Performance of selected superior bacterial isolates from biogas sludge on the growth of upland rice in ultisols

Desempenho dos isolados bacterianos superiores seleccionados das lamas de biogás no crescimento do arroz upland em ultisóis

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ABSTRACT

The study to obtain the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth, biomass, and nutrient uptake in upland rice in ultisols. This study used a Randomized Block Design within two factors and seven replications from October 2020 until April 2021. The first factor used selected SBI (B0= untreated, B1= nitrogen-fixing bacteria isolate (N3), B2= phosphate solubilizing bacteria isolate (P7), B3= isolates combination (N3+P7). The second factor was dosage of biogas sludge (S0= untreated, S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). The parameters were determined by ANOVA and followed by DMRT at $P< 0.05$. The results showed that the P7 isolate significantly increased total-N uptake and the highest crop growth rate (CGR) of upland rice by 20.77% and 2.81-folds, respectively. Biogas sludge dosage from 315 to 630 mL polybag^{-1} significantly increased plant height, uptake of total-N and available-P, total fresh and dry weight, and CGR of upland rice. The interaction of N3 with biogas sludge dosage of 630 mL polybag^{-1} significantly increased the CGR of upland rice. The application of N3 and P7 isolates and their combination within biogas sludge of 630 ml polybag^{-1} has the potential to archive the CGR of upland rice in acidic soils.

Keywords: acidic soil, crop growth rate, dosage, sludge potential, upland rice.

RESUMEN

O estudo para obter a influência de isolados bacterianos superiores selecionados (IBS), lamas de biogás e suas interações no crescimento, biomassa e absorção de nutrientes no arroz upland em ultisóis. Este estudo utilizou um projeto de bloco aleatorizado dentro de dois fatores e sete replicações de outubro de 2020 até abril de 2021. O primeiro factor utilizado foi o IBS seleccionado (B0= não tratado, B1= isolado de bactérias fixadoras de azoto (N3), B2= isolado de bactérias solubilizadoras de fosfato (P7), B3= combinação de isolados (N3+P7). O segundo factor foi a dosagem das lamas de biogás (S0= não tratadas, S1= 157.5; S2= 315; S3= 630 mL o polybag^{-1}). Os parâmetros foram determinados pela ANOVA e seguidos pela DMRT Em P< 0,05. Os resultados mostraram que o isolado P7 aumentou significativamente a captação total de N e a maior taxa de crescimento das culturas (TCC) de arroz das terras altas em 20,77% e 2,81 dobras, respectivamente. Dosagem das lamas de biogás de 315 a 630 mL o polibag^{-1} aumentou significativamente a altura da planta, a absorção do total-N e do Disponível-P, o peso total fresco e seco e o TCC do arroz das terras altas. Interacção de N3 com a dosagem de lamas de biogás de 630 mL o polibag^{-1} aumentou significativamente o TCC do arroz upland. A aplicação de isolados N3 e P7 e a sua combinação nas lamas de biogás de 630 mL o polibag^{-1} tem o potencial de arquivar o TCC do arroz upland em solos ácidos.

Palavras-chave: arroz de montanha, dosagem, potencial de lamas, solo ácido, taxa de crescimento das culturas.

Introduction

Biogas sludge is the waste by-product installation from an anaerobic processing system (Food and Agriculture Organization, 1997) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and plant yield (Adela *et al.*, 2014). It has been reported that the characteristics of biogas sludge from palm oil waste such as total-N of 490 mg L^{-1}; NH_3-N was 65 mg L^{-1}; total-P by 110 mg L^{-1}; K of 1.9 mg L^{-1}; Ca by 23 mg L^{-1}; Mg of 256 mg L^{-1}; As <0.01 mg L^{-1}; Zn was 0.61 mg L^{-1}; and Cr by 0.04 mg L^{-1} (Lubis *et al.*, 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al.*, 2019), NH_3-N ranged by 91 to 112 mg L^{-1} (Choorit & Wisarnwan, 2007), pH ranged by 6.8 to 8.3; and the highest bacterial populations was 7.21×10^7 cells per mL and the lowest was 3.15×10^7 cells per mL (Alvionita *et al.*, 2019). Mustamu & Triyanto (2020) also reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing which have the potential to availability of nitrogen and phosphate in the soil.

The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing has greater potential in increasing soil fertility and plant growth. Zhang *et al.* (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility, plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described that the *Bacillus* genera such as *B. circulans, B. cereus, B. fusiformis, B. pumilus, B. megaterium, B. mycoides, B. coagulans, B. chitinolyticus, B. subtilis* have been reported as phosphate solubilizing. Ambrosini *et al.* (2016) reported that *Bacillus cereus* showed the highest nitrogenase activity among 42 different strains of *Bacillus spp*. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, i.e. *Proteobacteria, Firmicutes, Bacteroidetes,* and *Thermotogae.*

The bacteria from biogas sludge has never been reported in Indonesia on the application of bacterial isolates from biogas sludge in improving upland rice growth on acidic soils. Thus, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge in increasing the availability of nitrogen, phosphate, and the response to the growth of upland rice due to the biogas sludge and selected isolate in ultisols. The study was aimed to obtain the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the growth of upland rice in ultisols.

Material and Methods

Study area

The concentration of total-N and available-P in ultisols and the plant tissue were analyzed in the Analytical Laboratory of PT. Socfin Indonesia, Medan. The bacterial isolates were applied to upland rice in Padang Bulan Village, Medan Selayang Subdistrict, Medan City, North Sumatra, Indonesia from October 2020 to April 2021.

Preparation of medium and upland rice seeds

The planting medium used a soil type of ultisols from the Simalingkar area, Medan Tuntungan Subdistrict, Medan City with a soil depth of 0 to 20 cm. 100 g of soil samples were taken and analyzed for chemical characteristics such as pH, organic-C, available-P, total-N, CEC, and base saturation (K, Ca, Na, Mg) (Table 1). The soil was sterilized by burning at 100^0C for 2 h. After being incubated for a day, the soil was put into a polybag with a size of 10 kg. A basic fertilizer of NPK Mutiara at a dose of 300 kg ha^{-1} was given by stirring evenly with the soil. In concurrently, the seeds of upland rice used was inbred variety of Inpago-8 then soaked in water for 24 h and followed by a propineb fungicide (70%) for two hours. Upland rice was planted after one day of basic fertilization with two seeds per polybag at a depth of 2 cm.

TABLE 1. The chemical characteristics of sterile ultisols.

Chemical characteristics	**Value**	**Category***
Soil pH (H_2O)	4.80	Acid
Organic-C (%)	0.44	Very low
Total-N (%)	0.04	Very low
Available-P (mg kg^{-1})	870.25	Very high
CEC (me/100 g)	28.31	High
Base saturation (%)	4.85	Very low
Exchangeable cations		
K (me/100 g)	0.60	High
Ca (me/100 g)	0.34	Very low
Mg (me/100 g)	0.32	Very low
Na (me/100 g)	0.09	Very low
Al (%)	0.02	Very low

Source: *Criteria for pH H_2O= 4.5-5.5 (acid); organik-C <1% (very low); total-N <0,1% (very low); available-P >60 mg kg^{-1} (very high); CEC= 25-40 me/100 g (high); base saturation <20% (very low); exchangeable-K= 0.60-1.00 me/100 g (high); exchangeable-Ca <2 me/100 g (very low); exchangeable-Mg <0.4 me/100 g (very low); exchangeable-Na <0.1 me/100 g (very low); exchangeable-Al <5% (very low) (Soil Research Institute, 2009).

Preparation of superior bacterial isolates suspension and biogas sludge

The selected superior bacterial isolates used phosphate solubilizing bacteria (P7) which has been confirmed by Mustamu *et al.* (2021) and nitrogen-fixing bacteria or N3 (data unpublished). The isolates were grown on NB medium, and incubated for 48 h. The microbial mass in the solution was measured using spectrophotometer with a density of 10^8 cells per mL. 10 mL was taken from solution containing nitrogen-fixing bacteria (N3) and phosphate solubilizing (P7).

The biogas sludge was taken from an identical location in the first phase of the study (palm oil mill of PT. Nubika Jaya, Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia). Bacterial isolate and biogas sludge were applied to the soil surface at the plants were one week after planting (WAP). Biogas sludge samples were taken 500 mL then analyzed the chemical and biological characteristics in the laboratory (Table 2).

TABLE 2. The chemical biological characteristics of biogas sludge.

Characteristics of biogas sludge	**Value**
Chemical	
pH	7.41
Chemical oxygen demand/COD (mg L^{-1})	4547.8
Biological oxygen demand/BOD (mg L^{-1})	1127.5
Total-N (%)	0.051
Total-P (%)	0.0097
Available-P (%)	0.013
Total-K (%)	0.18
Organic-C (%)	0.14
Ca (%)	0.04
Mg (%)	0.04
Na (ppm)	44.41
Cu (%)	0.0001
Biological	
Total nitrogen-fixing bacteria (CFU mL^{-1})	29.4×10^5
Total phosphate solubilizing bacteria (CFU mL^{-1})	7.0×10^4

Treatments Application

This study used a Randomized Block Design within two factors and seven replications. The first factor with the type of superior bacterial isolates at the similarly likewise dose, namely 10 mL polybag^{-1} (B0= un-treated; B1= nitrogen-fixing bacterial isolate (N3); B2= phosphate solubilizing bacteria isolate (P7); B3= combination isolates N3+P7). The second factor was dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Each replication was dismantled at 4, 8, and 12 Weeks After Isolate and Biogas Sludge Application (WAIBSA) for determination Crop Growth Rate (CGR).

Parameters and Data Analysis

Variable observations were conducted by measuring the growth of upland rice (plant height, total fresh and dry weight), total-N and available-P content in the shoots, total uptake-N, and available-P. CGR were conducted on plants at 4-8, 8-12, and 12-16 WAIBSA. Each polybag from each treatment and replication was dismantled at the plants were 4, 8, 12, and 16 WAIBSA, then measured the plant height, the total fresh weight was conducted by weighing the roots and shoots. The total dry weight was measured by oven at 60^0C for 48 hours and weighed by the analytical scales. A sample of the 2nd leaf from the shoots was taken by 200 g and analyzed for the total-N using the Kjeldahl method and available-P by the dry ashing method through UV-Vıs Spectrophotometer. The total-N and available-P absorption were measured using equation (1). The CGR was calculated by the dry weight per unit area using equation (2) (Shon *et al.*, 1997):

$$\text{Uptake nutrient} = \text{nutrient content in the shoots} \times \text{total dry weight} \qquad (1)$$

$$\text{CGR} = \frac{\Delta W}{\Delta t} = \frac{W2 - W1}{t2 - t1} \qquad (2)$$

Note:

CGR = crop growth rate

W1 = dry weight per unit area at t1

W2 = dry weight per unit area at t2

t1 = first sampling

t2 = second sampling

The parameters of the second phase of the study were analyzed by ANOVA and if the treatment had a significant effect, then continued by DMRT at $P<0.05$ with SPSS v.20 software.

Results and discussion

Plant height of upland rice (cm)

The effect of biogas sludge application was significant on the plant height of upland rice at 8, 12, and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect on the plant height of upland rice at 4, 8, 12, and 16 WAIBSA (Figure 1). A significant increase in plant height of upland rice along with increased doses of biogas sludge to 630 mL polybag^{-1} at 8, 12, and 16 WAIBSA with the highest increase of 14.81% compared to the control at 16 WAIBSA. Although the effect was insignificant, it was seen that the isolates combination of B3 and the interaction of B2S3 showed the highest increase plant height of upland rice by 2.94% and 22.06%, respectively, compared to the control.

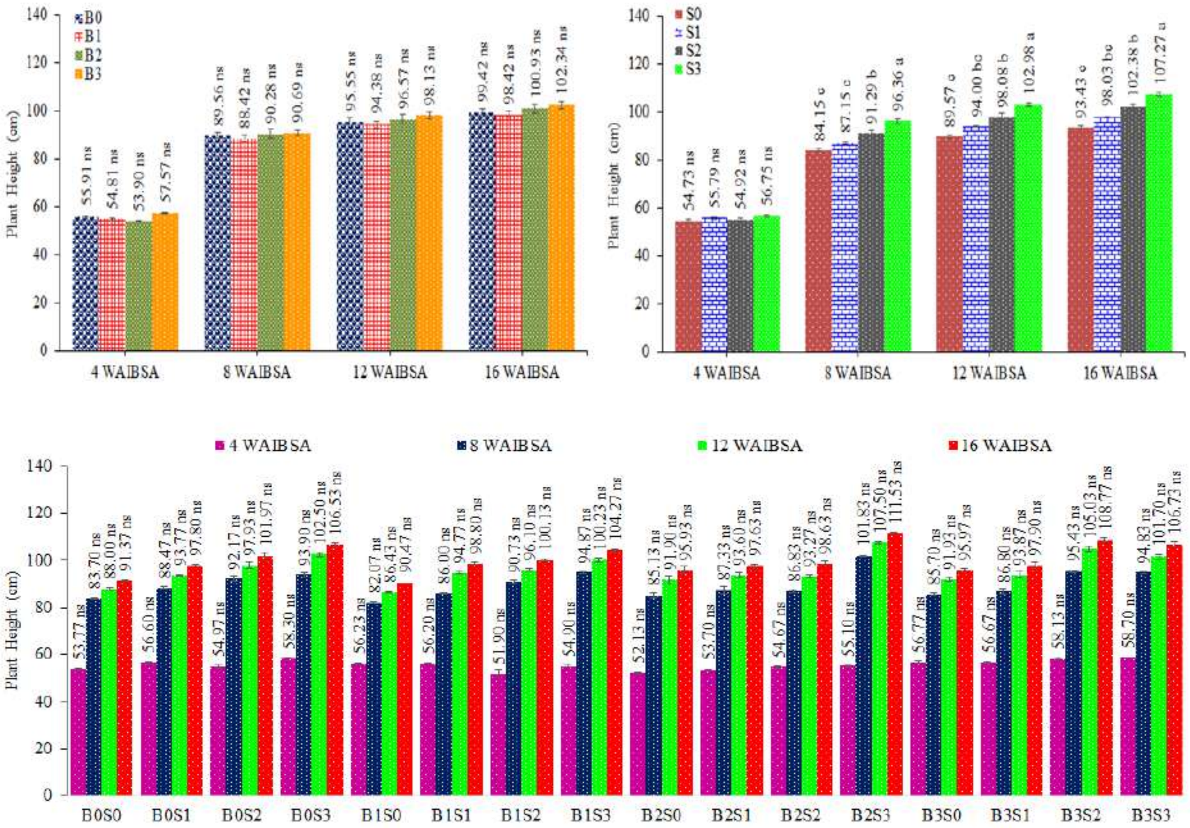

FIGURE 1. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on plant height of upland rice at 4, 8, 12, and 16 WAIBSA. Values followed by the different letter in the graph indicated significantly by DMRT at $P<0.05$. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

Biomass of upland rice (g)

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect on the total fresh weight of upland rice at 4-16 WAIBSA (Table 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on total fresh weight of upland rice at 4, 8, 12, and 16 WAIBSA.

Treatments	**Total fresh weight ± SE (g)**			
	4 WAIBSA	**8 WAIBSA**	**12 WAIBSA**	**16 WAIBSA**
Superior bacterial isolates (B)				
B0	4.15±0.21ns	169.31±8.90ns	215.27±8.42ns	229.82±8.94ns
B1	3.12±0.12ns	194.50±9.35ns	235.08±10.32 ns	252.02±10.22ns
B2	4.52±0.23ns	162.89±11.15ns	201.85±9.89ns	230.70±9.28ns
B3	3.30±0.25ns	173.91±12.55ns	220.40±15.96ns	245.03±16.32ns
Biogas sludge (S)				
S0	3.72±0.24ns	144.07±9.37b	182.67±7.14b	197.56±6.58b
S1	3.58±0.27ns	153.41±7.93b	190.70±8.90b	215.65±7.03b
S2	3.64±0.27ns	199.68±10.30a	258.70±9.63a	280.15±9.25a
S3	4.15±0.25ns	203.45±1.36a	240.52±2.81a	264.21±2.42a
Interactions (B×S)				
B0S0	4.99±0.33ns	124.08±5.60ns	185.64±3.32ns	192.78±2.96ns
B0S1	3.47±0.26ns	160.43±1.16ns	188.60±5.76ns	207.05±3.97ns
B0S2	3.42±0.42ns	185.97±6.80ns	232.60±8.75ns	250.84±7.40ns
B0S3	4.71±0.42ns	206.76±5.49ns	254.23±10.27ns	268.61±8.85ns
B1S0	2.80±0.18ns	155.79±1.12ns	183.96±5.20ns	202.88±2.88ns
B1S1	3.74±0.29ns	174.82±9.01ns	227.91±6.38ns	236.60±6.32ns
B1S2	3.28±0.40ns	241.17±5.25ns	283.60±7.76ns	296.08±8.05ns
B1S3	2.67±0.22ns	206.20±7.23ns	244.85±6.26ns	272.52±4.34ns
B2S0	3.19±0.18ns	190.90±7.77ns	215.36±7.67ns	229.11±6.75ns
B2S1	4.85±0.38ns	106.74±13.42ns	143.16±13.02ns	179.61±10.36ns
B2S2	5.20±0.24ns	148.40±11.59ns	219.65±5.26ns	248.72±6.94ns
B2S3	4.82±0.45ns	205.53±10.50ns	229.21±16.57ns	265.34±9.58ns
B3S0	3.91±0.30ns	105.53±3.94ns	145.72±1.96ns	165.45±1.11ns
B3S1	2.25±0.09ns	171.63±4.90ns	203.14±7.07ns	239.34±12.07ns
B3S2	2.66±0.14ns	223.17±7.84ns	298.95±1.51ns	324.94±3.03ns
B3S3	4.37±0.07ns	195.31±6.77ns	233.79±8.40ns	250.38±8.16ns
CV (%)	56.09	29.68	26.31	20.78

Note: values followed by the different letter in the column indicated significantly by DMRT at *P*<0.05±SE. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

A significant increase in total fresh weight of upland rice along with the increase in the dosage of biogas sludge to 315 mL polybag^{-1} at 16 WAIBSA with the highest increase by 41.81% compared to the control. Although the effect was insignificant, it was seen that the B1 and the interaction of B3S2 showed the highest increase in the total fresh weight of upland rice were 9.66% and 68.55%, respectively compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4-16 WAIBSA (Table 4).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight of upland rice at 4, 8, 12, and 16 WAIBSA.

Treatments	**Total dry weight ± SE (g)**			
	4 WAIBSA	**8 WAIBSA**	**12 WAIBSA**	**16 WAIBSA**
Superior bacterial isolates (B)				
B0	1.38±0.06ns	48.01±1.29ns	73.60±3.99ns	82.52±4.18ns
B1	1.13±0.05ns	54.09±2.41ns	76.83±2.66ns	99.72±4.15ns
B2	1.49±0.06ns	47.30±3.30ns	73.20±2.28ns	98.25±3.90ns
B3	1.15±0.07ns	52.32±3.39ns	77.18±4.90ns	98.47±4.56ns
Biogas sludge (S)				
S0	1.26±0.06ns	45.51±2.63ns	62.88±2.19b	76.78±1.63c
S1	1.23±0.08ns	44.47±1.71ns	68.52±2.00ab	87.65±2.84bc
S2	1.26±0.08ns	55.36±3.43ns	85.69±1.08a	98.95±1.86b
S3	1.40±0.06ns	56.38±1.05ns	83.73±3.44a	115.59±2.11a
Interactions (B×S)				
B0S0	1.58±0.08ns	41.73±2.78ns	58.08±1.54ns	67.23±0.96ns
B0S1	1.12±0.08ns	45.87±0.83ns	62.74±1.83ns	71.08±1.91ns
B0S2	1.20±0.12ns	52.25±2.07ns	81.39±5.48ns	88.28±5.02ns
B0S3	1.60±0.12ns	52.18±0.29ns	92.20±3.05ns	103.49±2.43ns
B1S0	0.97±0.04ns	46.64±1.39ns	69.53±4.90ns	80.30±4.51ns
B1S1	1.40±0.07ns	48.13±2.78ns	78.91±0.53ns	96.23±1.50ns
B1S2	1.12±0.10ns	67.79±1.44ns	91.05±2.25ns	101.80±2.40ns
B1S3	1.02±0.08ns	53.81±3.76ns	67.84±1.77ns	120.54±2.15ns
B2S0	1.17±0.05ns	59.32±2.33ns	70.92±4.20ns	81.43±3.82ns
B2S1	1.54±0.10ns	34.47±2.16ns	61.69±1.97ns	89.84±1.41ns
B2S2	1.73±0.05ns	37.37±3.74ns	83.10±1.19ns	105.46±1.37ns
B2S3	1.53±0.10ns	58.05±1.76ns	77.07±4.27ns	116.28±1.30ns
B3S0	1.30±0.07ns	34.35±7.04ns	52.98±0.73ns	78.16±0.48ns
B3S1	0.85±0.03ns	49.40±0.08ns	70.72±1.29ns	93.44±2.19ns
B3S2	0.99±0.05ns	64.05±4.68ns	87.22±2.90ns	100.26±1.93ns
B3S3	1.44±0.02ns	61.48±2.47ns	97.80±0.77ns	122.04±0.20ns
CV (%)	43.80	31.22	26.54	18.38

Note: values followed by the different letter in the column indicated significantly by DMRT at $P<0.05$±SE. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

A significant increase in total dry weight of upland rice along with the increase in the dosage of biogas sludge to 630 mL polybag^{-1} at 16 WAIBSA with the highest increase of 50.55%

compared to the control. Although the effect was insignificant, it was seen that the B1 and the interaction of B3S3 showed the highest increase in the total dry weight of upland rice were 20.84% and 81.53%, respectively compared to the control.

Content and uptake of total-N and available-P nutrient of upland rice

The effect of biogas sludge, superior bacterial isolates, and their interactions had an insignificant effect on the nutrient content of total-N and available-P in upland rice. The effect of biogas sludge significantly increased in the nutrient uptake of total-N and available-P. Superior bacterial isolates significantly increased in the nutrient uptake of total-N. The interaction of biogas sludge with superior bacterial isolates had an insignificant effect on the nutrient uptake of total-N and available-P of upland rice (Figure 2).

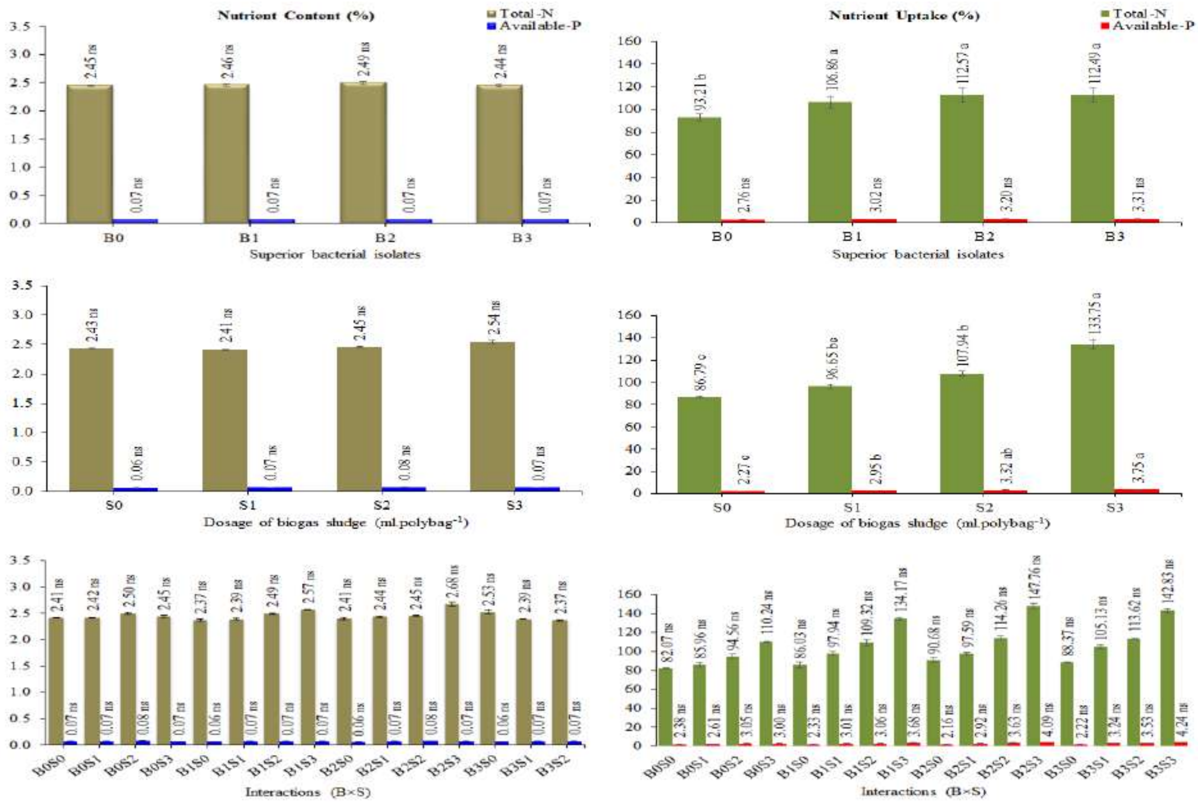

FIGURE 2. The effect of superior bacterial isolates, dosage of biogas sludge, and their interactions in the content and uptake of total-N and available-P nutrient of upland rice. Values followed by the different letter in graph indicated significantly by DMRT at *P*<0.05. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

A significant increase in the nutrient uptake of total-N and available-P in upland rice along with the increase in the dose of biogas sludge to 630 mL polybag^{-1} with the highest increase of 54.11% and 65.20%, respectively compared to the control. The superior bacterial isolates (B1-B3) also significantly increased the nutrient uptake of total-N for upland rice with the highest increase in the B2 by 20.77% compared to the control. Although the effect was insignificant, it was seen that the B3 showed the highest increase in nutrient uptake of available-P in upland rice was 19.93% compared to the control. Likewise, the interaction of B2S3 and B3S3 showed the highest increase in nutrient uptake of total-N and available-P in upland rice by 80.04% and 79.41%, respectively, compared to the control.

The biogas sludge doses of 315 and 630 mL polybag^{-1} (S2 and S3) explained that the nutrient content of available-P and total-N in the plant tissue of upland rice were higher by 33.33% and 4.53%, respectively compared to the control. The B2 isolate showed the highest nutrient content of total-N in the plant tissue of upland rice by 1.63% compared to the control, but all isolates (B1-B3) showed a similar level of available-P in the plant tissue of upland rice with the control. The interactions of B2S3 and B2S2 also showed the highest nutrient content of total-N and available-P compared to other interactions.

Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12 to 16 WAIBSA, but it had an insignificant effect at 4-8 and 8-12 WAIBSA (Table 5).

The biogas sludge dose of 630 mL polybag^{-1} (S3) significantly increased the highest crop growth rate for upland rice at 12 to 16 WAIBSA by 129.44% compared to the control. The ability of B1-B3 isolates significantly increased the crop growth rate of upland rice with the highest increase in the B2 by 181.45% compared to the controls at 12 to 16 WAIBSA. The interaction of the B1S3 significantly increased the crop growth rate of upland rice by 5.76-folds greater compared to the control.

TABLE 5. The effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of upland rice.

Superior bacterial isolates (B)	**Biogas sludge (S)**				**Average**
	S0	**S1**	**S2**	**S3**	
			4-8 WAIBSA		
B0	1.434ns	1.598ns	1.823ns	1.806ns	1.665ns
B1	1.631ns	1.669ns	2.381ns	1.885ns	1.892ns
B2	2.077ns	1.176ns	1.273ns	2.019ns	1.636ns
B3	1.180ns	1.734ns	2.252ns	2.144ns	1.828ns
Average	1.580ns	1.544ns	1.932ns	1.964ns	CV= 32.28%
			8-12 WAIBSA		
B0	0.584ns	0.602ns	1.041ns	1.430ns	0.914ns
B1	0.818ns	1.099ns	0.831ns	0.501ns	0.812ns
B2	0.414ns	0.972ns	1.633ns	0.679ns	0.925ns
B3	0.665ns	0.761ns	0.828ns	1.297ns	0.888ns
Average	0.620ns	0.859ns	1.083ns	0.977ns	CV= 56.17%
			12-16 WAIBSA		
B0	0.327fgh	0.298gh	0.246h	0.403b-h	0.318b
B1	0.385c-h	0.619a-h	0.384d-h	1.882a	0.817a
B2	0.375e-h	1.005a-h	0.798a-h	1.400a-h	0.895a
B3	0.899a-h	0.811a-h	0.466a-h	0.866a-h	0.761a
Average	0.496b	0.683b	0.474b	1.138a	CV= 51.07%

Note: values followed by the different letter in the column indicated significantly by DMRT at *P*<0.05. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

The effect of selected superior bacterial isolates

The selected superior bacterial isolates (N3 and P7) significantly increased the nutrient uptake of total-N and crop growth rate of upland rice on ultisols at 12 to 16 WAIBSA, but it had an insignificant effect on plant height, total fresh weight, total dry weight, nutrient content (total-N and available-P) in leaf tissue, nutrient uptake of available-P, and crop growth rate of upland rice at 4 to 8 and 8 to 12 WAIBSA. The superior bacterial isolates (N3, P7, and N3+P7) could increase the nutrient uptake of total-N in upland rice by 14.64%, 20.77%, and 20.68%, respectively compared to control (Figure 2). Similar results are also shown in Table 5, that the crop growth rate of upland rice at 12 to 16 WAIBSA due to selected superior bacterial isolates (N3, P7, N3+P7) has increased by 2.57; 2.81; and 2.39-folds, respectively compared to the control. The finding results indicated that the ability of a single isolate by P7 bacteria was greater in increasing total-N and crop growth rate of upland rice compared to a single isolate by N3 bacteria and a combination of N3+P7 bacterial isolates. It was due to the presence of several organic acids and hormones produced by P7 that can increase the nutrient uptake of total-N and crop growth rate of upland rice. This result was supported by Mustamu *et al.* (2021) that the phosphate solubilizing bacteria isolate (P7) from the biogas sludge contains organic acids produced such as lactic, oxalic, acetic, citric acids, and it had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate was 4.62 and 2.66-folds, respectively compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus slightly increased in the application of bio fertilization with *Bacillus cereus*, it was due to the production of organic acids and other chemicals such as citric, tartaric, and oxalic acids which can stimulate plant growth and nutrients availability. Youssef & Eissa (2017) reported that the increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that the hormone gibberellin, IAA, and other hormones can increase plant growth under stressful conditions. Kang *et al.* (2014) said that PGPB has several mechanisms to increase plant growth with nitrogen-fixation, phosphate solubilizing, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from anaerobic digester include: *Ruminococcus sp., Thiomargarita sp., Clostridium sp. Anaerobacter sp., Bacillus sp., Sporobacterium sp., Saccharofermentans sp., Oscillibacter sp., Sporobacter sp.,* and *Enterobacter sp.* Liaquat *et al.* (2017) also reported that an abundance of *Bacillus*, *Clostridium*, and *Enterobacter spp* in anaerobic digester of wastewater in producing biogas.

The effect of biogas sludge

The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and 16 WAIBSA), total dry weight (12 and 16 WAIBSA), nutrient uptake (total-N and available-P), and the crop growth rate of upland rice at 8 to 12 WAIBSA, but it had an insignificant effect on nutrient content (total-N and available-P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAIBSA). An increase in plant height, total dry weight, nutrient uptake of total-N and available-P, and also crop growth rate of upland rice on ultisols along with the increase in the dose of biogas sludge to 630 mL polybag^{-1} at the end of this study (16 WAIBSA). However, in contrast to the total fresh weight, an increase along with the increase in the biogas sludge dose to 315 mL polybag^{-1} then decreased at the dose of 630 mL polybag^{-1}. It was caused the biogas sludge had chemical characteristics such as pH (7.41), total-N (0.051%), available-P (0.013%), organic-C (0.14%), total-K (0.18%), and biological characteristics such as total nitrogen-fixing bacteria (29.4×10^{5} CFU mL^{-1}) and total phosphate solubilizing bacteria (7.0×10^{4} CFU mL^{-1}) (Table 2). C-organic content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could be increased the nutrient uptake of total-N and available-P in upland rice along with increasing the dose of biogas sludge to 630 mL polybag^{-1} (Figure 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the growth of plant height, biomass, and crop growth rate of upland rice. A similar result was reported by Mustamu & Triyanto (2020) that the macro and micronutrients from the biogas sludge and also had the population of nitrogen-fixing and phosphate solubilizing bacteria by 480×10^{4} and 42×10^{4} CFU mL^{-1}, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that total phosphate solubilizing bacteria (1.6 to 2.5 CFU mL^{-1}) was significantly higher compared to nitrogen-fixing bacteria (0.5–1.4 CFU mL^{-1}) and a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller & Müller (2012) reported that an increase in concentrations of NH_4^+-N ranged from 45 to 80% after anaerobic waste.

The interaction effect of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice on ultisols at 8 to 12 WAIBSA, but it had an insignificant effect on other parameters in this study. The interaction of NFB *Bacillus paramycoides* with biogas sludge at the dose of 630 mL polybag^{-1} (B1S3) showed the highest crop growth rate of upland rice compared to other interactions and 5.76-folds greater compared to the control. It was caused by the application of biogas sludge could be increased soil organic matter and the total population of beneficial bacteria. Likewise, the characteristics of the biogas sludge had the organic-C was 0.14%, total nitrogen-fixing bacteria was 29.4×10^5 CFU mL^{-1}, and total phosphate solubilizing bacteria was 7.0×10^4 CFU mL^{-1} (Table 2) could improve soil quality and support the crop growth rate. This result is supported by Urra *et al.* (2019) that the application of sewage sludge in the long-term significantly increases the organic matter in the soil, which causes a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of organic matter. Bhardwaj *et al.* (2014); Carvajal-Muñoz *et al.* (2012) reported that the application of biofertilizer had advantages in the plant such as availability of nutrients that are balanced for plant health, stimulating nutrient mobilization that can increase soil biological activity, availability of microbial food to encourage the growth of beneficial microorganisms, increasing the soil organic matter content thereby increasing the cation exchange capacity. Siswanti & Lestari (2019) reported that the interaction of biogas sludge+biofertilizer (36 mL+10 L ha^{-1})

significantly increased the plant height, number of leaves, and capcaisin content in chili compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

The isolates of N3, P7, N3+P7 from the biogas sludge significantly increased the nutrient uptake of total-N and crop growth rate of upland rice on ultisols with the highest increase found in the P7 isolate of 20.77% and 2.81-folds, respectively. The dose of biogas sludge significantly increased plant height, total dry weight, nutrient uptake of total-N and available-P, and also crop growth rate of upland rice on ultisols with the highest increase at a dose of 630 mL polybag^{-1} by 14.81%; 50.55%; 54.11%; 65.20%; and 129.44%, respectively. Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice with the highest increase at the dose of 315 mL polybag^{-1} by 41.81%. The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice on ultisols with the highest increase found in the B1S3 by 5.76-folds.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author’s contributions

Novilda Elizabeth Mustamu (NEM), Zulkifli Nasution (ZN), Irvan (I), and Mariani Sembiring (MS). All authors formulated the overarching research goals and aims, provided the study materials, developed or designed the methodology. NEM analyze and interpretation study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in field, data collection. ZN and I verified the overall reproducibility of results and other research outputs. All authors conducted the critical review/commentary/revision of the manuscript.

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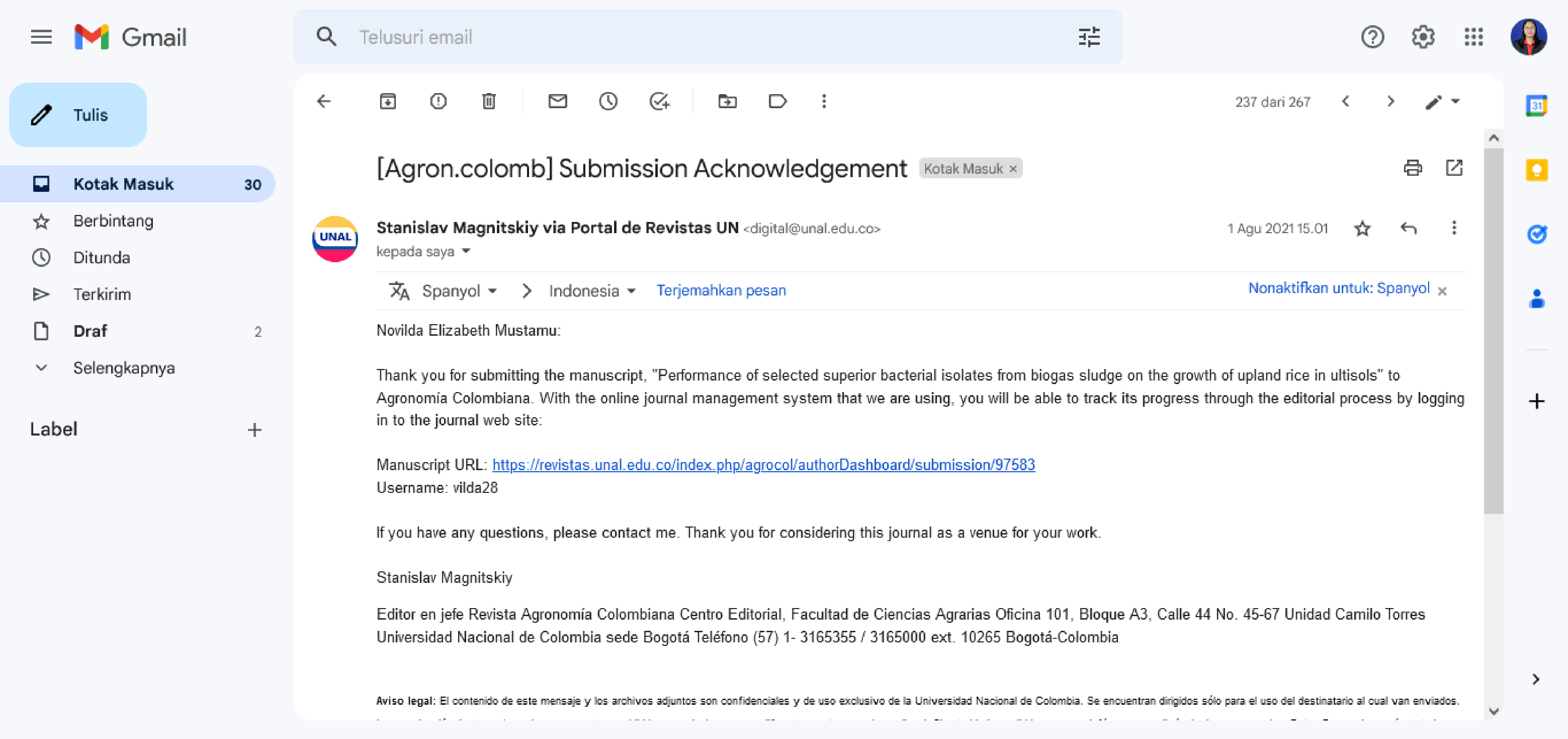

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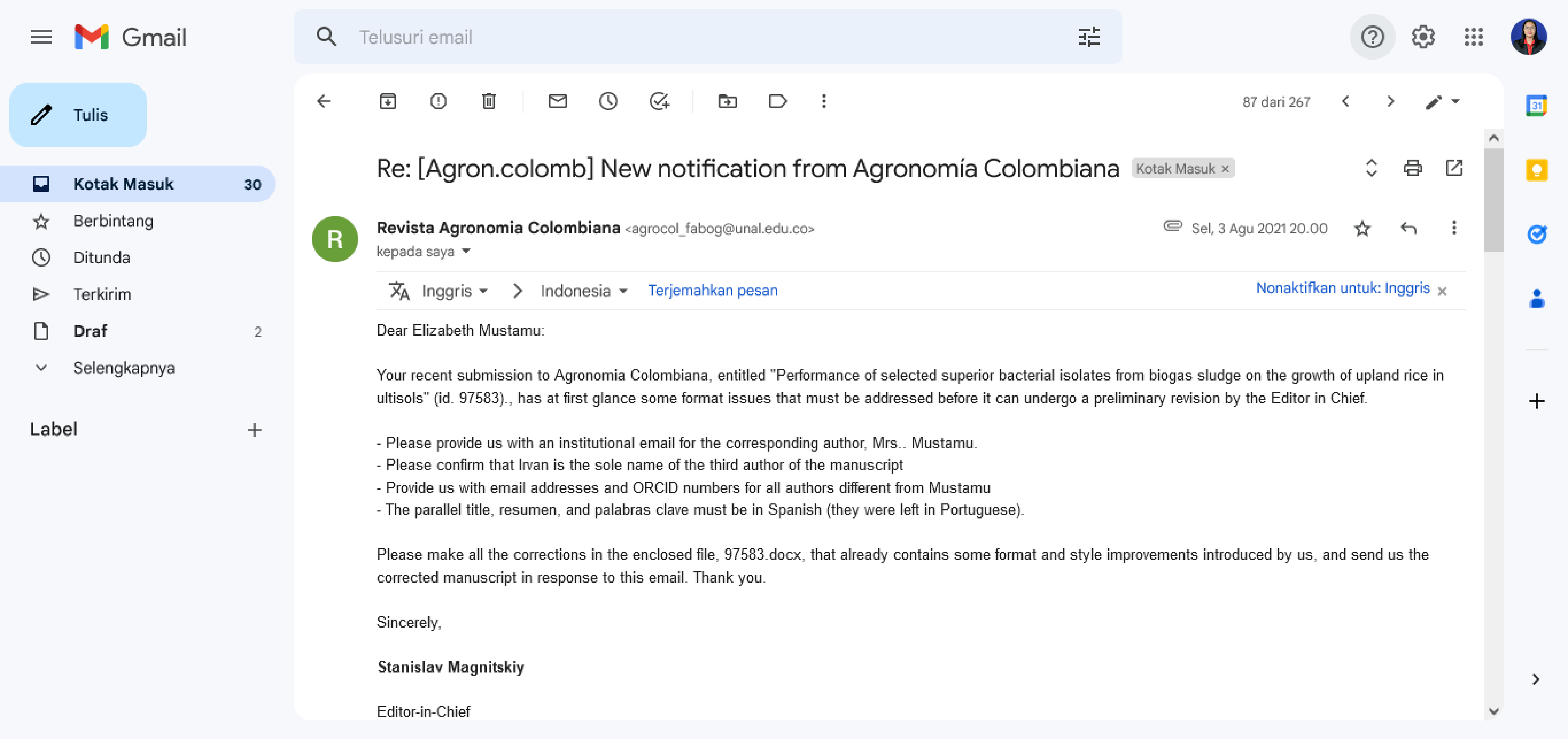

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- Please provide us with an institutional email for the corresponding author, Mrs.. Mustamu.
- Please confirm that Irvan is the sole name of the third author of the manuscript
- Provide us with email addresses and ORCID numbers for all authors different from Mustamu
- The parallel title, resumen, and palabras clave must be in Spanish (they were left in Portuguese).

Please make all the corrections in the enclosed file, 97583.docx, that already contains some format and style improvements introduced by us, and send us the corrected manuscript in response to this email. Thank you.

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Editor-in-Chief

Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la nutrición del arroz de secano

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ABSTRACT

The investigation evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth, biomass, and nutrient uptake in upland rice in ultisols. This study used a Randomized Block Design within two factors and seven replications from October 2020 until April 2021. The first factor used selected SBI (B0= untreated, B1= nitrogen-fixing bacteria isolate (N3), B2= phosphate solubilizing bacteria isolate (P7), B3= isolates combination (N3+P7). The second factor was dosage of biogas sludge (S0= untreated, S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). The parameters were determined by ANOVA and followed by Duncan's multiple range test (DMRT) at *P*< 0.05. The results showed that the P7 isolate significantly increased total-N uptake and the highest crop growth rate (CGR) of upland rice by 20.77% and 2.81-times, respectively. Biogas sludge dosage from 315 to 630 mL polybag^{-1} significantly increased plant height, uptake of total-N and available-P, total fresh and dry weight, and CGR of upland rice. The interaction of N3 with biogas sludge dosage of 630 mL polybag^{-1} significantly increased the CGR of upland rice. The application of N3 and P7 isolates and their combination within biogas sludge of 630 mL polybag^{-1} has the potential to archive the CGR of upland rice in acidic soils.

Keywords: acidic soil, crop growth rate, dosage, sludge potential, upland rice.

RESUMEN

La investigación evaluó la influencia de aislados bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones en el crecimiento, la biomasa y la absorción de nutrientes en el arroz de tierras altas en ultisoles. Este estudio utilizó un Diseño de Bloques Aleatorizados dentro de dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0= sin tratar, B1 = aislado de bacterias fijadoras de nitrógeno (N3), B2= aislado de bacterias solubilizantes de fosfato (P7), B3= combinación de aislados (N3+P7). El segundo factor fue la dosificación del lodo de biogás (S0= sin tratar, S1= 157,5; S2= 315; S3= 630 mL de polybag^{-1}). Los parámetros fueron determinados por ANOVA y seguidos de la prueba de rangos múltiples de Duncan (DMRT) en P< 0,05. Los resultados mostraron que el aislado P7 aumentó significativamente la captación total de N y la mayor tasa de crecimiento del cultivo (TCC) de arroz de tierras altas en 20.77% y 2.81-veces, respectivamente. La dosificación de fangos de biogás de 315 a 630 mL de polybag^{-1} aumentó significativamente la altura de la planta, la absorción de N total y P disponible, el peso fresco y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosificación de lodo de biogás de 630 mL de polybag^{-1} aumentó significativamente el TCC del arroz de tierras altas.

La aplicación de aislamientos de N3 y P7 y su combinación dentro de lodos de biogás de 630 mL de polybag^{-1} tiene el potencial de archivar el TCC de arroz de tierras altas en suelos ácidos.

Palavras-chave: arroz de tierras altas, dosis, potencial de lodo, suelo ácido, tasa de crecimiento de los cultivos.

Introduction

Biogas sludge is the waste by-product installation from an anaerobic processing system (Food and Agriculture Organization, 1997) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and plant yield (Adela *et al.*, 2014). It has been reported that the characteristics of biogas sludge from palm oil waste such as total-N of 490 mg L^{-1}; total-P by 110 mg L^{-1}; and K was 1.9 mg L^{-1} (Lubis *et al.*, 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al.*, 2019), NH_3-N ranged by 91 to 112 mg L^{-1} (Choorit & Wisarnwan, 2007), pH ranged by 6.8 to 8.3; and the highest bacterial populations was 7.21×10^{7} cells per mL and the lowest was 3.15×10^{7} cells per mL (Alvionita *et al.*, 2019). Mustamu & Triyanto (2020) also reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing which have the potential to availability of nitrogen and phosphate in the soil.

The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing has greater potential in increasing soil fertility and plant growth. Zhang *et al.* (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility, plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described that the *Bacillus* genera such as *B. circulans, B. cereus, B. fusiformis, B. pumilus, B. megaterium, B. mycoides, B. coagulans*, *B. chitinolyticus*, *B. subtilis* have been reported as phosphate solubilizing. Ambrosini *et al.* (2016) reported that *Bacillus cereus* showed the highest nitrogenase activity among 42 different strains of *Bacillus spp*. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, i.e. *Proteobacteria, Firmicutes, Bacteroidetes*, and *Thermotogae.*

The bacteria from biogas sludge has never been reported in Indonesia on the application of bacterial isolates from biogas sludge in improving upland rice growth on acidic soils. Thus, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge in increasing the availability of nitrogen, phosphate, and the response to the growth of upland rice due to the biogas sludge and selected isolate in ultisols. The study was aimed to evaluated the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the nutrition of upland rice in ultisols.

Materials and methods

Study area

The concentration of total-N and available-P in ultisols and the plant tissue were analyzed in the Analytical Laboratory of PT. Socfin Indonesia, Medan. The bacterial isolates were applied to upland rice in Padang Bulan Village (3°37.760'N; 98°38.898'E; altitude 18 m above sea level), Medan Selayang Subdistrict, Medan City, North Sumatra, Indonesia from October 2020 to April 2021. Furthermore the average humidity was 82%, temperature was 27.4°C and the average rainfall was recorded 228.5 mm by month.

Preparation of medium and upland rice seeds

The medium used ultisols order from Simalingkar area, Medan Tuntungan Subdistrict, Medan City with a depth of 0 to 20 cm. 100 g of soil samples were taken and analyzed for chemical characteristics such as pH using HCl 25% method with spectrophotometer, organic-C by Walkley-Black method with spectrophotometer, available-P by Bray-II method with spectrophotometer, total-N using Kjeldahl method with spectrophotometer, Cation Exchange Capacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH 7 method with atomic absorption spectrophotometry (Tab. 1). The soil was sterilized by burning at 100^0C for 2 hours. After being incubated for 1 day, the soil was put into a polybag with a size of 10 kg. A basic fertilizer of NPK Mutiara at a dose of 300 kg ha^{-1} was given by stirring evenly with the soil. Concurrently, the seeds of upland rice used was inbred variety of Inpago-8 then soaked in water for 24 hours and followed by a propineb fungicide (70%) for 2 hours. Upland rice was planted after one day of basic fertilization with two seeds per polybag at a depth of 2 cm.

TABLE 1. The chemical characteristics of sterile ultisols.

