treatment increased the moisture content of the plant by 15.34% higher than the N_2 treatment and 53.84% compared to the N_1 treatment. The moisture content of the plants increases with the treatment of nitrogen-fixing bacteria indicating that plant growth is better than with no nitrogen-fixing bacteria treatment. Nitrogen-fixing bacteria can increase nitrogen-fixation so that it increases plant growth[24-26]. The treatment of nitrogen-fixing bacteria can increase plant growth and affect soil fertility[27-30]. The treatment of urea as much as 5 g/plant (U_4) can increase the moisture content of plants by 17.7% compared with no treatment of urea fertilizer (U_0). The mix of nitrogen-fixing bacteria *D. japonica+E. cloacae* (N_3)+urea fertilizer 1.25 g/plant N_3U_1 can increase the moisture content of plants up to 112.9% compared to with no bacteria and urea fertilizer treatment (N_0U_0). The moisture content of the plants decreases in line with the increasing dose of urea fertilizer. In the treatment with no Nitrogen-fixing bacteria (N_0), the moisture content increases with the higher dose of urea (Fig. 4). It shows that nitrogen-fixing bacteria can reduce the application of urea fertilizer by 75%.

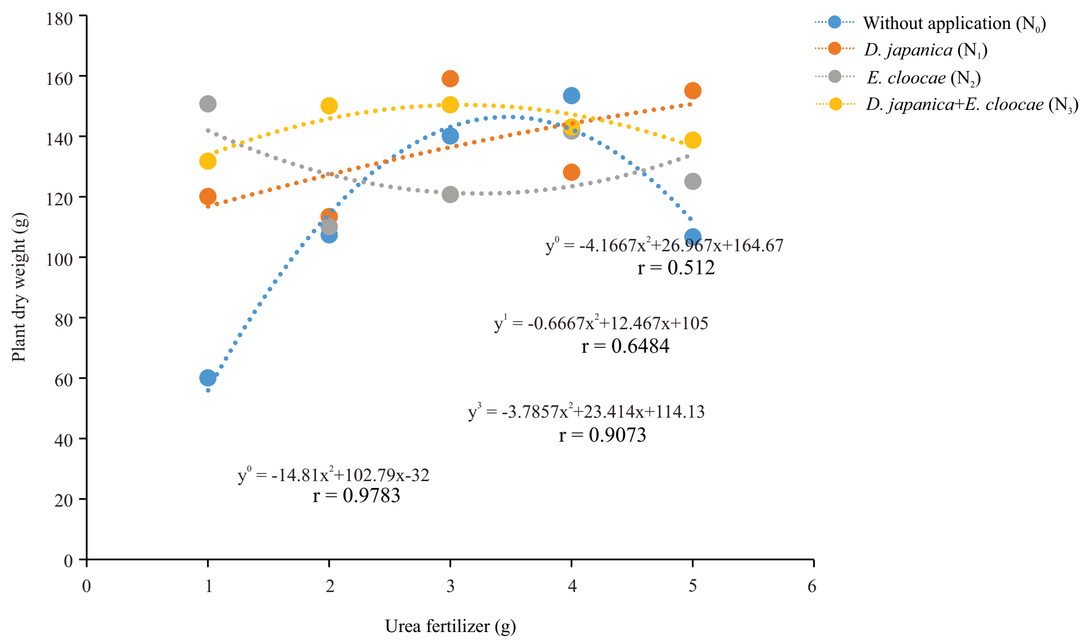

Fig. 4: Effect of nitrogen-fixing bacteria and urea fertilizer mixing on plant dry weight (g)

CONCLUSION

Based on the results of the study, it can be concluded that the treatment of *D. japonica*+*E. cloacae* (N_3)+urea fertilizer of 1.25 g/plant (N_3U_1) can increase soil N nutrient content up to 14.52%, plant dry weight up to 150% and plant moisture content up to 113%. It means that it can increase the growth of corn on Andisol soil with an acidic pH and urea fertilizer efficiency by 75%.

SIGNIFICANCE STATEMENT

This study found superior and environmental-specific nitrogen-fixing microorganisms that were useful for increasing the efficiency of nitrogen fertilization and increasing maize plant growth. This research will help researchers and farmers to overcome low nitrogen availability in the soil. Thus the new theory found that the interaction of treatment of *D. japonica*+*E. cloacae* with 1.25 g urea fertilizer per plant was the best treatment that could reduce the dose of chemical fertilizer (Urea) up to 75%.

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