Chemical characteristics	**Value**	**Category***
Soil pH (H_2O)	4.80	Acid
Organic-C (%)	0.44	Very low
Total-N (%)	0.04	Very low
Available-P (mg kg^{-1})	870.25	Very high
CEC (me/100 g)	28.31	High
Base saturation (%)	4.85	Very low
Exchangeable cations		
K (me/100 g)	0.60	High
Ca (me/100 g)	0.34	Very low
Mg (me/100 g)	0.32	Very low
Na (me/100 g)	0.09	Very low
Al (%)	0.02	Very low

Source: *Criteria for pH H_2O= 4.5-5.5 (acid); organik-C <1% (very low); total-N <0,1% (very low); available-P >60 mg kg^{-1} (very high); CEC= 25-40 me/100 g (high); base saturation <20% (very low); exchangeable-K= 0.60-1.00 me/100 g (high); exchangeable-Ca <2 me/100 g (very low); exchangeable-Mg <0.4 me/100 g (very low); exchangeable-Na <0.1 me/100 g (very low); exchangeable-Al <5% (very low) (Soil Research Institute, 2009).

Preparation of superior bacterial isolates suspension and biogas sludge

The selected superior bacterial isolates used phosphate solubilizing bacteria (P7) which has been confirmed by Mustamu *et al.* (2021a) and nitrogen-fixing bacteria or N3 (Mustamu *et al.*, 2021b). The isolates were grown on NB medium, and incubated for 48 h. The bacteria growth in the solution was measured using spectrophotometer with a density of 10^8 cells per mL. 10 mL was taken from solution containing nitrogen-fixing bacteria (N3) and phosphate solubilizing (P7).

The biogas sludge was taken from the digester tank at the palm oil mill of PT. Nubika Jaya, Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia. Bacterial isolate and biogas sludge were applied to the soil surface at the plants were one week after planting (WAP). Biogas sludge samples were taken 500 mL then analyzed the chemical and biological characteristics in the laboratory (Tab. 2).

TABLE 2. The chemical biological characteristics of biogas sludge.

Characteristics of biogas sludge	**Method**	**Value**
Chemical		
pH	Electrometry	7.41
Chemical oxygen demand/COD (mg L^{-1})	Spectrophotometry	4547.8
Biological oxygen demand/BOD (mg L^{-1})	Titrimetry	1127.5
Total-N (%)	Spectrophotometry	0.051
Total-P (%)	Spectrophotometry	0.0097
Available-P (%)	Spectrophotometry	0.013
Total-K (%)	Graphite furnace-AAS	0.18
Organic-C (%)	Atomic absorption spectrophotometry (AAS)	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (ppm)	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Biological		
Total nitrogen-fixing bacteria (CFU mL^{-1})	Plate count	29.4×10^5
Total phosphate solubilizing bacteria (CFU mL^{-1})	Plate count	7.0×10^4

Treatments application

This study used a Randomized Block Design within two factors and seven replications. The first factor was the type of superior bacterial isolates (B0= un-treated; B1= nitrogen-fixing bacterial isolate (N3); B2= phosphate solubilizing bacteria isolate (P7); B3= combination isolates N3+P7) at the similar dose, namely 10 mL polybag^{-1}. The second factor was dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Determination of biogas sludge based on the dose of liquid organic fertilizer at the oil palm was 126 m^3 ha^{-1} equal to 126,000 L ha^{-1} (Sutarta *et al.*, 2003) then converted to soil weight per polybag (equation 1).

Each replication was disassembled at 4, 8, and 12 Weeks After Isolate and Biogas Sludge Application (WAIBSA) for determination Crop Growth Rate (CGR).

$$\text{Biogas sludge} = \frac{\text{The dose of liquid organic fertilizer per ha}}{\text{soil weight per ha}} \text{ x soil weight per polybag} \quad (1)$$

$$= \frac{126{,}000 \text{ L ha}^{-1}}{2{,}000{,}000 \text{ kg ha}^{-1}} \text{ x } 10 \text{ kg} = 630 \text{ ml}$$

Parameters and data analysis

Variable observations were conducted by measuring the growth of upland rice (plant height, total fresh and dry weight), total-N and available-P content in the shoots, total uptake-N, and available-P. CGR were conducted on plants at 4-8, 8-12, and 12-16 WAIBSA. Each polybag from each treatment and replication was disassembled at the plants were 4, 8, 12, and 16 WAIBSA, then measured the plant height, the total fresh weight was conducted by weighing the roots and shoots. The total dry weight was measured by oven at 60^0C for 48 hours and weighed by the analytical scales. A sample of the second leaf from the shoots was taken by 200 g and analyzed for the total-N using the Kjeldahl method and available-P by the dry ashing method through UV-Vɪs Spectrophotometer. The total-N and available-P absorption were measured using equation (2). The CGR was calculated by the dry weight per unit area using equation (3) (Shon *et al.*, 1997):

$$\text{Uptake nutrient} = \text{nutrient content in the shoots} \times \text{total dry weight} \quad (2)$$

$$\text{CGR} = \frac{\Delta W}{\Delta t} = \frac{W2 - W1}{t2 - t1} \quad (3)$$

Note:

CGR = crop growth rate

W1 = dry weight per unit area at t1

W2 = dry weight per unit area at t2

t1 = first sampling

t2 = second sampling

The parameters of the second phase of the study were analyzed by ANOVA and if the treatment had a significant effect, then continued by Duncan's multiple range test (DMRT) at *P*<0.05 with SPSS v.20 software.

Results

Effect of bacterial isolates and biogas sludge on upland rice growth

Plant height of upland rice (cm)

The effect of biogas sludge application was significant on the plant height of upland rice at 8, 12, and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect on the plant height of upland rice at 4, 8, 12, and 16 WAIBSA (Fig. 1). A significant increase in plant height of upland rice along with increased doses of biogas sludge to 630 mL polybag^{-1} at 8, 12, and 16 WAIBSA with the highest increase of 14.81% compared to the control at 16 WAIBSA. Although the effect was insignificant, it was seen that the isolates combination of B3 and the interaction of B2S3 showed the highest increase plant height of upland rice by 2.94% and 22.06%, respectively, compared to the control.

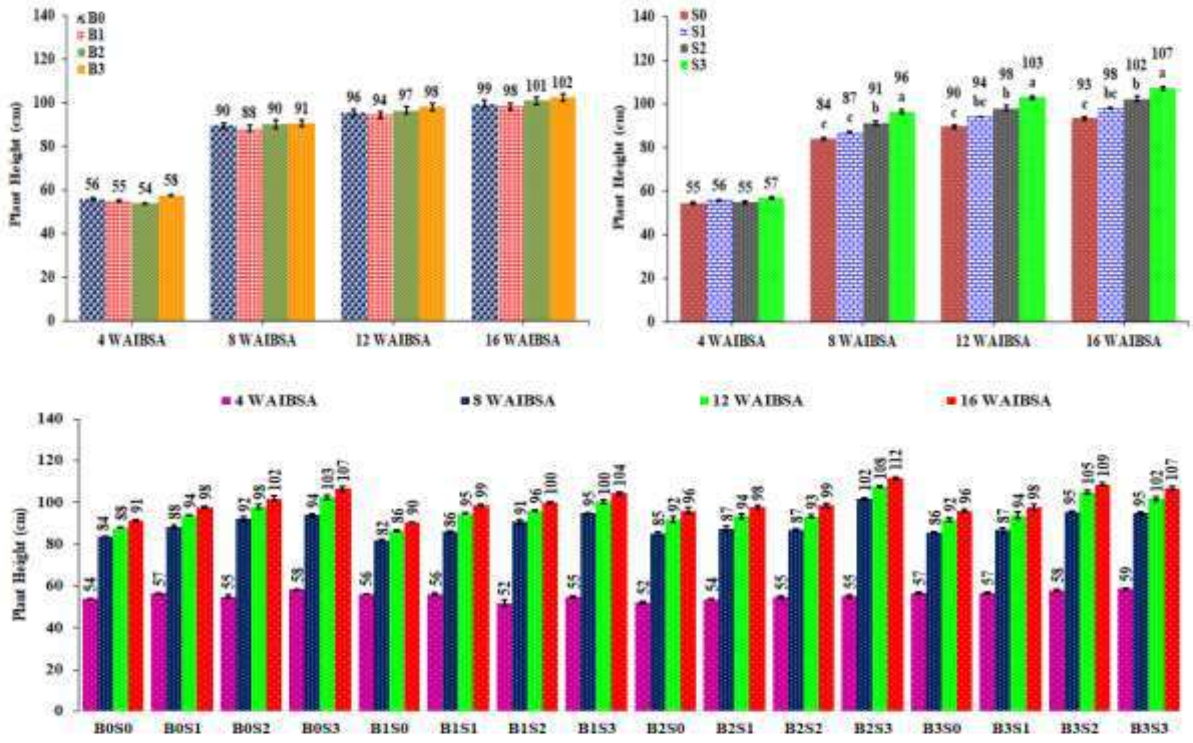

FIGURE 1. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on plant height of upland rice at 4, 8, 12, and 16 WAIBSA. Values followed by the different letter in the graph indicated significantly by DMRT at *P*<0.05. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

Biomass of upland rice (g)

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect on the total fresh weight of upland rice at 4-16 WAIBSA (Tab. 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on total fresh weight of upland rice at 4, 8, 12, and 16 WAIBSA.

Treatments	**Total fresh weight ± SE (g)**			
	4 WAIBSA	**8 WAIBSA**	**12 WAIBSA**	**16 WAIBSA**
Superior bacterial isolates (B)				
B0	4.15±0.21	169.31±8.90	215.27±8.42	229.82±8.94
B1	3.12±0.12	194.50±9.35	235.08±10.32	252.02±10.22
B2	4.52±0.23	162.89±11.15	201.85±9.89	230.70±9.28
B3	3.30±0.25	173.91±12.55	220.40±15.96	245.03±16.32
Biogas sludge (S)				
S0	3.72±0.24	144.07±9.37 b	182.67±7.14 b	197.56±6.58 b
S1	3.58±0.27	153.41±7.93 b	190.70±8.90 b	215.65±7.03 b
S2	3.64±0.27	199.68±10.30 a	258.70±9.63 a	280.15±9.25 a
S3	4.15±0.25	203.45±1.36 a	240.52±2.81 a	264.21±2.42 a
Interactions (B×S)				
B0S0	4.99±0.33	124.08±5.60	185.64±3.32	192.78±2.96
B0S1	3.47±0.26	160.43±1.16	188.60±5.76	207.05±3.97
B0S2	3.42±0.42	185.97±6.80	232.60±8.75	250.84±7.40
B0S3	4.71±0.42	206.76±5.49	254.23±10.27	268.61±8.85
B1S0	2.80±0.18	155.79±1.12	183.96±5.20	202.88±2.88
B1S1	3.74±0.29	174.82±9.01	227.91±6.38	236.60±6.32
B1S2	3.28±0.40	241.17±5.25	283.60±7.76	296.08±8.05
B1S3	2.67±0.22	206.20±7.23	244.85±6.26	272.52±4.34
B2S0	3.19±0.18	190.90±7.77	215.36±7.67	229.11±6.75
B2S1	4.85±0.38	106.74±13.42	143.16±13.02	179.61±10.36
B2S2	5.20±0.24	148.40±11.59	219.65±5.26	248.72±6.94
B2S3	4.82±0.45	205.53±10.50	229.21±16.57	265.34±9.58
B3S0	3.91±0.30	105.53±3.94	145.72±1.96	165.45±1.11
B3S1	2.25±0.09	171.63±4.90	203.14±7.07	239.34±12.07
B3S2	2.66±0.14	223.17±7.84	298.95±1.51	324.94±3.03
B3S3	4.37±0.07	195.31±6.77	233.79±8.40	250.38±8.16
CV (%)	56.09	29.68	26.31	20.78

Note: values followed by the different letter in the column indicated significantly by DMRT at *P*<0.05±SE. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

A significant increase in total fresh weight of upland rice along with the increase in the dosage of biogas sludge to 315 mL polybag^{-1} at 16 WAIBSA with the highest increase by 41.81% compared to the control. Although the effect was insignificant, it was seen that the B1 and the interaction of B3S2 showed the highest increase in the total fresh weight of upland rice were 9.66% and 68.55%, respectively compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4-16 WAIBSA (Tab. 4).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight of upland rice at 4, 8, 12, and 16 WAIBSA.

Treatments	**Total dry weight ± SE (g)**			
	4 WAIBSA	**8 WAIBSA**	**12 WAIBSA**	**16 WAIBSA**
Superior bacterial isolates (B)				
B0	1.38±0.06	48.01±1.29	73.60±3.99	82.52±4.18
B1	1.13±0.05	54.09±2.41	76.83±2.66	99.72±4.15
B2	1.49±0.06	47.30±3.30	73.20±2.28	98.25±3.90
B3	1.15±0.07	52.32±3.39	77.18±4.90	98.47±4.56
Biogas sludge (S)				
S0	1.26±0.06	45.51±2.63	62.88±2.19 b	76.78±1.63 c
S1	1.23±0.08	44.47±1.71	68.52±2.00 ab	87.65±2.84 bc
S2	1.26±0.08	55.36±3.43	85.69±1.08 a	98.95±1.86 b
S3	1.40±0.06	56.38±1.05	83.73±3.44 a	115.59±2.11 a
Interactions (B×S)				
B0S0	1.58±0.08	41.73±2.78	58.08±1.54	67.23±0.96
B0S1	1.12±0.08	45.87±0.83	62.74±1.83	71.08±1.91
B0S2	1.20±0.12	52.25±2.07	81.39±5.48	88.28±5.02
B0S3	1.60±0.12	52.18±0.29	92.20±3.05	103.49±2.43
B1S0	0.97±0.04	46.64±1.39	69.53±4.90	80.30±4.51
B1S1	1.40±0.07	48.13±2.78	78.91±0.53	96.23±1.50
B1S2	1.12±0.10	67.79±1.44	91.05±2.25	101.80±2.40
B1S3	1.02±0.08	53.81±3.76	67.84±1.77	120.54±2.15
B2S0	1.17±0.05	59.32±2.33	70.92±4.20	81.43±3.82
B2S1	1.54±0.10	34.47±2.16	61.69±1.97	89.84±1.41

B2S2	1.73±0.05	37.37±3.74	83.10±1.19	105.46±1.37
B2S3	1.53±0.10	58.05±1.76	77.07±4.27	116.28±1.30
B3S0	1.30±0.07	34.35±7.04	52.98±0.73	78.16±0.48
B3S1	0.85±0.03	49.40±0.08	70.72±1.29	93.44±2.19
B3S2	0.99±0.05	64.05±4.68	87.22±2.90	100.26±1.93
B3S3	1.44±0.02	61.48±2.47	97.80±0.77	122.04±0.20
CV (%)	43.80	31.22	26.54	18.38

Note: values followed by the different letter in the column indicated significantly by DMRT at *P*<0.05±SE. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

A significant increase in total dry weight of upland rice along with the increase in the dosage of biogas sludge to 630 mL polybag^{-1} at 16 WAIBSA with the highest increase of 50.55% compared to the control. Although the effect was insignificant, it was seen that the B1 and the interaction of B3S3 showed the highest increase in the total dry weight of upland rice were 20.84% and 81.53%, respectively compared to the control.

Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12 to 16 WAIBSA, but it had an insignificant effect at 4-8 and 8-12 WAIBSA (Tab. 5).

TABLE 5. The effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of upland rice.

Superior bacterial isolates (B)	**Biogas sludge (S)**				**Average**
	S0	**S1**	**S2**	**S3**	
4-8 WAIBSA					
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV= 32.28%
8-12 WAIBSA					
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888

Average	0.620	0.859	1.083	0.977	CV= 56.17%
			12-16 WAIBSA		
B0	0.327 fgh	0.298 gh	0.246 h	0.403b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV= 51.07%

Note: values followed by the different letter in the column indicated significantly by DMRT at $P<0.05$. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

The biogas sludge dose of 630 mL polybag^{-1} (S3) significantly increased the highest crop growth rate for upland rice at 12 to 16 WAIBSA by 129.44% compared to the control. The ability of B1-B3 isolates significantly increased the crop growth rate of upland rice with the highest increase in the B2 by 181.45% compared to the controls at 12 to 16 WAIBSA. The interaction of the B1S3 significantly increased the crop growth rate of upland rice by 5.76-times greater compared to the control.

Effect of bacterial isolates and biogas sludge on upland rice nutrition

Content of total-N and available-P nutrient of upland rice

The effect of biogas sludge, superior bacterial isolates, and their interactions had an insignificant effect on the nutrient content of total-N and available-P in upland rice (Fig. 2). The biogas sludge doses of 315 and 630 mL polybag^{-1} (S2 and S3) explained that the nutrient content of available-P and total-N in the plant tissue of upland rice were higher by 33.33% and 4.53%, respectively compared to the control. The B2 isolate showed the highest nutrient content of total-N in the plant tissue of upland rice by 1.63% compared to the control, but all isolates (B1-B3) showed a similar level of available-P in the plant tissue of upland rice with the control.

Uptake of total-N and available-P nutrient of upland rice

The effect of biogas sludge significantly increased in the nutrient uptake of total-N and available-P. Superior bacterial isolates significantly increased in the nutrient uptake of total-N. The interaction of biogas sludge with superior bacterial isolates had an insignificant effect on the nutrient uptake of total-N and available-P of upland rice (Fig. 2).

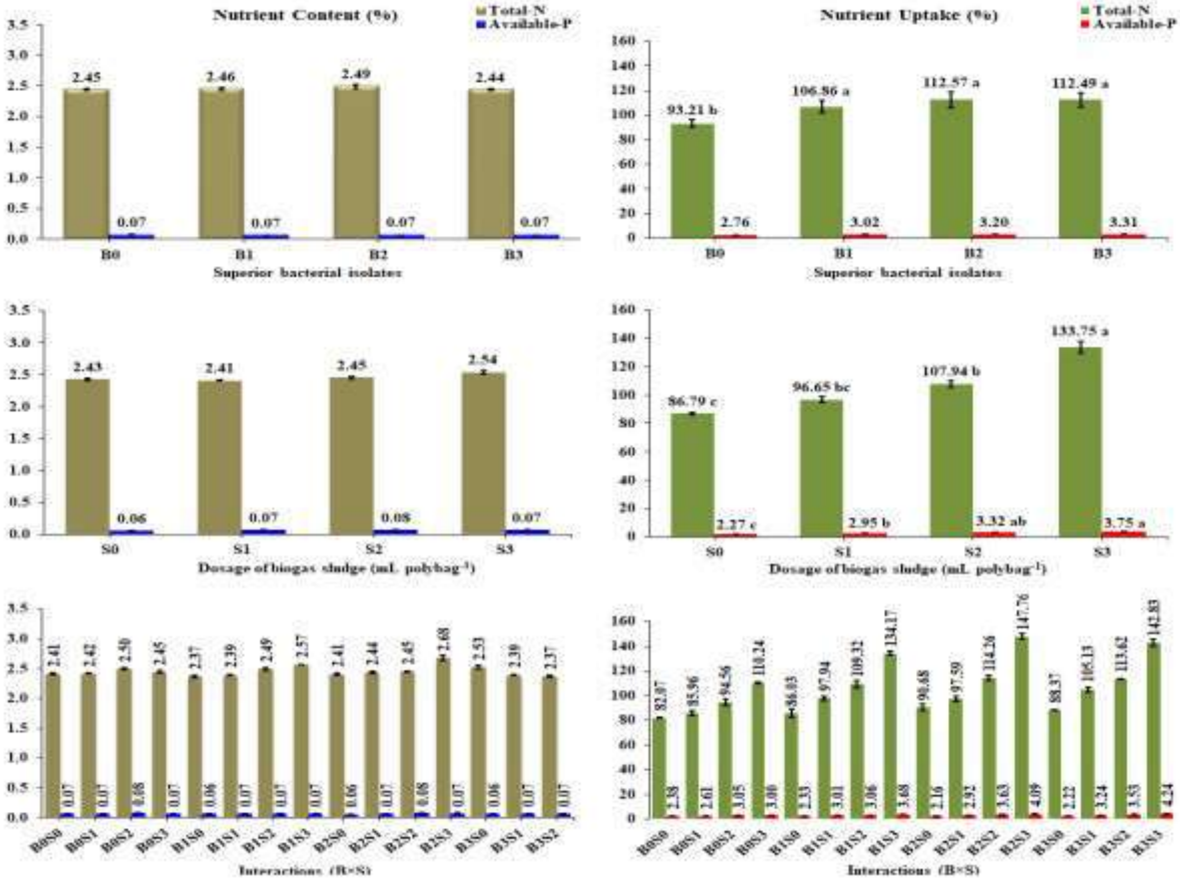

FIGURE 2. The effect of superior bacterial isolates, dosage of biogas sludge, and their interactions in the content and uptake of total-N and available-P nutrient of upland rice. Values followed by the different letter in graph indicated significantly by DMRT at *P*<0.05. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

A significant increase in the nutrient uptake of total-N and available-P in upland rice along with the increase in the dose of biogas sludge to 630 mL polybag^{-1} with the highest increase of 54.11% and 65.20%, respectively compared to the control. The superior bacterial isolates (B1-B3) also significantly increased the nutrient uptake of total-N for upland rice with the highest increase in the B2 by 20.77% compared to the control. Although the effect was insignificant, it was seen that the B3 showed the highest increase in nutrient uptake of available-P in upland rice was 19.93% compared to the control.

Discussion

The effect of selected superior bacterial isolates

The selected superior bacterial isolates (N3 and P7) significantly increased the nutrient uptake of total-N and crop growth rate of upland rice on ultisols at 12 to 16 WAIBSA, but it had an insignificant effect on plant height, total fresh weight, total dry weight, nutrient content (total-N and available-P) in leaf tissue, nutrient uptake of available-P, and crop growth rate of upland rice at 4 to 8 and 8 to 12 WAIBSA. The superior bacterial isolates (N3, P7, and N3+P7) could increase the nutrient uptake of total-N in upland rice by 14.64%, 20.77%, and 20.68%, respectively compared to control (Fig. 2). Similar results are also shown in Table 5, that the crop growth rate of upland rice at 12 to 16 WAIBSA due to selected superior bacterial isolates (N3, P7, N3+P7) has increased by 2.57; 2.81; and 2.39-times, respectively compared to the control. The finding results indicated that the ability of a single isolate by P7 bacteria was greater in increasing total-N and crop growth rate of upland rice compared to a single isolate by N3 bacteria and a combination of N3+P7 bacterial isolates. It was due to the presence of several organic acids and hormones produced by P7 that can increase the nutrient uptake of total-N and crop growth rate of upland rice. This result was supported by Mustamu *et al.* (2021) that the phosphate solubilizing bacteria isolate (P7) from the biogas sludge contains organic acids produced such as lactic, oxalic, acetic, citric acids, and it had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate was 4.62 and 2.66-times, respectively compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus slightly increased in the application of bio fertilization with *Bacillus cereus*, it was due to the production of organic acids and other chemicals such as citric, tartaric, and oxalic acids which can stimulate plant growth and nutrients availability. Youssef and Eissa (2017) reported that the increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that the hormone gibberellin, IAA, and other hormones can increase plant growth under stressful conditions. Kang *et al.* (2014) said that PGPB has several mechanisms to increase plant growth with nitrogen-fixation, phosphate solubilizing, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from anaerobic digester include: *Ruminococcus sp., Thiomargarita sp., Clostridium sp. Anaerobacter sp., Bacillus sp., Sporobacterium sp., Saccharofermentans sp., Oscillibacter sp., Sporobacter sp.,* and *Enterobacter sp.* Liaquat *et al.* (2017) also reported that an abundance of *Bacillus, Clostridium,* and *Enterobacter spp* in anaerobic digester of wastewater in producing biogas.

The effect of biogas sludge

The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and 16 WAIBSA), total dry weight (12 and 16 WAIBSA), nutrient uptake (total-N and available-P), and the crop growth rate of upland rice at 8 to 12 WAIBSA, but it had an insignificant effect on nutrient content (total-N and available-P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAIBSA). An increase in plant height, total dry weight, nutrient uptake of total-N and available-P, and also crop growth rate of upland rice on ultisols along with the increase in the dose of biogas sludge to 630 mL polybag^{-1} at the end of this study (16 WAIBSA). However, in contrast to the total fresh weight, an increase along with the increase in the biogas sludge dose to 315 mL polybag^{-1} then decreased at the dose of 630 mL polybag^{-1}. It was caused the biogas sludge had chemical characteristics such as pH (7.41), total-N (0.051%), available-

P (0.013%), organic-C (0.14%), total-K (0.18%), and biological characteristics such as total nitrogen-fixing bacteria (29.4×10^5 CFU mL^{-1}) and total phosphate solubilizing bacteria (7.0×10^4 CFU mL^{-1}) (Table 2). C-organic content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could be increased the nutrient uptake of total-N and available-P in upland rice along with increasing the dose of biogas sludge to 630 mL polybag^{-1} (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the growth of plant height, biomass, and crop growth rate of upland rice. A similar result was reported by Mustamu and Triyanto (2020) that the macro and micronutrients from the biogas sludge and also had the population of nitrogen-fixing and phosphate solubilizing bacteria by 480×10^4 and 42×10^4 CFU mL^{-1}, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that total phosphate solubilizing bacteria (1.6 to 2.5 CFU mL^{-1}) was significantly higher compared to nitrogen-fixing bacteria (0.5–1.4 CFU mL^{-1}) and a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported that an increase in concentrations of NH_4^+-N ranged from 45 to 80% after anaerobic waste.

The interaction effect of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice on ultisols at 12-16 WAIBSA, but it had an insignificant effect on other parameters in this study. The interaction of NFB *Bacillus paramycoides* with biogas sludge at the dose of 630 mL polybag^{-1} (B1S3) showed the highest crop growth rate of upland rice compared to other interactions and 5.76-times greater compared to the control. It was caused by the application of biogas sludge could be increased soil organic matter and the total population of beneficial bacteria. Likewise, the characteristics of the biogas sludge had the organic-C was 0.14%, total nitrogen-fixing bacteria was 29.4×10^5 CFU mL^{-1}, and total phosphate solubilizing bacteria was 7.0×10^4 CFU mL^{-1} (Tab. 2) could improve soil quality and support the crop growth rate. This result is supported by Urra *et al.* (2019) that the application of sewage sludge in the long-term significantly increases the organic matter in the soil, which causes a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of organic matter. Bhardwaj *et al.* (2014); Carvajal-Muñoz *et al.* (2012) reported that the application of biofertilizer had advantages in the plant such as availability of nutrients that are balanced for plant health, stimulating nutrient mobilization that can increase soil biological activity, availability of microbial food to encourage the growth of beneficial microorganisms, increasing the soil organic matter content thereby increasing the cation exchange capacity. Siswanti and Lestari (2019) reported that the interaction of biogas sludge+biofertilizer (36 mL+10 L ha^{-1}) significantly increased the plant height, number of leaves, and capcaisin content in chili compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

The isolates of N3, P7, N3+P7 from the biogas sludge significantly increased the nutrient uptake of total-N and crop growth rate of upland rice on ultisols with the highest increase found in the P7 isolate of 20.77% and 2.81-times, respectively. The dose of biogas sludge significantly increased plant height, total dry weight, nutrient uptake of total-N and available-P, and also crop growth rate of upland rice on ultisols with the highest increase at a dose of 630 mL $polybag^{-1}$ by 14.81%; 50.55%; 54.11%; 65.20%; and 129.44%, respectively. Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice with the highest increase at the dose of 315 mL $polybag^{-1}$ by 41.81%. The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice on ultisols with the highest increase found in the B1S3 by 5.76-times.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

All authors formulated the overarching research goals and aims, provided the study materials, developed or designed the methodology. NEM analyze and interpretation study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in field, data collection. ZN and I verified the overall reproducibility of results and other research outputs. All authors conducted the critical review/commentary/revision of the manuscript.

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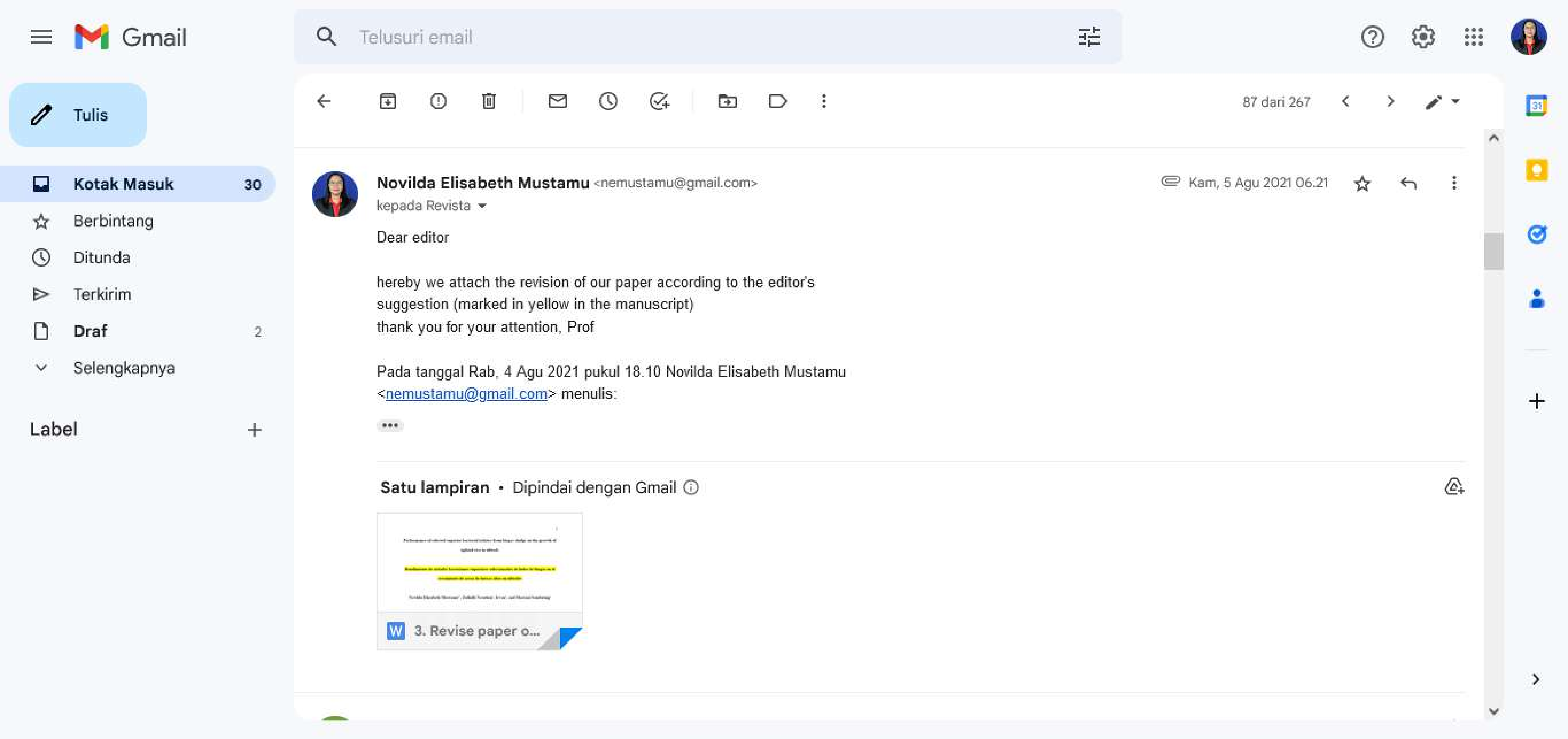

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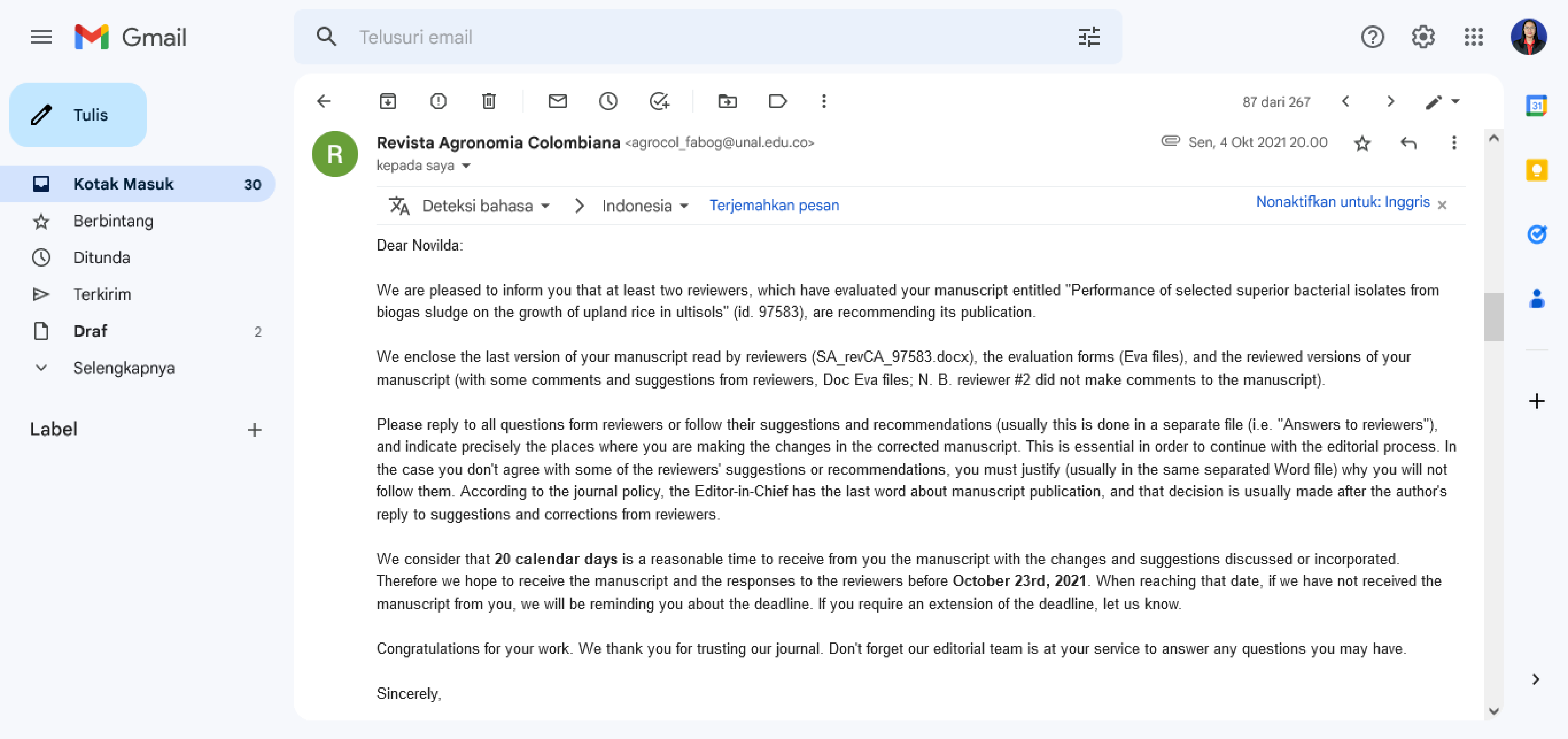

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Dear Novilda:

We are pleased to inform you that at least two reviewers, which have evaluated your manuscript entitled "Performance of selected superior bacterial isolates from biogas sludge on the growth of upland rice in ultisols" (id. 97583), are recommending its publication.

We enclose the last version of your manuscript read by reviewers (SA_revCA_97583.docx), the evaluation forms (Eva files), and the reviewed versions of your manuscript (with some comments and suggestions from reviewers, Doc Eva files; N. B. reviewer #2 did not make comments to the manuscript).

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Sincerely,

Performance of selected superior bacterial isolates from biogas sludge on the growth of upland rice in ultisols

Rendimiento de aislados bacterianos superiores seleccionados de lodos de biogás en el crecimiento de arroz de tierras altas en ultisoles

ABSTRACT

The study searched to obtain the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth, biomass, and nutrient uptake in upland rice in ultisols. This study was conducted from October 2020 to April 2021in aRandomized Block Design within two factors and seven replicates. The first factor was selected SBI (B0= untreated, B1= nitrogen-fixing bacteria isolate (N3), B2= phosphate solubilizing bacteria isolate (P7), B3= isolates combination (N3+P7)). The second factor was dosage of biogas sludge (S0= untreated, S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). The parameters were determined by ANOVA and followed by DMRT at $P < 0.05$. The results showed that the P7 isolate significantly increased total-N uptake and the highest crop growth rate (CGR) of upland rice by 20.77% and 2.81-folds, respectively. Biogas sludge dosage from 315 to 630 ml polybag^{-1} significantly increased plant height, uptake of total-N and available-P, total fresh and dry weight, and CGR of upland rice. The interaction of N3 with biogas sludge dosage of 630 ml polybag^{-1} significantly increased the CGR of upland rice. The application of N3 and P7 isolates and their combination within biogas sludge of 630 mL polybag^{-1} has the potential to archive the CGR of upland rice in acidic soils.

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Keywords: acidic soil, crop growth rate, dosage, sludge potential, upland rice.

RESUMEN

El estudio se realizó para obtener la influencia de aislados bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones en el crecimiento, la biomasa y la absorción de nutrientes en el arroz de tierras altas en ultisoles. Este estudio utilizó un Diseño de Bloques Aleatorizados dentro de dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0= sin tratar, B1 = aislado de bacterias fijadoras de nitrógeno (N3), B2= aislado de bacterias solubilizantes de fosfato (P7), B3= combinación de aislados (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (S0= sin tratar, S1= 157.5; S2= 315; S3= 630 mL de polybag^{-1}). Los parámetros fueron determinados por ANOVA y seguidos por DMRT en $P < 0.05$. Los resultados mostraron que el aislado P7 aumentó significativamente la captación total de N y la mayor tasa de crecimiento del cultivo (TCC) de arroz de tierras altas en 20.77% y 2.81-pliegues, respectivamente. La dosificación de fangos de biogás de 315 a 630 mL de polybag^{-1} aumentó significativamente la altura de la planta, la absorción de N total y P disponible, el peso fresco y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosificación de lodo de biogás de 630 mL de polybag^{-1} aumentó significativamente el TCC del arroz de tierras altas. La aplicación de aislamientos de N3 y P7 y su combinación dentro de lodos de biogás de 630 mL de polybag^{-1} tiene el potencial de archivar el TCC de arroz de tierras altas en suelos ácidos.

Palabras clave: arroz de tierras altas, dosis, potencial de lodo, suelo ácido, tasa de crecimiento de los cultivos.

Introduction

Biogas sludge is the waste by-product installation from an anaerobic processing system (Food and Agriculture Organization, 1997) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and plant yield (Adela *et al.*, 2014). The biogas sludge from palm oil waste contained total-N of 490 mg L^{-1}; NH_3-N was 65 mg L^{-1}; total-P by 110 mg L^{-1}; K of 1.9 mg L^{-1}; Ca by 23 mg L^{-1}; Mg of 256 mg L^{-1}; As <0.01 mg L^{-1}; Zn was 0.61 mg L^{-1}; and Cr by 0.04 mg L^{-1} (Lubis *et al.*, 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al.*, 2019), NH_3-N ranged by 91 to 112 mg L^{-1} (Choorit & Wisarnwan, 2007), pH ranged by 6.8 to 8.3; and the highest bacterial populations was 7.21×10^{7} cells per ml and the lowest one was 3.15×10^{7} cells per ml (Alvionita *et al.*, 2019). Mustamu and Triyanto (2020) also reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria which have the potential to increase availability of nitrogen and phosphate in soils.

The diversity of beneficial bacteria, such as nitrogen-fixing and phosphate solubilizing, has greater potential forincreasing soil fertility and plant growth. Zhang *et al.* (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility, plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described that the *Bacillus* genera such as *B. circulans, B. cereus, B. fusiformis, B. pumilus, B. megaterium, B. mycoides, B. coagulans, B. chitinolyticus,* and *B. subtilis* have been reported as having phosphate solubilizing activities. Ambrosini *et al.* (2016) reported that *B. cereus* showed the highest nitrogenase activity among 42 different strains of *Bacillus spp*. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, i.e. *Proteobacteria, Firmicutes, Bacteroidetes*, and *Thermotogae.*

Application of bacteriai isolates from biogas sludge for the improvement of upland rice growth on acidic soils has never been reported in Indonesia Thus, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge in increasing the availability of nitrogen, phosphate, and the growth responses of upland rice due to the biogas sludge and selected isolates in ultisols. The study was aimed at obtaining the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the growth of upland rice in ultisols.

Materials and methods

Study area

The concentration of total-N and available-P in ultisols and the plant tissue were analyzed in the Analytical Laboratory of PT. Socfin Indonesia, Medan. The bacterial isolates were applied to upland rice in Padang Bulan Village, Medan Selayang Subdistrict, Medan City, North Sumatra, Indonesia from October 2020 to April 2021.

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Preparation of medium and upland rice seeds

The planting medium used a soil type of ultisols from the Simalingkar area, Medan Tuntungan Subdistrict, Medan City with a soil depth of 0 to 20 cm. One hundreed grams of soil samples were taken and analyzed for chemical characteristics such as pH, organic-C, available-P, total-N, CEC, and base saturation (K, Ca, Na, Mg) (Tab. 1). The soil was sterilized by dryingat 100^{0}C for 2 hours. After being incubated for 1 day , the soil was put into a polybag of 10 kg. A basic fertilizer of NPK Mutiara at a dose of 300 kg ha^{-1} was given by stirring evenly with the soil. Concurrently, the seeds of upland rice were inbred varieties of Inpago-8 then soaked in water for 24 h and followed by a propineb fungicide (70%) for 2 h . Upland rice was planted after 1 d of basic fertilization with two seeds per polybag at a depth of 2 cm.

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TABLE 1. Chemical characteristics of sterile ultisols

Chemical characteristics	**Value**	**Category***
Soil pH (H_2O)	4.80	Acid
Organic-C (%)	0.44	Very low
Total-N (%)	0.04	Very low
Available-P (mg kg^{-1})	870.25	Very high
CEC (me/100 g)	28.31	High
Base saturation (%)	4.85	Very low
Exchangeable cations		
K (me/100 g)	0.60	High
Ca (me/100 g)	0.34	Very low
Mg (me/100 g)	0.32	Very low
Na (me/100 g)	0.09	Very low
Al (%)	0.02	Very low

Source: *Criteria for pH H_2O= 4.5-5.5 (acid); organik-C <1% (very low); total-N <0,1% (very low); available-P >60 mg kg^{-1} (very high); CEC= 25-40 me/100 g (high); base saturation <20% (very low); exchangeable-K= 0.60-1.00 me/100 g (high); exchangeable-Ca <2 me/100 g (very low); exchangeable-Mg <0.4 me/100 g (very low); exchangeable-Na <0.1 me/100 g (very low); exchangeable-Al <5% (very low) (Soil Research Institute, 2009).

Preparation of superior bacterial isolates suspension and biogas sludge

The selected superior bacterial isolates used phosphate solubilizing bacteria (P7) which has been confirmed by Mustamu *et al.* (2021) and nitrogen-fixing bacteria or N3 (data unpublished). The isolates were grown on an NB medium, and incubated for 48 h. The microbial mass in the solution was measured using a spectrophotometer with a density of 10^8 cells per mL. 10~~Ten~~ m~~m~~L~~l~~ was taken from the solution containing nitrogen-fixing bacteria (N3) and phosphate solubilizing bacteria (P7).

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The biogas sludge was taken from an identical location in the first phase of the study (palm oil mill of PT. Nubika Jaya, Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia). Bacterial isolate and biogas sludge were applied to the soil surface of the plants one week after planting (WAP). Biogas sludge of 500 mL~~l~~ samples were taken and then the chemical and biological characteristics were analyzed in the laboratory (Tab. 2).

TABLE 2. Chemical and biological characteristics of biogas sludge

Characteristics of biogas sludge	Value
Chemical	
pH	7.41
Chemical oxygen demand/COD (mg L^{-1})	4547.8
Biological oxygen demand/BOD (mg L^{-1})	1127.5
Total-N (%)	0.051
Total-P (%)	0.0097
Available-P (%)	0.013
Total-K (%)	0.18
Organic-C (%)	0.14
Ca (%)	0.04
Mg (%)	0.04
Na (ppm)	44.41
Cu (%)	0.0001
Biological	

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Total nitrogen-fixing bacteria (CFU mL^{-1})	29.4×10^5
Total phosphate solubilizing bacteria (CFU mL^{-1})	7.0×10^4

Treatments application

This study used a Randomized Block Design within two factors and seven replicates. The first factor was the type of superior bacterial isolates at the similarly likewise dose, namely 10 mL $polybag^{-1}$ (B0= un-treated; B1= nitrogen-fixing bacterial isolate (N3); B2= phosphate solubilizing bacteria isolate (P7); B3= combination isolates N3+P7). The second factor was dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL $polybag^{-1}$). Each replicate was dismantled at 4, 8, and 12 Weeks After Isolate and Biogas Sludge Application (WAIBSA) for determination of the CGR.

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Parameters and data analysis

Variable observations were conducted by measuring the growth of upland rice (plant height, total fresh and dry weight), total-N and available-P content in the shoots, total uptake-N, and available-P. CGR was determined on plants at 4-8, 8-12, and 12-16 WAIBSA. Each polybag from each treatment and replicate was dismantled at the plants at 4, 8, 12, and 16 WAIBSA, then the plant height was measured, the total fresh weight was determined by weighing the roots and shoots. The total dry weight was measured by oven at 60^0C for 48 h and weighed with analytical scales. A 200 g sample of the 2nd leaf from the shoots was taken by 200 g and analyzed for total-N using the Kjeldahl method and available-P by the dry ashing method with a UV-Vıs Spectrophotometer. The total-N and available-P absorption were measured using equation (1). The CGR was calculated by the dry weight per unit area using equation (2) (Shon *et al.*, 1997):

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$$\text{Uptake nutrient= nutrient content in the shoots} \times \text{total dry weight} \qquad (1)$$

$$\text{CGR}= \frac{\Delta W}{\Delta t} = \frac{W2-W1}{t2-t1} \qquad (2)$$

where:

CGR = crop growth rate

W1 = dry weight per unit area at t1

W2 = dry weight per unit area at t2

t1 = first sampling

t2 = second sampling

The parameters of the second phase of the study were analyzed by ANOVA and if the treatment had a significant effect, it was continued by DMRT at $P<0.05$ with SPSS v.20 software.

Results and discussion

Plant height of upland rice

The effect of biogas sludge application on the plant height of upland rice was significant at 8, 12, and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect on the plant height of upland rice at 4, 8, 12, and 16 WAIBSA (Fig. 1). A significant increase in plant height of upland rice along with increased doses of biogas sludge to 630 mL~~l~~ polybag^{-1} at 8, 12, and 16 WAIBSA with the highest increase of 14.81% compared to the control at 16 WAIBSA. Although the effect was insignificant, the isolates combination of B3 and the interaction of B2S3 showed the highest increase plant height of upland rice by 2.94% and 22.06%, respectively, compared to the control.

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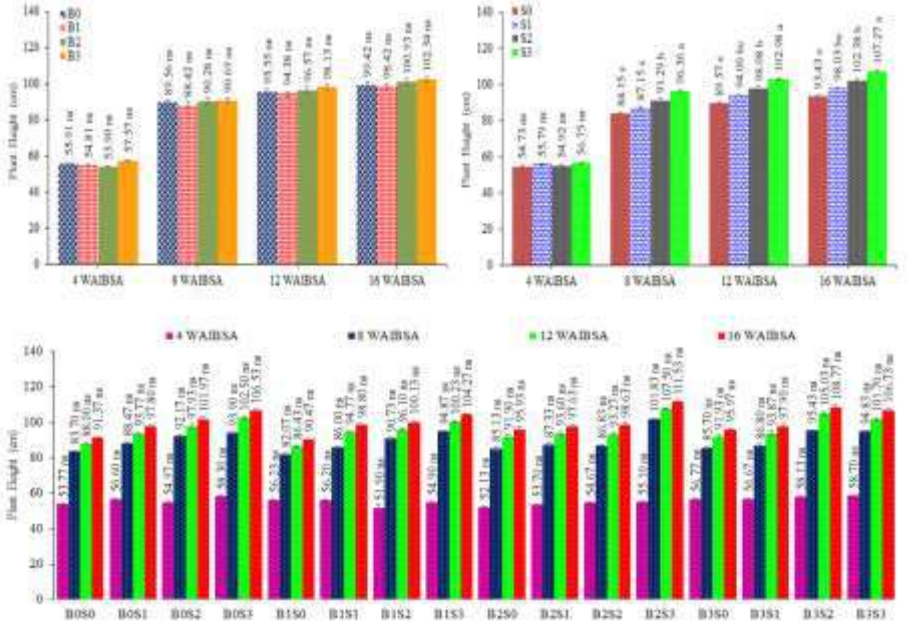

FIGURE 1. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on plant height of upland rice at 4, 8, 12, and 16 WAIBSA. Values followed by the different letter in the graph indicated significantly by DMRT at *P*<0.05. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

Biomass of upland rice (g)

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect on the total fresh weight of upland rice at 4-16 WAIBSA (Tab. 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on total fresh weight of upland rice at 4, 8, 12, and 16 WAIBSA.

Treatments	**Total fresh weight ± SE (g)**			
	4 WAIBSA	**8 WAIBSA**	**12 WAIBSA**	**16 WAIBSA**
Superior bacterial isolates (B)				
B0	4.15±0.21ns	169.31±8.90ns	215.27±8.42ns	229.82±8.94ns
B1	3.12±0.12ns	194.50±9.35ns	235.08±10.32 ns	252.02±10.22ns
B2	4.52±0.23ns	162.89±11.15ns	201.85±9.89ns	230.70±9.28ns

B3	3.30±0.25ns	173.91±12.55ns	220.40±15.96ns	245.03±16.32ns
Biogas sludge (S)				
S0	3.72±0.24ns	144.07±9.37b	182.67±7.14b	197.56±6.58b
S1	3.58±0.27ns	153.41±7.93b	190.70±8.90b	215.65±7.03b
S2	3.64±0.27ns	199.68±10.30a	258.70±9.63a	280.15±9.25a
S3	4.15±0.25ns	203.45±1.36a	240.52±2.81a	264.21±2.42a
Interactions (B×S)				
B0S0	4.99±0.33ns	124.08±5.60ns	185.64±3.32ns	192.78±2.96ns
B0S1	3.47±0.26ns	160.43±1.16ns	188.60±5.76ns	207.05±3.97ns
B0S2	3.42±0.42ns	185.97±6.80ns	232.60±8.75ns	250.84±7.40ns
B0S3	4.71±0.42ns	206.76±5.49ns	254.23±10.27ns	268.61±8.85ns
B1S0	2.80±0.18ns	155.79±1.12ns	183.96±5.20ns	202.88±2.88ns
B1S1	3.74±0.29ns	174.82±9.01ns	227.91±6.38ns	236.60±6.32ns
B1S2	3.28±0.40ns	241.17±5.25ns	283.60±7.76ns	296.08±8.05ns
B1S3	2.67±0.22ns	206.20±7.23ns	244.85±6.26ns	272.52±4.34ns
B2S0	3.19±0.18ns	190.90±7.77ns	215.36±7.67ns	229.11±6.75ns
B2S1	4.85±0.38ns	106.74±13.42ns	143.16±13.02ns	179.61±10.36ns
B2S2	5.20±0.24ns	148.40±11.59ns	219.65±5.26ns	248.72±6.94ns
B2S3	4.82±0.45ns	205.53±10.50ns	229.21±16.57ns	265.34±9.58ns
B3S0	3.91±0.30ns	105.53±3.94ns	145.72±1.96ns	165.45±1.11ns
B3S1	2.25±0.09ns	171.63±4.90ns	203.14±7.07ns	239.34±12.07ns
B3S2	2.66±0.14ns	223.17±7.84ns	298.95±1.51ns	324.94±3.03ns
B3S3	4.37±0.07ns	195.31±6.77ns	233.79±8.40ns	250.38±8.16ns
CV (%)	56.09	29.68	26.31	20.78

Note: values followed by the different letter in the column indicated significantly by DMRT at *P*<0.05±SE. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

Commented [MOU14]: This is not an interaction assessment, it is the treatments. In the ANOVA of this trial there are 16 treatments and 112 experimental units. The sources of variation evaluated would be (treatments, bacteria, sludge, and bacteria x sludge interaction). And there may be a significant difference in treatments without any interaction between the factors.

A significant increase in total fresh weight of upland rice along with the increase in the dosage of biogas sludge to 315 mL~~l~~ polybag^{-1} at 16 WAIBSA with the highest increase by 41.81% compared to the control. Although the effect was insignificant, the B1 and the interaction of B3S2 showed the highest increase in the total fresh weight of upland rice with 9.66% and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4-16 WAIBSA (Tab. 4).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight of upland rice at 4, 8, 12, and 16 WAIBSA.

Treatments	Total dry weight ± SE (g)			
	4 WAIBSA	**8 WAIBSA**	**12 WAIBSA**	**16 WAIBSA**
Superior bacterial isolates (B)				
B0	1.38±0.06ns	48.01±1.29ns	73.60±3.99ns	82.52±4.18ns
B1	1.13±0.05ns	54.09±2.41ns	76.83±2.66ns	99.72±4.15ns
B2	1.49±0.06ns	47.30±3.30ns	73.20±2.28ns	98.25±3.90ns
B3	1.15±0.07ns	52.32±3.39ns	77.18±4.90ns	98.47±4.56ns
Biogas sludge (S)				
S0	1.26±0.06ns	45.51±2.63ns	62.88±2.19b	76.78±1.63c
S1	1.23±0.08ns	44.47±1.71ns	68.52±2.00ab	87.65±2.84bc
S2	1.26±0.08ns	55.36±3.43ns	85.69±1.08a	98.95±1.86b
S3	1.40±0.06ns	56.38±1.05ns	83.73±3.44a	115.59±2.11a
Interactions (B×S)				
B0S0	1.58±0.08ns	41.73±2.78ns	58.08±1.54ns	67.23±0.96ns
B0S1	1.12±0.08ns	45.87±0.83ns	62.74±1.83ns	71.08±1.91ns
B0S2	1.20±0.12ns	52.25±2.07ns	81.39±5.48ns	88.28±5.02ns
B0S3	1.60±0.12ns	52.18±0.29ns	92.20±3.05ns	103.49±2.43ns
B1S0	0.97±0.04ns	46.64±1.39ns	69.53±4.90ns	80.30±4.51ns
B1S1	1.40±0.07ns	48.13±2.78ns	78.91±0.53ns	96.23±1.50ns
B1S2	1.12±0.10ns	67.79±1.44ns	91.05±2.25ns	101.80±2.40ns
B1S3	1.02±0.08ns	53.81±3.76ns	67.84±1.77ns	120.54±2.15ns
B2S0	1.17±0.05ns	59.32±2.33ns	70.92±4.20ns	81.43±3.82ns
B2S1	1.54±0.10ns	34.47±2.16ns	61.69±1.97ns	89.84±1.41ns
B2S2	1.73±0.05ns	37.37±3.74ns	83.10±1.19ns	105.46±1.37ns
B2S3	1.53±0.10ns	58.05±1.76ns	77.07±4.27ns	116.28±1.30ns
B3S0	1.30±0.07ns	34.35±7.04ns	52.98±0.73ns	78.16±0.48ns
B3S1	0.85±0.03ns	49.40±0.08ns	70.72±1.29ns	93.44±2.19ns
B3S2	0.99±0.05ns	64.05±4.68ns	87.22±2.90ns	100.26±1.93ns
B3S3	1.44±0.02ns	61.48±2.47ns	97.80±0.77ns	122.04±0.20ns
CV (%)	43.80	31.22	26.54	18.38

Commented [MOU15]: Idem table 3

Note: values followed by the different letter in the column indicated significantly by DMRT at *P*<0.05±SE. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

A significant increase in total dry weight of upland rice along with the increase in the dosage of biogas sludge to 630 mL~~l~~ polybag^{-1} at 16 WAIBSA with the highest increase of 50.55% compared to the control. Although the effect was insignificant, the B1 and the interaction of B3S3 showed the highest increase in the total dry weight of upland rice, with20.84% and 81.53%, respectively, compared to the control.

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Content and uptake of total-N and available-P nutrient of upland ri~~ce~~

The effect of biogas sludge, superior bacterial isolates, and their interactions had an insignificant effect on the nutrient content of total-N and available-P in upland rice. The effect of biogas sludge significantly increased the nutrient uptake of total-N and available-P. Superior bacterial isolates significantly increased the nutrient uptake of total-N. The interaction of biogas sludge with superior bacterial isolates had an insignificant effect on the nutrient uptake of total-N and available-P of upland rice (Fig. 2).

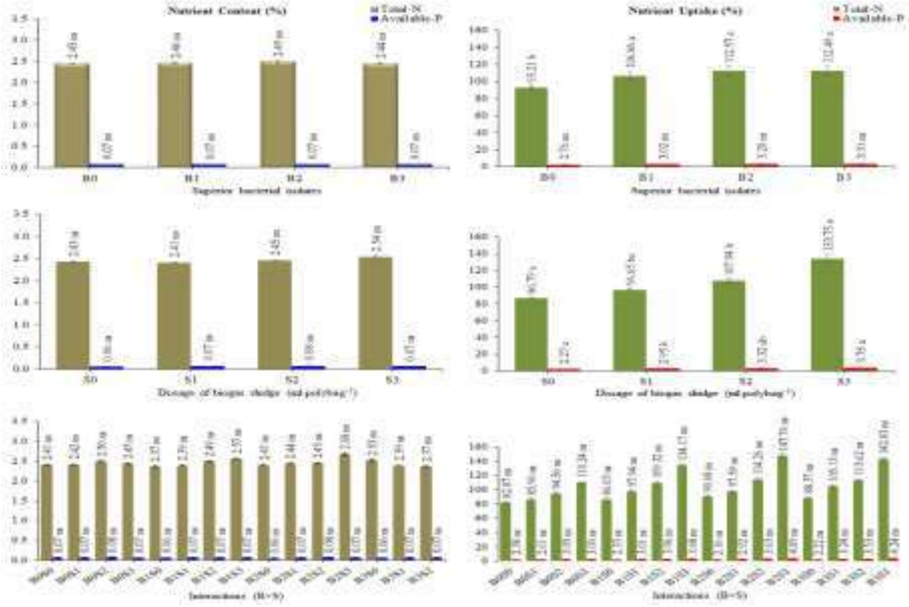

FIGURE 2. The effect of superior bacterial isolates, dosage of biogas sludge, and their interactions in the content and uptake of total-N and available-P nutrient of upland rice. Values followed by the different letter in graph indicated significantly by DMRT at *P*<0.05. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

A significant increase in the nutrient uptake of total-N and available-P in upland rice along with the increase in the dose of biogas sludge to 630 mL polybag^{-1} with the highest increase of 54.11% and 65.20%, respectively compared to the control. The superior bacterial isolates (B1-B3) also significantly increased the nutrient uptake of total-N for upland rice with the highest increase in the B2 by 20.77% compared to the control. Although the effect was insignificant, the B3 showed the highest increase in nutrient uptake of available-P in upland rice, with19.93% compared to the control. Likewise, the interaction of B2S3 and B3S3 showed the highest increase in nutrient uptake of total-N and available-P in upland rice by 80.04% and 79.41%, respectively, compared to the control.

Commented [MOU17]: there is no interaction, should not be analyzed, the results are a result of chance

The biogas sludge doses of 315 and 630 mL~~l~~ polybag^{-1} (S2 and S3) explained that the nutrient content of available-P and total-N in the plant tissue of upland rice were higher by 33.33% and 4.53%, respectively, compared to the control. The B2 isolate showed the highest nutrient content of total-N in the plant tissue of upland rice by 1.63% compared to the control, but all isolates (B1-B3) showed a similar level of available-P in the plant tissue of upland rice with the control. The interactions of B2S3 and B2S2 also showed the highest nutrient content of total-N and available-P compared to other interactions.

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Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12 to 16 WAIBSA, but it had an insignificant effect at 4-8 and 8-12 WAIBSA (Tab. 5).

The biogas sludge dose of 630 mL~~l~~ polybag^{-1} (S3) significantly increased the highest crop growth rate for upland rice at 12 to 16 WAIBSA by 129.44% compared to the control. The ability of B1-B3 isolates significantly increased the crop growth rate of upland rice with the highest increase in the B2 by 181.45% compared to the controls at 12 to 16 WAIBSA. The interaction of the B1S3 significantly increased the crop growth rate of upland rice by 5.76-folds greater compared to the control.

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TABLE 5. The effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of upland rice.

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Superior bacterial	Biogas sludge (S)	Average

isolates (B)	S0	S1	S2	S3	
4-8 WAIBSA					
B0	1.434ns	1.598ns	1.823ns	1.806ns	1.665ns
B1	1.631ns	1.669ns	2.381ns	1.885ns	1.892ns
B2	2.077ns	1.176ns	1.273ns	2.019ns	1.636ns
B3	1.180ns	1.734ns	2.252ns	2.144ns	1.828ns
Average	1.580ns	1.544ns	1.932ns	1.964ns	CV= 32.28%
8-12 WAIBSA					
B0	0.584ns	0.602ns	1.041ns	1.430ns	0.914ns
B1	0.818ns	1.099ns	0.831ns	0.501ns	0.812ns
B2	0.414ns	0.972ns	1.633ns	0.679ns	0.925ns
B3	0.665ns	0.761ns	0.828ns	1.297ns	0.888ns
Average	0.620ns	0.859ns	1.083ns	0.977ns	CV= 56.17%
12-16 WAIBSA					
B0	0.327fgh	0.298gh	0.246h	0.403b-h	0.318b
B1	0.385c-h	0.619a-h	0.384d-h	1.882a	0.817a
B2	0.375e-h	1.005a-h	0.798a-h	1.400a-h	0.895a
B3	0.899a-h	0.811a-h	0.466a-h	0.866a-h	0.761a
Average	0.496b	0.683b	0.474b	1.138a	CV= 51.07%

Note: values followed by the different letter in the column indicated significantly by DMRT at $P<0.05$. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

The effect of selected superior bacterial isolates

Commented [MOU21]: In this analysis, it is necessary to justify the reason why the bacteria achieve a greater absorption of nutrients such as nitrogen and a higher growth rate, but do not achieve a greater height, dry weight and content of N and P

The selected superior bacterial isolates (N3 and P7) significantly increased the nutrient uptake of total-N and crop growth rate of upland rice on ultisols at 12 to 16 WAIBSA, but it had an insignificant effect on plant height, total fresh weight, total dry weight, nutrient content (total-N and available-P) in leaf tissue, nutrient uptake of available-P, and crop growth rate of upland rice at 4 to 8 and 8 to 12 WAIBSA. The superior bacterial isolates (N3, P7, and N3+P7) could increase the nutrient uptake of total-N in upland rice by 14.64%, 20.77%, and 20.68%, respectively compared to control (Fig. 2). Similar results are also shown in Table 5, that the crop growth rate of upland rice at 12 to 16 WAIBSA due to selected superior bacterial isolates (N3, P7, N3+P7) has increased by 2.57; 2.81; and 2.39-folds, respectively compared to the control. The finding results indicated that the ability of a single isolate by P7 bacteria was greater in increasing total-N and crop growth rate of upland rice compared to a single isolate by N3 bacteria and a combination of N3+P7 bacterial isolates. It was due to the presence of several organic acids and hormones produced by P7 that can increase the nutrient uptake of total-N and crop growth rate of upland rice. This result was supported by Mustamu *et al.* (2021) that the phosphate solubilizing bacteria isolate (P7) from the biogas sludge contains organic acids produced such as lactic, oxalic, acetic, citric acids, and it had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate was 4.62 and 2.66-folds, respectively compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus slightly increased in the application of bio fertilization with *Bacillus cereus*, it was due to the production of organic acids and other chemicals such as citric, tartaric, and oxalic acids which can stimulate plant growth and nutrients availability. Youssef and Eissa (2017) reported that the increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the gibberellins, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that gibberellins, IAA, and other hormones can increase plant growth under stressful conditions. Kang *et al.* (2014) said that PGPB has several mechanisms to increase plant growth with nitrogen-fixation, phosphate solubilizing, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from anaerobic digester include: *Ruminococcus sp.*, *Thiomargarita sp.*, *Clostridium sp. Anaerobacter sp.*, *Bacillus sp.*, *Sporobacterium sp.*, *Saccharofermentans sp.*, *Oscillibacter sp.*, *Sporobacter sp.*, and *Enterobacter sp.* Liaquat *et al.* (2017) also reported that an abundance of *Bacillus*, *Clostridium*, and *Enterobacter spp* in anaerobic digester of wastewater in producing biogas.

The effect of biogas sludge

The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and 16 WAIBSA), total dry weight (12 and 16 WAIBSA), nutrient uptake (total-N and available-

P), and the crop growth rate of upland rice at 8 to 12 WAIBSA, but it had an insignificant effect on nutrient content (total-N and available-P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAIBSA). An increase in plant height, total dry weight, nutrient uptake of total-N and available-P, and also crop growth rate of upland rice on ultisols along with the increase in the dose of biogas sludge to 630 mL polybag^{-1} at the end of this study (16 WAIBSA). However, in contrast to the total fresh weight, an increase along with the increase in the biogas sludge dose to 315 mL polybag^{-1} then decreased at the dose of 630 mL polybag^{-1}. It was caused the biogas sludge had chemical characteristics such as pH (7.41), total-N (0.051%), available-P (0.013%), organic-C (0.14%), total-K (0.18%), and biological characteristics such as total nitrogen-fixing bacteria (29.4×10^{5} CFU mL^{-1}) and total phosphate solubilizing bacteria (7.0×10^{4} CFU mL^{-1}) (Tab. 2). C-organic content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could be increased the nutrient uptake of total-N and available-P in upland rice along with increasing the dose of biogas sludge to 630 mL polybag^{-1} (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the growth of plant height, biomass, and crop growth rate of upland rice. A similar result was reported by Mustamu and Triyanto (2020) that the macro and micronutrients from the biogas sludge and also had the population of nitrogen-fixing and phosphate solubilizing bacteria by 480×10^{4} and 42×10^{4} CFU mL^{-1}, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that total phosphate solubilizing bacteria (1.6 to 2.5 CFU mL^{-1}) was significantly higher compared to nitrogen-fixing bacteria (0.5–1.4 CFU mL^{-1}) and a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported that an increase in concentrations of NH_4^+-N ranged from 45 to 80% after anaerobic waste.

The interaction effect of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice on ultisols at 8 to 12 WAIBSA, but it had an insignificant effect on other parameters in this study. The interaction of NFB *Bacillus paramycoides* with biogas sludge at the dose of 630 mL~~l~~ polybag^{-1} (B1S3) showed the highest crop growth rate of upland rice compared to other interactions and 5.76-fold greater compared to the control. It was caused by the application of biogas sludge that could increase the contents of soil organic matter and the total population of beneficial bacteria. Likewise, the characteristics of the biogas sludge had the organic-C was 0.14%, total nitrogen-fixing bacteria was 29.4×10^5 CFU mL^{-1}, and total phosphate solubilizing bacteria was 7.0×10^4 CFU mL^{-1} (Tab. 2) could improve soil quality and support the crop growth rate. This result is supported by Urra *et al.* (2019) that the application of sewage sludge in the long-term significantly increases the organic matter in the soil, which causes a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of organic matter. Bhardwaj *et al.* (2014); Carvajal-Muñoz *et al.* (2012) reported that the application of biofertilizer had advantages for plants, such as availability of nutrients that are balanced for plant health, stimulating nutrient mobilization that can increase soil biological activity, availability of microbial food to encourage the growth of beneficial microorganisms, increasing the soil organic matter content thereby increasing the cation exchange capacity. Siswanti and Lestari (2019) reported that the interaction of biogas sludge+biofertilizer (36 mL+10 L ha^{-1}) significantly increased the plant height, number of leaves, and capcaisin content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

The isolates of N3, P7, N3+P7 from the biogas sludge significantly increased the nutrient uptake of total-N and crop growth rate of upland rice on ultisols with the highest increase

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Commented [MOU23]: This bacterium is named for the first time, what is its function and characteristics, because the characteristics of the bacteria do not appear in the methodology found in the P7 isolate of 20.77% and 2.81-folds, respectively. The dose of biogas sludge significantly increased plant height, total dry weight, nutrient uptake of total-N and available-P, and also crop growth rate of upland rice on ultisols with the highest increase at a dose of 630 mL polybag^{-1} by 14.81%; 50.55%; 54.11%; 65.20%; and 129.44%, respectively. Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice with the highest increase at the dose of 315 mL polybag^{-1} by 41.81%. The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice on ultisols with the highest increase found in the B1S3 by 5.76-folds.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author’s contributions

All authors formulated the overarching research goals and aims, provided the study materials, developed or designed the methodology. NEM analyze and interpretation study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in field, data collection. ZN and I verified the overall reproducibility of results and other research outputs. All authors conducted the critical review/commentary/revision of the manuscript.

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Performance of selected superior bacterial isolates from biogas sludge on the growth of upland rice in ultisols

Rendimiento de aislados bacterianos superiores seleccionados de lodos de biogás en el crecimiento de arroz de tierras altas en ultisoles

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ABSTRACT

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The study searched to obtain the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth, biomass, and nutrient uptake in upland rice in ultisols. This study was conducted from October 2020 to April 2021in aRandomized Block Design within two factors and seven replicates. The first factor was selected SBI (B0= untreated, B1= nitrogen-fixing bacteria isolate (N3), B2= phosphate solubilizing bacteria isolate (P7), B3= isolates combination (N3+P7)). The second factor was dosage of biogas sludge (S0= untreated, S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). The parameters were determined by ANOVA and followed by DMRT at $P < 0.05$. The results showed that the P7 isolate significantly increased total-N uptake and the highest crop growth rate (CGR) of upland rice by 20.77% and 2.81-folds, respectively. Biogas sludge dosage from 315 to 630 ml polybag^{-1} significantly increased plant height, uptake of total-N and available-P, total fresh and dry weight, and CGR of upland rice. The interaction of N3 with biogas sludge dosage of 630 ml polybag^{-1} significantly increased the CGR of upland rice. The application of N3 and P7 isolates and their combination within biogas sludge of 630 mL polybag^{-1} has the potential to archive the CGR of upland rice in acidic soils.

Keywords: acidic soil, crop growth rate, dosage, sludge potential, upland rice.

RESUMEN

El estudio se realizó para obtener la influencia de aislados bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones en el crecimiento, la biomasa y la

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absorción de nutrientes en el arroz de tierras altas en ultisoles. Este estudio utilizó un Diseño de Bloques Aleatorizados dentro de dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0= sin tratar, B1 = aislado de bacterias fijadoras de nitrógeno (N3), B2= aislado de bacterias solubilizantes de fosfato (P7), B3= combinación de aislados (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (S0= sin tratar, S1= 157.5; S2= 315; S3= 630 mL de polybag^{-1}). Los parámetros fueron determinados por ANOVA y seguidos por DMRT en $P < 0.05$. Los resultados mostraron que el aislado P7 aumentó significativamente la captación total de N y la mayor tasa de crecimiento del cultivo (TCC) de arroz de tierras altas en 20.77% y 2.81-pliegues, respectivamente. La dosificación de fangos de biogás de 315 a 630 mL de polybag^{-1} aumentó significativamente la altura de la planta, la absorción de N total y P disponible, el peso fresco y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosificación de lodo de biogás de 630 mL de polybag^{-1} aumentó significativamente el TCC del arroz de tierras altas. La aplicación de aislamientos de N3 y P7 y su combinación dentro de lodos de biogás de 630 mL de polybag^{-1} tiene el potencial de archivar el TCC de arroz de tierras altas en suelos ácidos.

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Palabras clave: arroz de tierras altas, dosis, potencial de lodo, suelo ácido, tasa de crecimiento de los cultivos.

Introduction

Biogas sludge is the waste by-product installation from an anaerobic processing system (Food and Agriculture Organization, 1997) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and plant yield (Adela *et al.*, 2014). The biogas sludge from palm oil waste contained total-N of 490 mg L^{-1}; NH_3-N was 65 mg L^{-1}; total-P by 110 mg L^{-1}; K of 1.9 mg L^{-1}; Ca by 23 mg L^{-1}; Mg of 256 mg L^{-1}; As <0.01 mg L^{-1}; Zn was 0.61 mg L^{-1}; and Cr by 0.04 mg L^{-1} (Lubis *et al.*, 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al.*, 2019), NH_3-N ranged by 91 to 112 mg L^{-1} (Choorit & Wisarnwan, 2007), pH ranged by 6.8 to 8.3; and the highest bacterial populations was 7.21×10^7 cells per ml and the lowest one was 3.15×10^7 cells per ml (Alvionita *et al.*, 2019). Mustamu and Triyanto (2020) also reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria which have the potential to increase availability of nitrogen and phosphate in soils.

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The diversity of beneficial bacteria, such as nitrogen-fixing and phosphate solubilizing, has greater potential forincreasing soil fertility and plant growth. Zhang *et al.* (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility, plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described that the *Bacillus* genera such as *B. circulans, B. cereus, B. fusiformis, B. pumilus, B. megaterium, B. mycoides, B. coagulans, B. chitinolyticus,* and *B. subtilis* have been reported as having phosphate solubilizing activities. Ambrosini *et al.* (2016) reported that *B. cereus* showed the highest nitrogenase activity among 42 different strains of *Bacillus spp*. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, i.e. *Proteobacteria, Firmicutes, Bacteroidetes*, and *Thermotogae.*

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Application of bacteria~~l~~ isolates from biogas sludge for the improvement of upland rice growth on acidic soils has never been reported in Indonesia. Thus, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge in increasing the availability of nitrogen, phosphate, and the growth responses of upland rice due to the biogas sludge and selected isolates in ultisols. The study was aimed at obtaining the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the growth of upland rice in ultisols.

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Materials and methods

Study area

The concentration of total-N and available-P in ultisols and the plant tissue were analyzed in the Analytical Laboratory of PT. Socfin Indonesia, Medan. The bacterial isolates were applied to upland rice in Padang Bulan Village, Medan Selayang Subdistrict, Medan City, North Sumatra, Indonesia from October 2020 to April 2021.

Preparation of medium and upland rice seeds

The planting medium used a soil type of ultisols from the Simalingkar area, Medan Tuntungan Subdistrict, Medan City with a soil depth of 0 to 20 cm. One hundreed grams of soil samples were taken and analyzed for chemical characteristics such as pH, organic-C, available-P, total-N, CEC, and base saturation (K, Ca, Na, Mg) (Tab. 1). The soil was sterilized by dryingat 100^0C for 2 h. After being incubated for 1 d , the soil was put into a polybag of 10 kg. A basic fertilizer of NPK Mutiara at a dose of 300 kg ha^{-1} was given by stirring evenly with the soil. Concurrently, the seeds of upland rice were inbred varieties of Inpago-8 then soaked in water for 24 h and followed by a propineb fungicide (70%) for 2 h . Upland rice was planted after 1 d of basic fertilization with two seeds per polybag at a depth of 2 cm.

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TABLE 1. Chemical characteristics of sterile ultisols

Chemical characteristics	**Value**	**Category***
Soil pH (H_2O)	4.80	Acid
Organic-C (%)	0.44	Very low
Total-N (%)	0.04	Very low
Available-P (mg kg^{-1})	870.25	Very high
CEC (me/100 g)	28.31	High
Base saturation (%)	4.85	Very low
Exchangeable cations		
K (me/100 g)	0.60	High
Ca (me/100 g)	0.34	Very low
Mg (me/100 g)	0.32	Very low
Na (me/100 g)	0.09	Very low
Al (%)	0.02	Very low

Source: *Criteria for pH H_2O= 4.5-5.5 (acid); organik-C <1% (very low); total-N <0,1% (very low); available-P >60 mg kg^{-1} (very high); CEC= 25-40 me/100 g (high); base saturation <20% (very low); exchangeable-K= 0.60-1.00 me/100 g (high); exchangeable-Ca <2 me/100 g (very low); exchangeable-Mg <0.4 me/100 g (very low); exchangeable-Na <0.1 me/100 g (very low); exchangeable-Al <5% (very low) (Soil Research Institute, 2009).

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Preparation of superior bacterial isolates suspension and biogas sludge

The selected superior bacterial isolates used phosphate solubilizing bacteria (P7) which has been confirmed by Mustamu *et al.* (2021) and nitrogen-fixing bacteria or N3 (data unpublished). The isolates were grown on an NB medium, and incubated for 48 h. The bacteria growth ~~microbial mass~~ in the solution was measured using a spectrophotometer with a density of 10^8 cells per mL. Ten ml was taken from the solution containing nitrogen-fixing bacteria (N3) and phosphate solubilizing bacteria (P7).

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The biogas sludge was taken from an identical location in the first phase of the study (palm oil mill of PT. Nubika Jaya, Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia). Bacterial isolate and biogas sludge were applied to the soil surface of the plants one week after planting (WAP). Biogas sludge of 500 ml samples were taken and then the chemical and biological characteristics were analyzed in the laboratory (Tab. 2).

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TABLE 2. Chemical and biological characteristics of biogas sludge

Characteristics of biogas sludge	**Value**
Chemical	
pH	7.41
Chemical oxygen demand/COD (mg L^{-1})	4547.8
Biological oxygen demand/BOD (mg L^{-1})	1127.5
Total-N (%)	0.051
Total-P (%)	0.0097
Available-P (%)	0.013
Total-K (%)	0.18
Organic-C (%)	0.14
Ca (%)	0.04
Mg (%)	0.04
Na (ppm)	44.41
Cu (%)	0.0001
Biological	

Total nitrogen-fixing bacteria (CFU mL^{-1})	29.4×10^{5}
Total phosphate solubilizing bacteria (CFU mL^{-1})	7.0×10^{4}

Treatments application

This study used a Randomized Block Design with~~in~~ two factors and seven replicates. The first factor was the type of superior bacterial isolates at the similarly likewise dose, namely 10 mL polybag^{-1} (B0= un-treated; B1= nitrogen-fixing bacterial isolate (N3); B2= phosphate solubilizing bacteria isolate (P7); B3= combination isolates N3+P7). The second factor was dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Each replicate was dismantled at 4, 8, and 12 Weeks After Isolate and Biogas Sludge Application (WAIBSA) for determination of the CGR.

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Parameters and data analysis

Variable observations were conducted by measuring the growth of upland rice (plant height, total fresh and dry weight), total-N and available-P content in the shoots, total uptake-N, and available-P. CGR was determined on plants at 4-8, 8-12, and 12-16 WAIBSA. Each polybag from each treatment and replicate was dismantled at the plants at 4, 8, 12, and 16 WAIBSA, then the plant height was measured, the total fresh weight was determined by weighing the roots and shoots. The total dry weight was measured by oven at 60^{0}C for 48 h and weighed with analytical scales. A 200 g sample of the 2nd leaf from the shoots was taken by 200 g and analyzed for total-N using the Kjeldahl method and available-P by the dry ashing method with a UV-Vıs Spectrophotometer. The total-N and available-P absorption were measured using equation (1). The CGR was calculated by the dry weight per unit area using equation (2) (Shon *et al.*, 1997):

$$\text{Uptake nutrient} = \text{nutrient content in the shoots} \times \text{total dry weight} \qquad (1)$$

$$\text{CGR} = \frac{\Delta W}{\Delta t} = \frac{W2 - W1}{t2 - t1} \qquad (2)$$

where:

CGR = crop growth rate

W1 = dry weight per unit area at t1

W2 = dry weight per unit area at t2

t1 = first sampling

t2 = second sampling

The parameters of the second phase of the study were analyzed by ANOVA and if the treatment had a significant effect, it was continued by DMRT at *P*<0.05 with SPSS v.20 software.

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Results and discussion

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-Plant height of upland rice
-Biomass of upland rice
-Crop growth rate of upland rice
The second point could include:
-Content of total-N and available-P nutrient of upland rice
-Uptake of total-N and available-P nutrient of upland rice
Furthermore, for both points the analysis made from lines 249-332 must be undertook.

Plant height of upland rice

The effect of biogas sludge application on the plant height of upland rice was significant at 8, 12, and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect on the plant height of upland rice at 4, 8, 12, and 16 WAIBSA (Fig. 1). A significant increase in plant height of upland rice along with increased doses of biogas sludge to 630 ml polybag^{-1} at 8, 12, and 16 WAIBSA with the highest increase of 14.81% compared to the control at 16 WAIBSA. Although the effect was insignificant, the isolates combination of B3 and the interaction of B2S3 showed the highest increase plant height of upland rice by 2.94% and 22.06%, respectively, compared to the control.

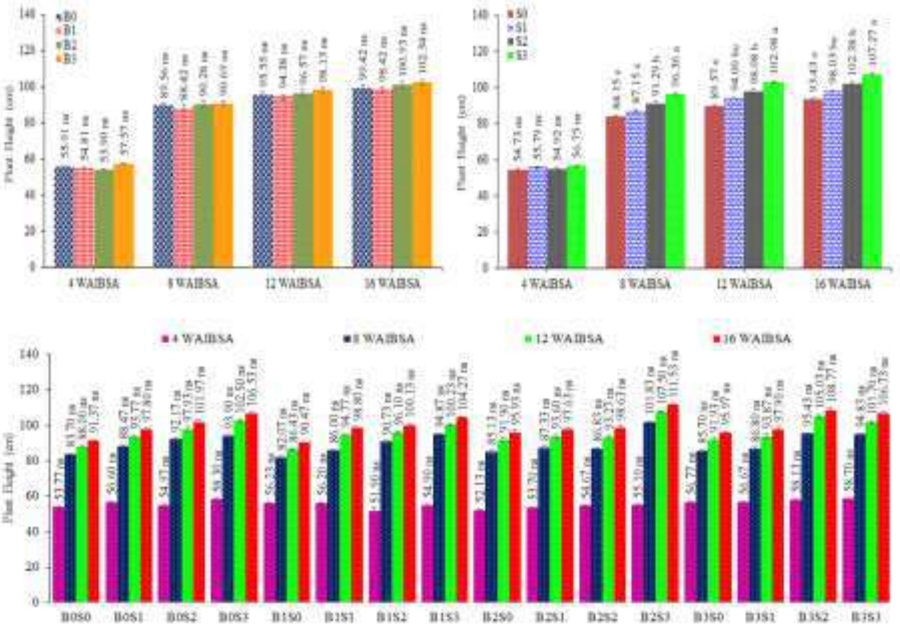

FIGURE 1. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on plant height of upland rice at 4, 8, 12, and 16 WAIBSA. Values followed by the different letter in the graph indicated significantly by DMRT at *P*<0.05. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

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Biomass of upland rice (g)

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect on the total fresh weight of upland rice at 4-16 WAIBSA (Tab. 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on total fresh weight of upland rice at 4, 8, 12, and 16 WAIBSA.

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Treatments	**Total fresh weight ± SE (g)**			
	4 WAIBSA	**8 WAIBSA**	**12 WAIBSA**	**16 WAIBSA**
Superior bacterial isolates (B)				
B0	4.15±0.21ns	169.31±8.90ns	215.27±8.42ns	229.82±8.94ns
B1	3.12±0.12ns	194.50±9.35ns	235.08±10.32 ns	252.02±10.22ns
B2	4.52±0.23ns	162.89±11.15ns	201.85±9.89ns	230.70±9.28ns

B3	3.30±0.25ns	173.91±12.55ns	220.40±15.96ns	245.03±16.32ns
Biogas sludge (S)				
S0	3.72±0.24ns	144.07±9.37b	182.67±7.14b	197.56±6.58b
S1	3.58±0.27ns	153.41±7.93b	190.70±8.90b	215.65±7.03b
S2	3.64±0.27ns	199.68±10.30a	258.70±9.63a	280.15±9.25a
S3	4.15±0.25ns	203.45±1.36a	240.52±2.81a	264.21±2.42a
Interactions (B×S)				
B0S0	4.99±0.33ns	124.08±5.60ns	185.64±3.32ns	192.78±2.96ns
B0S1	3.47±0.26ns	160.43±1.16ns	188.60±5.76ns	207.05±3.97ns
B0S2	3.42±0.42ns	185.97±6.80ns	232.60±8.75ns	250.84±7.40ns
B0S3	4.71±0.42ns	206.76±5.49ns	254.23±10.27ns	268.61±8.85ns
B1S0	2.80±0.18ns	155.79±1.12ns	183.96±5.20ns	202.88±2.88ns
B1S1	3.74±0.29ns	174.82±9.01ns	227.91±6.38ns	236.60±6.32ns
B1S2	3.28±0.40ns	241.17±5.25ns	283.60±7.76ns	296.08±8.05ns
B1S3	2.67±0.22ns	206.20±7.23ns	244.85±6.26ns	272.52±4.34ns
B2S0	3.19±0.18ns	190.90±7.77ns	215.36±7.67ns	229.11±6.75ns
B2S1	4.85±0.38ns	106.74±13.42ns	143.16±13.02ns	179.61±10.36ns
B2S2	5.20±0.24ns	148.40±11.59ns	219.65±5.26ns	248.72±6.94ns
B2S3	4.82±0.45ns	205.53±10.50ns	229.21±16.57ns	265.34±9.58ns
B3S0	3.91±0.30ns	105.53±3.94ns	145.72±1.96ns	165.45±1.11ns
B3S1	2.25±0.09ns	171.63±4.90ns	203.14±7.07ns	239.34±12.07ns
B3S2	2.66±0.14ns	223.17±7.84ns	298.95±1.51ns	324.94±3.03ns
B3S3	4.37±0.07ns	195.31±6.77ns	233.79±8.40ns	250.38±8.16ns
CV (%)	56.09	29.68	26.31	20.78

Note: values followed by the different letter in the column indicated significantly by DMRT at *P*<0.05±SE. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

A significant increase in total fresh weight of upland rice along with the increase in the dosage of biogas sludge to 315 ml polybag^{-1} at 16 WAIBSA with the highest increase by 41.81% compared to the control. Although the effect was insignificant, the B1 and the interaction of B3S2 showed the highest increase in the total fresh weight of upland rice with 9.66% and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4-16 WAIBSA (Tab. 4).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight of upland rice at 4, 8, 12, and 16 WAIBSA.

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Treatments	**Total dry weight ± SE (g)**			
	4 WAIBSA	**8 WAIBSA**	**12 WAIBSA**	**16 WAIBSA**
Superior bacterial isolates (B)				
B0	1.38±0.06ns	48.01±1.29ns	73.60±3.99ns	82.52±4.18ns
B1	1.13±0.05ns	54.09±2.41ns	76.83±2.66ns	99.72±4.15ns
B2	1.49±0.06ns	47.30±3.30ns	73.20±2.28ns	98.25±3.90ns
B3	1.15±0.07ns	52.32±3.39ns	77.18±4.90ns	98.47±4.56ns
Biogas sludge (S)				
S0	1.26±0.06ns	45.51±2.63ns	62.88±2.19b	76.78±1.63c
S1	1.23±0.08ns	44.47±1.71ns	68.52±2.00ab	87.65±2.84bc
S2	1.26±0.08ns	55.36±3.43ns	85.69±1.08a	98.95±1.86b
S3	1.40±0.06ns	56.38±1.05ns	83.73±3.44a	115.59±2.11a
Interactions (B×S)				
B0S0	1.58±0.08ns	41.73±2.78ns	58.08±1.54ns	67.23±0.96ns
B0S1	1.12±0.08ns	45.87±0.83ns	62.74±1.83ns	71.08±1.91ns
B0S2	1.20±0.12ns	52.25±2.07ns	81.39±5.48ns	88.28±5.02ns
B0S3	1.60±0.12ns	52.18±0.29ns	92.20±3.05ns	103.49±2.43ns
B1S0	0.97±0.04ns	46.64±1.39ns	69.53±4.90ns	80.30±4.51ns
B1S1	1.40±0.07ns	48.13±2.78ns	78.91±0.53ns	96.23±1.50ns
B1S2	1.12±0.10ns	67.79±1.44ns	91.05±2.25ns	101.80±2.40ns
B1S3	1.02±0.08ns	53.81±3.76ns	67.84±1.77ns	120.54±2.15ns
B2S0	1.17±0.05ns	59.32±2.33ns	70.92±4.20ns	81.43±3.82ns
B2S1	1.54±0.10ns	34.47±2.16ns	61.69±1.97ns	89.84±1.41ns
B2S2	1.73±0.05ns	37.37±3.74ns	83.10±1.19ns	105.46±1.37ns
B2S3	1.53±0.10ns	58.05±1.76ns	77.07±4.27ns	116.28±1.30ns
B3S0	1.30±0.07ns	34.35±7.04ns	52.98±0.73ns	78.16±0.48ns
B3S1	0.85±0.03ns	49.40±0.08ns	70.72±1.29ns	93.44±2.19ns
B3S2	0.99±0.05ns	64.05±4.68ns	87.22±2.90ns	100.26±1.93ns
B3S3	1.44±0.02ns	61.48±2.47ns	97.80±0.77ns	122.04±0.20ns
CV (%)	43.80	31.22	26.54	18.38

Note: values followed by the different letter in the column indicated significantly by DMRT at *P*<0.05±SE. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

A significant increase in total dry weight of upland rice along with the increase in the dosage of biogas sludge to 630 ml polybag^{-1} at 16 WAIBSA with the highest increase of 50.55% compared to the control. Although the effect was insignificant, the B1 and the interaction of B3S3 showed the highest increase in the total dry weight of upland rice, with 20.84% and 81.53%, respectively, compared to the control.

Content and uptake of total-N and available-P nutrient of upland rice

The apliccation ~~effect~~ of biogas sludge, superior bacterial isolates, and their interactions had an insignificant effect on the nutrient content of total-N and available-P in upland rice. The effect of biogas sludge significantly increased the nutrient uptake of total-N and available-P. Superior bacterial isolates significantly increased the nutrient uptake of total-N. The interaction of biogas sludge with superior bacterial isolates had an insignificant effect on the nutrient uptake of total-N and available-P of upland rice (Fig. 2).

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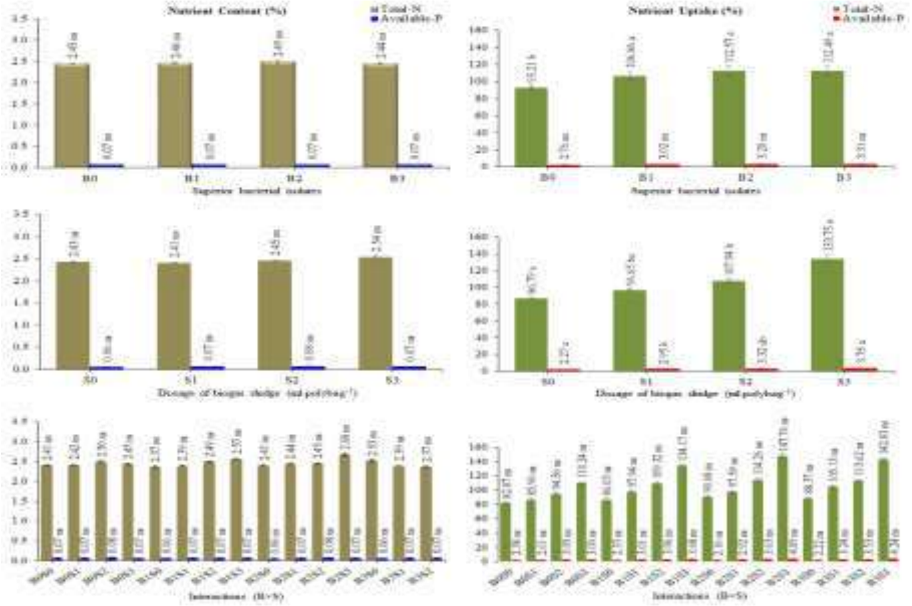

FIGURE 2. The effect of superior bacterial isolates, dosage of biogas sludge, and their interactions in the content and uptake of total-N and available-P nutrient of upland rice. Values followed by the different letter in graph indicated significantly by DMRT at *P*<0.05. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

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A significant increase in the nutrient uptake of total-N and available-P in upland rice along with the increase in the dose of biogas sludge to 630 mL polybag^{-1} with the highest increase of 54.11% and 65.20%, respectively compared to the control. The superior bacterial isolates (B1-B3) also significantly increased the nutrient uptake of total-N for upland rice with the highest increase in the B2 by 20.77% compared to the control. Although the effect was insignificant, the B3 showed the highest increase in nutrient uptake of available-P in upland rice, with19.93% compared to the control. Likewise, the interaction of B2S3 and B3S3 showed the highest increase in nutrient uptake of total-N and available-P in upland rice by 80.04% and 79.41%, respectively, compared to the control.

The biogas sludge doses of 315 and 630 ml polybag^{-1} (S2 and S3) explained that the nutrient content of available-P and total-N in the plant tissue of upland rice were higher by 33.33% and 4.53%, respectively, compared to the control. The B2 isolate showed the highest nutrient content of total-N in the plant tissue of upland rice by 1.63% compared to the control, but all isolates (B1-B3) showed a similar level of available-P in the plant tissue of upland rice with the control. The interactions of B2S3 and B2S2 also showed the highest nutrient content of total-N and available-P compared to other interactions.

Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12 to 16 WAIBSA, but it had an insignificant effect at 4-8 and 8-12 WAIBSA (Tab. 5).

The biogas sludge dose of 630 ml polybag^{-1} (S3) significantly increased the highest crop growth rate for upland rice at 12 to 16 WAIBSA by 129.44% compared to the control. The ability of B1-B3 isolates significantly increased the crop growth rate of upland rice with the highest increase in the B2 by 181.45% compared to the controls at 12 to 16 WAIBSA. The interaction of the B1S3 significantly increased the crop growth rate of upland rice by 5.76-folds greater compared to the control.

TABLE 5. The effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of upland rice.

Superior bacterial	Biogas sludge (S)	Average

isolates (B)	**S0**	**S1**	**S2**	**S3**	
4-8 WAIBSA					
B0	1.434ns	1.598ns	1.823ns	1.806ns	1.665ns
B1	1.631ns	1.669ns	2.381ns	1.885ns	1.892ns
B2	2.077ns	1.176ns	1.273ns	2.019ns	1.636ns
B3	1.180ns	1.734ns	2.252ns	2.144ns	1.828ns
Average	1.580ns	1.544ns	1.932ns	1.964ns	CV= 32.28%
8-12 WAIBSA					
B0	0.584ns	0.602ns	1.041ns	1.430ns	0.914ns
B1	0.818ns	1.099ns	0.831ns	0.501ns	0.812ns
B2	0.414ns	0.972ns	1.633ns	0.679ns	0.925ns
B3	0.665ns	0.761ns	0.828ns	1.297ns	0.888ns
Average	0.620ns	0.859ns	1.083ns	0.977ns	CV= 56.17%
12-16 WAIBSA					
B0	0.327fgh	0.298gh	0.246h	0.403b-h	0.318b
B1	0.385c-h	0.619a-h	0.384d-h	1.882a	0.817a
B2	0.375e-h	1.005a-h	0.798a-h	1.400a-h	0.895a
B3	0.899a-h	0.811a-h	0.466a-h	0.866a-h	0.761a
Average	0.496b	0.683b	0.474b	1.138a	CV= 51.07%

Note: values followed by the different letter in the column indicated significantly by DMRT at *P*<0.05. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag^{-1}). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).

The effect of selected superior bacterial isolates

The selected superior bacterial isolates (N3 and P7) significantly increased the nutrient uptake of total-N and crop growth rate of upland rice on ultisols at 12 to 16 WAIBSA, but it had an insignificant effect on plant height, total fresh weight, total dry weight, nutrient content (total-N and available-P) in leaf tissue, nutrient uptake of available-P, and crop growth rate of upland rice at 4 to 8 and 8 to 12 WAIBSA. The superior bacterial isolates (N3, P7, and N3+P7) could increase the nutrient uptake of total-N in upland rice by 14.64%, 20.77%, and 20.68%, respectively compared to control (Fig. 2). Similar results are also shown in Table 5, that the crop growth rate of upland rice at 12 to 16 WAIBSA due to selected superior bacterial isolates (N3, P7, N3+P7) has increased by 2.57; 2.81; and 2.39-folds, respectively compared to the control. The finding results indicated that the ability of a single isolate by P7 bacteria was greater in increasing total-N and crop growth rate of upland rice compared to a single isolate by N3 bacteria and a combination of N3+P7 bacterial isolates. It was due to the presence of several organic acids and hormones produced by P7 that can increase the nutrient uptake of total-N and crop growth rate of upland rice. This result was supported by Mustamu *et al.* (2021) that the phosphate solubilizing bacteria isolate (P7) from the biogas sludge contains organic acids produced such as lactic, oxalic, acetic, citric acids, and it had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate was 4.62 and 2.66-folds, respectively compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus slightly increased in the application of bio fertilization with *Bacillus cereus*, it was due to the production of organic acids and other chemicals such as citric, tartaric, and oxalic acids which can stimulate plant growth and nutrients availability. Youssef and Eissa (2017) reported that the increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the gibberellins, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that gibberellins, IAA, and other hormones can increase plant growth under stressful conditions. Kang *et al.* (2014) said that PGPB has several mechanisms to increase plant growth with nitrogen-fixation, phosphate solubilizing, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from anaerobic digester include: *Ruminococcus sp.*, *Thiomargarita sp.*, *Clostridium sp. Anaerobacter sp.*, *Bacillus sp.*, *Sporobacterium sp.*, *Saccharofermentans sp.*, *Oscillibacter sp.*, *Sporobacter sp.*, and *Enterobacter sp.* Liaquat *et al.* (2017) also reported that an abundance of *Bacillus*, *Clostridium*, and *Enterobacter spp* in anaerobic digester of wastewater in producing biogas.

The effect of biogas sludge

The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and 16 WAIBSA), total dry weight (12 and 16 WAIBSA), nutrient uptake (total-N and available-

P), and the crop growth rate of upland rice at 8 to 12 WAIBSA, but it had an insignificant effect on nutrient content (total-N and available-P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAIBSA). An increase in plant height, total dry weight, nutrient uptake of total-N and available-P, and also crop growth rate of upland rice on ultisols along with the increase in the dose of biogas sludge to 630 mL polybag^{-1} at the end of this study (16 WAIBSA). However, in contrast to the total fresh weight, an increase along with the increase in the biogas sludge dose to 315 mL polybag^{-1} then decreased at the dose of 630 mL polybag^{-1}. It was caused the biogas sludge had chemical characteristics such as pH (7.41), total-N (0.051%), available-P (0.013%), organic-C (0.14%), total-K (0.18%), and biological characteristics such as total nitrogen-fixing bacteria (29.4×10^{5} CFU mL^{-1}) and total phosphate solubilizing bacteria (7.0×10^{4} CFU mL^{-1}) (Tab. 2). C-organic content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could be increased the nutrient uptake of total-N and available-P in upland rice along with increasing the dose of biogas sludge to 630 mL polybag^{-1} (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the growth of plant height, biomass, and crop growth rate of upland rice. A similar result was reported by Mustamu and Triyanto (2020) that the macro and micronutrients from the biogas sludge and also had the population of nitrogen-fixing and phosphate solubilizing bacteria by 480×10^{4} and 42×10^{4} CFU mL^{-1}, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that total phosphate solubilizing bacteria (1.6 to 2.5 CFU mL^{-1}) was significantly higher compared to nitrogen-fixing bacteria (0.5–1.4 CFU mL^{-1}) and a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported that an increase in concentrations of NH_4^+-N ranged from 45 to 80% after anaerobic waste.

The interaction effect of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice on ultisols at 8 to 12 WAIBSA, but it had an insignificant effect on other parameters in this study. The interaction of NFB *Bacillus paramycoides* with biogas sludge at the dose of 630 ml polybag^{-1} (B1S3) showed the highest crop growth rate of upland rice compared to other interactions and 5.76-fold greater compared to the control. It was caused by the application of biogas sludge that could increase the contents of soil organic matter and the total population of beneficial bacteria. Likewise, the characteristics of the biogas sludge had the organic-C was 0.14%, total nitrogen-fixing bacteria was 29.4×10^5 CFU mL^{-1}, and total phosphate solubilizing bacteria was 7.0×10^4 CFU mL^{-1} (Tab. 2) could improve soil quality and support the crop growth rate. This result is supported by Urra *et al.* (2019) that the application of sewage sludge in the long-term significantly increases the organic matter in the soil, which causes a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of organic matter. Bhardwaj *et al.* (2014); Carvajal-Muñoz *et al.* (2012) reported that the application of biofertilizer had advantages for plants, such as availability of nutrients that are balanced for plant health, stimulating nutrient mobilization that can increase soil biological activity, availability of microbial food to encourage the growth of beneficial microorganisms, increasing the soil organic matter content thereby increasing the cation exchange capacity. Siswanti and Lestari (2019) reported that the interaction of biogas sludge+biofertilizer (36 mL+10 L ha^{-1}) significantly increased the plant height, number of leaves, and capcaisin content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

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The conclusions aren't the repetition of results. The authors could make an effort to show emerging properties accounted for in the results of this work and enhance the practical application and the investigation novelty.

The isolates of N3, P7, N3+P7 from the biogas sludge significantly increased the nutrient uptake of total-N and crop growth rate of upland rice on ultisols with the highest increase found in the P7 isolate of 20.77% and 2.81-folds, respectively. The dose of biogas sludge significantly increased plant height, total dry weight, nutrient uptake of total-N and available-P, and also crop growth rate of upland rice on ultisols with the highest increase at a dose of 630 mL polybag^{-1} by 14.81%; 50.55%; 54.11%; 65.20%; and 129.44%, respectively. Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice with the highest increase at the dose of 315 mL polybag^{-1} by 41.81%. The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice on ultisols with the highest increase found in the B1S3 by 5.76-folds.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

All authors formulated the overarching research goals and aims, provided the study materials, developed or designed the methodology. NEM analyze and interpretation study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in field, data collection. ZN and I verified the overall reproducibility of results and other research outputs. All authors conducted the critical review/commentary/revision of the manuscript.

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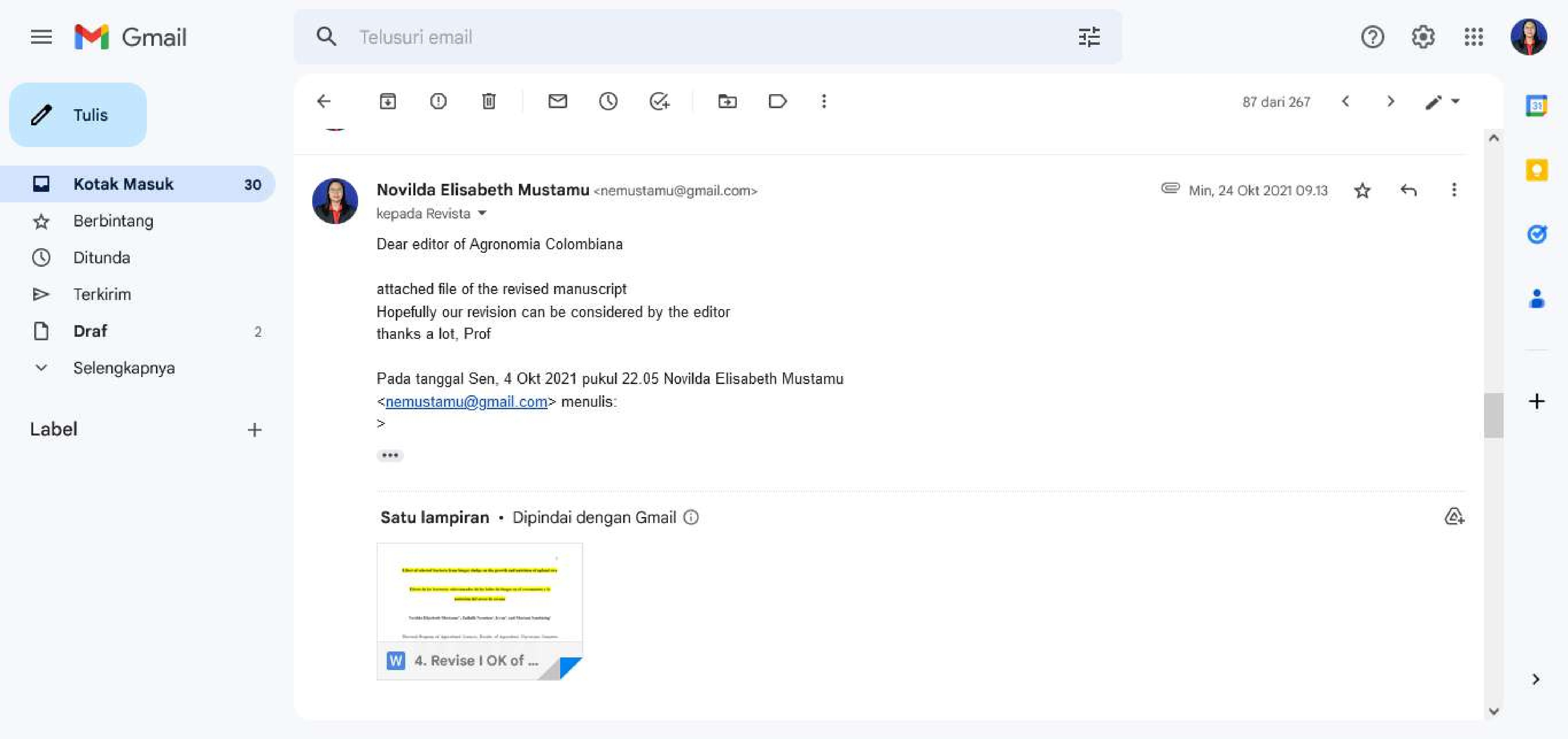

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Hopefully our revision can be considered by the editor
thanks a lot, Prof

Pada tanggal Sen, 4 Okt 2021 pukul 22.05 Novilda Elisabeth Mustamu
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Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la nutrición del arroz de secano

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ABSTRACT

This study ~~e investigation ~~evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth, biomass, and nutrient uptake in upland rice grown? in ultisols. ~~This study~~We used a r~~R~~andomized b~~B~~lock d~~D~~esign with~~in~~ two factors and seven replicates~~ions~~ from October 2020 to~~until~~ April 2021. The first factor used selected SBI (B0 = untreated, B1 = nitrogen-fixing bacteria isolate (N3), B2 = phosphate solubilizing bacteria isolate (P7), B3 = isolate~~s~~ combination (N3+P7). The second factor was the dosage of biogas sludge (S0 = untreated, S1 = 157.5; S2 = 315; S3 = 630 ~~mL~~ml/~~ ~~polybag~~$^{-1}$~~). The parameters were determined by ANOVA and followed by Duncan's multiple range test ~~(DMRT) ~~at $P<$~~ ~~0.05. The results showed that the isolate P7 ~~isolate ~~significantly increased the total~~-~~N uptake by 20.77% and~~-~~ the highest crop growth rate (CGR) of upland rice ~~by 20.77% and~~ 2.81 ~~-~~times~~, respectively~~. Biogas sludge doses~~dosage~~ from 315 to 630 ~~mL~~ml/~~ ~~polybag~~$^{-1}$~~ significantly increased plant height, uptake of total~~-~~N and available~~-~~P, total fresh and dry weight, and CGR of upland rice. The interaction of N3 with biogas sludge dosage of 630 ~~mL~~ml/ polybag~~$^{-1}$~~ significantly increased the CGR of upland rice. The application of isolates N3 and P7

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Reviewer 3 wrote: The title don't show effectively the results of investigation. I recommend the title: Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice.
Regarding the term "superior bacterial isolates" reviewer 3 wrote: What the authors wanted to say? I don't understand this term.
This reviewer also wrote: The authors evaluated only the effect of bacteria and biogas sludge on rice growth. They evaluated also the effect of these treatments on rice nutrition!
Finally, regarding the word "ultisols" reviewer 3 wrote: Why is it necessary to specify the soil type?

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~~isolates~~ and their combination within biogas sludge of 630 ~~mL~~ml/polybag~~$^{-1}$~~ has the potential to archive the CGR of upland rice in acidic soils.

Key words: acidic soil, crop growth rate, dosage, sludge potential.~~,~~ ~~upland rice.~~

RESUMEN

~~La investigación~~El presente estudio evaluó la influencia de aislamientos~~dos~~ bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones sobre~~en~~ el crecimiento, la biomasa y la absorción de nutrientes en el arroz de tierras altas cultivado en ultisoles. ~~Este estudio~~ Se utilizó un d~~D~~iseño de b~~B~~loques al azar ~~Aleatorizados~~ ~~dentro de~~con dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0 = sin tratamiento~~r~~, B1 = aislamiento~~do~~ de bacterias fijadoras de nitrógeno (N3), B2 = aislamiento~~do~~ de bacterias solubilizantes de fosfato (P7), B3 = combinación de aislados (N3+P7))). El segundo factor fue la dosificación del lodo de biogás (S0 = sin tratamiento~~r~~, S1 = 157.~~,~~5; S2 = 315; S3 = 630 ~~mL~~ml/~~de~~ polybag~~$^{-1}$~~). Los parámetros fueron determinados por ~~ANOVA~~ análisis de varianza y seguidos de la prueba de rangos múltiples de Duncan ~~(DMRT)~~ a~~en~~ *P*<0~~,~~.05. Los resultados mostraron que el aislamiento~~do~~ P7 aumentó significativamente la captación total de N en 20.77% y la mayor tasa de crecimiento del cultivo (TCC) de arroz de tierras altas ~~en 20.77% y~~ 2.81-veces~~, respectivamente~~. Las dosis ~~dosificación~~ de ~~fangos~~ lodos de biogás de 315 a 630 ~~mL~~ml/~~de~~ polybag~~$^{-1}$~~ aumentaron~~ó~~ significativamente la altura de la planta, la absorción de N total y P disponible, el peso fresco y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la ~~dosificación~~ dosis de lodos de biogás de 630 ~~mL~~ml/~~de~~ polybag~~$^{-1}$~~ aumentó significativamente el TCC del arroz de tierras altas. La aplicación de los aislamientos ~~de~~ N3 y P7 y su combinación dentro de lodos de biogás de 630

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I recommend: "Los resultados mostraron que la inoculación aumentó… pues el aislado por sí solo no produce el efecto, sino que hay que inocularlo".

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~~mL~~ml/~~ de ~~polybag~~$^{-1}$~~ tiene el potencial de archivar el TCC de arroz de tierras altas en suelos ácidos.

~~**Palavras chave**~~**Palabras clave**: ~~arroz de tierras altas, ~~suelo ácido, tasa de crecimiento de cultivos, dosis, potencial de lodo~~, suelo ácido~~, ~~tasade crecimiento de los cultivos~~.

Introduction

Biogas sludge is the waste by-product ~~installation ~~from an anaerobic processing system (~~Food and Agriculture Organization~~FAO, 1997) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and plant yields (Adela *et al.*, 2014). ~~It has been reported that t~~The following characteristics of the biogas sludge from palm oil waste have been ~~were ~~reported: ~~such as ~~total ~~-~~N of 490 mg L^{-1},~~;~~ total ~~-~~P ~~by~~of 110 mg L^{-1},~~;~~ ~~and ~~total K of ~~was~~ 1.9 mg L^{-1} (Lubis *et al.*, 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al.*, 2019), and NH_3-N of ~~ranged by ~~91 ~~to ~~–112 mg L^{-1} (Choorit & Wisarnwan, 2007).~~,~~ The pH may ~~could ~~range~~d~~ ~~by~~from 6.8 to 8.3,~~; and ~~with the highest bacterial population~~s~~ ~~was~~of~~ ~~7.21×10^7 cells per ~~mL~~ml and the lowest one of ~~was ~~3.15×10^7 cells per ~~mL~~ml (Alvionita *et al.*, 2019). Additionally, Mustamu ~~&~~and Triyanto (2020) ~~also ~~reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria? That ~~which~~ have the potential to availability of nitrogen and phosphate in~~ the~~ soils.

The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing bacteria has a greater potential to~~in~~ increase~~ing~~ soil fertility and plant growth. Zhang *et al.* (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility, and plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described ~~that the ~~different *Bacillus* species, ~~genera ~~such as *B. circulans, B. cereus, B. fusiformis, B. pumilus, B. megaterium, B. mycoides, B. coagulans, B. chitinolyticus*, and *B. subtilis*~~,~~ ~~have been reported~~ as phosphate solubilizing microorganisms. Ambrosini *et al.* (2016) ~~reported that *Bacillus cereus*~~

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Furthermore, the authors must enhance the investigation novelty.

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showed the highest nitrogenase activity in *Bacillus cereus* among 42 different strains of *Bacillus* spp. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, *i.e.*, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Thermotogae*.

The application of bacteria from biogas sludge has never been reported in Indonesia ~~on the application of bacterial isolates from biogas sludge in~~ for improving upland rice growth on acidic soils. Thus, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge to increase ~~in increasing~~ the availability of nitrogen and~~,~~ phosphate, and the growth response ~~to the growth~~ of upland rice due to application of the biogas sludge and selected isolates in ultisols. The study ~~was~~ aimed to evaluate ~~d~~ the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the mineral nutrition of the upland rice grown in ultisols.

Materials and methods

Study area

The concentration of total-N and available-P in ultisols and the plant tissue were analyzed in the Analytical Laboratory of PT. Socfin Indonesia, Medan (Indonesia). The bacterial isolates were applied to upland rice in the village of Padang Bulan ~~Village~~ (3°37.760' N; 98°38.898' E; altitude 18 m a.s.l.~~above sea level~~), Medan Selayang Subdistrict, Medan City, ~~North Sumatra,~~ Indonesia, from October 2020 to April 2021. ~~Furthermore~~ The average temperature was 27.4°C, the average air humidity was 82%, ~~temperature was 27.4°C~~ and the average rainfall was ~~recorded~~ 228.5 mm ~~by~~per month.

Preparation of medium and upland rice seeds

The medium to grow upland rice plants? used ultisols ~~order~~ from the Simalingkar area, Medan Tuntungan Subdistrict, Medan City, ~~with~~ at a depth of 0 to 20 cm. One hundred ~~100~~ g of soil

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Commented [EDTR31]: Reviewer 3 wrote: Are acid soils representative in Indonesia? What percentage of rice is cultivated upland in Indonesia? Is it representative? I think that the authors must state this information here for the enhancement of the investigation novelty. Furthermore, the authors must show the importance of using bio-products as an alternative to mineral fertilization in rice.

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samples were taken and analyzed for chemical characteristics such as pH using HCl 25% ~~method with spectrophotometer~~, organic ~~-~~C by Walkley-Black ~~method with spectrophotometer~~, available ~~-~~P by Bray-II ~~method with spectrophotometer~~, total ~~-~~N using Kjeldahl method ~~with spectrophotometer~~, and c~~C~~ation e~~E~~xchange c~~C~~apacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH 7 method ~~with atomic absorption spectrophotometry~~ (Tab. 1). The soil was sterilized by ~~burning~~drying at 100°~~0~~C for 2 ~~hours~~h. After being incubated for 1 d~~ay~~, the soil was ~~put~~ placed into a 10 kg polybag ~~with a size of 10 kg~~. A basic NPK fertilizer of ~~NPK~~ Mutiara at a dose of 300 kg ha^{-1} was applied ~~given~~ by stirring evenly with the soil. ~~Concurrently,~~ T~~t~~he seeds of upland rice (*Oryza sativa* L. (?)) used ~~was~~ were of an? inbred variety of Inpago-8, then soaked in water for 24 ~~hours~~h, ~~and~~ followed by a ~~p~~Propineb fungicide (70%) application for 2 ~~hours~~h. Upland rice was planted after 1 d ~~one day~~ of basic fertilization with two seeds per polybag at a depth of 2 cm.

TABLE 1. ~~The c~~Chemical characteristics of the ~~sterile~~ ultisols soil samples after sterilization at 100°~~0~~C.~~.~~

Chemical characteristics	**Value**	**Category***
Soil pH (H_2O)	4.80	Acid
Organic ~~-~~C (%)	0.44	Very low
Total ~~-~~N (%)	0.04	Very low
Available ~~-~~P (mg kg^{-1})	870.25	Very high
CEC (meq/100 g)	28.31	High
Base saturation (%)	4.85	Very low
Exchangeable cations		
K (meq/100 g)	0.60	High
Ca (meq/100 g)	0.34	Very low
Mg (meq/100 g)	0.32	Very low
Na (meq/100 g)	0.09	Very low
Al (%)	0.02	Very low

~~Source:~~ *Criteria for pH H_2O = 4.5-5.5 (acid); organic ~~k-~~C <1% (very low); total ~~-~~N <0~~,~~.1% (very low); available ~~-~~P >60 mg kg^{-1} (very high); Cation exchange capacity (CEC) = 25-40 meq ~~/~~100 g^{-1} (high); base saturation <20% (very low); exchangeable ~~-~~K= 0.60-1.00 meq ~~/~~100 g^{-1} (high); exchangeable ~~-~~Ca <2 meq ~~/~~100 g^{-1} (very low); exchangeable ~~-~~Mg <0.4 meq ~~/~~100 g^{-1} (very low); exchangeable ~~-~~Na <0.1 meq ~~/~~100 g^{-1} (very low); exchangeable ~~-~~Al <5% (very low) (Soil Research Institute, 2009).

Preparation of superior bacterial isolates suspension and biogas sludge

The selected superior bacterial isolates used phosphate solubilizing bacteria (P7) which has been confirmed by Mustamu *et al.* (2021a) and nitrogen-fixing bacteria or N3 (Mustamu *et al.*,

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Commented [SM47]: According to which criteria these are called "superior"?

Commented [SM48]: The species or the genera of the microorganisms should be named for each of the treatments P7, N3, etc., so that the differences between the treatments could be appreciated.
Without these data, this research may not be reproduced in future since no information is provided about the bacterial composition of these isolates.

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2021b). The isolates were grown on NB medium, and incubated for 48 h. ~~The~~ B~~b~~acterial growth in the solution was measured using an spectrophotometer with a density of 10^8 cells per ~~mL~~ml. Ten~~10~~ ~~mL~~ml was taken from solution containing nitrogen-fixing ~~bacteria~~ (N3) and phosphate solubilizing (P7)~~.~~ bacteria.

The biogas sludge was ~~taken~~ collected from the digester tank at the palm oil mill of PT. Nubika Jaya, Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia. Bacterial isolates and biogas sludge were applied to the soil surface at the base of? the plants ~~were~~ one week after planting. ~~(WAP).~~ Biogas sludge samples ~~were taken~~ at 500 ~~mL~~ml volume were used to analyze the ~~then analyzed the~~ chemical and biological characteristics in the laboratory (Tab. 2).

TABLE 2. The chemical and biological characteristics of the biogas sludge.

Characteristics ~~of biogas sludge~~	**Method**	**Value**
~~Chemical~~		
pH	Electrometry	7.41
Chemical oxygen demand~~/COD~~ (mg L^{-1})	Spectrophotometry	4547.8
Biological oxygen demand~~/BOD~~ (mg L^{-1})	Titrimetry	1127.5
Total ~~-~~N (%)	Spectrophotometry	0.051
Total ~~-~~P (%)	Spectrophotometry	0.0097
Available-P (%)	Spectrophotometry	0.013
Total ~~-~~K (%)	Graphite furnace - a~~A~~tomic absorption spectrophotometry ~~AAS~~	0.18
Organic ~~-~~C (%)	Atomic absorption spectrophotometry (AAS)	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (~~ppm~~ mg L^{-1})	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
~~Biological~~		
Total nitrogen-fixing bacteria (CFU ~~mL~~ml^{-1})	Plate count	29.4×10^5
Total phosphate solubilizing bacteria (CFU ~~mL~~ml^{-1})	Plate count	7.0×10^4

Treatment~~s~~ application

This study used a r~~R~~andomized b~~B~~lock d~~D~~esign with~~in~~ two factors and seven replicates~~ions~~. The first factor was the type of superior bacterial isolates (B0 = un~~-~~treated; B1 = nitrogen-fixing bacterial isolate (N3); B2 = phosphate solubilizing bacteria isolate (P7); B3 = combination of isolates N3+P7) at ~~the~~ a similar dose, namely 10 ~~mL~~ml/ polybag~~$^{-1}$~~. The second factor was

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Commented [SM58]: Could you indicate which species or hybrid of palm was employed? This may have an effect on the sludge composition

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Commented [EDTR60]: Reviewer 3 wrote: I think that the authors must give more details about how biogas sludge was taken.

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~~dosage~~ dose of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ~~mL~~ml polybag^{-1}). Determination of biogas sludge based on the dose of liquid organic fertilizer at the oil palm was 126 m^3 ha^{-1} equal to 126,000 L ha^{-1} (Sutarta *et al*., 2003), then converted to soil weight per polybag (E~~e~~q.~~uation~~ 1). Each replicat~~eion~~ was disassembled at 4, 8, and 12 weeks after the application of the isolates and biogas sludge ~~application~~ (~~WAIBSA~~WAA) for determination of the crop growth rate (CGR).

$$\text{Biogas sludge} = \frac{\text{The dose of liquid organic fertilizer per ha}}{\text{soil weight per ha}} \text{ x soil weight per polybag} \qquad (1)$$

$$= \frac{126{,}000 \text{ L ha}^{-1}}{2{,}000{,}000 \text{ kg ha}^{-1}} \text{ x 10 kg} = 630 \text{ }\cancel{\text{ml}}\text{ml}$$

Parameters and data analysis

~~Variable~~ The observations of the variables were conducted by measuring the growth of upland rice (plant height, and total fresh and dry weight), total ~~-~~N and available ~~-~~P contents in the shoots, total N uptake ~~-N~~, and available ~~-~~P. The CGR ~~were conducted~~was determined on plants at 4-8, 8-12, and 12-16 ~~WAIBSA~~WAA. Each polybag from each treatment and replicat~~eion~~ was disassembled ~~at~~when the plants were 4, 8, 12, and 16 ~~WAIBSA~~WAA, then ~~measured~~ the plant height was measured, and the total fresh weight was ~~conducted~~ obtained by weighing the roots and shoots. The total dry weight (roots + shoots) was measured after using an~~by~~ oven at 60~~^{0}~~$^\circ$C for 48 ~~hours~~h and weighed using ~~by~~ the analytical scales. A 200 g sample of the second leaf from the shoots was collected ~~taken by 200 g~~ and analyzed to determine~~for~~ the total ~~-~~N using the Kjeldahl method and the available ~~-~~P was estimated using~~by~~ the dry ashing method ~~through UV-Vis Spectrophotometer~~. The total ~~-~~N and available ~~-~~P absorption were measured using E~~e~~quation ~~(~~2~~)~~. The CGR was calculated ~~by~~as the dry weight related to the ~~per~~ unit area using E~~e~~quation ~~(~~3~~)~~ (Shon *et al*., 1997):

~~Uptake~~ N~~n~~utrient uptake = nutrient content in the shoots × total dry weight (2)

$$\text{CGR} = \frac{\Delta W}{\Delta t} = \frac{W2 - W1}{t2 - t1} \qquad (3)$$

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Also, add spaces between the words because they are written together
Please use the equation editor in Word to write these equations. It is not possible to edit them.

Commented [EDTR74]: Delete the space between ha and $^{-1}$

Commented [SM75]: How exactly the plant height was measured?

Commented [SM76]: The total weight includes aerial parts + plant roots?

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How it is possible to absorb the total nitrogen?
Please check the English spelling

where~~Note~~:

CGR = crop growth rate;

W1 = dry weight per unit area at t1;

W2 = dry weight per unit area at t2;

t1 = first sampling;

t2 = second sampling;

The parameters of the second phase of the study were analyzed by an ANOVA and if the treatment had a significant effect, ~~then continued~~ followed by Duncan's multiple range test ~~(DMRT)~~ at $P<0.05$ using~~with~~ SPSS v.20 software.

Results

Effect of bacterial isolates and biogas sludge on upland rice growth

Plant height of upland rice ~~(cm)~~

The effect of biogas sludge application was significant on the plant height of upland rice at 8, 12, and 16 ~~WAIBSA~~WAA. Superior bacterial isolates and their interactions did not have a significant ~~had an insignificant~~ effect on the plant height of upland rice at 4, 8, 12, and 16 ~~WAIBSA~~WAA (Fig. 1). A significant increase in plant height of upland rice was observed ~~along~~ with increased doses of biogas sludge of~~to~~ 630 ~~mL~~ml~~-~~/polybag~~$^{-1}$~~ at 8, 12, and 16 ~~WAIBSA~~WAA with the highest increase of 14.81% compared to the control at 16 ~~WAIBSA~~WAA. Although the effect was ~~insignificant,~~not significant, ~~it was seen that~~ the ~~isolates~~ combination of isolates B3 and the interaction of B2S3 showed the highest increase in plant height of upland rice by 2.94~~%~~ and 22.06%, respectively, compared to the control.

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Reviewer 3 wrote: It is necessary write Results and discussion again in other form. All the information of this section must be re-organized. Account of the statistical analysis made and some information in figures and tables is irrelevant. The figures have repeated information.

Commented [EDTR84R83]: The authors did not reply to these comments.

Commented [EDTR85]: Reviewer 3 wrote: I consider that it is necessary to write again Results and discussion in other form. The information of this section must be re-organized in two aspects: (1) Effect of bacterial isolates and biogas sludge on upland rice growth. (2) Effect of bacterial isolates and biogas sludge on upland rice nutrition. The first point could include:
- Plant height of upland rice
-.Biomass of upland rice
- Crop growth rate of upland rice
The second point could include:
-.Content of total-N and available-P nutrient of upland rice
- Uptake of total-N and available-P nutrient of upland rice
Furthermore, for both points the analysis made from lines 249-332 must be undertaken.

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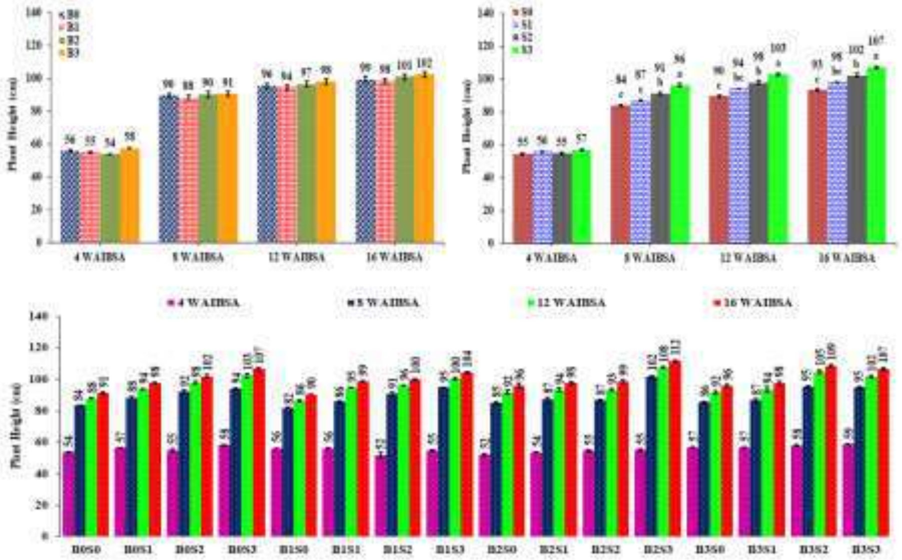

FIGURE 1. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on plant height of upland rice at 4, 8, 12, and 16 ~~WAIBSA~~WAA. Values followed by the different letter in the graph significantly differed according to the Duncan test ~~indicated significantly by DMRT~~ at $P<0.05$. ns -~~=~~ not significant~~ly~~. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ~~mL~~ml/~~-~~polybag~~$^{-1}$~~). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = combination of isolates N3+P7).

Biomass of upland rice ~~(g)~~

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 ~~WAIBSA~~WAA. Superior bacterial isolates and their interactions did not have a ~~had an~~ ~~in~~significant effect on the total fresh weight of upland rice at 4-16 ~~WAIBSA~~WAA (Tab. 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total fresh weight (~~aerial parts~~shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application of the isolate and biogase sludge (WAA). ~~WAIBSA.~~

Treatments	**Total fresh weight ± standard error ~~SE~~ (g)**			
	4 ~~WAIBSA~~WAA	**8 ~~WAIBSA~~WAA**	**12 ~~WAIBSA~~WAA**	**16 ~~WAIBSA~~WAA**
Superior bacterial isolates (B)				
B0	4.15 ± 0.21	169.31 ± 8.90	215.27 ± 8.42	229.82 ± 8.94
B1	3.12 ± 0.12	194.50 ± 9.35	235.08 ± 10.32	252.02 ± 10.22
B2	4.52 ± 0.23	162.89 ± 11.15	201.85 ± 9.89	230.70 ± 9.28
B3	3.30 ± 0.25	173.91 ± 12.55	220.40 ± 15.96	245.03 ± 16.32
Biogas sludge (S)				
S0	3.72±0.24	144.07±9.37 b	182.67±7.14 b	197.56±6.58 b
S1	3.58±0.27	153.41±7.93 b	190.70±8.90 b	215.65±7.03 b
S2	3.64±0.27	199.68±10.30 a	258.70±9.63 a	280.15±9.25 a

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Please add these graphs as independent files to ensure they have the appropriate resolution and quality.

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S3	4.15±0.25	203.45±1.36 a	240.52±2.81 a	264.21±2.42 a
Interactions (B×S)				
B0S0	4.99±0.33	124.08±5.60	185.64±3.32	192.78±2.96
B0S1	3.47±0.26	160.43±1.16	188.60±5.76	207.05±3.97
B0S2	3.42±0.42	185.97±6.80	232.60±8.75	250.84±7.40
B0S3	4.71±0.42	206.76±5.49	254.23±10.27	268.61±8.85
B1S0	2.80±0.18	155.79±1.12	183.96±5.20	202.88±2.88
B1S1	3.74±0.29	174.82±9.01	227.91±6.38	236.60±6.32
B1S2	3.28±0.40	241.17±5.25	283.60±7.76	296.08±8.05
B1S3	2.67±0.22	206.20±7.23	244.85±6.26	272.52±4.34
B2S0	3.19±0.18	190.90±7.77	215.36±7.67	229.11±6.75
B2S1	4.85±0.38	106.74±13.42	143.16±13.02	179.61±10.36
B2S2	5.20±0.24	148.40±11.59	219.65±5.26	248.72±6.94
B2S3	4.82±0.45	205.53±10.50	229.21±16.57	265.34±9.58
B3S0	3.91±0.30	105.53±3.94	145.72±1.96	165.45±1.11
B3S1	2.25±0.09	171.63±4.90	203.14±7.07	239.34±12.07
B3S2	2.66±0.14	223.17±7.84	298.95±1.51	324.94±3.03
B3S3	4.37±0.07	195.31±6.77	233.79±8.40	250.38±8.16
CV (%)	56.09	29.68	26.31	20.78

~~Note: v~~Values followed by the different letter in the column significantly differed according to the Duncan test ~~indicated significantly by DMRT~~ at *P*<0.05~~±SE~~. ns ~~-~~= not significantly. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ~~mL~~ml/~~-~~polybag~~$^{-1}$~~). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = combination of isolates N3+P7).

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A significant increase in the total fresh weight of upland rice ~~along~~ was observed with the higher ~~increase in the dosage~~ dose of biogas sludge of~~to~~ 315 ~~mL~~ml/~~-~~polybag~~$^{-1}$~~ at 16 ~~WAIBSA~~WAA, with the highest increase ~~by~~ of 41.81% compared to the control. Although the effect was not ~~in~~significant, ~~it was seen that the~~ B1 and the interaction of B3S2 showed the highest increase in the total fresh weight of upland rice ~~were~~with -9.66~~%~~ and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 ~~WAIBSA~~WAA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4 -16 ~~WAIBSA~~WAA (Tab. 4).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight (~~aerial parts~~shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application of isolate and biogase sludge (WAA). ~~the total dry weight of upland rice at 4, 8, 12, and 16 WAIBSA.~~

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Treatments	**Total dry weight ± standard error~~SE~~ (g)**			
	4 ~~WAIBSA~~WAA	**8 ~~WAIBSA~~WAA**	**12 ~~WAIBSA~~WAA**	**16 ~~WAIBSA~~WAA**
Superior bacterial isolates (B)				
B0	1.38±0.06	48.01±1.29	73.60±3.99	82.52±4.18
B1	1.13±0.05	54.09±2.41	76.83±2.66	99.72±4.15

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B2	1.49±0.06	47.30±3.30	73.20±2.28	98.25±3.90
B3	1.15±0.07	52.32±3.39	77.18±4.90	98.47±4.56
Biogas sludge (S)				
S0	1.26±0.06	45.51±2.63	62.88±2.19 b	76.78±1.63 c
S1	1.23±0.08	44.47±1.71	68.52±2.00 ab	87.65±2.84 bc
S2	1.26±0.08	55.36±3.43	85.69±1.08 a	98.95±1.86 b
S3	1.40±0.06	56.38±1.05	83.73±3.44 a	115.59±2.11 a
Interactions (B×S)				
B0S0	1.58±0.08	41.73±2.78	58.08±1.54	67.23±0.96
B0S1	1.12±0.08	45.87±0.83	62.74±1.83	71.08±1.91
B0S2	1.20±0.12	52.25±2.07	81.39±5.48	88.28±5.02
B0S3	1.60±0.12	52.18±0.29	92.20±3.05	103.49±2.43
B1S0	0.97±0.04	46.64±1.39	69.53±4.90	80.30±4.51
B1S1	1.40±0.07	48.13±2.78	78.91±0.53	96.23±1.50
B1S2	1.12±0.10	67.79±1.44	91.05±2.25	101.80±2.40
B1S3	1.02±0.08	53.81±3.76	67.84±1.77	120.54±2.15
B2S0	1.17±0.05	59.32±2.33	70.92±4.20	81.43±3.82
B2S1	1.54±0.10	34.47±2.16	61.69±1.97	89.84±1.41
B2S2	1.73±0.05	37.37±3.74	83.10±1.19	105.46±1.37
B2S3	1.53±0.10	58.05±1.76	77.07±4.27	116.28±1.30
B3S0	1.30±0.07	34.35±7.04	52.98±0.73	78.16±0.48
B3S1	0.85±0.03	49.40±0.08	70.72±1.29	93.44±2.19
B3S2	0.99±0.05	64.05±4.68	87.22±2.90	100.26±1.93
B3S3	1.44±0.02	61.48±2.47	97.80±0.77	122.04±0.20
CV (%)	43.80	31.22	26.54	18.38

~~Note: v~~Values followed by the different letter in the column significantly differed according to the Duncan test ~~indicated significantly by DMRT~~ at *P*<0.05~~±SE~~. ns= not significantly. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ~~mL~~ml/ polybag~~-1~~). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = combination of isolates N3+P7).

A significant increase in total dry weight of upland rice was observed ~~along~~ with the increase in the dosage of biogas sludge of~~to~~ 630 ~~mL~~ml/ polybag~~-1~~ at 16 ~~WAIBSA~~WAA, with the highest increase of 50.55% compared to the control. Although the effect was not ~~in~~significant, ~~it was seen that the~~ B1 and the interaction of B3S3 showed the highest increase in the total dry weight of upland rice~~-~~ with ~~were 20~~.84~~%~~ and 81.53%, respectively, compared to the control.

Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12 to 16 ~~WAIBSA~~WAA, but it did not have ~~had~~ a ~~n in~~significant effect at 4-8 and 8-12 ~~WAIBSA~~WAA (Tab. 5).

TABLE 5. E~~The e~~ffect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of the upland rice.

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Superior bacterial isolates (B)	Biogas sludge (S)				Average
	S0	S1	S2	S3	
		4-8 ~~WAIBSA~~WAA			
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV = 32.28%
		8-12 ~~WAIBSA~~WAA			
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888
Average	0.620	0.859	1.083	0.977	CV = 56.17%
		12-16 ~~WAIBSA~~WAA			
B0	0.327 fgh	0.298 gh	0.246 h	0.403b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV = 51.07%

~~Note: v~~Values followed by the different letter in the column significantly differed according to the Duncan test ~~indicated significantly by DMRT~~ at *P*<0.05. ns ~~-~~= not significantly. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ~~mL~~ml polybag^{-1}). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = combination of isolates N3+P7).

The biogas sludge dose of 630 ~~mL~~ml/~~-~~polybag~~$^{-1}$~~ (S3) significantly increased the highest crop growth rate for upland rice at 12 to 16 ~~WAIBSA~~WAA by 129.44% compared to the control. The ~~ability of~~ isolates B1-B3 ~~isolates~~ significantly increased the crop growth rate of upland rice with the highest increase ~~in the~~for B2 of~~by~~ 181.45% compared to the controls at 12 to 16 ~~WAIBSA~~WAA. The interaction of the B1S3 significantly increased the crop growth rate of upland rice, showing values ~~by~~ 5.76-times greater ~~compared to~~than those of the control.

Effect of bacterial isolates and biogas sludge on upland rice nutrition

Content of total ~~-~~N and available ~~-~~P ~~nutrient~~ of upland rice

The effect of biogas sludge, superior bacterial isolates, and their interactions ~~had an~~did not have a ~~in~~significant effect on the nutrient content of total ~~-~~N and available ~~-~~P in the upland rice (Fig. 2). The biogas sludge doses of 315 and 630 ~~mL~~ml/~~-~~polybag~~$^{-1}$~~ (S2 and S3) explained that the ~~nutrient~~ contents of available ~~-~~P and total ~~-~~N in the plant tissue of upland rice were ~~higher by~~ 33.33% and 4.53% higher, respectively, compared to the control. The isolate B2 ~~isolate~~ showed

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the highest~~ nutrient~~ content of total ~~-~~N in the plant tissue of upland rice with values ~~by~~ 1.63% higher than those of ~~compared to ~~the control; however,~~, but~~ all isolates (B1-B3) showed a similar level of available ~~-~~P in the plant tissue of upland rice ~~with ~~compared to? the control.

Uptake of total-N and available-P nutrient of upland rice

The effect of biogas sludge significantly increased in the ~~nutrient ~~uptake of total ~~-~~N and available ~~-~~P. The ~~S~~superior bacterial isolates significantly increased in the ~~nutrient ~~uptake of total ~~-~~N. The interaction of biogas sludge with superior bacterial isolates did not show a~~had an~~ ~~in~~significant effect on the ~~nutrient ~~uptake of total ~~-~~N and available ~~-~~P of the upland rice (Fig. 2).

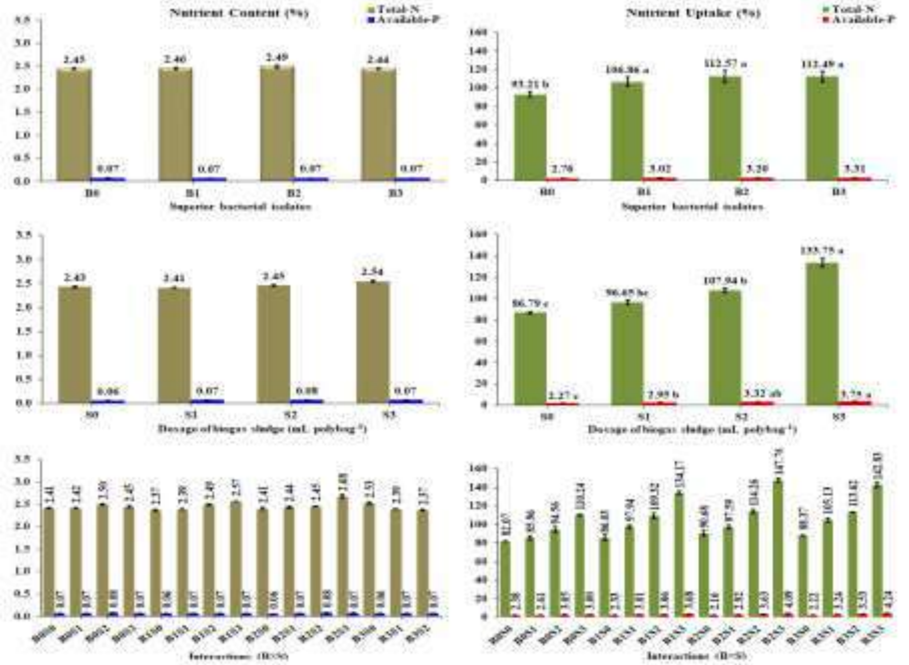

FIGURE 2. The effect of superior bacterial isolates, dosage of biogas sludge, and their interactions o~~i~~n the content and uptake of total ~~-~~N and available ~~-~~P ~~nutrient ~~of the upland rice. Values followed by ~~the~~ different letters ~~in graph~~ significantly differed according to the Duncan test ~~indicated significantly by DMRT ~~at *P*<0.05. ns ~~-~~= not significant~~ly~~. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ~~mL~~ml/~~-~~polybag~~$^{-1}$~~). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = combination of isolates N3+P7).

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Please add these graphs as independent files to ensure they have the appropriate quality and resolution.

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A significant increase in the ~~nutrient~~ uptake of total -N and available -P in upland rice was observed ~~along~~ with ~~the increase~~a higher ~~in the~~ dose of biogas sludge of~~to~~ 630 ~~mL~~ml/polybag, ~~-1~~ with the highest increases of 54.11~~%~~ and 65.20%, respectively, compared to the control. The superior bacterial isolates (B1-B3) also significantly increased the ~~nutrient~~ uptake of total -N ~~for~~ in upland rice with the highest increase ~~in~~ with the B2 of ~~by~~ 20.77% compared to the control. Although the effect was not ~~in~~significant, ~~it was seen that the~~ B3 showed the highest increase in ~~nutrient~~ uptake of available -P in upland rice ~~was~~ of 19.93% compared to the control.

Discussion

~~The e~~Effect of selected superior bacterial isolates

The selected superior bacterial isolates (N3 and P7) significantly increased the ~~nutrient~~ uptake of total -N and crop growth rate of upland rice on ultisols at 12 to 16 ~~WAIBSA~~WAA, but it did not have~~d~~ a~~n~~ ~~in~~significant effect on plant height, total fresh weight, total dry weight, nutrient content (total -N and available -P) in leaf tissue, nutrient uptake of available -P, and crop growth rate of upland rice at 4 to 8 and 8 to 12 ~~WAIBSA~~WAA. The superior bacterial isolates (N3, P7, and N3+P7) could increase the ~~nutrient~~ uptake of total -N in upland rice by 14.64%, 20.77%, and 20.68%, respectively, compared to the control (Fig. 2). Similar results are also shown in Table 5, ~~that~~ where can be observed that the crop growth rate of upland rice at 12 to 16 ~~WAIBSA~~WAA ~~due to selected superior bacterial isolates (N3, P7, N3+P7)~~ has increased ~~by~~ 2.57,~~;~~ 2.81,~~;~~ and 2.39 -times, respectively due to the selected superior bacterial isolates (N3, P7, N3+P7), compared to the control. The ~~finding~~ results indicate~~d~~ that the ability of a single ~~isolate by~~ P7 bacterial isolate was greater in increasing total -N and crop growth rate of upland rice compared to a single ~~isolate by~~ N3 bacterial isolate and the~~a~~ combination of N3+P7 bacterial isolates. This~~It~~ was due to the presence of several organic acids and hormones produced by P7 that can increase the nutrient uptake of total -N and crop growth rate of upland rice. This result

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Commented [SM121]: The phrase “nutrient uptake of total-N” has no physiological sense. Please re-write these phrases using different terms i~~wa~~s supported by Mustamu *et al.* (2021) who found that the phosphate solubilizing bacterial isolate (P7) from the biogas sludge contains organic acids~~ produced~~ such as lactic, oxalic, acetic, and citric acids, and~~ it~~ had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate with values ~~was ~~4.62 and 2.66 ~~-~~times higher, respectively, compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus in soils slightly increased with~~in~~ the application of bio fertilization with *Bacillus cereus*; this~~, it~~ was due to the production of organic acids and other chemicals such as citric, tartaric, and oxalic acids ~~which ~~that can stimulate plant growth and nutrient~~s~~ availability. Youssef and Eissa (2017) reported that the increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of mineral nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that the hormone gibberellin and~~,~~ IAA, ~~and other hormones ~~can increase plant growth under stressful conditions. Kang *et al.* (2014) said that PGPB has several mechanisms to increase plant growth with nitrogen-fixation and~~,~~ phosphate solubilization~~ing~~, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from an anaerobic digester include~~:~~ *Ruminococcus* sp., *Thiomargarita* sp., *Clostridium* sp. *Anaerobacter* sp., *Bacillus* sp., *Sporobacterium* sp., *Saccharofermentans* sp., *Oscillibacter* sp., *Sporobacter* sp., and *Enterobacter* sp. Liaquat *et al.* (2017) also reported ~~that an~~ abundance of *Bacillus*, *Clostridium*, and *Enterobacter* spp. in an anaerobic digester of wastewater when~~in~~ producing biogas.

~~The e~~Effect of biogas sludge

The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and 16 ~~WAIBSA~~WAA), total dry weight (12 and 16 ~~WAIBSA~~WAA), nutrient uptake (total ~~-~~N and available ~~-~~P), and the crop growth rate of upland rice at 8 to 12 ~~WAIBSA~~WAA. However,~~,~~ ~~but~~

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What does the “available P” stand for in relation to the nutrient uptake by the plants in this experiment?

it did not have~~ had~~ a~~n~~ ~~in~~significant effect on nutrient content (total ~~-~~N and available ~~-~~P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 ~~WAIBSA~~WAA). An increase in plant height, total dry weight, nutrient uptake in terms of ~~of ~~total ~~-~~N and available ~~-~~P, and also crop growth rate of upland rice on ultisols~~ along~~ with ~~the increase in~~a higher~~ the~~ dose of biogas sludge ~~to ~~of 630 ~~mL~~ml/~~-~~polybag~~$^{-1}$~~ at the end of this study (16 ~~WAIBSA~~WAA). However, in contrast to the total fresh weight, an increase along with the increase in the biogas sludge dose to 315 ~~mL~~ml/~~-~~polybag~~$^{-1}$~~ then decreased at the dose of 630 ~~mL~~ml/~~-~~polybag~~$^{-1}$~~. ~~It was caused~~ T~~t~~he biogas sludge had chemical characteristics such as pH (7.41), total ~~-~~N (0.051%), available ~~-~~P (0.013%), organic ~~-~~C (0.14%), total ~~-~~K (0.18%), and biological characteristics such as total nitrogen-fixing bacteria (29.4×10^5 CFU ~~mL~~ml^{-1}) and total phosphate solubilizing bacteria (7.0×10^4 CFU ~~mL~~ml^{-1}) (Tab.~~le~~ 2). The ~~C ~~organic C content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could ~~be ~~increase~~d~~ the nutrient uptake in terms of ~~of ~~total ~~-~~N and available ~~-~~P in upland rice ~~along ~~with an increasing ~~the ~~dose of biogas sludge of~~to~~ 630 ~~mL~~ml/~~-~~polybag~~$^{-1}$~~ (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the ~~growth of ~~plant height, biomass, and crop growth rate of the upland rice. A similar result was reported by Mustamu and Triyanto (2020) who determined? ~~that ~~the macro and micronutrients from the biogas sludge and ~~also had~~ the population of nitrogen-fixing and phosphate solubilizing bacteria of~~by~~ 480×10^4 and 42×10^4 CFU ~~mL~~ml^{-1}, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that the total phosphate solubilizing bacteria (1.6 to 2.5 CFU ~~mL~~ml^{-1}) was significantly higher compared to nitrogen-fixing bacteria (0.5-~~–~~1.4 CFU ~~mL~~ml^{-1}) showing~~ and~~ a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported that an increase in concentrations of NH_4^+-N ranged from 45 to 80% after anaerobic waste.

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~~The i~~Interaction ~~effect~~ of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice on ultisols at 12-16 ~~WAIBSA~~WAA, but it did not have~~had~~ a~~n~~ ~~in~~significant effect on the other parameters in this study. The interaction of NFB *Bacillus paramycoides* with biogas sludge at the dose of 630 ~~mL~~ml/~~–~~polybag~~$^{-1}$~~ (B1S3) showed the highest crop growth rate of upland rice compared to other interactions and was 5.76-times greater compared to the control. This ~~It~~ was caused by the application of biogas sludge that could have~~be~~ increased soil organic matter and the total population of beneficial bacteria. Likewise, ~~the characteristics of~~ the biogas sludge contained ~~had~~ ~~the~~ organic ~~-~~C ~~was~~ (0.14%), total nitrogen-fixing bacteria ~~was~~ (29.4×10^{5} CFU ~~mL~~ml^{-1}), and total phosphate solubilizing bacteria ~~was~~ (7.0×10^{4} CFU ~~mL~~ml^{-1}) (Tab. 2) that could improve soil quality and support the crop growth rate. This result is supported by Urra *et al.* (2019) who found that the application of sewage sludge in the long ~~-~~term significantly increases the organic matter contents in the soil, causing ~~which causes~~ a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of the organic matter. Bhardwaj *et al.* (2014)~~;~~ and Carvajal-Muñoz and Carmona-Garcia~~*et al.*~~ (2012) showed~~reported~~ that the application of a biofertilizer had advantages in the plant such as availability of nutrients that are balanced for plant health. It also~~,~~ stimulate~~s~~~~ing~~ nutrient mobilization that can increase soil biological activity and the~~,~~ availability of microbial food to encourage the growth of beneficial microorganisms, increasing the soil organic matter content and, therefore, ~~thereby increasing~~ the cation exchange capacity. Siswanti and Lestari (2019) indicated~~reported~~ that the interaction of biogas sludge+biofertilizer (36 ~~mL~~ml+10 L ha^{-1}) significantly increased the plant height, number of leaves, and capcai~~s~~cin content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

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The conclusions aren't the repetition of results. The authors could make an effort to show emerging properties accounted for in the results of this work and enhance the practical application and the investigation novelty.

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The isolates ~~of~~ N3, P7, N3+P7 from the biogas sludge significantly increased the ~~nutrient~~ uptake of total -N (20.77%) and crop growth rate (2.81 times higher than the control?) of upland rice on ultisols with the highest increase found ~~in~~ with the P7 isolate. ~~of 20.77% and 2.81 times, respectively.~~ The dose of biogas sludge significantly increased plant height (14.81%), total dry weight (50.55%), ~~nutrient~~ uptake of total -N (54.11%) and available -P (65.20%), and also crop growth rate (129.44%) of upland rice on ultisols with the highest increase at a dose of 630 ~~mL~~ml /polybag~~$^{-1}$~~. ~~by 14.81,%; 50.55,%; 54.11,%; 65.20,%; and 129.44%, respectively.~~ Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice by 41.81% with the highest increase at the dose of 315 ~~mL~~ml/~~-~~polybag. ~~$^{-1}$~~ ~~by 41.81%.~~ The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice on ultisols 5.76 times with the highest increase found with~~in the~~ B1S3. ~~by 5.76 times.~~

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

All authors formulated the overarching research goals and aims, provided the study materials, developed or designed the methodology. NEM analyzed and ~~interpretation~~ interpreted the study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in the field, ~~data collection~~and collected the data. ZN and I verified the overall reproducibility of results and the other research outputs. All authors conducted the critical review/commentary/revision of the manuscript.

Literature cited

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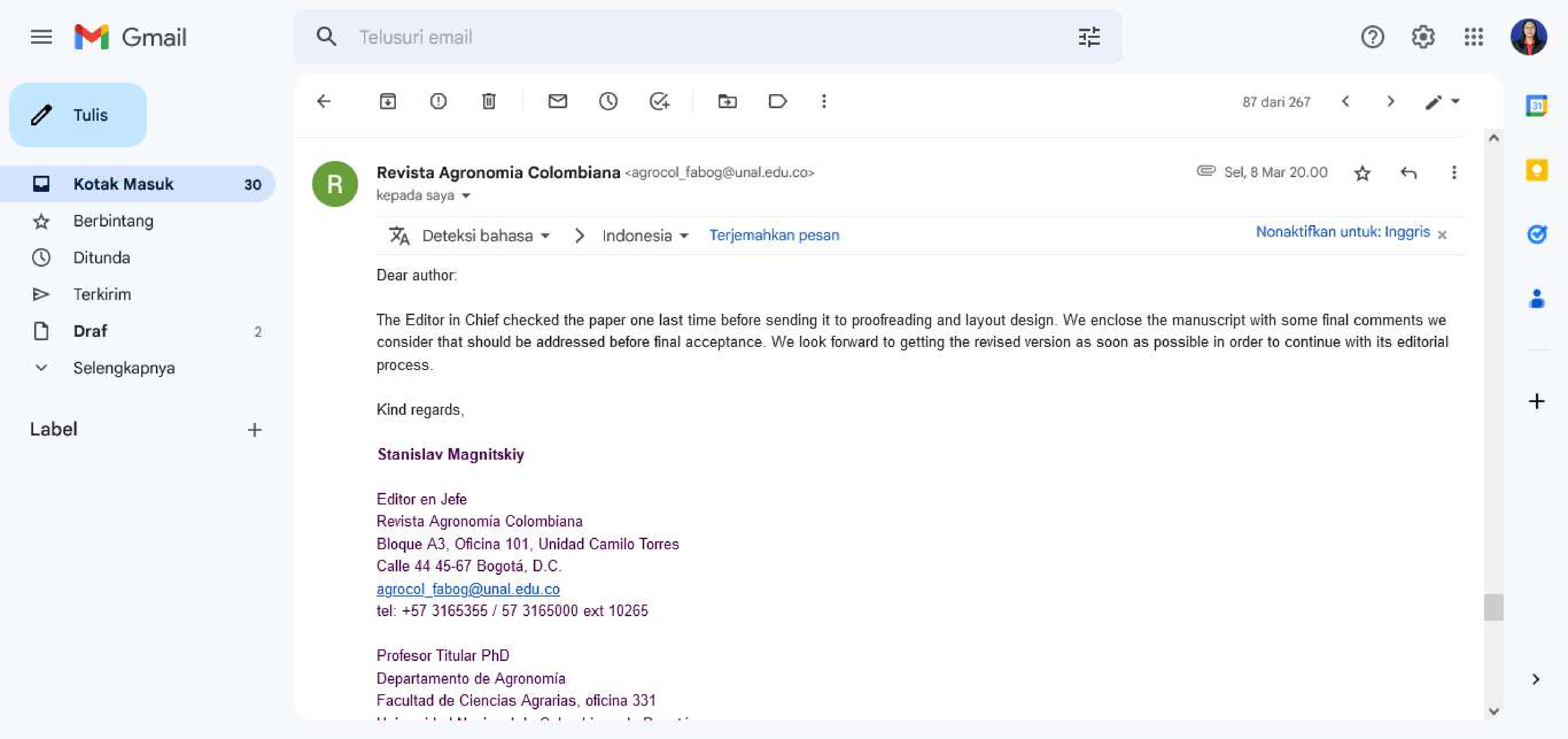

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Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Commented [EDTR1]: Reviewer 1 wrote: I recommend "Evaluation of selected bacterial isolates from biogas sludge in upland rice"

Reviewer 3 wrote: The title don't show effectively the results of investigation. I recommend the title: Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice.
Regarding the term "superior bacterial isolates" reviewer 3 wrote: What the authors wanted to say? I don't understand this term.
This reviewer also wrote: The authors evaluated only the effect of bacteria and biogas sludge on rice growth. They evaluated also the effect of these treatments on rice nutrition!
Finally, regarding the word "ultisols" reviewer 3 wrote: Why is it necessary to specify the soil type?

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Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la nutrición del arroz de secano

Novilda Elizabeth Mustamu[1*], Zulkifli Nasution[2~~*~~], Irvan[3], and Mariani Sembiring[2]

[1]Doctoral Program in Agricultural Sciences, Faculty of Agriculture, Universitas Sumatera Utara, Sumatera Utara (Indonesia).
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ABSTRACT

This study evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth and nutrient uptake of upland rice grown in Ultisols. We used a randomized block design with two factors and seven replicates from October 2020 to April 2021. The first factor used selected SBI (B0 = untreated, B1 = nitrogen-fixing bacteria isolate (N3), B2 = phosphate solubilizing bacteria isolate (P7), B3 = isolate combination (N3+P7)). The second factor was the dosage of biogas sludge (S0 = untreated, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). The parameters were determined by ANOVA and followed by Duncan's multiple range test at *P*<0.05. The results showed that the isolate P7 significantly increased the N uptake by 20.77% and ~~the highest~~ crop growth rate (CGR) of upland rice 2.81 times. Biogas sludge doses from 315 to 630 ml/polybag significantly increased plant height, ~~nutrient~~ uptake of N and P, total fresh and dry weight, and CGR of upland rice. The interaction ~~of~~ between N3 ~~with~~ and biogas sludge dosage of 630 ml/polybag significantly increased the CGR of upland rice. The application of isolates N3 and P7 and their combination within biogas sludge of 630 ml/polybag has the potential to increase the CGR of upland rice in acidic soils.

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Key words: acidic soil, crop growth rate, dosage, sludge potential.

RESUMEN

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El presente estudio evaluó la influencia de aislamientos bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones sobre el crecimiento y la absorción de nutrientes en el arroz de tierras altas cultivado en ultisoles. Se utilizó un diseño de bloques al azar con dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0 = sin tratamiento, B1 = aislamiento de bacterias fijadoras de nitrógeno (N3), B2 = aislamiento de bacterias solubilizantes de fosfato (P7), B3 = combinación de aislados (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (S0 = sin tratamiento, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Los parámetros fueron determinados por análisis de varianza y seguidos de la prueba de rangos múltiples de Duncan a $P<0.05$. Los resultados mostraron que el aislamiento P7 aumentó significativamente la absorción de N en un 20.~~,~~77% y la ~~mayor~~ tasa de crecimiento del cultivo (TCC) de arroz de tierras altas 2.81 ~~-~~veces. Las dosis de lodos de biogás de 315 a 630 ml/polybag aumentaron significativamente la altura de la planta, la absorción ~~de nutrientes~~ de N y P, el peso fresco y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosis de lodos de biogás de 630 ml/polybag aumentó significativamente la TCC del arroz de tierras altas. La aplicación de los aislamientos N3 y P7 y su combinación dentro de lodos de biogás de 630 ml/polybag tiene el potencial de aumentar el TCC de arroz de tierras altas en suelos ácidos.

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I recommend: "Los resultados mostraron que la inoculación aumentó… pues el aislado por sí solo no produce el efecto, sino que hay que inocularlo".

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Palabras clave: suelo ácido, tasa de crecimiento de cultivos, dosis, potencial de lodo~~,~~.

Introduction

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Furthermore, the authors must enhance the investigation novelty.

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Biogas sludge is the waste by-product from an anaerobic processing system (FAO, 1977) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and the plants yield (Adela *et al.*, 2014). The following characteristics of the biogas sludge from palm oil waste have been reported: total N of 490 mg L^{-1}, total P of 110 mg L^{-1}, total K of 1.9 mg L^{-1} (Lubis *et al.*, 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al.*, 2019), and NH_3-N of 91 -112 mg L^{-1} (Choorit & Wisarnwan, 2007). The pH may range from 6.8 to 8.3, with the highest bacterial population of 7.21×10^{7} cells per ml and the lowest one of 3.15×10^{7} cells per ml (Alvionita *et al.*, 2019). Additionally, Mustamu and Triyanto (2020) reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria that have the potential to increase the availability of nitrogen and phosphate in soils.

The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing bacteria has a greater potential to increase soil fertility and plant growth. Zhang *et al.* (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility, and plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described different *Bacillus* species, such as *B. circulans, B. cereus, B. fusiformis, B. pumilus, B. megaterium, B. mycoides, B. coagulans, B. chitinolyticus,* and *B. subtilis* as phosphate solubilizing microorganisms. Ambrosini *et al.* (2016) showed the highest nitrogenase activity in *Bacillus cereus* among 42 different strains of *Bacillus* spp. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, *i.e*., *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Thermotogae*.

The application of bacteria from biogas sludge has never been reported in Indonesia for improving upland rice growth on acidic soils, including~~e~~ Ultisols. According to the Pusat Penelitian Tanah dan Agroklimat (Center for Soil and Agro-climate Research)~~,~~ (2000), ~~found that~~ the area in Indonesia covered by Ultisols was 45.8 million ha, or 24% of the total area of Indonesia. Furthermore, according to the Ministry of Agriculture, the area ~~of~~ dedicated to rice

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~~growing~~ cultivation in Indonesia was 15,712,025 ha with a ~~the~~ yield of 81,148,617 t~~on~~ ha^{-1} in 2017 and the contribution of upland rice yield reached~~s~~ 4.66% (Kementerian Pertanian, ~~Ministry of Agriculture,~~ 2017). The yield contribution of upland rice was classified as low and, therefore, it is necessary to ~~develop~~find options in order to~~ ~~increase ~~yield~~ it~~through biogas sludge~~. Thus, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge to increase the availability of nitrogen and phosphate, and the growth response of upland rice due to application of the biogas sludge and selected isolates in Ultisols. The study aimed to evaluate the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the mineral nutrition of the upland rice grown in Ultisols.

Materials and methods

Study area

The concentration of total N and available P in Ultisols and in the plant tissue (N and P uptake) were analyzed in the Analytical Laboratory of Socfin Indonesia Inc., Medan (Indonesia). The bacterial isolates were applied to upland rice in the village of Padang Bulan (3°37.760' N; 98°38.898' E; altitude 18 m a.s.l.), Medan Selayang Subdistrict, Medan City, Indonesia, from October 2020 to April 2021. The average temperature was 27.4°C, the average air humidity, was 82% and average rainfall was ~~in this study were 27.4°C, 82%, and~~ 228.5 mm per month~~, respectively~~.

Preparation of medium and upland rice seeds

The medium to grow upland rice plants used the Ultisols from the Simalingkar area, Medan Tuntungan Subdistrict, Medan City, at a depth of 0 to 20 cm. One hundred g of soil samples were taken and analyzed for chemical characteristics such as pH using H_2O, organic C by Walkley-Black, available P by Bray-II, total N using Kjeldahl method, and cation exchange

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capacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH 7 method (Tab. 1). The soil was sterilized by drying at 100°C for 2 h. For preventing heat from the sterilization process, the soil was~~is~~ incubated for 1 d and then placed into a 10 kg polybag (18 cm × 18 cm). A basic NPK fertilizer (16-16-16) by Meroke Tetap Jaya Inc., at a dose of 1.5 g/polybag was applied by stirring evenly with the soil. The seeds of upland rice (*Oryza sativa* L.) ~~used in the~~were of the inbred variety Inpago-8 ~~inbred variety were of~~from the ~~-~~Indonesian Agency for Agricultural Research and Development~~,~~ ~~then~~ were soaked in water for 24 h, followed by the~~a~~ application of the fungicide Propineb ~~fungicide~~ (70%) ~~application~~ for 2 h. Upland rice was planted after 1 d of basic fertilization with two seeds per polybag at a depth of 2 cm.

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TABLE 1. Chemical characteristics of the Ultisols soil samples after sterilization at 100°C.

Chemical characteristics	Value	Category*
Soil pH (H_2O)	4.80	Acid
Organic C (%)	0.44	Very low
Total N (%)	0.04	Very low
Available P (mg kg^{-1})	870.25	Very high
CEC (meq 100 g^{-1})	28.31	High
Base saturation (%)	4.85	Very low
Exchangeable cations		
K (meq 100 g^{-1})	0.60	High
Ca (meq 100 g^{-1})	0.34	Very low
Mg (meq 100 g^{-1})	0.32	Very low
Na (meq 100 g^{-1})	0.09	Very low
Al (%)	0.02	Very low

*Criteria for pH (H_2O) = 4.5-5.5 (acid); organic C <1% (very low); total N <0.1% (very low); available P >60 mg kg^{-1} (very high); Cation exchange capacity (CEC) = 25-40 meq 100 g^{-1} (high); base saturation <20% (very low); exchangeable K= 0.60-1.00 meq 100 g^{-1} (high); exchangeable Ca <2 meq 100 g^{-1} (very low); exchangeable Mg <0.4 meq 100 g^{-1} (very low); exchangeable Na <0.1 meq 100 g^{-1} (very low); exchangeable Al <5% (very low) (Balai Penelitian Tanah (Indonesia Soil Research Institute), 2009).

Commented [EDTR21]: Reviewer 3 wrote: I think that it is irrelevant. I recommend to write the techniques that allow the soil characteristics and then state the bibliographic cites correspondingly.

Commented [EDTR22R21]: The authors neither replied to this comment nor changed the table's footnote.

Preparation of superior bacterial isolates suspension and biogas sludge

A total of 1 ml of the bacterial isolate suspension obtained from the characteristic stage was put into a test tube containing 9 ml of distilled water and homogenized. ~~It put a total of 1 ml of the suspension from the dilution into 9 ml of distilled water.~~ The dilution was made to 10^{-5}. A total of 0.1 ml of the suspension from the last dilution was spread over the James n~~N~~itrogen f~~F~~ree m~~M~~alat b~~B~~romothymol b~~B~~lue (JNFB) medium for the nitrogen-fixing bacterial isolates test.

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While the suspension phosphate solubilizing bacteria isolates, the test was spread over Pikovskaya (PVK) medium. The culture medium was incubated for 2 to 3 d~~ays~~ at room temperature. The nitrogen-fixing bacterial isolate test was characterized by the presence of colonies growing on the JNFB medium. The growth of phosphate solubilizing bacterial isolates was~~is~~ indicated by a halo zone around the microbial colonies on the PVK medium. ~~The result was found in~~ S~~s~~even nitrogen-fixing and seven phosphate-solubilizing isolates were found to produce total-N and available-P. The isolates ~~of superior bacteria were selected which~~that ~~had~~ showed the highest phosphate and nitrogen increasing abilities were selected, namely phosphate solubilizing bacteria (P7) and nitrogen-fixing bacteria (N3), which were confirmed by Mustamu *et al.* (2021a, 2021b).

The biogas sludge was collected by the fixed tank of digester at the palm oil mill of Nubika Jaya Inc., Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia, and then handled with tongs. Bacterial isolates and biogas sludge were applied to the soil surface at the base of the plants at one week after planting. Biogas sludge samples at a 500 ml volume were used to analyze the chemical and biological characteristics (Tab. 2).

TABLE 2. The chemical and biological characteristics of the biogas sludge.

Characteristics	**Method**	**Value**
pH	Electrometry	7.41
Chemical oxygen demand (mg L^{-1})	Spectrophotometry	4547.8
Biological oxygen demand (mg L^{-1})	Titrimetry	1127.5
Total N (%)	Kjeldahl	0.051
Total P (%)	Spectrophotometry	0.0097
Available-P (%)	Spectrophotometry	0.013
Total K (%)	Graphite furnace - atomic absorption spectrophotometry (AAS)	0.18
Organic C (%)	Walkley-Black	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (mg L^{-1})	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Total nitrogen-fixing bacteria (CFU ml^{-1})	Plate count	29.4×10^5
Total phosphate solubilizing bacteria (CFU ml^{-1})	Plate count	7.0×10^4

Source: laboratory analysis

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Commented [EDTR26]: What medium is this?

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The isolates were grown on NB medium, and incubated for 48 h. The bacterial growth in the solution was measured using an spectrophotometer with a density of 108 cells per ml.

Commented [EDTR28]: The Editor in Chief asked: Could you indicate which species or hybrid of palm was employed? This may have an effect on the sludge composition.

Commented [EDTR29R28]: The authors did not reply.

Commented [EDTR30]: Reviewer 3 wrote: I think that the authors must give more details about how biogas sludge was taken.

Commented [EDTR31R30]: The authors did not add any information.

Commented [EDTR32]: Reviewer 3 wrote: The authors must say how the chemical and biological characteristics of biogas sludge were determined, or make a bibliography cite.

The Editor in Chief also wrote: Please, provide the bibliographic reference of the manual used for the biogas sludge analysis.

Commented [EDTR33R32]: The authors did not add a citation.

Commented [EDTR34]: Reviewer 1 asked: Is it correct? It can be a very high amount of sodium.

Commented [EDTR35R34]: The authors did not answer.

Treatment application

This study used a randomized block design with two factors and seven replicates. The first factor was the type of superior bacterial isolates (B0 = untreated; B1 = nitrogen-fixing bacterial isolate (N3); B2 = phosphate solubilizing bacteria isolate (P7); B3 = combination of isolates N3+P7) at a similar dose, namely 10 ml/polybag. The second factor was dose of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Determination of biogas sludge based on the dose of liquid organic fertilizer at the oil palm was 126 m^3 ha^{-1} equal to 126,000 L ha^{-1} (Sutarta *et al.*, 2003), then converted to soil weight per polybag (Eq. 1). Each replicate was disassembled at 4, 8, and 12 weeks after ~~the ~~application (WAA) of what? for determination of the crop growth rate (CGR).

Commented [EDTR36]: Reviewer 3 wrote: Why the authors use these biogas sludge concentrations. I think it is important!

Commented [EDTR37R36]: The authors did not reply.

Commented [EDTR38]: What do you mean by disassembled?

$$\text{Biogas sludge} = \frac{\text{The dose of liquid organic fertilizer ha}^{-1}}{\text{Soil weight ha}^{-1}} \times \text{soil weight per polybag} \qquad (1)$$

$$= \frac{26{,}000\ \text{L ha}^{-1}}{2{,}000{,}000\ \text{kg ha}^{-1}} \times 10\ \text{kg} = 630\ \text{ml}$$

Parameters and data analysis

The observations of the variables were conducted by measuring the growth of upland rice (plant height, and total fresh and dry weight), ~~nutrient ~~contents and uptake of N and P in the shoots, and CGR. The plant height was measured from~~by~~ the base of the roots to the tip of leaves using a measuring tape?, and the total fresh weight was obtained by weighing the roots and shoots. The total dry weight (roots + shoots) was measured after using an oven (model VS-1202D3, Vision Scientific Co., ~~Ltd., ~~Korea) at 60°C for 48 h and weighed using the analytical scales. A 200 g sample of the second leaf from the shoots was collected and analyzed to determine the N content using the Kjeldahl and the P content was estimated using the destruction method through~~t~~ dry ashing. The N and P uptake were measured using Equation 2. The CGR was calculated as the dry weight related to the unit area at 4-8, 8-12, and 12-16 WAA using Equation 3 (Shon *et al.*, 1997):

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Nutrient uptake = nutrient content in the shoots × total dry weight (2)

$$\text{CGR} = \frac{\Delta W}{\Delta t} = \frac{W2 - W1}{t2 - t1} \qquad (3)$$

where:

CGR = crop growth rate;

W1 = dry weight per unit area at t1;

W2 = dry weight per unit area at t2;

t1 = first sampling;

t2 = second sampling;

The parameters of the second phase of the study were analyzed by an ANOVA and if the treatment had a significant effect, followed by Duncan's multiple range test at *P*<0.05 using SPSS v.20 software (IBM, 2011).

Results

Effect of bacterial isolates and biogas sludge on upland rice growth

Plant height of upland rice

The effect of biogas sludge application was significant on the plant height of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the plant height of upland rice at 4, 8, 12, and 16 WAA (Fig. 1). A significant increase in plant height of upland rice was observed with ~~increased~~ higher doses of biogas sludge of 630 ml/polybag at 8, 12, and 16 WAA with the highest increase of 14.81% compared to the control at 16 WAA. Although the effect was not significant, the combination of isolates B3 and the interaction of B2S3 showed the highest in plant height of upland rice by 2.94 and 22.06%, respectively, compared to the control.

Commented [EDTR41]: Reviewer 1 wrote: I recommend that the results part be restructured in its figures and tables. It could only show figures that show a significant difference, without showing those that do not have a significant difference.
Reviewer 3 wrote: It is necessary write Results and discussion again in other form. All the information of this section must be re-organized. Account of the statistical analysis made and some information in figures and tables is irrelevant. The figures have repeated information.

Commented [EDTR42R41]: The authors did not reply to these comments.

Commented [EDTR43]: Reviewer 3 wrote: I consider that it is necessary to write again Results and discussion in other form. The information of this section must be re-organized in two aspects: (1) Effect of bacterial isolates and biogas sludge on upland rice growth. (2) Effect of bacterial isolates and biogas sludge on upland rice nutrition. The first point could include:
- Plant height of upland rice
- Biomass of upland rice
- Crop growth rate of upland rice
The second point could include:
- Content of total-N and available-P nutrient of upland rice
- Uptake of total-N and available-P nutrient of upland rice
Furthermore, for both points the analysis made from lines 249-332 must be undertaken.

Commented [EDTR44R43]: It seems the authors reorganized these sections following the reviewer's suggestion. However, they did not reply to the comment.

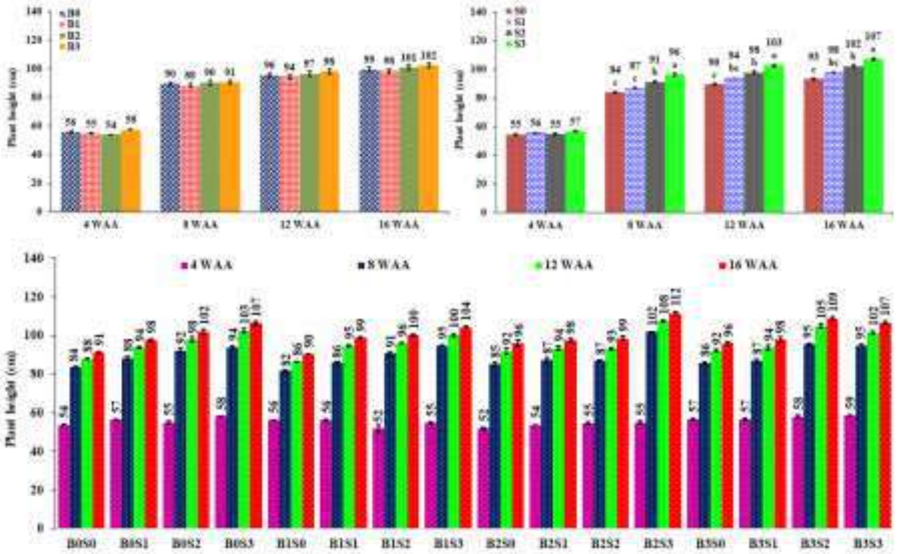

FIGURE 1. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on plant height of upland rice at 4, 8, 12, and 16 WAA. Values followed by the different letter in the graph significantly differed according to the Duncan test at *P*<0.05. ns - not significant. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un~~-~~treated; B1 = isolate N3, B2 = isolate P7;~~,~~ B3 = isolates N3+P7 ~~isolates~~).

Commented [EDTR45]: Please add these graphs as independent files to ensure they have the appropriate resolution and quality.

Commented [EDTR46]: Reviewer 3 wrote: If all the treatments (B x S) are NS then there was not interaction between the factors. Then it is unnecessary to show the third figure. I recommend to show only first and second figures. The authors could explore the possibility that gives them not interaction between the factors for discussing the result.

Commented [EDTR47R46]: The authors did not reply to this comment.

Biomass of upland rice

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the total fresh weight of upland rice at 4-16 WAA (Tab. 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total fresh weight (shoot+roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Commented [EDTR48]: Reviewer 3 wrote: If all the treatments (B x S) are NS then there was not interaction between the factors. Then it is unnecessary to show the interaction.

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Treatments	**Total fresh weight ± standard error (g)**			
	4 WAA	**8 WAA**	**12 WAA**	**16 WAA**
Superior bacterial isolates (B)				
B0	4.15 ± 0.21	169.31 ± 8.90	215.27 ± 8.42	229.82 ± 8.94
B1	3.12 ± 0.12	194.50 ± 9.35	235.08 ± 10.32	252.02 ± 10.22
B2	4.52 ± 0.23	162.89 ± 11.15	201.85 ± 9.89	230.70 ± 9.28
B3	3.30 ± 0.25	173.91 ± 12.55	220.40 ± 15.96	245.03 ± 16.32
Biogas sludge (S)				
S0	3.72 ± 0.24	144.07 ± 9.37 b	182.67 ± 7.14 b	197.56 ± 6.58 b
S1	3.58 ± 0.27	153.41 ± 7.93 b	190.70 ± 8.90 b	215.65 ± 7.03 b
S2	3.64 ± 0.27	199.68 ± 10.30 a	258.70 ± 9.63 a	280.15 ± 9.25 a
S3	4.15 ± 0.25	203.45 ± 1.36 a	240.52 ± 2.81 a	264.21 ± 2.42 a
Interactions (B×S)				
B0S0	4.99 ± 0.33	124.08 ± 5.60	185.64 ± 3.32	192.78 ± 2.96

B0S1	3.47 ± 0.26	160.43 ± 1.16	188.60 ± 5.76	207.05 ± 3.97
B0S2	3.42 ± 0.42	185.97 ± 6.80	232.60 ± 8.75	250.84 ± 7.40
B0S3	4.71 ± 0.42	206.76 ± 5.49	254.23 ± 10.27	268.61 ± 8.85
B1S0	2.80 ± 0.18	155.79 ± 1.12	183.96 ± 5.20	202.88 ± 2.88
B1S1	3.74 ± 0.29	174.82 ± 9.01	227.91 ± 6.38	236.60 ± 6.32
B1S2	3.28 ± 0.40	241.17 ± 5.25	283.60 ± 7.76	296.08 ± 8.05
B1S3	2.67 ± 0.22	206.20 ± 7.23	244.85 ± 6.26	272.52 ± 4.34
B2S0	3.19 ± 0.18	190.90 ± 7.77	215.36 ± 7.67	229.11 ± 6.75
B2S1	4.85 ± 0.38	106.74 ± 13.42	143.16 ± 13.02	179.61 ± 10.36
B2S2	5.20 ± 0.24	148.40 ± 11.59	219.65 ± 5.26	248.72 ± 6.94
B2S3	4.82 ± 0.45	205.53 ± 10.50	229.21 ± 16.57	265.34 ± 9.58
B3S0	3.91 ± 0.30	105.53 ± 3.94	145.72 ± 1.96	165.45 ± 1.11
B3S1	2.25 ± 0.09	171.63 ± 4.90	203.14 ± 7.07	239.34 ± 12.07
B3S2	2.66 ± 0.14	223.17 ± 7.84	298.95 ± 1.51	324.94 ± 3.03
B3S3	4.37 ± 0.07	195.31±6.77	233.79 ± 8.40	250.38 ± 8.16
CV (%)	56.09	29.68	26.31	20.78

Commented [EDTR50]: Reviewer 1 wrote: This is not an interaction assessment, it is the treatments. In the ANOVA of this trial there are 16 treatments and 112 experimental units. The sources of variation evaluated would be (treatments, bacteria, sludge, and bacteria x sludge interaction). And there may be a significant difference in treatments without any interaction between the factors.

Commented [EDTR51R50]: The authors did not reply to this comment.

Values followed by the different letter in the column significantly differed according to the Duncan test at *P*<0.05. ns - not significant~~ly;~~, CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7 ~~isolates~~).

A significant increase in the total fresh weight of upland rice was observed with the higher dose of biogas sludge of 315 ml/polybag at 16 WAA, with the highest increase of 41.81% compared to the control. Although the effect was not significant, B1 and the interaction of B3S2 showed the highest in the total fresh weight of upland rice with 9.66 and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4 -16 WAA (Tab. 4).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight (shoot+roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Commented [EDTR52]: Reviewer 3 wrote: If all the treatments (B x S) are NS, there was not interaction between the factors. Then it is unnecessary to show the interaction.

Commented [EDTR53R52]: The authors did not reply to this comment.

Treatments	**Total dry weight ± standard error (g)**			
	4 WAA	**8 WAA**	**12 WAA**	**16 WAA**
Superior bacterial isolates (B)				
B0	1.38 ± 0.06	48.01 ± 1.29	73.60 ± 3.99	82.52 ± 4.18
B1	1.13 ± 0.05	54.09 ± 2.41	76.83 ± 2.66	99.72 ± 4.15
B2	1.49 ± 0.06	47.30 ± 3.30	73.20 ± 2.28	98.25 ± 3.90
B3	1.15 ± 0.07	52.32 ± 3.39	77.18 ± 4.90	98.47 ± 4.56
Biogas sludge (S)				
S0	1.26 ± 0.06	45.51 ± 2.63	62.88 ± 2.19 b	76.78 ± 1.63 c
S1	1.23 ± 0.08	44.47 ± 1.71	68.52 ± 2.00 ab	87.65 ± 2.84 bc
S2	1.26 ± 0.08	55.36 ± 3.43	85.69 ± 1.08 a	98.95 ± 1.86 b
S3	1.40 ± 0.06	56.38 ± 1.05	83.73 ± 3.44 a	115.59 ± 2.11 a
Interactions (B×S)				

B0S0	1.58 ± 0.08	41.73 ± 2.78	58.08 ± 1.54	67.23 ± 0.96
B0S1	1.12 ± 0.08	45.87 ± 0.83	62.74 ± 1.83	71.08 ± 1.91
B0S2	1.20 ± 0.12	52.25 ± 2.07	81.39 ± 5.48	88.28 ± 5.02
B0S3	1.60 ± 0.12	52.18 ± 0.29	92.20 ± 3.05	103.49 ± 2.43
B1S0	0.97 ± 0.04	46.64 ± 1.39	69.53 ± 4.90	80.30 ± 4.51
B1S1	1.40 ± 0.07	48.13 ± 2.78	78.91 ± 0.53	96.23 ± 1.50
B1S2	1.12 ± 0.10	67.79 ± 1.44	91.05 ± 2.25	101.80 ± 2.40
B1S3	1.02 ± 0.08	53.81 ± 3.76	67.84 ± 1.77	120.54 ± 2.15
B2S0	1.17 ± 0.05	59.32 ± 2.33	70.92 ± 4.20	81.43 ± 3.82
B2S1	1.54 ± 0.10	34.47 ± 2.16	61.69 ± 1.97	89.84 ± 1.41
B2S2	1.73 ± 0.05	37.37 ± 3.74	83.10 ± 1.19	105.46 ± 1.37
B2S3	1.53 ± 0.10	58.05 ± 1.76	77.07 ± 4.27	116.28 ± 1.30
B3S0	1.30 ± 0.07	34.35 ± 7.04	52.98 ± 0.73	78.16 ± 0.48
B3S1	0.85 ± 0.03	49.40 ± 0.08	70.72 ± 1.29	93.44 ± 2.19
B3S2	0.99 ± 0.05	64.05 ± 4.68	87.22 ± 2.90	100.26 ± 1.93
B3S3	1.44 ± 0.02	61.48 ± 2.47	97.80 ± 0.77	122.04 ± 0.20
CV (%)	43.80	31.22	26.54	18.38

Values followed by the different letter in the column significantly differed according to the Duncan test at *P*<0.05. ns= not significant~~ly;,~~ CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7~~ isolates~~).

A significant increase in total dry weight of upland rice was observed with the increase in the dosage of biogas sludge of 630 ml/polybag at 16 WAA, with the highest increase of 50.55% compared to the control. Although the effect was not significant, B1 and the interaction of B3S3 showed the highest in the total dry weight of upland rice with 20.84 and 81.53%, respectively, compared to the control.

Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12 to 16 WAA, but it did not have a significant effect at 4-8 and 8-12 WAA (Tab. 5).

TABLE 5. Effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of the upland rice~~.~~ 4, 8, 12, and 16 weeks after the application (WAA).

Superior bacterial isolates (B)	**Biogas sludge (S)**				**Average**
	S0	**S1**	**S2**	**S3**	
	4-8 WAA				
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV = 32.28%

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			8-12 WAA		
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888
Average	0.620	0.859	1.083	0.977	CV = 56.17%
			12-16 WAA		
B0	0.327 fgh	0.298 gh	0.246 h	0.403b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV = 51.07%

Values followed by the different letter in the column significantly differed according to the Duncan test at *P*<0.05. ns - not significant~~ly;~~, CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7~~ isolates~~).

The biogas sludge dose of 630 ml/polybag (S3) significantly increased the highest crop growth rate for upland rice at 12 to 16 WAA by 129.44% compared to the control. The isolates B1-B3 significantly increased the crop growth rate of upland rice with the highest increase for B2 of 181.45% compared to the controls at 12 to 16 WAA. The interaction of the B1S3 significantly increased the crop growth rate of upland rice, showing values 5.76 ~~-~~times greater than those of the control.

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Effect of bacterial isolates and biogas sludge on upland rice nutrition

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~~Nutrient~~ C~~c~~ontent of N and P in the upland rice

Commented [EDTR64]: This expression does not make sense. The right phrase is content of N and P.

The effect of biogas sludge, superior bacterial isolates, and their interactions did not have a significant effect on the~~ nutrient~~ content of N and P in the upland rice (Fig. 2). The biogas sludge doses of 315 and 630 ml/polybag (S2 and S3) explained that the contents of P and N in the plant tissue of upland rice were 33.33 and 4.53% higher, respectively, compared to the control. The isolate B2 showed the highest content of N in the plant tissue of upland rice with values 1.63% higher than those of the control; however, all isolates (B1-B3) showed a similar level of P in the plant tissue of upland rice compared to the control.

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Commented [EDTR67]: Reviewer 1 wrote: there is no significant difference should not be analyzed, the differences are given only by chance.

Commented [EDTR68R67]: The authors did not reply to this comment.

~~Nutrient u~~Uptake of N and P in the upland rice

The effect of biogas sludge significantly increased in the ~~nutrient~~ uptake of N and P. The superior bacterial isolates significantly increased in the ~~nutrient~~ uptake of nitrogen. The interaction of biogas sludge with superior bacterial isolates did not show a significant effect on the nutrient uptake of N and P in the upland rice (Fig. 2).

A significant increase in the ~~nutrient~~ uptake of N and P in upland rice was observed with a higher dose of biogas sludge of 630 ml/polybag, with the highest increases of 54.11 and 65.20%, respectively, compared to the control. The ~~superior~~ bacterial isolates ~~(~~B1-B3~~)~~ also significantly increased the ~~nutrient~~ uptake of N in the upland rice with the highest increase with the B2 of 20.77% compared to the control. Although the effect was not significant, B3 showed the highest ~~in nutrient~~ uptake of P in the upland rice of 19.93% compared to the control.

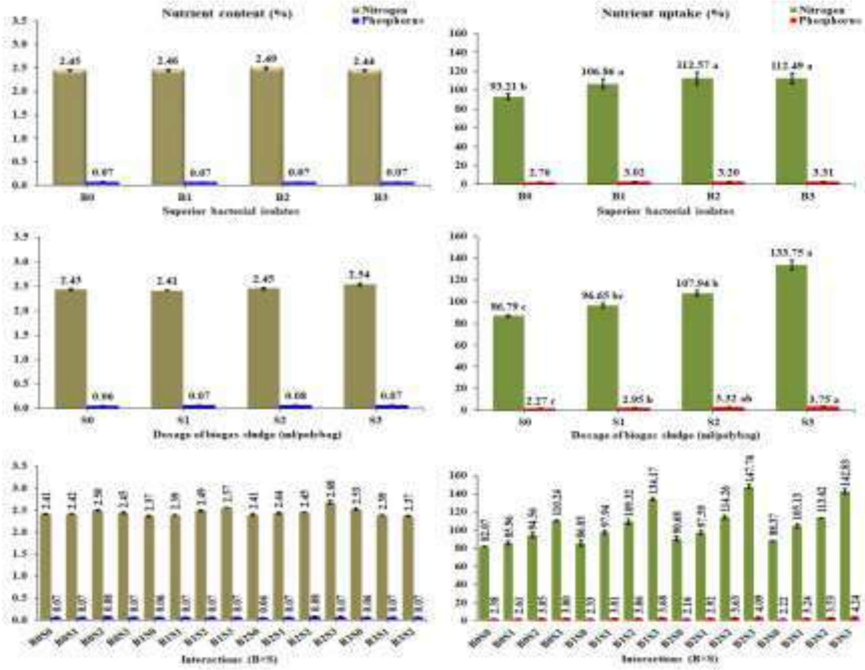

FIGURE 2. The effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on the nutrient content and uptake of N and P in the upland rice. Values followed by different letters significantly differed according to the Duncan test at $P<0.05$. ns - not significant. Dosage of biogas sludge (S0 = untreated; S1 = 157.5;

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You can use the phrase "nutrient uptake" when referring to the nutrients the plant absorbs in general. Nutrient uptake of N and P is redundant and does not make any sense. Please use only "uptake of N and/or P"

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Please add these graphs as independent files to ensure they have the appropriate quality and resolution.

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S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = N3+P7 isolates).

Discussion

Effect of selected superior bacterial isolates

The selected superior bacterial isolates (N3 and P7) significantly increased the ~~nutrient~~ uptake of nitrogen and crop growth rate of upland rice on Ultisols at 12 to 16 WAA, but it did not have a significant effect on plant height, total fresh weight, total dry weight, nutrient content (N and P) in leaf tissue, ~~nutrient~~ uptake of phosphorus, and crop growth rate of upland rice at 4 to 8 and 8 to 12 WAA. The superior bacterial isolates (N3, P7, and N3+P7) could increase the ~~nutrient~~ uptake of nitrogen in upland rice by 14.64%, 20.77%, and 20.68%, respectively, compared to the control (Fig. 2). Similar results are also shown in Table 5, where can be observed that the crop growth rate of upland rice at 12 to 16 WAA has increased 2.57, 2.81, and 2.39 times, respectively due to the selected superior bacterial isolates (N3, P7, N3+P7), compared to the control. The results indicate that the ability of a single P7 bacterial isolate was greater in increasing the nitrogen and crop growth rate of upland rice compared to a single N3 isolate and the combination of N3+P7 isolates. This was due to the presence of several organic acids and hormones produced by P7 that can increase the ~~nutrient~~ uptake of nitrogen and crop growth rate of upland rice. This result is supported by Mustamu *et al.* (2021a) who found that the phosphate solubilizing bacterial isolate (P7) from the biogas sludge contains organic acids such as lactic, oxalic, acetic, and citric acids, and had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate with values 4.62 and 2.66 times higher, respectively, compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus in soils slightly increased with the application of bio fertilization with *Bacillus cereus*; this was due to the production of organic acids and other chemicals such as citric, tartaric, and oxalic acids that can stimulate plant growth and nutrient availability. Youssef

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Commented [EDTR74]: The phrase "nutrient uptake of N" has no physiological sense. Please re-write these phrases using different terms and Eissa (2017) reported that the increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of mineral nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that the hormone gibberellin and IAA, can increase plant growth under stressful conditions. Kang *et al.* (2014) said that the p~~P~~lant g~~G~~rowth-p~~P~~romoting b~~B~~acteria (PGPB) has several mechanisms to increase plant growth with nitrogen-fixation and phosphate solubilization, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from an anaerobic digester include *Ruminococcus* sp., *Thiomargarita* sp., *Clostridium* sp. *Anaerobacter* sp., *Bacillus* sp., *Sporobacterium* sp., *Saccharofermentans* sp., *Oscillibacter* sp., *Sporobacter* sp., and *Enterobacter* sp. Liaquat *et al.* (2017) also reported abundance of *Bacillus, Clostridium*, and *Enterobacter* spp. in an anaerobic digester of wastewater when producing biogas.

Effect of biogas sludge

The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and 16 WAA), total dry weight (12 and 16 WAA), nutrient uptake (N and P), and the crop growth rate of upland rice at 8 to 12 WAA. However, it did not have a significant effect on nutrient content (N and P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAA). An increase in plant height, total dry weight, nutrient uptake in terms of nitrogen and phosphorus, and also crop growth rate of upland rice on Ultisols with a higher dose of biogas sludge of 630 ml/polybag at the end of this study (16 WAA). In contrast, the total fresh weight ~~had an~~ increased~~ing~~ along with the ~~increase at the~~higher dose of biogas sludge to 315 ml/polybag and then decreased at the dose of 630 ml/polybag. This result is supported the biogas sludge had chemical characteristics such as pH (7.41), total N (0.051%), available P (0.013%), organic C (0.14%), total K (0.18%), and biological characteristics such as total nitrogen-fixing bacteria

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(29.4×10^5 CFU ml^{-1}) and total phosphate solubilizing bacteria (7.0×10^4 CFU ml^{-1}) (Tab. 2). The organic C content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could increase the nutrient uptake in terms of nitrogen and phosphorus in upland rice with an increasing dose of biogas sludge of 630 ml/polybag (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the plant height, biomass, and crop growth rate of the upland rice. A similar result was reported by Mustamu and Triyanto (2020) who determined the macro and micronutrients from the biogas sludge and the population of nitrogen-fixing and phosphate solubilizing bacteria of 480×10^4 and 42×10^4 CFU ml^{-1}, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that the total phosphate solubilizing bacteria (1.6 to 2.5 CFU ml^{-1}) was significantly higher compared to nitrogen-fixing bacteria (0.5-1.4 CFU ml^{-1}) showing a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported that an increase in concentrations of NH_4^+-N ranged from 45 to 80% in the anaerobic waste.

Interaction of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice on Ultisols at 12-16 WAA, but it did not have a significant effect on the other parameters in this study. The interaction of B1 with biogas sludge at the dose of 630 ml/polybag (B1S3) showed the highest crop growth rate of upland rice compared to other interactions and was 5.76 -times greater compared to the control. This was caused by the application of biogas sludge that could have increased soil organic matter and the total population of beneficial bacteria. Likewise, the biogas sludge contained organic C (0.14%), total nitrogen-fixing bacteria (29.4×10^5 CFU ml^{-1}), and total phosphate solubilizing bacteria (7.0×10^4 CFU ml^{-1}) (Tab. 2) that could improve soil quality and support the crop growth rate.

This result is supported by Urra *et al.* (2019) who found that the application of sewage sludge in the long term significantly increases the organic matter contents in the soil, causing a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of the organic matter. Bhardwaj *et al.* (2014); Carvajal-Muñoz and Carmona-Garcia (2012) showed that the application of a biofertilizer had advantages in the plant such as availability of nutrients that are balanced for plant health. It also stimulates nutrient mobilization that can increase soil biological activity and the availability of microbial food to encourage the growth of beneficial microorganisms, increasing the soil organic matter content and, therefore, the cation exchange capacity. Siswanti and Lestari (2019) indicated that the interaction of biogas sludge+biofertilizer (36 ml+10 L ha^{-1}) significantly increased the plant height, number of leaves, and caps~~c~~aicin content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

The isolates N3, P7, N3+P7 from the biogas sludge significantly increased the ~~nutrient~~ uptake of nitrogen (20.77%) and crop growth rate (2.81 times higher than the control) of upland rice on Ultisols with the highest increase found with the P7 isolate. The dose of biogas sludge significantly increased plant height (14.81%), total dry weight (50.55%), ~~nutrient~~ uptake of nitrogen (54.11%) and phosphorus (65.20%), and also crop growth rate (129.44%) of upland rice on Ultisols with the highest increase at a dose of 630 ml/polybag. Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice by 41.81% with the highest increase at the dose of 315 ml/polybag. The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice on Ultisols 5.76 times with the highest increase found with B1S3.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

All authors formulated the overarching research goals and aims, provided the study materials, developed or designed the methodology. NEM analyzed and interpreted the study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in the field, and collected the data. ZN and I verified the overall reproducibility of results and the other research outputs. All authors conducted the critical review/commentary/revision of the manuscript.

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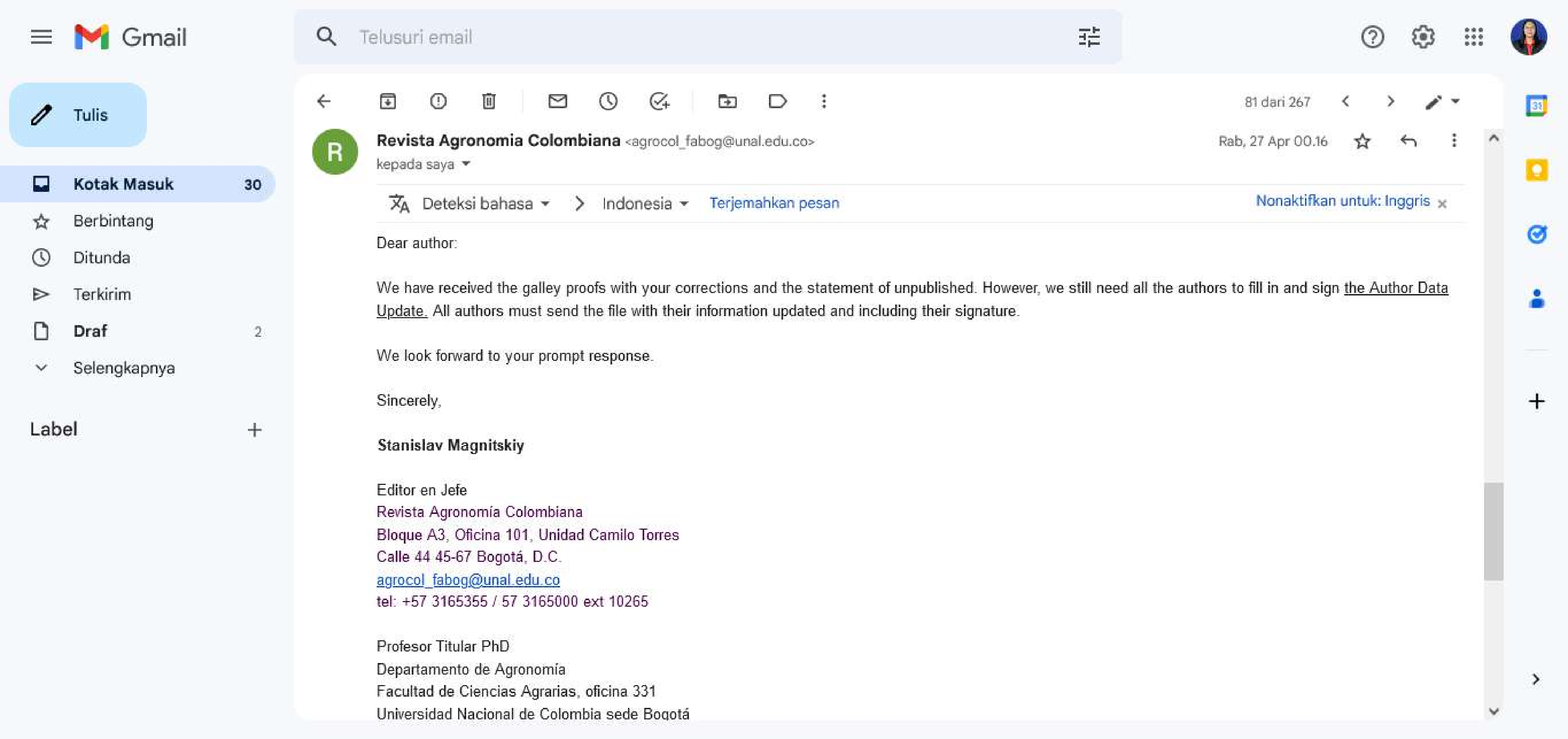

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Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la nutrición del arroz de secano

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ABSTRACT

This study evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth and nutrient uptake of upland rice grown in Ultisols. We used a randomized block design with two factors and seven replicates from October 2020 to April 2021. The first factor used selected SBI (B0 = untreated, B1 = nitrogen-fixing bacteria isolate (N3), B2 = phosphate solubilizing bacteria isolate (P7), B3 = isolate combination (N3+P7)). The second factor was the dosage of biogas sludge (S0 = untreated, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). The parameters were determined by ANOVA and followed by Duncan's multiple range test at *P*<0.05. The results showed that the isolate P7 significantly increased the N uptake by 20.77% and crop growth rate (CGR) of upland rice 2.81 times. Biogas sludge doses from 315 to 630 ml/polybag significantly increased plant height, uptake of N and P, total fresh and dry weight, and CGR of upland rice. The interaction between N3 and biogas sludge dosage of 630 ml/polybag significantly increased the CGR of upland rice. The application of isolates N3 and P7 and their combination within biogas sludge of 630 ml/polybag has the potential to increase the CGR of upland rice in acidic soils.

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Reviewer 3 wrote: The title don't show effectively the results of investigation. I recommend the title: Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice.
Regarding the term "superior bacterial isolates" reviewer 3 wrote: What the authors wanted to say? I don't understand this term.
This reviewer also wrote: The authors evaluated only the effect of bacteria and biogas sludge on rice growth. They evaluated also the effect of these treatments on rice nutrition!
Finally, regarding the word "ultisols" reviewer 3 wrote: Why is it necessary to specify the soil type?

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We agree with reviewer 3 suggestion, the title of the manuscript doesn't need to have the word superior and soil type because it is clarified in the materials and methods

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Correspondending author is required by my promoter, so that this manuscript can be used in completing my doctoral education

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Key words: acidic soil, crop growth rate, dosage, sludge potential.

RESUMEN

El presente estudio evaluó la influencia de aislamientos bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones sobre el crecimiento y la absorción de nutrientes en el arroz de tierras altas cultivado en ultisoles. Se utilizó un diseño de bloques al azar con dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0 = sin tratamiento, B1 = aislamiento de bacterias fijadoras de nitrógeno (N3), B2 = aislamiento de bacterias solubilizantes de fosfato (P7), B3 = combinación de aislados (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (S0 = sin tratamiento, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Los parámetros fueron determinados por análisis de varianza y seguidos de la prueba de rangos múltiples de Duncan a $P<0.05$. Los resultados mostraron que el aislamiento P7 aumentó significativamente la absorción de N en un 20.77% y la tasa de crecimiento del cultivo (TCC) de arroz de tierras altas 2.81 veces. Las dosis de lodos de biogás de 315 a 630 ml/polybag aumentaron significativamente la altura de la planta, la absorción de N y P, el peso fresco y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosis de lodos de biogás de 630 ml/polybag aumentó significativamente la TCC del arroz de tierras altas. La aplicación de los aislamientos N3 y P7 y su combinación dentro de lodos de biogás de 630 ml/polybag tiene el potencial de aumentar el TCC de arroz de tierras altas en suelos ácidos.

Palabras clave: suelo ácido, tasa de crecimiento de cultivos, dosis, potencial de lodo.

Introduction

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Biogas sludge is the waste by-product from an anaerobic processing system (FAO, 1977) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and the plants yield (Adela *et al.*, 2014). The following characteristics of the biogas sludge from palm oil waste have been reported: total N of 490 mg L^{-1}, total P of 110 mg L^{-1}, total K of 1.9 mg L^{-1} (Lubis *et al.*, 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al.*, 2019), and NH_3-N of 91 -112 mg L^{-1} (Choorit & Wisarnwan, 2007). The pH may range from 6.8 to 8.3, with the highest bacterial population of 7.21×10^7 cells per ml and the lowest one of 3.15×10^7 cells per ml (Alvionita *et al.*, 2019). Additionally, Mustamu and Triyanto (2020) reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria that have the potential to increase the availability of nitrogen and phosphate in soils.

The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing bacteria has a greater potential to increase soil fertility and plant growth. Zhang *et al.* (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility, and plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described different *Bacillus* species, such as *B. circulans, B. cereus, B. fusiformis, B. pumilus, B. megaterium, B. mycoides, B. coagulans, B. chitinolyticus,* and *B. subtilis* as phosphate solubilizing microorganisms. Ambrosini *et al.* (2016) showed the highest nitrogenase activity in *Bacillus cereus* among 42 different strains of *Bacillus* spp. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, *i.e.*, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Thermotogae*.

The application of bacteria from biogas sludge has never been reported in Indonesia for improving upland rice growth on acidic soils, including Ultisols. According to the Pusat Penelitian Tanah dan Agroklimat (Center for Soil and Agro-climate Research) (2000), the area in Indonesia covered by Ultisols was 45.8 million ha, or 24% of the total area of Indonesia. Furthermore, according to the Ministry of Agriculture, the area dedicated to rice cultivation in

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Indonesia was 15,712,025 ha with a yield of 81,148,617 t ha^{-1} in 2017 and the contribution of upland rice yield reached 4.66% (Kementerian Pertanian, 2017). The yield contribution of upland rice was classified as low and, therefore, it is necessary to find options in order to increase it. Thus, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge to increase the availability of nitrogen and phosphate, and the growth response of upland rice due to application of the biogas sludge and selected isolates in Ultisols. The study aimed to evaluate the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the mineral nutrition of the upland rice grown in Ultisols.

Materials and methods

Study area

The concentration of total N and available P in Ultisols and in the plant tissue (N and P uptake) were analyzed in the Analytical Laboratory of Socfin Indonesia Inc., Medan (Indonesia). The bacterial isolates were applied to upland rice in the village of Padang Bulan (3°37.760' N; 98°38.898' E; altitude 18 m a.s.l.), Medan Selayang Subdistrict, Medan City, Indonesia, from October 2020 to April 2021. The average temperature was 27.4°C, the average air humidity, was 82% and average rainfall was 228.5 mm per month.

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Preparation of medium and upland rice seeds

The medium to grow upland rice plants used the Ultisols from the Simalingkar area, Medan Tuntungan Subdistrict, Medan City, at a depth of 0 to 20 cm. One hundred g of soil samples were taken and analyzed for chemical characteristics such as pH using H_2O, organic C by Walkley-Black, available P by Bray-II, total N using Kjeldahl method, and cation exchange capacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH 7 method (Tab. 1). The soil was sterilized by drying at 100°C for 2 h. For preventing heat from the sterilization process, the soil was incubated for 1 d and then placed into a 10 kg polybag (18 cm × 18 cm). A basic NPK fertilizer (16-16-16) by Meroke Tetap Jaya Inc., Medan (Indonesia) at a dose of 1.5 g/polybag was applied by stirring evenly with the soil. The seeds of upland rice (*Oryza sativa* L.) were of the inbred variety Inpago-8 from the Indonesian Agency for Agricultural Research and Development were soaked in water for 24 h, followed by the application of the fungicide Propineb (70%) for 2 h. Upland rice was planted after 1 d of basic fertilization with two seeds per polybag at a depth of 2 cm.

TABLE 1. Chemical characteristics of the Ultisols soil samples after sterilization at 100°C.

Chemical characteristics	**Methods***	**Value**	**Category***
Soil pH (H_2O)	Electrometry	4.80	Acid
Organic C (%)	Walkley-Black	0.44	Very low
Total N (%)	Kjeldahl	0.04	Very low
Available P (mg kg^{-1})	Spectrophotometry	870.25	Very high
CEC (meq 100 g^{-1})	Ammonium acetate pH 7	28.31	High
Base saturation (%)	Ammonium acetate pH 7	4.85	Very low
Exchangeable cations			
K (meq 100 g^{-1})	Ammonium acetate pH 7	0.60	High
Ca (meq 100 g^{-1})	Ammonium acetate pH 7	0.34	Very low
Mg (meq 100 g^{-1})	Ammonium acetate pH 7	0.32	Very low
Na (meq 100 g^{-1})	Ammonium acetate pH 7	0.09	Very low
Al (%)	Ammonium acetate pH 7	0.02	Very low

*Criteria for pH (H_2O) = 4.5-5.5 (acid); organic C <1% (very low); total N <0.1% (very low); available P >60 mg kg^{-1} (very high); Cation exchange capacity (CEC) = 25-40 meq 100 g^{-1} (high); base saturation <20% (very low); exchangeable K= 0.60-1.00 meq 100 g^{-1} (high); exchangeable Ca <2 meq 100 g^{-1} (very low); exchangeable Mg <0.4 meq 100 g^{-1} (very low); exchangeable Na <0.1 meq 100 g^{-1} (very low); exchangeable Al <5% (very low) (Balai Penelitian Tanah (Indonesia Soil Research Institute), 2009).

Preparation of superior bacterial isolates suspension and biogas sludge

A total of 1 ml of the bacterial isolate suspension obtained from the characteristic stage was put into a test tube containing 9 ml of distilled water and homogenized. The dilution was made to 10^{-5}. A total of 0.1 ml of the suspension from the last dilution was spread over the James nitrogen free malat bromothymol blue (JNFB) medium for the nitrogen-fixing bacterial isolates test and Pikovskaya (PVK) medium for the phosphate solubilizing bacteria isolates. The culture medium was incubated for 2 to 3 d at room temperature. The nitrogen-fixing bacterial isolate test was characterized by the presence of colonies growing on the JNFB medium. The growth

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The biogas sludge was collected from Nubika Jaya Inc., Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia. The procedure for processing biogas sludge can be explained that the palm oil mill removes POME (Palm Oil Mill Effluent) waste from the second pond which has been mixed with oil and then separated at an optimal temperature of 35°C. Liquid waste is pumped into the receiver tank with a volume of 10 m^3 and filtered on a fiber tank screen for separated the solid waste such as fiber and others. Liquid waste from the receiver tank is pumped to the tower tank. Then it is distributed evenly to the fixed tank with a temperature of 35 to 37℃ and a flow rate of 20 to 30 m^3/h. The biogas sludge is taken from a fixed tank. Bacterial isolates and biogas sludge were applied to the soil surface at the base of the plants at one week after planting. Biogas sludge samples at a 500 ml volume were used to analyze the chemical and biological characteristics (Tab. 2).

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The Editor in Chief also wrote: Please, provide the bibliographic reference of the manual used for the biogas sludge analysis.

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TABLE 2. The chemical and biological characteristics of the biogas sludge.

Characteristics	Method*	Value
pH	Electrometry	7.41
Chemical oxygen demand (mg L^{-1})	Spectrophotometry	4547.8
Biological oxygen demand (mg L^{-1})	Titrimetry	1127.5
Total N (%)	Kjeldahl	0.051
Total P (%)	Spectrophotometry	0.0097
Available-P (%)	Spectrophotometry	0.013
Total K (%)	Graphite furnace - atomic absorption spectrophotometry (AAS)	0.18
Organic C (%)	Walkley-Black	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (ppm)	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Total nitrogen-fixing bacteria (CFU ml^{-1})	Plate count	29.4×10^5

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Total phosphate solubilizing bacteria (CFU ml^{-1})	Plate count	7.0×10^4

Note: *laboratory analysis based on the Balai Penelitian Tanah (Indonesia Soil Research Institute), 2009).

Treatment application

This study used a randomized block design with two factors and seven replicates. The first factor was the type of superior bacterial isolates (B0 = untreated; B1 = nitrogen-fixing bacterial isolate (N3); B2 = phosphate solubilizing bacteria isolate (P7); B3 = combination of isolates N3+P7) at a similar dose, namely 10 ml/polybag. The second factor was dose of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Determination of biogas sludge based on the dose of liquid organic fertilizer at the oil palm was 126 m^3 ha^{-1} equal to 126,000 L ha^{-1} (Sutarta *et al.*, 2003), then converted to soil weight per polybag (Eq. 1). Each replicate was harvested at 4, 8, and 12 weeks after application (WAA) for determination of the crop growth rate (CGR).

$$\text{Biogas sludge} = \frac{\text{The dose of liquid organic fertilizer ha}^{-1}}{\text{Soil weight ha}^{-1}} \times \text{soil weight per polybag} \qquad (1)$$

$$= \frac{26{,}000\ \text{L ha}^{-1}}{2{,}000{,}000\ \text{kg ha}^{-1}} \times 10\ \text{kg} = 630\ \text{ml}$$

Parameters and data analysis

The observations of the variables were conducted by measuring the growth of upland rice (plant height, and total fresh and dry weight), contents and uptake of N and P in the shoots, and CGR. The plant height was measured from the base of the roots to the tip of leaves using a meter, and the total fresh weight was obtained by weighing the roots and shoots. The total dry weight (roots + shoots) was measured after using an oven (model VS-1202D3, Vision Scientific Co., Korea) at 60°C for 48 h and weighed using the analytical scales. A 200 g sample of the second leaf from the shoots was collected and analyzed to determine the N content using the Kjeldahl and the P content was estimated using the destruction method through dry ashing. The N and P

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uptake were measured using Equation 2. The CGR was calculated as the dry weight related to the unit area at 4-8, 8-12, and 12-16 WAA using Equation 3 (Shon *et al.*, 1997):

$$\text{Nutrient uptake} = \text{nutrient content in the shoots} \times \text{total dry weight} \qquad (2)$$

$$\text{CGR} = \frac{\Delta W}{\Delta t} = \frac{W2 - W1}{t2 - t1} \qquad (3)$$

where:

CGR = crop growth rate;

W1 = dry weight per unit area at t1;

W2 = dry weight per unit area at t2;

t1 = first sampling;

t2 = second sampling;

The parameters of the second phase of the study were analyzed by an ANOVA and if the treatment had a significant effect, followed by Duncan's multiple range test at *P*<0.05 using SPSS v.20 software (IBM, 2011).

Results

Effect of bacterial isolates and biogas sludge on upland rice growth

Plant height of upland rice

The effect of biogas sludge application was significant on the plant height of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the plant height of upland rice at 4, 8, 12, and 16 WAA (Fig. 1). A significant increase in plant height of upland rice was observed with higher doses of biogas sludge of 630 ml/polybag at 8, 12, and 16 WAA with the highest increase of 14.81% compared to the control at 16 WAA. Although the effect was not significant, the combination of isolates B3 and the interaction of B2S3 showed the highest in plant height of upland rice by 2.94 and 22.06%, respectively, compared to the control.

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Reviewer 3 wrote: It is necessary write Results and discussion again in other form. All the information of this section must be re-organized. Account of the statistical analysis made and some information in figures and tables is irrelevant. The figures have repeated information.

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Commented [EDTR46]: Reviewer 3 wrote: I consider that it is necessary to write again Results and discussion in other form. The information of this section must be re-organized in two aspects: (1) Effect of bacterial isolates and biogas sludge on upland rice growth. (2) Effect of bacterial isolates and biogas sludge on upland rice nutrition. The first point could include:
- Plant height of upland rice
-.Biomass of upland rice
- Crop growth rate of upland rice
The second point could include:
-.Content of total-N and available-P nutrient of upland rice
- Uptake of total-N and available-P nutrient of upland rice
Furthermore, for both points the analysis made from lines 249-332 must be undertaken.

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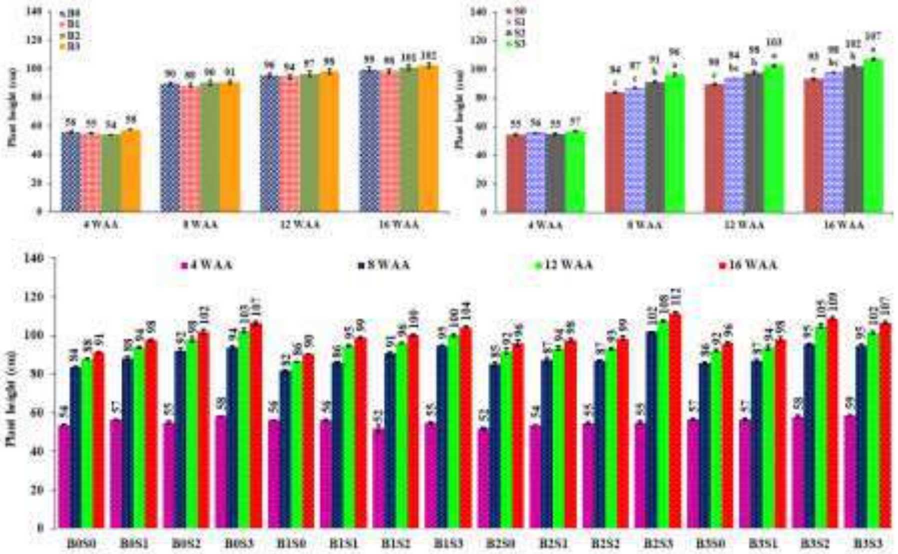

FIGURE 1. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on plant height of upland rice at 4, 8, 12, and 16 WAA. Values followed by the different letter in the graph significantly differed according to the Duncan test at *P*<0.05. ns - not significant. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7; B3 = isolates N3+P7).

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Biomass of upland rice

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the total fresh weight of upland rice at 4-16 WAA (Tab. 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total fresh weight (shoot+roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

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Treatments	**Total fresh weight ± standard error (g)**			
	4 WAA	**8 WAA**	**12 WAA**	**16 WAA**
Superior bacterial isolates (B)				
B0	4.15 ± 0.21	169.31 ± 8.90	215.27 ± 8.42	229.82 ± 8.94
B1	3.12 ± 0.12	194.50 ± 9.35	235.08 ± 10.32	252.02 ± 10.22
B2	4.52 ± 0.23	162.89 ± 11.15	201.85 ± 9.89	230.70 ± 9.28
B3	3.30 ± 0.25	173.91 ± 12.55	220.40 ± 15.96	245.03 ± 16.32
Biogas sludge (S)				
S0	3.72 ± 0.24	144.07 ± 9.37 b	182.67 ± 7.14 b	197.56 ± 6.58 b
S1	3.58 ± 0.27	153.41 ± 7.93 b	190.70 ± 8.90 b	215.65 ± 7.03 b
S2	3.64 ± 0.27	199.68 ± 10.30 a	258.70 ± 9.63 a	280.15 ± 9.25 a
S3	4.15 ± 0.25	203.45 ± 1.36 a	240.52 ± 2.81 a	264.21 ± 2.42 a
Interactions (B×S)				
B0S0	4.99 ± 0.33	124.08 ± 5.60	185.64 ± 3.32	192.78 ± 2.96

B0S1	3.47 ± 0.26	160.43 ± 1.16	188.60 ± 5.76	207.05 ± 3.97
B0S2	3.42 ± 0.42	185.97 ± 6.80	232.60 ± 8.75	250.84 ± 7.40
B0S3	4.71 ± 0.42	206.76 ± 5.49	254.23 ± 10.27	268.61 ± 8.85
B1S0	2.80 ± 0.18	155.79 ± 1.12	183.96 ± 5.20	202.88 ± 2.88
B1S1	3.74 ± 0.29	174.82 ± 9.01	227.91 ± 6.38	236.60 ± 6.32
B1S2	3.28 ± 0.40	241.17 ± 5.25	283.60 ± 7.76	296.08 ± 8.05
B1S3	2.67 ± 0.22	206.20 ± 7.23	244.85 ± 6.26	272.52 ± 4.34
B2S0	3.19 ± 0.18	190.90 ± 7.77	215.36 ± 7.67	229.11 ± 6.75
B2S1	4.85 ± 0.38	106.74 ± 13.42	143.16 ± 13.02	179.61 ± 10.36
B2S2	5.20 ± 0.24	148.40 ± 11.59	219.65 ± 5.26	248.72 ± 6.94
B2S3	4.82 ± 0.45	205.53 ± 10.50	229.21 ± 16.57	265.34 ± 9.58
B3S0	3.91 ± 0.30	105.53 ± 3.94	145.72 ± 1.96	165.45 ± 1.11
B3S1	2.25 ± 0.09	171.63 ± 4.90	203.14 ± 7.07	239.34 ± 12.07
B3S2	2.66 ± 0.14	223.17 ± 7.84	298.95 ± 1.51	324.94 ± 3.03
B3S3	4.37 ± 0.07	195.31±6.77	233.79 ± 8.40	250.38 ± 8.16
CV (%)	56.09	29.68	26.31	20.78

Values followed by the different letter in the column significantly differed according to the Duncan test at *P*<0.05. ns - not significant; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

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A significant increase in the total fresh weight of upland rice was observed with the higher dose of biogas sludge of 315 ml/polybag at 16 WAA, with the highest increase of 41.81% compared to the control. Although the effect was not significant, B1 and the interaction of B3S2 showed the highest in the total fresh weight of upland rice with 9.66 and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4 -16 WAA (Tab. 4).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight (shoot+roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	Total dry weight ± standard error (g)			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	1.38 ± 0.06	48.01 ± 1.29	73.60 ± 3.99	82.52 ± 4.18
B1	1.13 ± 0.05	54.09 ± 2.41	76.83 ± 2.66	99.72 ± 4.15
B2	1.49 ± 0.06	47.30 ± 3.30	73.20 ± 2.28	98.25 ± 3.90
B3	1.15 ± 0.07	52.32 ± 3.39	77.18 ± 4.90	98.47 ± 4.56
Biogas sludge (S)				
S0	1.26 ± 0.06	45.51 ± 2.63	62.88 ± 2.19 b	76.78 ± 1.63 c
S1	1.23 ± 0.08	44.47 ± 1.71	68.52 ± 2.00 ab	87.65 ± 2.84 bc
S2	1.26 ± 0.08	55.36 ± 3.43	85.69 ± 1.08 a	98.95 ± 1.86 b
S3	1.40 ± 0.06	56.38 ± 1.05	83.73 ± 3.44 a	115.59 ± 2.11 a
Interactions (B×S)				

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B0S0	1.58 ± 0.08	41.73 ± 2.78	58.08 ± 1.54	67.23 ± 0.96
B0S1	1.12 ± 0.08	45.87 ± 0.83	62.74 ± 1.83	71.08 ± 1.91
B0S2	1.20 ± 0.12	52.25 ± 2.07	81.39 ± 5.48	88.28 ± 5.02
B0S3	1.60 ± 0.12	52.18 ± 0.29	92.20 ± 3.05	103.49 ± 2.43
B1S0	0.97 ± 0.04	46.64 ± 1.39	69.53 ± 4.90	80.30 ± 4.51
B1S1	1.40 ± 0.07	48.13 ± 2.78	78.91 ± 0.53	96.23 ± 1.50
B1S2	1.12 ± 0.10	67.79 ± 1.44	91.05 ± 2.25	101.80 ± 2.40
B1S3	1.02 ± 0.08	53.81 ± 3.76	67.84 ± 1.77	120.54 ± 2.15
B2S0	1.17 ± 0.05	59.32 ± 2.33	70.92 ± 4.20	81.43 ± 3.82
B2S1	1.54 ± 0.10	34.47 ± 2.16	61.69 ± 1.97	89.84 ± 1.41
B2S2	1.73 ± 0.05	37.37 ± 3.74	83.10 ± 1.19	105.46 ± 1.37
B2S3	1.53 ± 0.10	58.05 ± 1.76	77.07 ± 4.27	116.28 ± 1.30
B3S0	1.30 ± 0.07	34.35 ± 7.04	52.98 ± 0.73	78.16 ± 0.48
B3S1	0.85 ± 0.03	49.40 ± 0.08	70.72 ± 1.29	93.44 ± 2.19
B3S2	0.99 ± 0.05	64.05 ± 4.68	87.22 ± 2.90	100.26 ± 1.93
B3S3	1.44 ± 0.02	61.48 ± 2.47	97.80 ± 0.77	122.04 ± 0.20
CV (%)	43.80	31.22	26.54	18.38

Values followed by the different letter in the column significantly differed according to the Duncan test at *P*<0.05. ns= not significant; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

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A significant increase in total dry weight of upland rice was observed with the increase in the dosage of biogas sludge of 630 ml/polybag at 16 WAA, with the highest increase of 50.55% compared to the control. Although the effect was not significant, B1 and the interaction of B3S3 showed the highest in the total dry weight of upland rice with 20.84 and 81.53%, respectively, compared to the control.

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Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12 to 16 WAA, but it did not have a significant effect at 4-8 and 8-12 WAA (Tab. 5).

TABLE 5. Effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of the upland rice 4, 8, 12, and 16 weeks after the application (WAA).

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Superior bacterial isolates (B)	**Biogas sludge (S)**				**Average**
	S0	**S1**	**S2**	**S3**	
	4-8 WAA				
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV = 32.28%

			8-12 WAA		
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888
Average	0.620	0.859	1.083	0.977	CV = 56.17%
			12-16 WAA		
B0	0.327 fgh	0.298 gh	0.246 h	0.403b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV = 51.07%

Values followed by the different letter in the column significantly differed according to the Duncan test at $P<0.05$. ns - not significant; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

The biogas sludge dose of 630 ml/polybag (S3) significantly increased the highest crop growth rate for upland rice at 12 to 16 WAA by 129.44% compared to the control. The isolates B1-B3 significantly increased the crop growth rate of upland rice with the highest increase for B2 of 181.45% compared to the controls at 12 to 16 WAA. The interaction of the B1S3 significantly increased the crop growth rate of upland rice, showing values 5.76 times greater than those of the control.

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Effect of bacterial isolates and biogas sludge on upland rice nutrition

Content of N and P in the upland rice

The effect of biogas sludge, superior bacterial isolates, and their interactions did not have a significant effect on the content of N and P in the upland rice (Fig. 2). The biogas sludge doses of 315 and 630 ml/polybag (S2 and S3) explained that the contents of P and N in the plant tissue of upland rice were 33.33 and 4.53% higher, respectively, compared to the control. The isolate B2 showed the highest content of N in the plant tissue of upland rice with values 1.63% higher than those of the control; however, all isolates (B1-B3) showed a similar level of P in the plant tissue of upland rice compared to the control.

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Commented [EDTR79]: The Editor in Chief asked: Do you refer to the contents of available P in soil or in the plant tissue? If plant tissue, what was the analytical method to determine the "available" P in the plant tissues?

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Uptake of N and P in the upland rice

The effect of biogas sludge significantly increased in the uptake of N and P. The superior bacterial isolates significantly increased in the uptake of nitrogen. The interaction of biogas sludge with superior bacterial isolates did not show a significant effect on the uptake of N and P in the upland rice (Fig. 2).

A significant increase in the uptake of N and P in upland rice was observed with a higher dose of biogas sludge of 630 ml/polybag, with the highest increases of 54.11 and 65.20%, respectively, compared to the control. The bacterial isolates B1-B3 also significantly increased the uptake of N in the upland rice with the highest increase with the B2 of 20.77% compared to the control. Although the effect was not significant, B3 showed the highest uptake of P in the upland rice of 19.93% compared to the control.

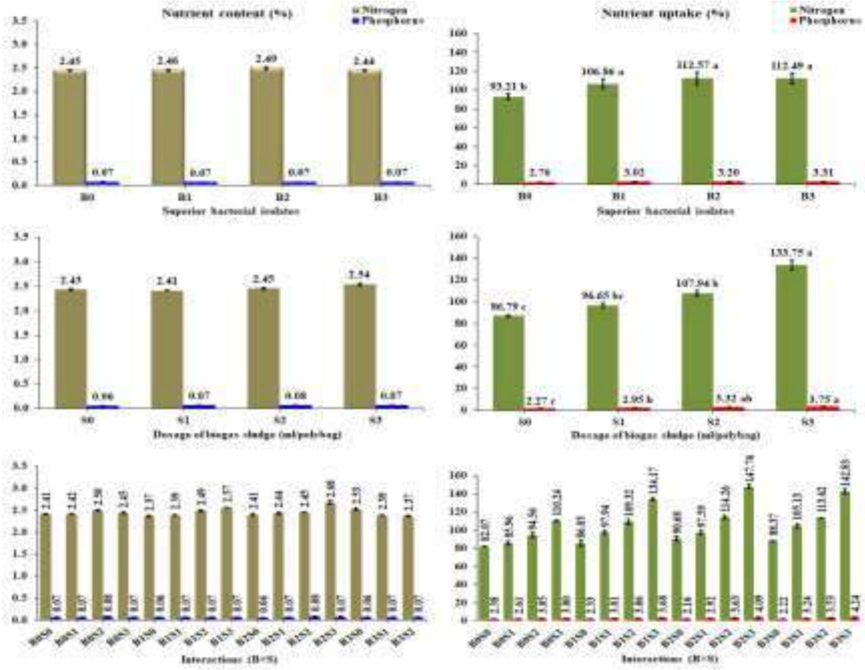

FIGURE 2. The effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on the nutrient content and uptake of N and P in the upland rice. Values followed by different letters significantly differed according to the Duncan test at $P<0.05$. ns - not significant. Dosage of biogas sludge (S0 = untreated; S1 = 157.5;

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You can use the phrase “nutrient uptake” when referring to the nutrients the plant absorbs in general. Nutrient uptake of N and P is redundant and does not make any sense. Please use only “uptake of N and/or P”

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Please add these graphs as independent files to ensure they have the appropriate quality and resolution.

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S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = N3+P7 isolates).

Discussion

Effect of selected superior bacterial isolates

The selected superior bacterial isolates (N3 and P7) significantly increased the uptake of nitrogen and crop growth rate of upland rice on Ultisols at 12 to 16 WAA, but it did not have a significant effect on plant height, total fresh weight, total dry weight, content (N and P) in leaf tissue, uptake of phosphorus, and crop growth rate of upland rice at 4 to 8 and 8 to 12 WAA. The superior bacterial isolates (N3, P7, and N3+P7) could increase the uptake of nitrogen in upland rice by 14.64%, 20.77%, and 20.68%, respectively, compared to the control (Fig. 2). Similar results are also shown in Table 5, where can be observed that the crop growth rate of upland rice at 12 to 16 WAA has increased 2.57, 2.81, and 2.39 times, respectively due to the selected superior bacterial isolates (N3, P7, N3+P7), compared to the control. The results indicate that the ability of a single P7 bacterial isolate was greater in increasing the nitrogen and crop growth rate of upland rice compared to a single N3 isolate and the combination of N3+P7 isolates. This was due to the presence of several organic acids and hormones produced by P7 that can increase the uptake of nitrogen and crop growth rate of upland rice. This result is supported by Mustamu *et al.* (2021a) who found that the phosphate solubilizing bacterial isolate (P7) from the biogas sludge contains organic acids such as lactic, oxalic, acetic, and citric acids, and had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate with values 4.62 and 2.66 times higher, respectively, compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus in soils slightly increased with the application of bio fertilization with *Bacillus cereus*; this was due to the production of organic acids and other chemicals such as citric, tartaric, and oxalic acids that can stimulate plant growth and nutrient availability. Youssef and Eissa (2017) reported that the

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Commented [NEM93R92]: This paper has been corrected by a professional English increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of mineral nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that the hormone gibberellin and IAA, can increase plant growth under stressful conditions. Kang *et al.* (2014) said that the plant growth-promoting bacteria (PGPB) has several mechanisms to increase plant growth with nitrogen-fixation and phosphate solubilization, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from an anaerobic digester include *Ruminococcus* sp., *Thiomargarita* sp., *Clostridium* sp. *Anaerobacter* sp., *Bacillus* sp., *Sporobacterium* sp., *Saccharofermentans* sp., *Oscillibacter* sp., *Sporobacter* sp., and *Enterobacter* sp. Liaquat *et al.* (2017) also reported abundance of *Bacillus*, *Clostridium*, and *Enterobacter* spp. in an anaerobic digester of wastewater when producing biogas.

Effect of biogas sludge

The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and 16 WAA), total dry weight (12 and 16 WAA), uptake (N and P), and the crop growth rate of upland rice at 8 to 12 WAA. However, it did not have a significant effect on content (N and P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAA). An increase in plant height, total dry weight, uptake in terms of nitrogen and phosphorus, and also crop growth rate of upland rice on Ultisols with a higher dose of biogas sludge of 630 ml/polybag at the end of this study (16 WAA). In contrast, the total fresh weight increased along with the higher dose of biogas sludge to 315 ml/polybag and then decreased at the dose of 630 ml/polybag. This result is supported the biogas sludge had chemical characteristics such as pH (7.41), total N (0.051%), available P (0.013%), organic C (0.14%), total K (0.18%), and biological characteristics such as total nitrogen-fixing bacteria (29.4×10^5 CFU ml^{-1}) and total phosphate solubilizing bacteria

(7.0×10^4 CFU ml^{-1}) (Tab. 2). The organic C content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could increase the uptake in terms of nitrogen and phosphorus in upland rice with an increasing dose of biogas sludge of 630 ml/polybag (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the plant height, biomass, and crop growth rate of the upland rice. A similar result was reported by Mustamu and Triyanto (2020) who determined the macro and micronutrients from the biogas sludge and the population of nitrogen-fixing and phosphate solubilizing bacteria of 480×10^4 and 42×10^4 CFU ml^{-1}, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that the total phosphate solubilizing bacteria (1.6 to 2.5 CFU ml^{-1}) was significantly higher compared to nitrogen-fixing bacteria (0.5-1.4 CFU ml^{-1}) showing a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported that an increase in concentrations of NH_4^+-N ranged from 45 to 80% in the anaerobic waste.

Interaction of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice on Ultisols at 12-16 WAA, but it did not have a significant effect on the other parameters in this study. The interaction of B1 with biogas sludge at the dose of 630 ml/polybag (B1S3) showed the highest crop growth rate of upland rice compared to other interactions and was 5.76 times greater compared to the control. This was caused by the application of biogas sludge that could have increased soil organic matter and the total population of beneficial bacteria. Likewise, the biogas sludge contained organic C (0.14%), total nitrogen-fixing bacteria (29.4×10^5 CFU ml^{-1}), and total phosphate solubilizing bacteria (7.0×10^4 CFU ml^{-1}) (Tab. 2) that could improve soil quality and support the crop growth rate. This result is supported by Urra *et al.* (2019) who found that the application of sewage sludge in the long term significantly increases the organic matter contents in the soil, causing a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of the organic matter. Bhardwaj *et al.* (2014); Carvajal-Muñoz and Carmona-Garcia (2012) showed that the application of a biofertilizer had advantages in the plant such as availability of nutrients that are balanced for plant health. It also stimulates nutrient mobilization that can increase soil biological activity and the availability of microbial food to encourage the growth of beneficial microorganisms, increasing the soil organic matter content and, therefore, the cation exchange capacity. Siswanti and Lestari (2019) indicated that the interaction of biogas sludge+biofertilizer (36 ml+10 L ha^{-1}) significantly increased the plant height, number of leaves, and capsaicin content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

The isolates N3, P7, N3+P7 from the biogas sludge significantly increased the uptake of nitrogen (20.77%) and crop growth rate (2.81 times higher than the control) of upland rice on Ultisols with the highest increase found with the P7 isolate. The dose of biogas sludge significantly increased plant height (14.81%), total dry weight (50.55%), uptake of nitrogen (54.11%) and phosphorus (65.20%), and also crop growth rate (129.44%) of upland rice on Ultisols with the highest increase at a dose of 630 ml/polybag. Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice by 41.81% with the highest increase at the dose of 315 ml/polybag. The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice on Ultisols 5.76 times with the highest increase found with B1S3.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

All authors formulated the overarching research goals and aims, provided the study materials, developed or designed the methodology. NEM analyzed and interpreted the study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in the field, and collected the data. ZN and I verified the overall reproducibility of results and the other research outputs. All authors conducted the critical review/commentary/revision of the manuscript.

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Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la nutrición del arroz de tierras altas

Novilda Elizabeth Mustamu[1], Zulkifli Nasution[2*], Irvan[3], and Mariani Sembiring[2]

ABSTRACT

This study evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth and nutrient uptake of upland rice grown in Ultisols. We used a randomized block design with two factors and seven replicates from October 2020 to April 2021. The first factor used selected SBI (B0 = untreated, B1 = nitrogen-fixing bacteria isolate (N3), B2 = phosphate solubilizing bacteria isolate (P7), B3 = isolate combination (N3+P7)). The second factor was the dosage of biogas sludge (S0 = untreated, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). The parameters were determined by ANOVA and followed by Duncan's multiple range test at $P<0.05$. The results showed that the isolate P7 significantly increased the N uptake by 20.77% and crop growth rate (CGR) of upland rice 2.81 times. Biogas sludge doses from 315 to 630 ml/polybag significantly increased plant height, uptake of N and P, total fresh and dry weight, and CGR of upland rice. The interaction between N3 and biogas sludge dosage of 630 ml/polybag significantly increased the CGR of upland rice. The application of isolates N3 and P7 and their combination with biogas sludge of 630 ml/polybag has the potential to increase the CGR of upland rice in acidic soils.

Key words: acidic soil, crop growth rate, dosage, sludge potential.

RESUMEN

El presente estudio evaluó la influencia de aislamientos bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones sobre el crecimiento y la absorción de nutrientes del arroz de tierras altas cultivado en ultisoles. Se utilizó un diseño de bloques al azar con dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0 = sin tratamiento, B1 = aislamiento de bacterias fijadoras de nitrógeno (N3), B2 = aislamiento de bacterias solubilizadoras de fosfato (P7), B3 = combinación de aislamientos (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (S0 = sin tratamiento, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Los parámetros fueron determinados por análisis de varianza y seguidos de la prueba de rangos múltiples de Duncan a $P<0.05$. Los resultados mostraron que el aislamiento P7 aumentó significativamente la absorción de N en un 20.77% y la tasa de crecimiento del cultivo (TCC) de arroz de tierras altas 2.81 veces. Las dosis de lodos de biogás de 315 a 630 ml/polybag aumentaron significativamente la altura de la planta, la absorción de N y P, el peso fresco y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosis de lodos de biogás de 630 ml/polybag aumentó significativamente la TCC del arroz de tierras altas. La aplicación de los aislamientos N3 y P7 y su combinación con lodos de biogás de 630 ml/polybag tiene el potencial de aumentar la TCC de arroz de tierras altas en suelos ácidos.

Palabras clave: suelo ácido, tasa de crecimiento de cultivos, dosis, potencial de lodo.

Introduction

Biogas sludge is the waste by-product from an anaerobic processing system (FAO, 1977) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and plant yield (Adela *et al.,* 2014). The following characteristics of the biogas sludge from palm oil waste have been reported: total N of 490 mg L^{-1}, total P of 110 mg L^{-1}, total K of 1.9 mg L^{-1} (Lubis *et al.,* 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al.,* 2019), and N-NH_3 of 91-112 mg L^{-1} (Choorit & Wisarnwan, 2007). The pH may range from 6.8 to 8.3, with the highest bacterial population of 7.21×10^7 cells ml^{-1} and the lowest of 3.15×10^7 cells ml^{-1} (Alvionita *et al.,* 2019). Additionally, Mustamu and Triyanto (2020) reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria that have the potential to increase the availability of nitrogen and phosphate in soils.

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The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing bacteria has a greater potential to increase soil fertility and plant growth. Zhang *et al.* (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility and plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described different *Bacillus* species, such as *B. circulans, B. cereus, B. fusiformis, B. pumilus, B. megaterium, B. mycoides, B. coagulans, B. chitinolyticus,* and *B. subtilis* as phosphate solubilizing microorganisms. Ambrosini *et al.* (2016) showed the highest nitrogenase activity in *Bacillus cereus* among 42 different strains of *Bacillus* spp. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, *i.e., Proteobacteria, Firmicutes, Bacteroidetes*, and *Thermotogae.*

The application of bacteria from biogas sludge has never been reported in Indonesia for improving upland rice growth on acidic soils, including Ultisols. According to the Pusat Penelitian Tanah dan Agroklimat (Center for Soil and Agro-climate Research) (2000), the area in Indonesia covered by Ultisols is 45.8 million ha or 24% of the total area of the country. Furthermore, according to the Ministry of Agriculture, the area dedicated to rice cultivation in Indonesia is 15,712,025 ha with a yield of 81,148,617 t in 2017, and the contribution of upland rice yield is 4.66% (Kementerian Pertanian, 2017). The yield contribution of upland rice is classified as low and, therefore, it is necessary to find options to increase it. Therefore, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge to increase the availability of nitrogen and phosphate and, thus, the growth response of upland rice in Ultisols. This study aimed to evaluate the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the mineral nutrition of the upland rice grown in Ultisols.

Materials and methods

Study area

The concentration of total N and available P in Ultisols and in the plant tissue (N and P uptake) were analyzed in the Analytical Laboratory of Socfin Indonesia Inc., Medan (Indonesia). The bacterial isolates were applied to upland rice in the village of Padang Bulan (3°37.760' N, 98°38.898' E, and altitude of 18 m a.s.l.), Medan Selayang Subdistrict, Medan City, Indonesia, from October 2020 to April 2021. The average temperature was 27.4°C, the average air humidity was 82%, and the average rainfall was 228.5 mm per month.

Preparation of medium and upland rice seeds

The medium to grow upland rice plants was the Ultisol soil from the Simalingkar area, Medan Tuntungan Subdistrict, Medan City, collected at a depth of 0 to 20 cm. One hundred g of soil sample were taken and analyzed for chemical characteristics of pH H_2O, organic C by Walkley-Black, available P by Bray-II, total N using the Kjeldahl method, and cation exchange capacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH7 method (Tab. 1). The soil was sterilized by drying at 100°C for 2 h. To prevent heat from the sterilization process, the soil was incubated for 1 d and then placed into a 10 kg polybag (18 cm × 18 cm). A basic NPK fertilizer (16-16-16) by Meroke Tetap Jaya Inc. (Medan, Indonesia) at a dose of 1.5 g/polybag was applied by stirring evenly with the soil. The seeds of upland rice (*Oryza sativa* L.) of the inbred variety Inpago-8 from the Indonesian Agency for Agricultural Research and Development were soaked in water for 24 h, followed by the application of the fungicide Propineb (70%) for 2 h. Upland rice was planted after 1 d of basic fertilization with two seeds per polybag at a depth of 2 cm.

TABLE 1. Chemical characteristics of the Ultisol soil samples after sterilization at 100°C.

Chemical characteristics	Method	Value	Category
Soil pH (H_2O)	Electrometry	4.80	Acid
Organic C (%)	Walkley-Black	0.44	Very low
Total N (%)	Kjeldahl	0.04	Very low
Available P (mg kg^{-1})	Spectrophotometry	870.25	Very high
CEC (meq 100 g^{-1})	Ammonium acetate pH 7	28.31	High
Base saturation (%)	Ammonium acetate pH 7	4.85	Very low
	Exchangeable cations		
K (meq 100 g^{-1})	Ammonium acetate pH 7	0.60	High
Ca (meq 100 g^{-1})	Ammonium acetate pH 7	0.34	Very low
Mg (meq 100 g^{-1})	Ammonium acetate pH 7	0.32	Very low
Na (meq 100 g^{-1})	Ammonium acetate pH 7	0.09	Very low
Al (%)	Ammonium acetate pH 7	0.02	Very low

Criteria for pH (H_2O) = 4.5-5.5 (acid); organic C <1% (very low); total N <0.1% (very low); available P >60 mg kg^{-1} (very high); cation exchange capacity (CEC) = 25-40 meq 100 g^{-1} (high); base saturation <20% (very low); exchangeable K = 0.60-1.00 meq 100 g^{-1} (high); exchangeable Ca <2 meq 100 g^{-1} (very low); exchangeable Mg <0.4 meq 100 g^{-1} (very low); exchangeable Na <0.1 meq 100 g^{-1} (very low); exchangeable Al <5% (very low) (Balai Penelitian Tanah (Indonesia Soil Research Institute), 2009).

Preparation of superior bacterial isolates suspension and biogas sludge

One ml of the bacterial isolate suspension obtained from the characteristic stage was put into a test tube containing 9 ml of distilled water, homogenized and then diluted to 10^{-5}. A total of 0.1 ml of the suspension from the last dilution was spread over James nitrogen free malat bromothymol blue

(JNFB) medium (Kirchhof *et al.,* 1997) for the nitrogen-fixing bacterial isolates test and Pikovskaya (PVK) medium (Pikovskaya, 1948) for the phosphate solubilizing bacteria isolates. The culture medium was incubated for 2 to 3 d at 37°C. The nitrogen-fixing bacterial isolate test was characterized by the presence of colonies growing on the JNFB medium. The growth of phosphate solubilizing bacterial isolates was indicated by a halo zone around the microbial colonies on the PVK medium. Seven nitrogen-fixing and seven phosphate-solubilizing isolates were found to produce total N and available P. The isolates that showed the highest phosphate and nitrogen increasing abilities were selected, namely phosphate solubilizing bacteria (P7) and nitrogen-fixing bacteria (N3), which were confirmed by Mustamu *et al.* (2021a; 2021b).

The biogas sludge was collected from Nubika Jaya Inc., Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia. The procedure for processing biogas sludge was the following: the palm oil mill removed the palm oil mill effluent (POME) waste from the second pond which has been mixed with oil and then separated at an optimal temperature of 35°C. Liquid waste was then pumped into the receiver tank of 10 m^3 volume and filtered on a fiber tank screen to separate solid waste such as fiber and other materials. Liquid waste from the receiver tank was further pumped into the tower tank. Then, it was distributed evenly to the fixed tank at a temperature of 35 to 37°C and a flow rate of 20 to 30 m^3/h. Finally, the biogas sludge was taken from the fixed tank. Bacterial isolates and biogas sludge were applied to the soil surface at the base of the plants at one week after planting. Biogas sludge samples of 500 ml volume were used to analyze the chemical and biological characteristics (Tab. 2).

Treatment application

This study used a randomized block design with two factors and seven replicates. The first factor was the type of superior bacterial isolates (B0 = untreated; B1 = nitrogen-fixing bacterial isolate (N3); B2 = phosphate solubilizing bacteria isolate (P7); B3 = combination of isolates N3+P7) at a similar dose, namely 10 ml/polybag. The second factor was the dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Determination of biogas sludge based on the dose of liquid organic fertilizer at the oil palm was 126 m^3 ha^{-1} equal to 126,000 L ha^{-1} (Sutarta *et al.,* 2000), then converted to soil weight per polybag (Eq. 1). Each replicate was harvested at 4, 8, and 12 weeks after application (WAA) for determination of the crop growth rate (CGR).

$$\begin{aligned}\text{Biogas sludge} &= \frac{\text{Dose of liquid organic fertilizer ha}^{-1}}{\text{Soil weight ha}^{-1}} \times \text{soil weight per polybag} \quad (1)\\ &= \frac{26{,}000 \text{ L ha}^{-1}}{2{,}000{,}000 \text{ kg ha}^{-1}} \times 10 \text{ kg} = 130 \text{ ml}\end{aligned}$$

Plant growth variables and data analysis

The growth variables were assessed by measuring the growth of upland rice (plant height, and total fresh and dry weights), contents and uptake of N and P in the aerial

TABLE 2. Chemical and biological characteristics of the biogas sludge.

Characteristics	Method	Value
pH	Electrometry	7.41
Chemical oxygen demand (mg L^{-1})	Spectrophotometry	4547.8
Biological oxygen demand (mg L^{-1})	Titrimetry	1127.5
Total N (%)	Kjeldahl	0.051
Total P (%)	Spectrophotometry	0.0097
Available P (%)	Spectrophotometry	0.013
Total K (%)	Graphite furnace - atomic absorption spectrophotometry (AAS)	0.18
Organic C (%)	Walkley-Black	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (mg L^{-1})	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Total nitrogen-fixing bacteria (CFU ml^{-1})	Plate count	29.4×10^5
Total phosphate solubilizing bacteria (CFU ml^{-1})	Plate count	7.0×10^4

Laboratory analysis based on the Balai Penelitian Tanah (Indonesia Soil Research Institute) (2009).

parts, and crop growth rate (CGR). The plant height was measured from the root apex to the tip of leaves using a measuring tape, and the total fresh weight was obtained by weighing the roots and shoots. The total dry weight (roots + shoots) was obtained after plant drying in an oven (VS-1202D3, Vision Scientific Co., Korea) at 60°C for 48 h and weighed using analytical scales. A 200 g sample of the second leaf from the shoots was collected and analyzed to determine the N content using the Kjeldahl method, and the P content was recorded using the destruction method through dry ashing (Bertramson, 1942). The N and P uptake were measured using Equation 2. The CGR was calculated as the dry weight related to the unit area at 4-8, 8-12, and 12-16 WAA using Equation 3 (Shon *et al.*, 1997):

$$\begin{array}{c}\text{Nutrient}\\\text{uptake}\end{array} = \begin{array}{c}\text{nutrient content}\\\text{in the shoots}\end{array} \times \begin{array}{c}\text{total dry}\\\text{weight}\end{array} \qquad (2)$$

$$\text{CGR} = \frac{\Delta \text{W}}{\Delta \text{t}} \times \frac{\text{W2-W1}}{\text{t2-t1}} \qquad (3)$$

where:

CGR = crop growth rate;

W1 = dry weight per unit area at t1;

W2 = dry weight per unit area at t2;

t_1 = first sampling;

t_2 = second sampling.

The parameters of the second phase of the study were analyzed by an ANOVA. If the treatment had a significant effect, the Duncan's multiple range test was applied at *P*<0.05 using SPSS v.20 software (IBM, 2011).

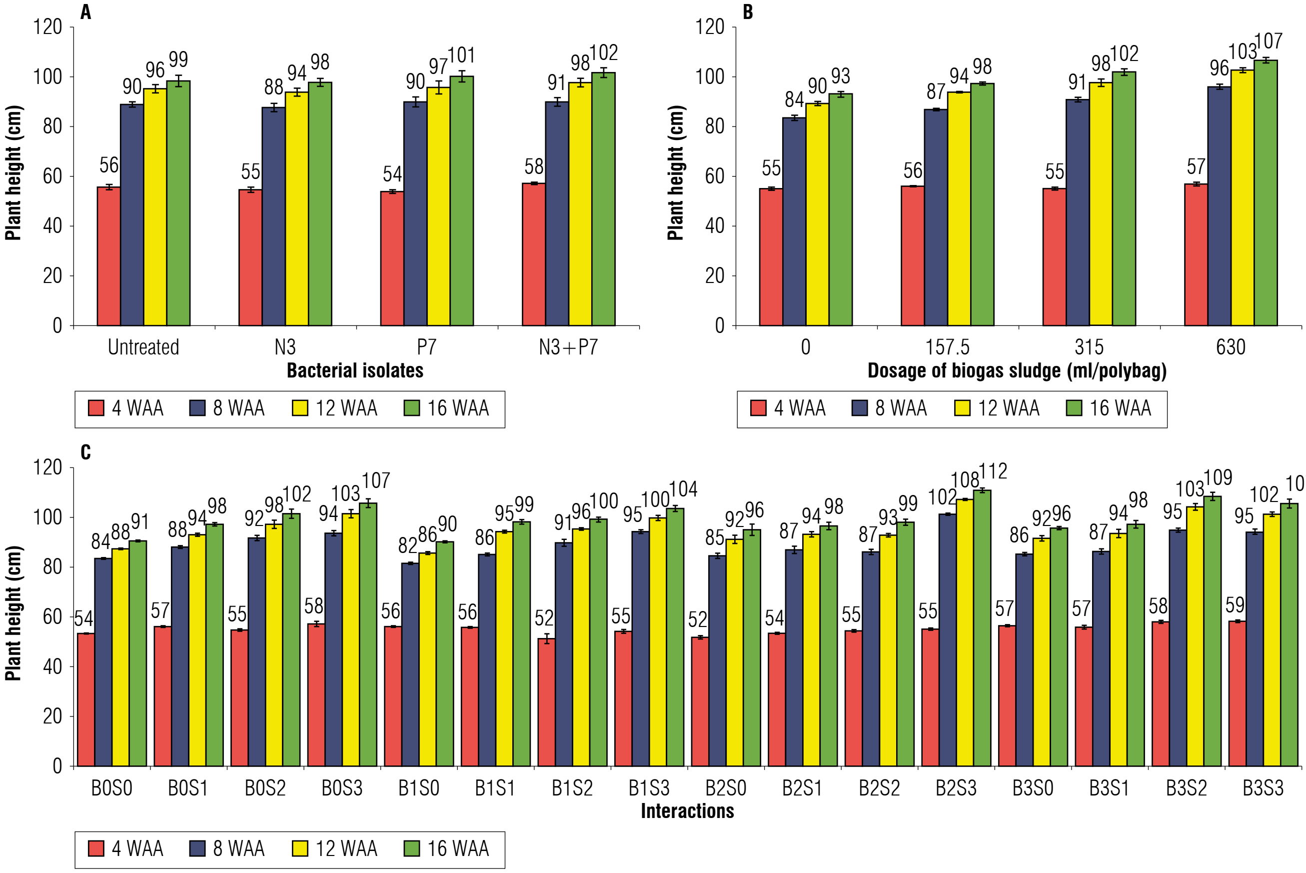

FIGURE 1. A) Effect of superior bacterial isolates, B) dosage of biogas sludge, and C) their interactions on plant height of upland rice at 4, 8, 12, and 16 weeks after application (WAA). Values followed by a different letter in the graph significantly differed according to the Duncan test at *P*<0.05. Vertical bars indicate the standard error. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7; B3 = isolates N3+P7).

Results

Effect of bacterial isolates and biogas sludge on upland rice growth

Plant height of upland rice

The effect of biogas sludge application was significant on the plant height of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the plant height of upland rice at 4, 8, 12, and 16 WAA (Fig. 1A-C). A significant increase in plant height of upland rice was observed with higher doses of biogas sludge of 630 ml/polybag at 8, 12, and 16 WAA, with the highest increase of 14.81% compared to the control at 16 WAA. Although the effect was not significant, the combination of isolates B3 and the interaction of B2S3 showed the highest increase in plant height of upland rice by 2.94% and 22.06%, respectively, compared to the control.

Biomass of upland rice

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the total fresh weight of upland rice at 4-16 WAA (Tab. 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total fresh weight (shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	**Total fresh weight (g) ± standard error**			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	4.15 ± 0.21	169.31 ± 8.90	215.27 ± 8.42	229.82 ± 8.94
B1	3.12 ± 0.12	194.50 ± 9.35	235.08 ± 10.32	252.02 ± 10.22
B2	4.52 ± 0.23	162.89 ± 11.15	201.85 ± 9.89	230.70 ± 9.28
B3	3.30 ± 0.25	173.91 ± 12.55	220.40 ± 15.96	245.03 ± 16.32
Biogas sludge (S)				
S0	3.72 ± 0.24	144.07 ± 9.37 b	182.67 ± 7.14 b	197.56 ± 6.58 b
S1	3.58 ± 0.27	153.41 ± 7.93 b	190.70 ± 8.90 b	215.65 ± 7.03 b
S2	3.64 ± 0.27	199.68 ± 10.30 a	258.70 ± 9.63 a	280.15 ± 9.25 a
S3	4.15 ± 0.25	203.45 ± 1.36 a	240.52 ± 2.81 a	264.21 ± 2.42 a
Interactions (B×S)				
B0S0	4.99 ± 0.33	124.08 ± 5.60	185.64 ± 3.32	192.78 ± 2.96
B0S1	3.47 ± 0.26	160.43 ± 1.16	188.60 ± 5.76	207.05 ± 3.97
B0S2	3.42 ± 0.42	185.97 ± 6.80	232.60 ± 8.75	250.84 ± 7.40
B0S3	4.71 ± 0.42	206.76 ± 5.49	254.23 ± 10.27	268.61 ± 8.85
B1S0	2.80 ± 0.18	155.79 ± 1.12	183.96 ± 5.20	202.88 ± 2.88
B1S1	3.74 ± 0.29	174.82 ± 9.01	227.91 ± 6.38	236.60 ± 6.32
B1S2	3.28 ± 0.40	241.17 ± 5.25	283.60 ± 7.76	296.08 ± 8.05
B1S3	2.67 ± 0.22	206.20 ± 7.23	244.85 ± 6.26	272.52 ± 4.34
B2S0	3.19 ± 0.18	190.90 ± 7.77	215.36 ± 7.67	229.11 ± 6.75
B2S1	4.85 ± 0.38	106.74 ± 13.42	143.16 ± 13.02	179.61 ± 10.36
B2S2	5.20 ± 0.24	148.40 ± 11.59	219.65 ± 5.26	248.72 ± 6.94
B2S3	4.82 ± 0.45	205.53 ± 10.50	229.21 ± 16.57	265.34 ± 9.58
B3S0	3.91 ± 0.30	105.53 ± 3.94	145.72 ± 1.96	165.45 ± 1.11
B3S1	2.25 ± 0.09	171.63 ± 4.90	203.14 ± 7.07	239.34 ± 12.07
B3S2	2.66 ± 0.14	223.17 ± 7.84	298.95 ± 1.51	324.94 ± 3.03
B3S3	4.37 ± 0.07	195.31 ± 6.77	233.79 ± 8.40	250.38 ± 8.16
CV (%)	56.09	29.68	26.31	20.78

Values followed by a different letter in the column significantly differed according to the Duncan test at $P<0.05$. CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

A significant increase in the total fresh weight of upland rice was observed with the higher dose of biogas sludge of 315 ml/polybag at 16 WAA, with the highest increase of 41.81% compared to the control. Although the effect was not significant, B1 and the interaction of B3S2 showed the highest increase in the total fresh weight of upland rice with 9.66% and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4-16 WAA (Tab. 4).

A significant increase in the total dry weight of upland rice was observed with the increase in the dosage of biogas sludge of 630 ml/polybag at 16 WAA, with the highest increase of 50.55% compared to the control. Although the effect was not significant, B1 and the interaction of B3S3 showed the highest increase in the total dry weight of upland rice with 20.84% and 81.53%, respectively, compared to the control.

Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12-16 WAA but did not have a significant effect at 4-8 and 8-12 WAA (Tab. 5).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight (shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	Total dry weight (g) ± standard error			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	1.38 ± 0.06	48.01 ± 1.29	73.60 ± 3.99	82.52 ± 4.18
B1	1.13 ± 0.05	54.09 ± 2.41	76.83 ± 2.66	99.72 ± 4.15
B2	1.49 ± 0.06	47.30 ± 3.30	73.20 ± 2.28	98.25 ± 3.90
B3	1.15 ± 0.07	52.32 ± 3.39	77.18 ± 4.90	98.47 ± 4.56
Biogas sludge (S)				
S0	1.26 ± 0.06	45.51 ± 2.63	62.88 ± 2.19 b	76.78 ± 1.63 c
S1	1.23 ± 0.08	44.47 ± 1.71	68.52 ± 2.00 ab	87.65 ± 2.84 bc
S2	1.26 ± 0.08	55.36 ± 3.43	85.69 ± 1.08 a	98.95 ± 1.86 b
S3	1.40 ± 0.06	56.38 ± 1.05	83.73 ± 3.44 a	115.59 ± 2.11 a
Interactions (B×S)				
B0S0	1.58 ± 0.08	41.73 ± 2.78	58.08 ± 1.54	67.23 ± 0.96
B0S1	1.12 ± 0.08	45.87 ± 0.83	62.74 ± 1.83	71.08 ± 1.91
B0S2	1.20 ± 0.12	52.25 ± 2.07	81.39 ± 5.48	88.28 ± 5.02
B0S3	1.60 ± 0.12	52.18 ± 0.29	92.20 ± 3.05	103.49 ± 2.43
B1S0	0.97 ± 0.04	46.64 ± 1.39	69.53 ± 4.90	80.30 ± 4.51
B1S1	1.40 ± 0.07	48.13 ± 2.78	78.91 ± 0.53	96.23 ± 1.50
B1S2	1.12 ± 0.10	67.79 ± 1.44	91.05 ± 2.25	101.80 ± 2.40
B1S3	1.02 ± 0.08	53.81 ± 3.76	67.84 ± 1.77	120.54 ± 2.15
B2S0	1.17 ± 0.05	59.32 ± 2.33	70.92 ± 4.20	81.43 ± 3.82
B2S1	1.54 ± 0.10	34.47 ± 2.16	61.69 ± 1.97	89.84 ± 1.41
B2S2	1.73 ± 0.05	37.37 ± 3.74	83.10 ± 1.19	105.46 ± 1.37
B2S3	1.53 ± 0.10	58.05 ± 1.76	77.07 ± 4.27	116.28 ± 1.30
B3S0	1.30 ± 0.07	34.35 ± 7.04	52.98 ± 0.73	78.16 ± 0.48
B3S1	0.85 ± 0.03	49.40 ± 0.08	70.72 ± 1.29	93.44 ± 2.19
B3S2	0.99 ± 0.05	64.05 ± 4.68	87.22 ± 2.90	100.26 ± 1.93
B3S3	1.44 ± 0.02	61.48 ± 2.47	97.80 ± 0.77	122.04 ± 0.20
CV (%)	43.80	31.22	26.54	18.38

Values followed by a different letter in the column significantly differed according to the Duncan test at $P<0.05$. CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

TABLE 5. Effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of the upland rice 4, 8, 12, and 16 weeks after the application (WAA).

Superior bacterial isolates (B)	Biogas sludge (S)				Average
	S0	S1	S2	S3	
			4-8 WAA		
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV = 32.28%
			8-12 WAA		
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888
Average	0.620	0.859	1.083	0.977	CV = 56.17%
			12-16 WAA		
B0	0.327 fgh	0.298 gh	0.246 h	0.403 b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882 a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400 a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866 a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV = 51.07%

Values followed by a different letter in the column significantly differed according to the Duncan test at $P<0.05$; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

The biogas sludge dose of 630 ml/polybag (S3) significantly increased the highest crop growth rate for upland rice at 12-16 WAA by 129.44% compared to the control. The isolates B1-B3 significantly increased the crop growth rate of upland rice with the highest increase for B2 of 181.45% compared to the controls at 12-16 WAA. The interaction of B1S3 significantly increased the crop growth rate of upland rice, showing values 5.76 times greater than those of the control.

Effect of bacterial isolates and biogas sludge on upland rice nutrition

Contents of N and P in the upland rice

The effect of biogas sludge, superior bacterial isolates, and their interactions did not have a significant effect on the content of N and P in the upland rice (Fig. 2A-C). The biogas sludge doses of 315 and 630 ml/polybag (S2 and S3) increased P and N in the plant tissue of upland rice by 33.33% and 4.53%, respectively, compared to the control. The isolate B2 showed the highest content of N in the plant tissues of upland rice with values 1.63% higher than those of the control. However, all isolates (B1-B3) showed a similar level of P in the plant tissues of upland rice compared to the control.

Uptake of N and P in the upland rice

The effect of biogas sludge significantly increased the uptake of N and P. The superior bacterial isolates significantly increased the uptake of nitrogen. The interaction of biogas sludge with superior bacterial isolates did not show a significant effect on the uptake of N and P in the upland rice (Fig. 2D-F).

A significant increase in the uptake of N and P in upland rice was observed with a higher dose of biogas sludge of 630 ml/polybag, with the highest increases of 54.11% and 65.20%, respectively, compared to the control. The bacterial isolates B1-B3 also significantly increased the uptake of N in the upland rice with the highest increase with B2 of 20.77% compared to the control. Although the effect was not significant, B3 showed the highest uptake of P in the upland rice of 19.93% compared to the control.

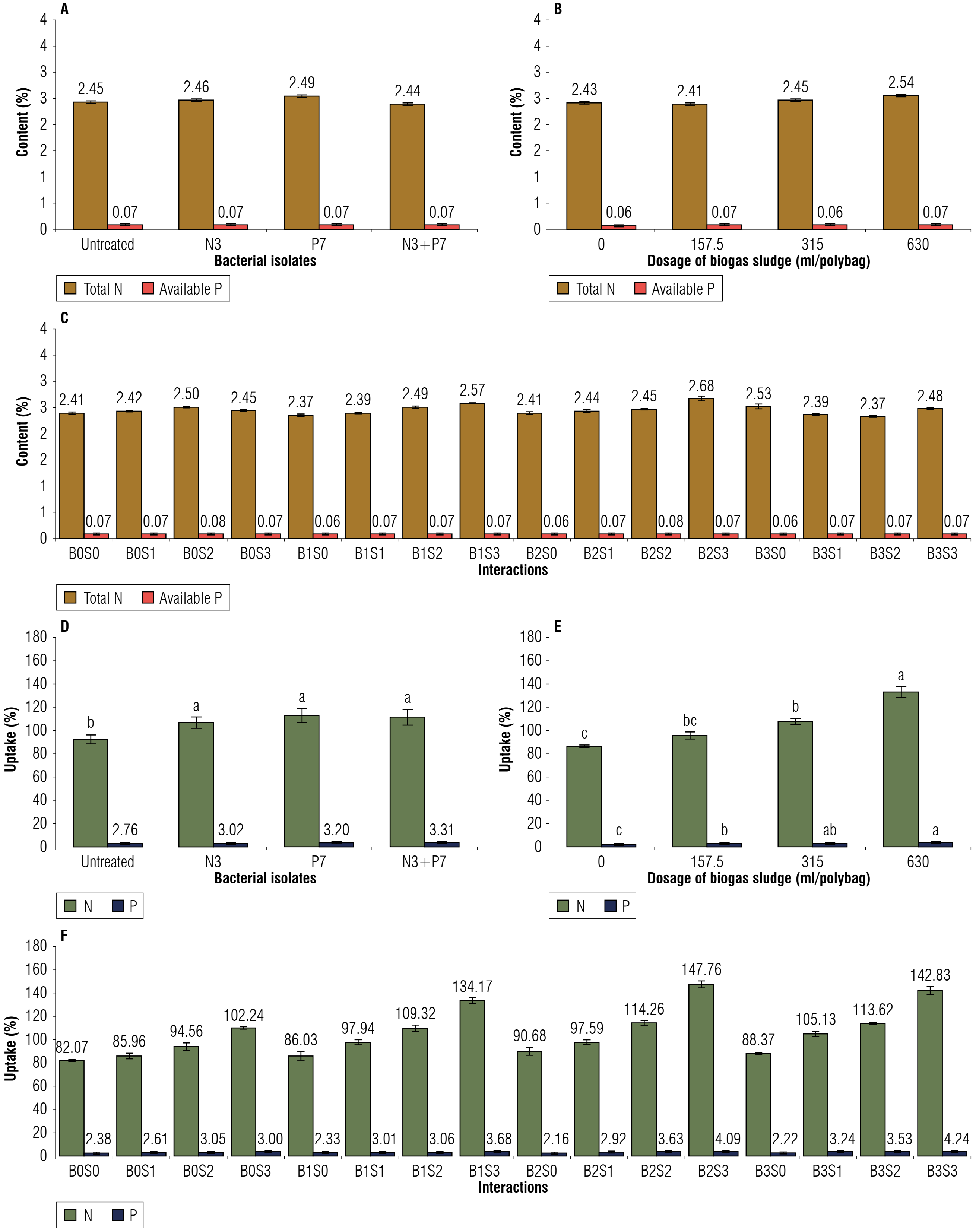

FIGURE 2. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on the nutrient content (A-C) and uptake of N and P (D-F) of upland rice. Values followed by different letters significantly differed according to the Duncan test at $P<0.05$. Vertical bars indicate the standard error. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = N3+P7 isolates).

Discussion

Effect of selected superior bacterial isolates

The selected superior bacterial isolates (N3 and P7) significantly increased the uptake of nitrogen and the crop growth rate of upland rice in an Ultisol at 12-16 WAA but did not have a significant effect on plant height, total fresh weight, total dry weight, content of N and P in leaves, uptake of phosphorus by the plants, and crop growth rate of upland rice from 4 to 12 WAA. The superior bacterial isolates (N3, P7, and N3+P7) increased the uptake of nitrogen in upland rice by 14.64%, 20.77%, and 20.68%, respectively, compared to the control (Fig. 2). Similar results are also shown in Table 5, with the crop growth rate of upland rice at 12-16 WAA increased by 2.57, 2.81, and 2.39 times, respectively, due to the selected superior bacterial isolates (N3, P7, N3+P7) compared to the control. The results indicate that a single P7 bacterial isolate increased the nitrogen uptake and crop growth rate of upland rice compared to a single N3 isolate and the combination of N3+P7 isolates. This was due to the presence of several organic acids and hormones produced by the isolate P7 that can increase the uptake of nitrogen and crop growth rate of upland rice. This result is supported by Mustamu *et al.* (2021a) who found that the phosphate solubilizing bacterial isolate (P7) from the biogas sludge contains organic acids, such as lactic, oxalic, acetic, and citric, and had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate with values 4.62 and 2.66 times higher, respectively, compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus in soils slightly increased with the application of bio fertilization with *Bacillus cereus*. This was due to the production of organic acids and other substances, including citric, tartaric, and oxalic acids, that can stimulate plant growth and nutrient availability. Youssef and Eissa (2017) reported that the increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of mineral nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that gibberellin and IAA can increase plant growth under stressful conditions. Kang *et al.* (2014) indicated that the plant growth-promoting bacteria (PGPB) has several mechanisms to increase plant growth with nitrogen-fixation and phosphate solubilization, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from an anaerobic digester include *Ruminococcus* sp., *Thiomargarita* sp., *Clostridium* sp. *Anaerobacter* sp., *Bacillus* sp., *Sporobacterium* sp., *Saccharofermentans* sp., *Oscillibacter* sp., *Sporobacter* sp., and *Enterobacter* sp. Liaquat *et al.* (2017) also reported abundance of *Bacillus, Clostridium*, and *Enterobacter* spp. in an anaerobic digester of wastewater when producing biogas.

Effect of biogas sludge

The dose of biogas sludge significantly increased the plant height, total fresh weight (8, 12, and 16 WAA), total dry weight (12 and 16 WAA), uptake (N and P), and crop growth rate of upland rice at 8-12 WAA. However, it did not have a significant effect on the content (N and P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAA). An increase in plant height, total dry weight, uptake of nitrogen and phosphorus, and also crop growth rate of upland rice in an Ultisol with a higher dose of biogas sludge of 630 ml/polybag was seen at the end of this study (16 WAA). In contrast, the total fresh weight increased along with the higher dose of biogas sludge of 315 ml/polybag and then decreased at the dose of 630 ml/polybag. This result is due to the chemical characteristics of the biogas sludge such as pH (7.41), total N (0.051%), available P (0.013%), organic C (0.14%), total K (0.18%), and the biological characteristics such as total nitrogen-fixing bacteria (29.4×10^5 CFU ml^{-1}) and total phosphate solubilizing bacteria (7.0×10^4 CFU ml^{-1}) (Tab. 2). The organic C content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could increase the uptake of nitrogen and phosphorus in upland rice with an increasing dose of biogas sludge of 630 ml/polybag (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the plant height, biomass, and crop growth rate of the upland rice. A similar result was reported by Mustamu and Triyanto (2020), who determined the macro and micronutrients from the biogas sludge and the population of nitrogen-fixing and phosphate solubilizing bacteria of 480×10^4 and 42×10^4 CFU ml^{-1}, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that the total phosphate solubilizing bacteria (1.6 to 2.5 CFU ml^{-1}) was significantly higher than nitrogen-fixing bacteria (0.5-1.4 CFU ml^{-1}), showing a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported an increase in concentrations of NH_4^+-N from 45% to 80% in the anaerobic waste.

Interaction of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice in Ultisols at 12-16 WAA but did not have a significant effect on the other parameters in this study. The interaction of B1 with biogas sludge at the dose of 630 ml/polybag (B1S3) showed the highest crop growth rate of

upland rice compared to other interactions and was 5.76 times greater compared to the control. This was caused by the application of biogas sludge that could have increased soil organic matter and the total population of beneficial bacteria. Likewise, the biogas sludge contained organic C (0.14%), total nitrogen-fixing bacteria (29.4×10^5 CFU ml^{-1}), and total phosphate solubilizing bacteria (7.0×10^4 CFU ml^{-1}) (Tab. 2) that could improve soil quality and increase the CGR. This result is supported by Urra *et al.* (2019) who found that the application of sewage sludge in the long term significantly increases the organic matter contents in the soil, causing a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of the organic matter. Bhardwaj *et al.* (2014) and Carvajal-Muñoz and Carmona-Garcia (2012) showed that the application of a biofertilizer had advantages for the plants such as availability of nutrients balanced for plant health. It also stimulates nutrient mobilization that can increase soil biological activity and nutrient availability for microorganisms to encourage the growth of beneficial microorganisms, increasing the soil organic matter content and, therefore, the cation exchange capacity. Siswanti and Lestari (2019) indicated that the interaction of biogas sludge + biofertilizer (36 ml + 10 L ha^{-1}) significantly increased the plant height, number of leaves, and capsaicin content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

The isolates N3, P7, N3+P7 from the biogas sludge significantly increased the uptake of nitrogen (20.77%) and crop growth rate (2.81 times higher than the control) of upland rice in Ultisols with the highest increase found with the P7 isolate. The dose of biogas sludge significantly increased plant height (14.81%), total dry weight (50.55%), uptake of nitrogen (54.11%) and phosphorus (65.20%), and also crop growth rate (129.44%) of upland rice in Ultisols with the highest increase at a dose of 630 ml/polybag. Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice by 41.81% with the highest increase at the dose of 315 ml/polybag. The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice in Ultisols 5.76 times with the highest increase found with B1S3.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

All authors formulated the overarching research goals and aims, provided the study materials, and developed the methodology. NEM analyzed and interpreted the study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in the field, and collected the data. ZN and I verified the overall reproducibility of the results and the other research outputs. All authors conducted the critical review of the manuscript.

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Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la nutrición del arroz de tierras altas

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ABSTRACT

This study evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth and nutrient uptake of upland rice grown in Ultisols. We used a randomized block design with two factors and seven replicates from October 2020 to April 2021. The first factor used selected SBI (B0 = untreated, B1 = nitrogen-fixing bacteria isolate (N3), B2 = phosphate solubilizing bacteria isolate (P7), B3 = isolate combination (N3+P7)). The second factor was the dosage of biogas sludge (S0 = untreated, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). The parameters were determined by ANOVA and followed by Duncan's multiple range test at $P<0.05$. The results showed that the isolate P7 significantly increased the N uptake by 20.77% and crop growth rate (CGR) of upland rice 2.81 times. Biogas sludge doses from 315 to 630 ml/polybag significantly increased plant height, uptake of N and P, total fresh and dry weight, and CGR of upland rice. The interaction between N3 and biogas sludge dosage of 630 ml/polybag significantly increased the CGR of upland rice. The application of isolates N3 and P7 and their combination with biogas sludge of 630 ml/polybag has the potential to increase the CGR of upland rice in acidic soils.

Key words: acidic soil, crop growth rate, dosage, sludge potential.

RESUMEN

El presente estudio evaluó la influencia de aislamientos bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones sobre el crecimiento y la absorción de nutrientes del arroz de tierras altas cultivado en ultisoles. Se utilizó un diseño de bloques al azar con dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0 = sin tratamiento, B1 = aislamiento de bacterias fijadoras de nitrógeno (N3), B2 = aislamiento de bacterias solubilizadoras de fosfato (P7), B3 = combinación de aislamientos (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (S0 = sin tratamiento, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Los parámetros fueron determinados por análisis de varianza y seguidos de la prueba de rangos múltiples de Duncan a $P<0.05$. Los resultados mostraron que el aislamiento P7 aumentó significativamente la absorción de N en un 20.77% y la tasa de crecimiento del cultivo (TCC) de arroz de tierras altas 2.81 veces. Las dosis de lodos de biogás de 315 a 630 ml/polybag aumentaron significativamente la altura de la planta, la absorción de N y P, el peso fresco y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosis de lodos de biogás de 630 ml/polybag aumentó significativamente la TCC del arroz de tierras altas. La aplicación de los aislamientos N3 y P7 y su combinación con lodos de biogás de 630 ml/polybag tiene el potencial de aumentar la TCC de arroz de tierras altas en suelos ácidos.

Palabras clave: suelo ácido, tasa de crecimiento de cultivos, dosis, potencial de lodo.

Introduction

Biogas sludge is the waste by-product from an anaerobic processing system (FAO, 1977) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and plant yield (Adela *et al.,* 2014). The following characteristics of the biogas sludge from palm oil waste have been reported: total N of 490 mg L^{-1}, total P of 110 mg L^{-1}, total K of 1.9 mg L^{-1} (Lubis *et al.,* 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al.,* 2019), and N-NH_3 of 91-112 mg L^{-1} (Choorit & Wisarnwan, 2007). The pH may range from 6.8 to 8.3, with the highest bacterial population of 7.21×10^7 cells ml^{-1} and the lowest of 3.15×10^7 cells ml^{-1} (Alvionita *et al.,* 2019). Additionally, Mustamu and Triyanto (2020) reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria that have the potential to increase the availability of nitrogen and phosphate in soils.

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The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing bacteria has a greater potential to increase soil fertility and plant growth. Zhang *et al.* (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility and plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described different *Bacillus* species, such as *B. circulans, B. cereus, B. fusiformis, B. pumilus, B. megaterium, B. mycoides, B. coagulans, B. chitinolyticus,* and *B. subtilis* as phosphate solubilizing microorganisms. Ambrosini *et al.* (2016) showed the highest nitrogenase activity in *Bacillus cereus* among 42 different strains of *Bacillus* spp. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, *i.e., Proteobacteria, Firmicutes, Bacteroidetes*, and *Thermotogae.*

The application of bacteria from biogas sludge has never been reported in Indonesia for improving upland rice growth on acidic soils, including Ultisols. According to the Pusat Penelitian Tanah dan Agroklimat (Center for Soil and Agro-climate Research) (2000), the area in Indonesia covered by Ultisols is 45.8 million ha or 24% of the total area of the country. Furthermore, according to the Ministry of Agriculture, the area dedicated to rice cultivation in Indonesia is 15,712,025 ha with a yield of 81,148,617 t in 2017, and the contribution of upland rice yield is 4.66% (Kementerian Pertanian, 2017). The yield contribution of upland rice is classified as low and, therefore, it is necessary to find options to increase it. Therefore, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge to increase the availability of nitrogen and phosphate and, thus, the growth response of upland rice in Ultisols. This study aimed to evaluate the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the mineral nutrition of the upland rice grown in Ultisols.

Materials and methods

Study area

The concentration of total N and available P in Ultisols and in the plant tissue (N and P uptake) were analyzed in the Analytical Laboratory of Socfin Indonesia Inc., Medan (Indonesia). The bacterial isolates were applied to upland rice in the village of Padang Bulan (3°37.760' N, 98°38.898' E, and altitude of 18 m a.s.l.), Medan Selayang Subdistrict, Medan City, Indonesia, from October 2020 to April 2021. The average temperature was 27.4°C, the average air humidity was 82%, and the average rainfall was 228.5 mm per month.

Preparation of medium and upland rice seeds

The medium to grow upland rice plants was the Ultisol soil from the Simalingkar area, Medan Tuntungan Subdistrict, Medan City, collected at a depth of 0 to 20 cm. One hundred g of soil sample were taken and analyzed for chemical characteristics of pH H_2O, organic C by Walkley-Black, available P by Bray-II, total N using the Kjeldahl method, and cation exchange capacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH7 method (Tab. 1). The soil was sterilized by drying at 100°C for 2 h. To prevent heat from the sterilization process, the soil was incubated for 1 d and then placed into a 10 kg polybag (18 cm × 18 cm). A basic NPK fertilizer (16-16-16) by Meroke Tetap Jaya Inc. (Medan, Indonesia) at a dose of 1.5 g/polybag was applied by stirring evenly with the soil. The seeds of upland rice (*Oryza sativa* L.) of the inbred variety Inpago-8 from the Indonesian Agency for Agricultural Research and Development were soaked in water for 24 h, followed by the application of the fungicide Propineb (70%) for 2 h. Upland rice was planted after 1 d of basic fertilization with two seeds per polybag at a depth of 2 cm.

TABLE 1. Chemical characteristics of the Ultisol soil samples after sterilization at 100°C.

Chemical characteristics	Method	Value	Category
Soil pH (H_2O)	Electrometry	4.80	Acid
Organic C (%)	Walkley-Black	0.44	Very low
Total N (%)	Kjeldahl	0.04	Very low
Available P (mg kg^{-1})	Spectrophotometry	870.25	Very high
CEC (meq 100 g^{-1})	Ammonium acetate pH 7	28.31	High
Base saturation (%)	Ammonium acetate pH 7	4.85	Very low
	Exchangeable cations		
K (meq 100 g^{-1})	Ammonium acetate pH 7	0.60	High
Ca (meq 100 g^{-1})	Ammonium acetate pH 7	0.34	Very low
Mg (meq 100 g^{-1})	Ammonium acetate pH 7	0.32	Very low
Na (meq 100 g^{-1})	Ammonium acetate pH 7	0.09	Very low
Al (%)	Ammonium acetate pH 7	0.02	Very low

Criteria for pH (H_2O) = 4.5-5.5 (acid); organic C <1% (very low); total N <0.1% (very low); available P >60 mg kg^{-1} (very high); cation exchange capacity (CEC) = 25-40 meq 100 g^{-1} (high); base saturation <20% (very low); exchangeable K = 0.60-1.00 meq 100 g^{-1} (high); exchangeable Ca <2 meq 100 g^{-1} (very low); exchangeable Mg <0.4 meq 100 g^{-1} (very low); exchangeable Na <0.1 meq 100 g^{-1} (very low); exchangeable Al <5% (very low) (Balai Penelitian Tanah (Indonesia Soil Research Institute), 2009).

Preparation of superior bacterial isolates suspension and biogas sludge

One ml of the bacterial isolate suspension obtained from the characteristic stage was put into a test tube containing 9 ml of distilled water, homogenized and then diluted to 10^{-5}. A total of 0.1 ml of the suspension from the last dilution was spread over James nitrogen free malat bromothymol blue

(JNFB) medium (Kirchhof *et al.*, 1997) for the nitrogen-fixing bacterial isolates test and Pikovskaya (PVK) medium (Pikovskaya, 1948) for the phosphate solubilizing bacteria isolates. The culture medium was incubated for 2 to 3 d at 37°C. The nitrogen-fixing bacterial isolate test was characterized by the presence of colonies growing on the JNFB medium. The growth of phosphate solubilizing bacterial isolates was indicated by a halo zone around the microbial colonies on the PVK medium. Seven nitrogen-fixing and seven phosphate-solubilizing isolates were found to produce total N and available P. The isolates that showed the highest phosphate and nitrogen increasing abilities were selected, namely phosphate solubilizing bacteria (P7) and nitrogen-fixing bacteria (N3), which were confirmed by Mustamu *et al.* (2021a; 2021b).

The biogas sludge was collected from Nubika Jaya Inc., Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia. The procedure for processing biogas sludge was the following: the palm oil mill removed the palm oil mill effluent (POME) waste from the second pond which has been mixed with oil and then separated at an optimal temperature of 35°C. Liquid waste was then pumped into the receiver tank of 10 m^3 volume and filtered on a fiber tank screen to separate solid waste such as fiber and other materials. Liquid waste from the receiver tank was further pumped into the tower tank. Then, it was distributed evenly to the fixed tank at a temperature of 35 to 37°C and a flow rate of 20 to 30 m^3/h. Finally, the biogas sludge was taken from the fixed tank. Bacterial isolates and biogas sludge were applied to the soil surface at the base of the plants at one week after planting. Biogas sludge samples of 500 ml volume were used to analyze the chemical and biological characteristics (Tab. 2).

Treatment application

This study used a randomized block design with two factors and seven replicates. The first factor was the type of superior bacterial isolates (B0 = untreated; B1 = nitrogen-fixing bacterial isolate (N3); B2 = phosphate solubilizing bacteria isolate (P7); B3 = combination of isolates N3+P7) at a similar dose, namely 10 ml/polybag. The second factor was the dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Determination of biogas sludge based on the dose of liquid organic fertilizer at the oil palm was 126 m^3 ha^{-1} equal to 126,000 L ha^{-1} (Sutarta *et al.*, 2000), then converted to soil weight per polybag (Eq. 1). Each replicate was harvested at 4, 8, and 12 weeks after application (WAA) for determination of the crop growth rate (CGR).

$$\begin{array}{c}\text{Biogas}\\\text{sludge}\end{array} = \frac{\begin{array}{c}\text{Dose of liquid}\\\text{organic fertilizer ha}^{-1}\end{array}}{\text{Soil weight ha}^{-1}} \times \begin{array}{c}\text{soil weight}\\\text{per polybag}\end{array} \qquad (1)$$

$$= \frac{\cancel{26{,}000}\ \text{L ha}^{-1}}{2{,}000{,}000\ \text{kg ha}^{-1}} \times 10\ \text{kg} = \cancel{130}\ \text{ml}$$

Plant growth variables and data analysis

The growth variables were assessed by measuring the growth of upland rice (plant height, and total fresh and dry weights), contents and uptake of N and P in the aerial

TABLE 2. Chemical and biological characteristics of the biogas sludge.

Characteristics	Method	Value
pH	Electrometry	7.41
Chemical oxygen demand (mg L^{-1})	Spectrophotometry	4547.8
Biological oxygen demand (mg L^{-1})	Titrimetry	1127.5
Total N (%)	Kjeldahl	0.051
Total P (%)	Spectrophotometry	0.0097
Available P (%)	Spectrophotometry	0.013
Total K (%)	Graphite furnace - atomic absorption spectrophotometry (AAS)	0.18
Organic C (%)	Walkley-Black	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (mg L^{-1})	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Total nitrogen-fixing bacteria (CFU ml^{-1})	Plate count	29.4×10^5
Total phosphate solubilizing bacteria (CFU ml^{-1})	Plate count	7.0×10^4

Laboratory analysis based on the Balai Penelitian Tanah (Indonesia Soil Research Institute) (2009).

parts, and crop growth rate (CGR). The plant height was measured from the root apex to the tip of leaves using a measuring tape, and the total fresh weight was obtained by weighing the roots and shoots. The total dry weight (roots + shoots) was obtained after plant drying in an oven (VS-1202D3, Vision Scientific Co., Korea) at 60°C for 48 h and weighed using analytical scales. A 200 g sample of the second leaf from the shoots was collected and analyzed to determine the N content using the Kjeldahl method, and the P content was recorded using the destruction method through dry ashing (Bertramson, 1942). The N and P uptake were measured using Equation 2. The CGR was calculated as the dry weight related to the unit area at 4-8, 8-12, and 12-16 WAA using Equation 3 (Shon *et al.*, 1997):

$$\text{Nutrient uptake} = \text{nutrient content in the shoots} \times \text{total dry weight} \qquad (2)$$

$$\text{CGR} = \frac{\Delta W}{\Delta t} \times \frac{W2\text{-}W1}{t2\text{-}t1} \qquad (3)$$

where:

CGR = crop growth rate;

W1 = dry weight per unit area at t1;

W2 = dry weight per unit area at t2;

t_1 = first sampling;

t_2 = second sampling.

The parameters of the second phase of the study were analyzed by an ANOVA. If the treatment had a significant effect, the Duncan's multiple range test was applied at *P*<0.05 using SPSS v.20 software (IBM, 2011).

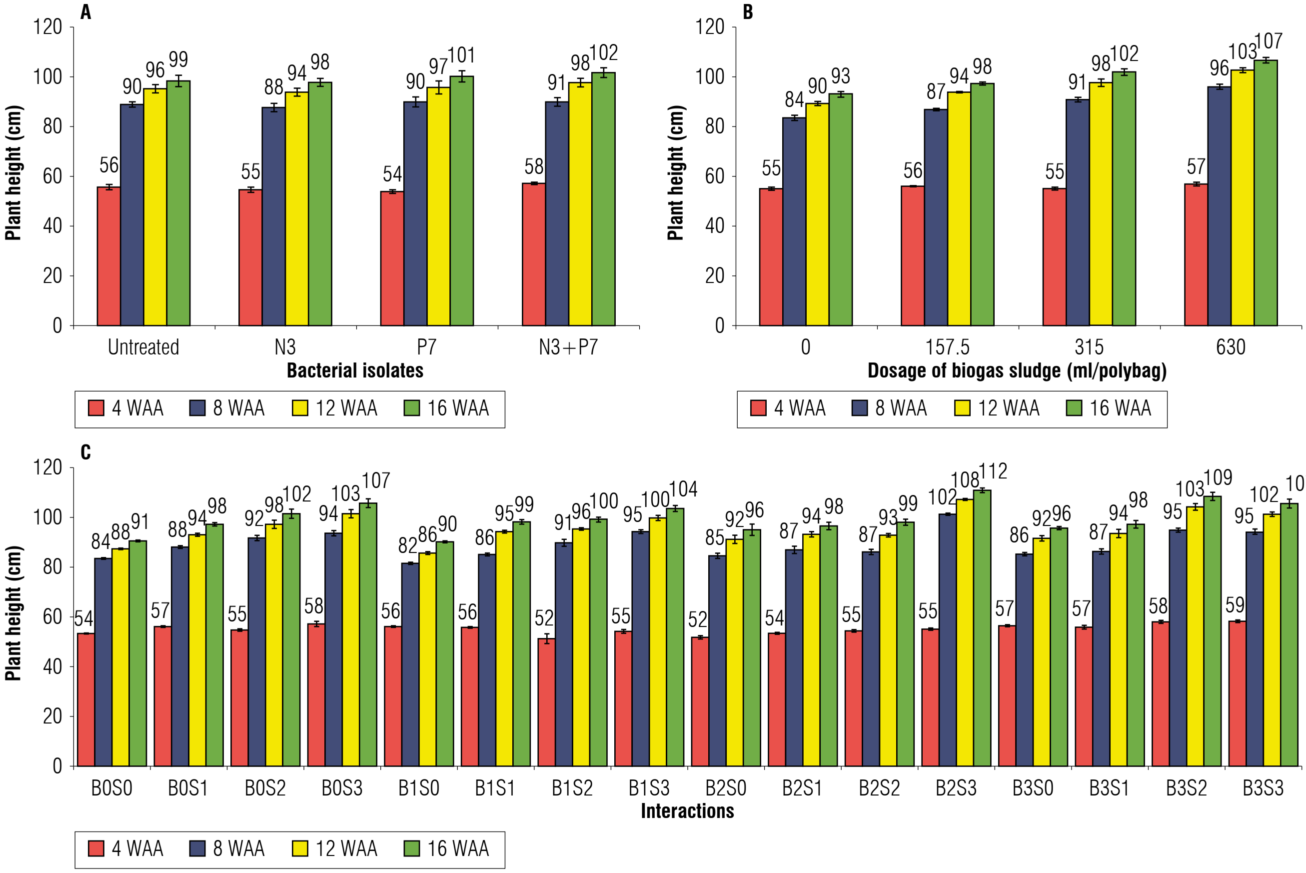

FIGURE 1. A) Effect of superior bacterial isolates, B) dosage of biogas sludge, and C) their interactions on plant height of upland rice at 4, 8, 12, and 16 weeks after application (WAA). Values followed by a different letter in the graph significantly differed according to the Duncan test at *P*<0.05. Vertical bars indicate the standard error. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7; B3 = isolates N3+P7).

Results

Effect of bacterial isolates and biogas sludge on upland rice growth

Plant height of upland rice

The effect of biogas sludge application was significant on the plant height of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the plant height of upland rice at 4, 8, 12, and 16 WAA (Fig. 1A-C). A significant increase in plant height of upland rice was observed with higher doses of biogas sludge of 630 ml/polybag at 8, 12, and 16 WAA, with the highest increase of 14.81% compared to the control at 16 WAA. Although the effect was not significant, the combination of isolates B3 and the interaction of B2S3 showed the highest increase in plant height of upland rice by 2.94% and 22.06%, respectively, compared to the control.

Biomass of upland rice

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the total fresh weight of upland rice at 4-16 WAA (Tab. 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total fresh weight (shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	**Total fresh weight (g) ± standard error**			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	4.15 ± 0.21	169.31 ± 8.90	215.27 ± 8.42	229.82 ± 8.94
B1	3.12 ± 0.12	194.50 ± 9.35	235.08 ± 10.32	252.02 ± 10.22
B2	4.52 ± 0.23	162.89 ± 11.15	201.85 ± 9.89	230.70 ± 9.28
B3	3.30 ± 0.25	173.91 ± 12.55	220.40 ± 15.96	245.03 ± 16.32
Biogas sludge (S)				
S0	3.72 ± 0.24	144.07 ± 9.37 b	182.67 ± 7.14 b	197.56 ± 6.58 b
S1	3.58 ± 0.27	153.41 ± 7.93 b	190.70 ± 8.90 b	215.65 ± 7.03 b
S2	3.64 ± 0.27	199.68 ± 10.30 a	258.70 ± 9.63 a	280.15 ± 9.25 a
S3	4.15 ± 0.25	203.45 ± 1.36 a	240.52 ± 2.81 a	264.21 ± 2.42 a
Interactions (B×S)				
B0S0	4.99 ± 0.33	124.08 ± 5.60	185.64 ± 3.32	192.78 ± 2.96
B0S1	3.47 ± 0.26	160.43 ± 1.16	188.60 ± 5.76	207.05 ± 3.97
B0S2	3.42 ± 0.42	185.97 ± 6.80	232.60 ± 8.75	250.84 ± 7.40
B0S3	4.71 ± 0.42	206.76 ± 5.49	254.23 ± 10.27	268.61 ± 8.85
B1S0	2.80 ± 0.18	155.79 ± 1.12	183.96 ± 5.20	202.88 ± 2.88
B1S1	3.74 ± 0.29	174.82 ± 9.01	227.91 ± 6.38	236.60 ± 6.32
B1S2	3.28 ± 0.40	241.17 ± 5.25	283.60 ± 7.76	296.08 ± 8.05
B1S3	2.67 ± 0.22	206.20 ± 7.23	244.85 ± 6.26	272.52 ± 4.34
B2S0	3.19 ± 0.18	190.90 ± 7.77	215.36 ± 7.67	229.11 ± 6.75
B2S1	4.85 ± 0.38	106.74 ± 13.42	143.16 ± 13.02	179.61 ± 10.36
B2S2	5.20 ± 0.24	148.40 ± 11.59	219.65 ± 5.26	248.72 ± 6.94
B2S3	4.82 ± 0.45	205.53 ± 10.50	229.21 ± 16.57	265.34 ± 9.58
B3S0	3.91 ± 0.30	105.53 ± 3.94	145.72 ± 1.96	165.45 ± 1.11
B3S1	2.25 ± 0.09	171.63 ± 4.90	203.14 ± 7.07	239.34 ± 12.07
B3S2	2.66 ± 0.14	223.17 ± 7.84	298.95 ± 1.51	324.94 ± 3.03
B3S3	4.37 ± 0.07	195.31 ± 6.77	233.79 ± 8.40	250.38 ± 8.16
CV (%)	56.09	29.68	26.31	20.78

Values followed by a different letter in the column significantly differed according to the Duncan test at $P<0.05$. CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

A significant increase in the total fresh weight of upland rice was observed with the higher dose of biogas sludge of 315 ml/polybag at 16 WAA, with the highest increase of 41.81% compared to the control. Although the effect was not significant, B1 and the interaction of B3S2 showed the highest increase in the total fresh weight of upland rice with 9.66% and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4-16 WAA (Tab. 4).

A significant increase in the total dry weight of upland rice was observed with the increase in the dosage of biogas sludge of 630 ml/polybag at 16 WAA, with the highest increase of 50.55% compared to the control. Although the effect was not significant, B1 and the interaction of B3S3 showed the highest increase in the total dry weight of upland rice with 20.84% and 81.53%, respectively, compared to the control.

Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12-16 WAA but did not have a significant effect at 4-8 and 8-12 WAA (Tab. 5).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight (shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	Total dry weight (g) ± standard error			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	1.38 ± 0.06	48.01 ± 1.29	73.60 ± 3.99	82.52 ± 4.18
B1	1.13 ± 0.05	54.09 ± 2.41	76.83 ± 2.66	99.72 ± 4.15
B2	1.49 ± 0.06	47.30 ± 3.30	73.20 ± 2.28	98.25 ± 3.90
B3	1.15 ± 0.07	52.32 ± 3.39	77.18 ± 4.90	98.47 ± 4.56
Biogas sludge (S)				
S0	1.26 ± 0.06	45.51 ± 2.63	62.88 ± 2.19 b	76.78 ± 1.63 c
S1	1.23 ± 0.08	44.47 ± 1.71	68.52 ± 2.00 ab	87.65 ± 2.84 bc
S2	1.26 ± 0.08	55.36 ± 3.43	85.69 ± 1.08 a	98.95 ± 1.86 b
S3	1.40 ± 0.06	56.38 ± 1.05	83.73 ± 3.44 a	115.59 ± 2.11 a
Interactions (B×S)				
B0S0	1.58 ± 0.08	41.73 ± 2.78	58.08 ± 1.54	67.23 ± 0.96
B0S1	1.12 ± 0.08	45.87 ± 0.83	62.74 ± 1.83	71.08 ± 1.91
B0S2	1.20 ± 0.12	52.25 ± 2.07	81.39 ± 5.48	88.28 ± 5.02
B0S3	1.60 ± 0.12	52.18 ± 0.29	92.20 ± 3.05	103.49 ± 2.43
B1S0	0.97 ± 0.04	46.64 ± 1.39	69.53 ± 4.90	80.30 ± 4.51
B1S1	1.40 ± 0.07	48.13 ± 2.78	78.91 ± 0.53	96.23 ± 1.50
B1S2	1.12 ± 0.10	67.79 ± 1.44	91.05 ± 2.25	101.80 ± 2.40
B1S3	1.02 ± 0.08	53.81 ± 3.76	67.84 ± 1.77	120.54 ± 2.15
B2S0	1.17 ± 0.05	59.32 ± 2.33	70.92 ± 4.20	81.43 ± 3.82
B2S1	1.54 ± 0.10	34.47 ± 2.16	61.69 ± 1.97	89.84 ± 1.41
B2S2	1.73 ± 0.05	37.37 ± 3.74	83.10 ± 1.19	105.46 ± 1.37
B2S3	1.53 ± 0.10	58.05 ± 1.76	77.07 ± 4.27	116.28 ± 1.30
B3S0	1.30 ± 0.07	34.35 ± 7.04	52.98 ± 0.73	78.16 ± 0.48
B3S1	0.85 ± 0.03	49.40 ± 0.08	70.72 ± 1.29	93.44 ± 2.19
B3S2	0.99 ± 0.05	64.05 ± 4.68	87.22 ± 2.90	100.26 ± 1.93
B3S3	1.44 ± 0.02	61.48 ± 2.47	97.80 ± 0.77	122.04 ± 0.20
CV (%)	43.80	31.22	26.54	18.38

Values followed by a different letter in the column significantly differed according to the Duncan test at $P<0.05$. CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

TABLE 5. Effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of the upland rice 4, 8, 12, and 16 weeks after the application (WAA).

Superior bacterial isolates (B)	Biogas sludge (S)				Average
	S0	S1	S2	S3	
			4-8 WAA		
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV = 32.28%
			8-12 WAA		
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888
Average	0.620	0.859	1.083	0.977	CV = 56.17%
			12-16 WAA		
B0	0.327 fgh	0.298 gh	0.246 h	0.403 b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882 a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400 a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866 a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV = 51.07%

Values followed by a different letter in the column significantly differed according to the Duncan test at $P<0.05$; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

The biogas sludge dose of 630 ml/polybag (S3) significantly increased the highest crop growth rate for upland rice at 12-16 WAA by 129.44% compared to the control. The isolates B1-B3 significantly increased the crop growth rate of upland rice with the highest increase for B2 of 181.45% compared to the controls at 12-16 WAA. The interaction of B1S3 significantly increased the crop growth rate of upland rice, showing values 5.76 times greater than those of the control.

Effect of bacterial isolates and biogas sludge on upland rice nutrition

Contents of N and P in the upland rice

The effect of biogas sludge, superior bacterial isolates, and their interactions did not have a significant effect on the content of N and P in the upland rice (Fig. 2A-C). The biogas sludge doses of 315 and 630 ml/polybag (S2 and S3) increased P and N in the plant tissue of upland rice by 33.33% and 4.53%, respectively, compared to the control. The isolate B2 showed the highest content of N in the plant tissues of upland rice with values 1.63% higher than those of the control. However, all isolates (B1-B3) showed a similar level of P in the plant tissues of upland rice compared to the control.

Uptake of N and P in the upland rice

The effect of biogas sludge significantly increased the uptake of N and P. The superior bacterial isolates significantly increased the uptake of nitrogen. The interaction of biogas sludge with superior bacterial isolates did not show a significant effect on the uptake of N and P in the upland rice (Fig. 2D-F).

A significant increase in the uptake of N and P in upland rice was observed with a higher dose of biogas sludge of 630 ml/polybag, with the highest increases of 54.11% and 65.20%, respectively, compared to the control. The bacterial isolates B1-B3 also significantly increased the uptake of N in the upland rice with the highest increase with B2 of 20.77% compared to the control. Although the effect was not significant, B3 showed the highest uptake of P in the upland rice of 19.93% compared to the control.

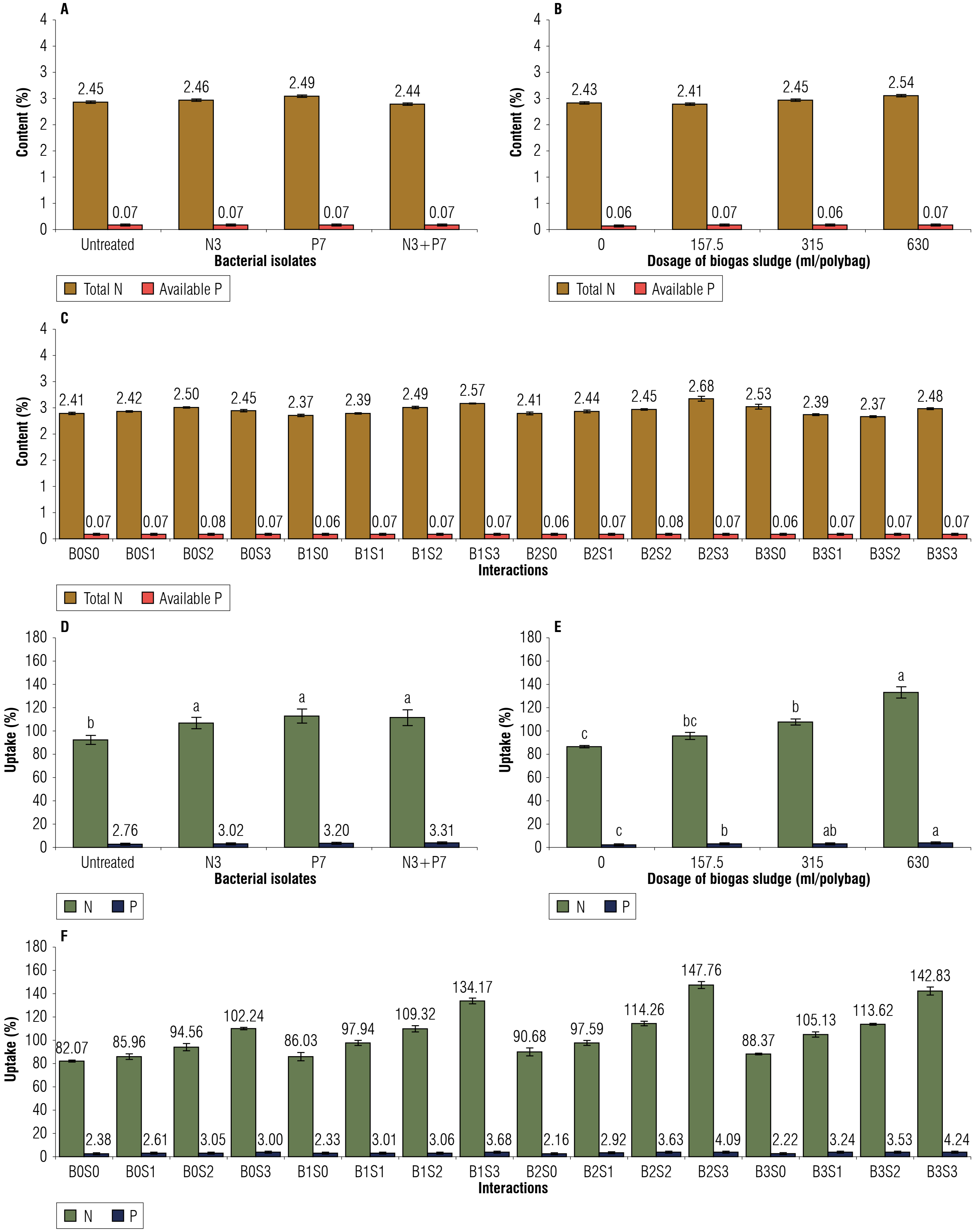

FIGURE 2. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on the nutrient content (A-C) and uptake of N and P (D-F) of upland rice. Values followed by different letters significantly differed according to the Duncan test at $P<0.05$. Vertical bars indicate the standard error. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = N3+P7 isolates).

Discussion

Effect of selected superior bacterial isolates

The selected superior bacterial isolates (N3 and P7) significantly increased the uptake of nitrogen and the crop growth rate of upland rice in an Ultisol at 12-16 WAA but did not have a significant effect on plant height, total fresh weight, total dry weight, content of N and P in leaves, uptake of phosphorus by the plants, and crop growth rate of upland rice from 4 to 12 WAA. The superior bacterial isolates (N3, P7, and N3+P7) increased the uptake of nitrogen in upland rice by 14.64%, 20.77%, and 20.68%, respectively, compared to the control (Fig. 2). Similar results are also shown in Table 5, with the crop growth rate of upland rice at 12-16 WAA increased by 2.57, 2.81, and 2.39 times, respectively, due to the selected superior bacterial isolates (N3, P7, N3+P7) compared to the control. The results indicate that a single P7 bacterial isolate increased the nitrogen uptake and crop growth rate of upland rice compared to a single N3 isolate and the combination of N3+P7 isolates. This was due to the presence of several organic acids and hormones produced by the isolate P7 that can increase the uptake of nitrogen and crop growth rate of upland rice. This result is supported by Mustamu *et al.* (2021a) who found that the phosphate solubilizing bacterial isolate (P7) from the biogas sludge contains organic acids, such as lactic, oxalic, acetic, and citric, and had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate with values 4.62 and 2.66 times higher, respectively, compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus in soils slightly increased with the application of bio fertilization with *Bacillus cereus*. This was due to the production of organic acids and other substances, including citric, tartaric, and oxalic acids, that can stimulate plant growth and nutrient availability. Youssef and Eissa (2017) reported that the increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of mineral nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that gibberellin and IAA can increase plant growth under stressful conditions. Kang *et al.* (2014) indicated that the plant growth-promoting bacteria (PGPB) has several mechanisms to increase plant growth with nitrogen-fixation and phosphate solubilization, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from an anaerobic digester include *Ruminococcus* sp., *Thiomargarita* sp., *Clostridium* sp. *Anaerobacter* sp., *Bacillus* sp., *Sporobacterium* sp., *Saccharofermentans* sp., *Oscillibacter* sp., *Sporobacter* sp., and *Enterobacter* sp. Liaquat *et al.* (2017) also reported abundance of *Bacillus, Clostridium*, and *Enterobacter* spp. in an anaerobic digester of wastewater when producing biogas.

Effect of biogas sludge

The dose of biogas sludge significantly increased the plant height, total fresh weight (8, 12, and 16 WAA), total dry weight (12 and 16 WAA), uptake (N and P), and crop growth rate of upland rice at 8-12 WAA. However, it did not have a significant effect on the content (N and P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAA). An increase in plant height, total dry weight, uptake of nitrogen and phosphorus, and also crop growth rate of upland rice in an Ultisol with a higher dose of biogas sludge of 630 ml/polybag was seen at the end of this study (16 WAA). In contrast, the total fresh weight increased along with the higher dose of biogas sludge of 315 ml/polybag and then decreased at the dose of 630 ml/polybag. This result is due to the chemical characteristics of the biogas sludge such as pH (7.41), total N (0.051%), available P (0.013%), organic C (0.14%), total K (0.18%), and the biological characteristics such as total nitrogen-fixing bacteria (29.4×10^5 CFU ml^{-1}) and total phosphate solubilizing bacteria (7.0×10^4 CFU ml^{-1}) (Tab. 2). The organic C content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could increase the uptake of nitrogen and phosphorus in upland rice with an increasing dose of biogas sludge of 630 ml/polybag (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the plant height, biomass, and crop growth rate of the upland rice. A similar result was reported by Mustamu and Triyanto (2020), who determined the macro and micronutrients from the biogas sludge and the population of nitrogen-fixing and phosphate solubilizing bacteria of 480×10^4 and 42×10^4 CFU ml^{-1}, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that the total phosphate solubilizing bacteria (1.6 to 2.5 CFU ml^{-1}) was significantly higher than nitrogen-fixing bacteria (0.5-1.4 CFU ml^{-1}), showing a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported an increase in concentrations of NH_4^+-N from 45% to 80% in the anaerobic waste.

Interaction of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice in Ultisols at 12-16 WAA but did not have a significant effect on the other parameters in this study. The interaction of B1 with biogas sludge at the dose of 630 ml/polybag (B1S3) showed the highest crop growth rate of

upland rice compared to other interactions and was 5.76 times greater compared to the control. This was caused by the application of biogas sludge that could have increased soil organic matter and the total population of beneficial bacteria. Likewise, the biogas sludge contained organic C (0.14%), total nitrogen-fixing bacteria (29.4×10^5 CFU ml^{-1}), and total phosphate solubilizing bacteria (7.0×10^4 CFU ml^{-1}) (Tab. 2) that could improve soil quality and increase the CGR. This result is supported by Urra *et al.* (2019) who found that the application of sewage sludge in the long term significantly increases the organic matter contents in the soil, causing a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of the organic matter. Bhardwaj *et al.* (2014) and Carvajal-Muñoz and Carmona-Garcia (2012) showed that the application of a biofertilizer had advantages for the plants such as availability of nutrients balanced for plant health. It also stimulates nutrient mobilization that can increase soil biological activity and nutrient availability for microorganisms to encourage the growth of beneficial microorganisms, increasing the soil organic matter content and, therefore, the cation exchange capacity. Siswanti and Lestari (2019) indicated that the interaction of biogas sludge + biofertilizer (36 ml + 10 L ha^{-1}) significantly increased the plant height, number of leaves, and capsaicin content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

The isolates N3, P7, N3+P7 from the biogas sludge significantly increased the uptake of nitrogen (20.77%) and crop growth rate (2.81 times higher than the control) of upland rice in Ultisols with the highest increase found with the P7 isolate. The dose of biogas sludge significantly increased plant height (14.81%), total dry weight (50.55%), uptake of nitrogen (54.11%) and phosphorus (65.20%), and also crop growth rate (129.44%) of upland rice in Ultisols with the highest increase at a dose of 630 ml/polybag. Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice by 41.81% with the highest increase at the dose of 315 ml/polybag. The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice in Ultisols 5.76 times with the highest increase found with B1S3.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

All authors formulated the overarching research goals and aims, provided the study materials, and developed the methodology. NEM analyzed and interpreted the study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in the field, and collected the data. ZN and I verified the overall reproducibility of the results and the other research outputs. All authors conducted the critical review of the manuscript.

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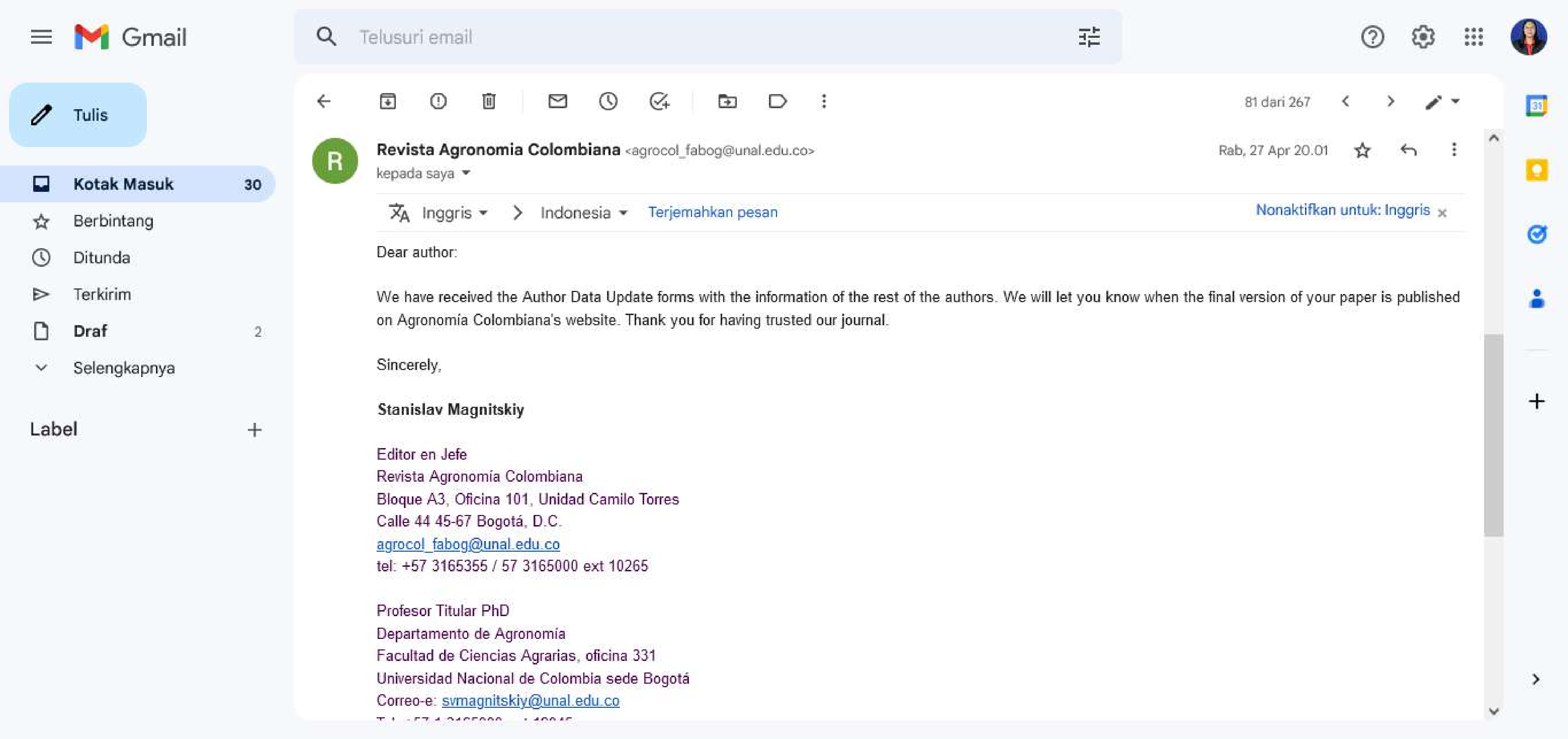

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Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la nutrición del arroz de tierras altas

Novilda Elizabeth Mustamu[1], Zulkifli Nasution[2*], Irvan[3], and Mariani Sembiring[2]

ABSTRACT

This study evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth and nutrient uptake of upland rice grown in Ultisols. We used a randomized block design with two factors and seven replicates from October 2020 to April 2021. The first factor used selected SBI (B0 = untreated, B1 = nitrogen-fixing bacteria isolate (N3), B2 = phosphate solubilizing bacteria isolate (P7), B3 = isolate combination (N3+P7)). The second factor was the dosage of biogas sludge (S0 = untreated, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). The parameters were determined by ANOVA and followed by Duncan's multiple range test at $P<0.05$. The results showed that the isolate P7 significantly increased the N uptake by 20.77% and crop growth rate (CGR) of upland rice 2.81 times. Biogas sludge doses from 315 to 630 ml/polybag significantly increased plant height, uptake of N and P, total fresh and dry weight, and CGR of upland rice. The interaction between N3 and biogas sludge dosage of 630 ml/polybag significantly increased the CGR of upland rice. The application of isolates N3 and P7 and their combination with biogas sludge of 630 ml/polybag has the potential to increase the CGR of upland rice in acidic soils.

Key words: acidic soil, crop growth rate, dosage, sludge potential.

RESUMEN

El presente estudio evaluó la influencia de aislamientos bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones sobre el crecimiento y la absorción de nutrientes del arroz de tierras altas cultivado en ultisoles. Se utilizó un diseño de bloques al azar con dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0 = sin tratamiento, B1 = aislamiento de bacterias fijadoras de nitrógeno (N3), B2 = aislamiento de bacterias solubilizadoras de fosfato (P7), B3 = combinación de aislamientos (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (S0 = sin tratamiento, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Los parámetros fueron determinados por análisis de varianza y seguidos de la prueba de rangos múltiples de Duncan a $P<0.05$. Los resultados mostraron que el aislamiento P7 aumentó significativamente la absorción de N en un 20.77% y la tasa de crecimiento del cultivo (TCC) de arroz de tierras altas 2.81 veces. Las dosis de lodos de biogás de 315 a 630 ml/polybag aumentaron significativamente la altura de la planta, la absorción de N y P, el peso fresco y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosis de lodos de biogás de 630 ml/polybag aumentó significativamente la TCC del arroz de tierras altas. La aplicación de los aislamientos N3 y P7 y su combinación con lodos de biogás de 630 ml/polybag tiene el potencial de aumentar la TCC de arroz de tierras altas en suelos ácidos.

Palabras clave: suelo ácido, tasa de crecimiento de cultivos, dosis, potencial de lodo.

Introduction

Biogas sludge is the waste by-product from an anaerobic processing system (FAO, 1977) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and plant yield (Adela *et al.,* 2014). The following characteristics of the biogas sludge from palm oil waste have been reported: total N of 490 mg L^{-1}, total P of 110 mg L^{-1}, total K of 1.9 mg L^{-1} (Lubis *et al.,* 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al.,* 2019), and N-NH_3 of 91-112 mg L^{-1} (Choorit & Wisarnwan, 2007). The pH may range from 6.8 to 8.3, with the highest bacterial population of 7.21×10^7 cells ml^{-1} and the lowest of 3.15×10^7 cells ml^{-1} (Alvionita *et al.,* 2019). Additionally, Mustamu and Triyanto (2020) reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria that have the potential to increase the availability of nitrogen and phosphate in soils.

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The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing bacteria has a greater potential to increase soil fertility and plant growth. Zhang *et al.* (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility and plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described different *Bacillus* species, such as *B. circulans, B. cereus, B. fusiformis, B. pumilus, B. megaterium, B. mycoides, B. coagulans, B. chitinolyticus,* and *B. subtilis* as phosphate solubilizing microorganisms. Ambrosini *et al.* (2016) showed the highest nitrogenase activity in *Bacillus cereus* among 42 different strains of *Bacillus* spp. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, *i.e.*, *Proteobacteria, Firmicutes, Bacteroidetes*, and *Thermotogae.*

The application of bacteria from biogas sludge has never been reported in Indonesia for improving upland rice growth on acidic soils, including Ultisols. According to the Pusat Penelitian Tanah dan Agroklimat (Center for Soil and Agro-climate Research) (2000), the area in Indonesia covered by Ultisols is 45.8 million ha or 24% of the total area of the country. Furthermore, according to the Ministry of Agriculture, the area dedicated to rice cultivation in Indonesia is 15,712,025 ha with a yield of 81,148,617 t in 2017, and the contribution of upland rice yield is 4.66% (Kementerian Pertanian, 2017). The yield contribution of upland rice is classified as low and, therefore, it is necessary to find options to increase it. Therefore, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge to increase the availability of nitrogen and phosphate and, thus, the growth response of upland rice in Ultisols. This study aimed to evaluate the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the mineral nutrition of the upland rice grown in Ultisols.

Materials and methods

Study area

The concentration of total N and available P in Ultisols and in the plant tissue (N and P uptake) were analyzed in the Analytical Laboratory of Socfin Indonesia Inc., Medan (Indonesia). The bacterial isolates were applied to upland rice in the village of Padang Bulan (3°37.760' N, 98°38.898' E, and altitude of 18 m a.s.l.), Medan Selayang Subdistrict, Medan City, Indonesia, from October 2020 to April 2021. The average temperature was 27.4°C, the average air humidity was 82%, and the average rainfall was 228.5 mm per month.

Preparation of medium and upland rice seeds

The medium to grow upland rice plants was the Ultisol soil from the Simalingkar area, Medan Tuntungan Subdistrict, Medan City, collected at a depth of 0 to 20 cm. One hundred g of soil sample were taken and analyzed for chemical characteristics of pH H_2O, organic C by Walkley-Black, available P by Bray-II, total N using the Kjeldahl method, and cation exchange capacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH7 method (Tab. 1). The soil was sterilized by drying at 100°C for 2 h. To prevent heat from the sterilization process, the soil was incubated for 1 d and then placed into a 10 kg polybag (18 cm × 18 cm). A basic NPK fertilizer (16-16-16) by Meroke Tetap Jaya Inc. (Medan, Indonesia) at a dose of 1.5 g/polybag was applied by stirring evenly with the soil. The seeds of upland rice (*Oryza sativa* L.) of the inbred variety Inpago-8 from the Indonesian Agency for Agricultural Research and Development were soaked in water for 24 h, followed by the application of the fungicide Propineb (70%) for 2 h. Upland rice was planted after 1 d of basic fertilization with two seeds per polybag at a depth of 2 cm.

TABLE 1. Chemical characteristics of the Ultisol soil samples after sterilization at 100°C.

Chemical characteristics	Method	Value	Category
Soil pH (H_2O)	Electrometry	4.80	Acid
Organic C (%)	Walkley-Black	0.44	Very low
Total N (%)	Kjeldahl	0.04	Very low
Available P (mg kg^{-1})	Spectrophotometry	870.25	Very high
CEC (meq 100 g^{-1})	Ammonium acetate pH 7	28.31	High
Base saturation (%)	Ammonium acetate pH 7	4.85	Very low
	Exchangeable cations		
K (meq 100 g^{-1})	Ammonium acetate pH 7	0.60	High
Ca (meq 100 g^{-1})	Ammonium acetate pH 7	0.34	Very low
Mg (meq 100 g^{-1})	Ammonium acetate pH 7	0.32	Very low
Na (meq 100 g^{-1})	Ammonium acetate pH 7	0.09	Very low
Al (%)	Ammonium acetate pH 7	0.02	Very low

Criteria for pH (H_2O) = 4.5-5.5 (acid); organic C <1% (very low); total N <0.1% (very low); available P >60 mg kg^{-1} (very high); cation exchange capacity (CEC) = 25-40 meq 100 g^{-1} (high); base saturation <20% (very low); exchangeable K = 0.60-1.00 meq 100 g^{-1} (high); exchangeable Ca <2 meq 100 g^{-1} (very low); exchangeable Mg <0.4 meq 100 g^{-1} (very low); exchangeable Na <0.1 meq 100 g^{-1} (very low); exchangeable Al <5% (very low) (Balai Penelitian Tanah (Indonesia Soil Research Institute), 2009).

Preparation of superior bacterial isolates suspension and biogas sludge

One ml of the bacterial isolate suspension obtained from the characteristic stage was put into a test tube containing 9 ml of distilled water, homogenized and then diluted to 10^{-5}. A total of 0.1 ml of the suspension from the last dilution was spread over James nitrogen free malat bromothymol blue

(JNFB) medium (Kirchhof *et al.,* 1997) for the nitrogen-fixing bacterial isolates test and Pikovskaya (PVK) medium (Pikovskaya, 1948) for the phosphate solubilizing bacteria isolates. The culture medium was incubated for 2 to 3 d at 37°C. The nitrogen-fixing bacterial isolate test was characterized by the presence of colonies growing on the JNFB medium. The growth of phosphate solubilizing bacterial isolates was indicated by a halo zone around the microbial colonies on the PVK medium. Seven nitrogen-fixing and seven phosphate-solubilizing isolates were found to produce total N and available P. The isolates that showed the highest phosphate and nitrogen increasing abilities were selected, namely phosphate solubilizing bacteria (P7) and nitrogen-fixing bacteria (N3), which were confirmed by Mustamu *et al.* (2021a; 2021b).

The biogas sludge was collected from Nubika Jaya Inc., Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia. The procedure for processing biogas sludge was the following: the palm oil mill removed the palm oil mill effluent (POME) waste from the second pond which has been mixed with oil and then separated at an optimal temperature of 35°C. Liquid waste was then pumped into the receiver tank of 10 m^3 volume and filtered on a fiber tank screen to separate solid waste such as fiber and other materials. Liquid waste from the receiver tank was further pumped into the tower tank. Then, it was distributed evenly to the fixed tank at a temperature of 35 to 37°C and a flow rate of 20 to 30 m^3/h. Finally, the biogas sludge was taken from the fixed tank. Bacterial isolates and biogas sludge were applied to the soil surface at the base of the plants at one week after planting. Biogas sludge samples of 500 ml volume were used to analyze the chemical and biological characteristics (Tab. 2).

Treatment application

This study used a randomized block design with two factors and seven replicates. The first factor was the type of superior bacterial isolates (B0 = untreated; B1 = nitrogen-fixing bacterial isolate (N3); B2 = phosphate solubilizing bacteria isolate (P7); B3 = combination of isolates N3+P7) at a similar dose, namely 10 ml/polybag. The second factor was the dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Determination of biogas sludge based on the dose of liquid organic fertilizer at the oil palm was 126 m^3 ha^{-1} equal to 126,000 L ha^{-1} (Sutarta *et al.,* 2000), then converted to soil weight per polybag (Eq. 1). Each replicate was harvested at 4, 8, and 12 weeks after application (WAA) for determination of the crop growth rate (CGR).

$$\begin{aligned}\text{Biogas sludge} &= \frac{\text{Dose of liquid organic fertilizer ha}^{-1}}{\text{Soil weight ha}^{-1}} \times \text{soil weight per polybag} \qquad (1)\\ &= \frac{126{,}000 \text{ L ha}^{-1}}{2{,}000{,}000 \text{ kg ha}^{-1}} \times 10 \text{ kg} = 630 \text{ ml}\end{aligned}$$

Plant growth variables and data analysis

The growth variables were assessed by measuring the growth of upland rice (plant height, and total fresh and dry weights), contents and uptake of N and P in the aerial

TABLE 2. Chemical and biological characteristics of the biogas sludge.

Characteristics	Method	Value
pH	Electrometry	7.41
Chemical oxygen demand (mg L^{-1})	Spectrophotometry	4547.8
Biological oxygen demand (mg L^{-1})	Titrimetry	1127.5
Total N (%)	Kjeldahl	0.051
Total P (%)	Spectrophotometry	0.0097
Available P (%)	Spectrophotometry	0.013
Total K (%)	Graphite furnace - atomic absorption spectrophotometry (AAS)	0.18
Organic C (%)	Walkley-Black	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (mg L^{-1})	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Total nitrogen-fixing bacteria (CFU ml^{-1})	Plate count	29.4×10^5
Total phosphate solubilizing bacteria (CFU ml^{-1})	Plate count	7.0×10^4

Laboratory analysis based on the Balai Penelitian Tanah (Indonesia Soil Research Institute) (2009).

parts, and crop growth rate (CGR). The plant height was measured from the root apex to the tip of leaves using a measuring tape, and the total fresh weight was obtained by weighing the roots and shoots. The total dry weight (roots + shoots) was obtained after plant drying in an oven (VS-1202D3, Vision Scientific Co., Korea) at 60°C for 48 h and weighed using analytical scales. A 200 g sample of the second leaf from the shoots was collected and analyzed to determine the N content using the Kjeldahl method, and the P content was recorded using the destruction method through dry ashing (Bertramson, 1942). The N and P uptake were measured using Equation 2. The CGR was calculated as the dry weight related to the unit area at 4-8, 8-12, and 12-16 WAA using Equation 3 (Shon *et al.*, 1997):

$$\text{Nutrient uptake} = \text{nutrient content in the shoots} \times \text{total dry weight} \qquad (2)$$

$$\text{CGR} = \frac{\Delta W}{\Delta t} \times \frac{\text{W2-W1}}{\text{t2-t1}} \qquad (3)$$

where:

CGR = crop growth rate;

W1 = dry weight per unit area at t1;

W2 = dry weight per unit area at t2;

t_1 = first sampling;

t_2 = second sampling.

The parameters of the second phase of the study were analyzed by an ANOVA. If the treatment had a significant effect, the Duncan's multiple range test was applied at P<0.05 using SPSS v.20 software (IBM, 2011).

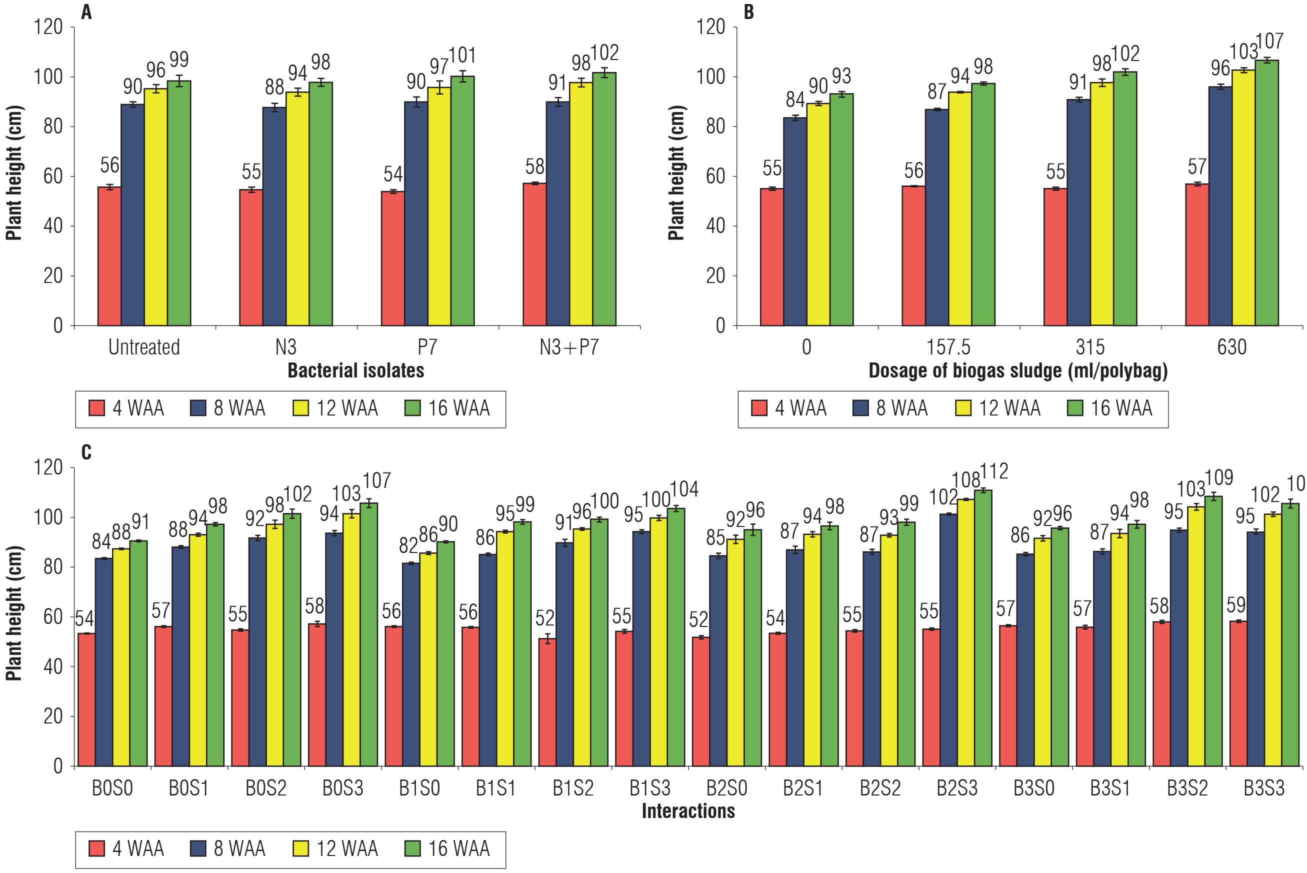

FIGURE 1. A) Effect of superior bacterial isolates, B) dosage of biogas sludge, and C) their interactions on plant height of upland rice at 4, 8, 12, and 16 weeks after application (WAA). Values followed by a different letter in the graph significantly differed according to the Duncan test at P<0.05. Vertical bars indicate the standard error. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7; B3 = isolates N3+P7).

Results

Effect of bacterial isolates and biogas sludge on upland rice growth

Plant height of upland rice

The effect of biogas sludge application was significant on the plant height of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the plant height of upland rice at 4, 8, 12, and 16 WAA (Fig. 1A-C). A significant increase in plant height of upland rice was observed with higher doses of biogas sludge of 630 ml/polybag at 8, 12, and 16 WAA, with the highest increase of 14.81% compared to the control at 16 WAA. Although the effect was not significant, the combination of isolates B3 and the interaction of B2S3 showed the highest increase in plant height of upland rice by 2.94% and 22.06%, respectively, compared to the control.

Biomass of upland rice

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the total fresh weight of upland rice at 4-16 WAA (Tab. 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total fresh weight (shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	**Total fresh weight (g) ± standard error**			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	4.15 ± 0.21	169.31 ± 8.90	215.27 ± 8.42	229.82 ± 8.94
B1	3.12 ± 0.12	194.50 ± 9.35	235.08 ± 10.32	252.02 ± 10.22
B2	4.52 ± 0.23	162.89 ± 11.15	201.85 ± 9.89	230.70 ± 9.28
B3	3.30 ± 0.25	173.91 ± 12.55	220.40 ± 15.96	245.03 ± 16.32
Biogas sludge (S)				
S0	3.72 ± 0.24	144.07 ± 9.37 b	182.67 ± 7.14 b	197.56 ± 6.58 b
S1	3.58 ± 0.27	153.41 ± 7.93 b	190.70 ± 8.90 b	215.65 ± 7.03 b
S2	3.64 ± 0.27	199.68 ± 10.30 a	258.70 ± 9.63 a	280.15 ± 9.25 a
S3	4.15 ± 0.25	203.45 ± 1.36 a	240.52 ± 2.81 a	264.21 ± 2.42 a
Interactions (B×S)				
B0S0	4.99 ± 0.33	124.08 ± 5.60	185.64 ± 3.32	192.78 ± 2.96
B0S1	3.47 ± 0.26	160.43 ± 1.16	188.60 ± 5.76	207.05 ± 3.97
B0S2	3.42 ± 0.42	185.97 ± 6.80	232.60 ± 8.75	250.84 ± 7.40
B0S3	4.71 ± 0.42	206.76 ± 5.49	254.23 ± 10.27	268.61 ± 8.85
B1S0	2.80 ± 0.18	155.79 ± 1.12	183.96 ± 5.20	202.88 ± 2.88
B1S1	3.74 ± 0.29	174.82 ± 9.01	227.91 ± 6.38	236.60 ± 6.32
B1S2	3.28 ± 0.40	241.17 ± 5.25	283.60 ± 7.76	296.08 ± 8.05
B1S3	2.67 ± 0.22	206.20 ± 7.23	244.85 ± 6.26	272.52 ± 4.34
B2S0	3.19 ± 0.18	190.90 ± 7.77	215.36 ± 7.67	229.11 ± 6.75
B2S1	4.85 ± 0.38	106.74 ± 13.42	143.16 ± 13.02	179.61 ± 10.36
B2S2	5.20 ± 0.24	148.40 ± 11.59	219.65 ± 5.26	248.72 ± 6.94
B2S3	4.82 ± 0.45	205.53 ± 10.50	229.21 ± 16.57	265.34 ± 9.58
B3S0	3.91 ± 0.30	105.53 ± 3.94	145.72 ± 1.96	165.45 ± 1.11
B3S1	2.25 ± 0.09	171.63 ± 4.90	203.14 ± 7.07	239.34 ± 12.07
B3S2	2.66 ± 0.14	223.17 ± 7.84	298.95 ± 1.51	324.94 ± 3.03
B3S3	4.37 ± 0.07	195.31 ± 6.77	233.79 ± 8.40	250.38 ± 8.16
CV (%)	56.09	29.68	26.31	20.78

Values followed by a different letter in the column significantly differed according to the Duncan test at $P<0.05$. CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

A significant increase in the total fresh weight of upland rice was observed with the higher dose of biogas sludge of 315 ml/polybag at 16 WAA, with the highest increase of 41.81% compared to the control. Although the effect was not significant, B1 and the interaction of B3S2 showed the highest increase in the total fresh weight of upland rice with 9.66% and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4-16 WAA (Tab. 4).

A significant increase in the total dry weight of upland rice was observed with the increase in the dosage of biogas sludge of 630 ml/polybag at 16 WAA, with the highest increase of 50.55% compared to the control. Although the effect was not significant, B1 and the interaction of B3S3 showed the highest increase in the total dry weight of upland rice with 20.84% and 81.53%, respectively, compared to the control.

Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12-16 WAA but did not have a significant effect at 4-8 and 8-12 WAA (Tab. 5).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight (shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	Total dry weight (g) ± standard error			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	1.38 ± 0.06	48.01 ± 1.29	73.60 ± 3.99	82.52 ± 4.18
B1	1.13 ± 0.05	54.09 ± 2.41	76.83 ± 2.66	99.72 ± 4.15
B2	1.49 ± 0.06	47.30 ± 3.30	73.20 ± 2.28	98.25 ± 3.90
B3	1.15 ± 0.07	52.32 ± 3.39	77.18 ± 4.90	98.47 ± 4.56
Biogas sludge (S)				
S0	1.26 ± 0.06	45.51 ± 2.63	62.88 ± 2.19 b	76.78 ± 1.63 c
S1	1.23 ± 0.08	44.47 ± 1.71	68.52 ± 2.00 ab	87.65 ± 2.84 bc
S2	1.26 ± 0.08	55.36 ± 3.43	85.69 ± 1.08 a	98.95 ± 1.86 b
S3	1.40 ± 0.06	56.38 ± 1.05	83.73 ± 3.44 a	115.59 ± 2.11 a
Interactions (B×S)				
B0S0	1.58 ± 0.08	41.73 ± 2.78	58.08 ± 1.54	67.23 ± 0.96
B0S1	1.12 ± 0.08	45.87 ± 0.83	62.74 ± 1.83	71.08 ± 1.91
B0S2	1.20 ± 0.12	52.25 ± 2.07	81.39 ± 5.48	88.28 ± 5.02
B0S3	1.60 ± 0.12	52.18 ± 0.29	92.20 ± 3.05	103.49 ± 2.43
B1S0	0.97 ± 0.04	46.64 ± 1.39	69.53 ± 4.90	80.30 ± 4.51
B1S1	1.40 ± 0.07	48.13 ± 2.78	78.91 ± 0.53	96.23 ± 1.50
B1S2	1.12 ± 0.10	67.79 ± 1.44	91.05 ± 2.25	101.80 ± 2.40
B1S3	1.02 ± 0.08	53.81 ± 3.76	67.84 ± 1.77	120.54 ± 2.15
B2S0	1.17 ± 0.05	59.32 ± 2.33	70.92 ± 4.20	81.43 ± 3.82
B2S1	1.54 ± 0.10	34.47 ± 2.16	61.69 ± 1.97	89.84 ± 1.41
B2S2	1.73 ± 0.05	37.37 ± 3.74	83.10 ± 1.19	105.46 ± 1.37
B2S3	1.53 ± 0.10	58.05 ± 1.76	77.07 ± 4.27	116.28 ± 1.30
B3S0	1.30 ± 0.07	34.35 ± 7.04	52.98 ± 0.73	78.16 ± 0.48
B3S1	0.85 ± 0.03	49.40 ± 0.08	70.72 ± 1.29	93.44 ± 2.19
B3S2	0.99 ± 0.05	64.05 ± 4.68	87.22 ± 2.90	100.26 ± 1.93
B3S3	1.44 ± 0.02	61.48 ± 2.47	97.80 ± 0.77	122.04 ± 0.20
CV (%)	43.80	31.22	26.54	18.38

Values followed by a different letter in the column significantly differed according to the Duncan test at $P<0.05$. CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

TABLE 5. Effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of the upland rice 4, 8, 12, and 16 weeks after the application (WAA).

Superior bacterial isolates (B)	Biogas sludge (S)				Average
	S0	S1	S2	S3	
			4-8 WAA		
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV = 32.28%
			8-12 WAA		
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888
Average	0.620	0.859	1.083	0.977	CV = 56.17%
			12-16 WAA		
B0	0.327 fgh	0.298 gh	0.246 h	0.403 b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882 a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400 a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866 a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV = 51.07%

Values followed by a different letter in the column significantly differed according to the Duncan test at $P<0.05$; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

The biogas sludge dose of 630 ml/polybag (S3) significantly increased the highest crop growth rate for upland rice at 12-16 WAA by 129.44% compared to the control. The isolates B1-B3 significantly increased the crop growth rate of upland rice with the highest increase for B2 of 181.45% compared to the controls at 12-16 WAA. The interaction of B1S3 significantly increased the crop growth rate of upland rice, showing values 5.76 times greater than those of the control.

Effect of bacterial isolates and biogas sludge on upland rice nutrition

Contents of N and P in the upland rice

The effect of biogas sludge, superior bacterial isolates, and their interactions did not have a significant effect on the content of N and P in the upland rice (Fig. 2A-C). The biogas sludge doses of 315 and 630 ml/polybag (S2 and S3) increased P and N in the plant tissue of upland rice by 33.33% and 4.53%, respectively, compared to the control. The isolate B2 showed the highest content of N in the plant tissues of upland rice with values 1.63% higher than those of the control. However, all isolates (B1-B3) showed a similar level of P in the plant tissues of upland rice compared to the control.

Uptake of N and P in the upland rice

The effect of biogas sludge significantly increased the uptake of N and P. The superior bacterial isolates significantly increased the uptake of nitrogen. The interaction of biogas sludge with superior bacterial isolates did not show a significant effect on the uptake of N and P in the upland rice (Fig. 2D-F).

A significant increase in the uptake of N and P in upland rice was observed with a higher dose of biogas sludge of 630 ml/polybag, with the highest increases of 54.11% and 65.20%, respectively, compared to the control. The bacterial isolates B1-B3 also significantly increased the uptake of N in the upland rice with the highest increase with B2 of 20.77% compared to the control. Although the effect was not significant, B3 showed the highest uptake of P in the upland rice of 19.93% compared to the control.

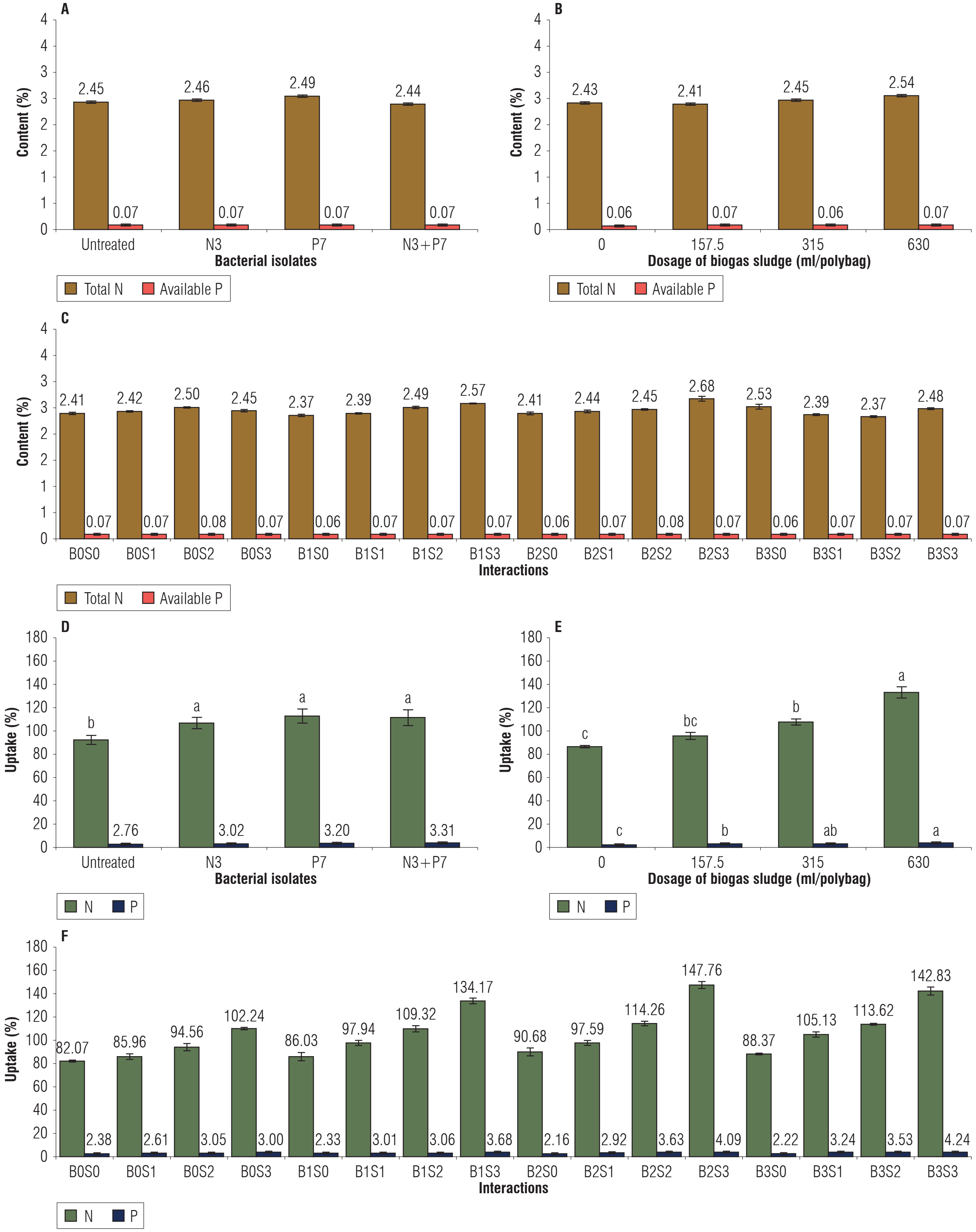

FIGURE 2. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on the nutrient content (A-C) and uptake of N and P (D-F) of upland rice. Values followed by different letters significantly differed according to the Duncan test at $P<0.05$. Vertical bars indicate the standard error. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = N3+P7 isolates).

Discussion

Effect of selected superior bacterial isolates

The selected superior bacterial isolates (N3 and P7) significantly increased the uptake of nitrogen and the crop growth rate of upland rice in an Ultisol at 12-16 WAA but did not have a significant effect on plant height, total fresh weight, total dry weight, content of N and P in leaves, uptake of phosphorus by the plants, and crop growth rate of upland rice from 4 to 12 WAA. The superior bacterial isolates (N3, P7, and N3+P7) increased the uptake of nitrogen in upland rice by 14.64%, 20.77%, and 20.68%, respectively, compared to the control (Fig. 2). Similar results are also shown in Table 5, with the crop growth rate of upland rice at 12-16 WAA increased by 2.57, 2.81, and 2.39 times, respectively, due to the selected superior bacterial isolates (N3, P7, N3+P7) compared to the control. The results indicate that a single P7 bacterial isolate increased the nitrogen uptake and crop growth rate of upland rice compared to a single N3 isolate and the combination of N3+P7 isolates. This was due to the presence of several organic acids and hormones produced by the isolate P7 that can increase the uptake of nitrogen and crop growth rate of upland rice. This result is supported by Mustamu *et al.* (2021a) who found that the phosphate solubilizing bacterial isolate (P7) from the biogas sludge contains organic acids, such as lactic, oxalic, acetic, and citric, and had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate with values 4.62 and 2.66 times higher, respectively, compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus in soils slightly increased with the application of bio fertilization with *Bacillus cereus*. This was due to the production of organic acids and other substances, including citric, tartaric, and oxalic acids, that can stimulate plant growth and nutrient availability. Youssef and Eissa (2017) reported that the increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of mineral nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that gibberellin and IAA can increase plant growth under stressful conditions. Kang *et al.* (2014) indicated that the plant growth-promoting bacteria (PGPB) has several mechanisms to increase plant growth with nitrogen-fixation and phosphate solubilization, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from an anaerobic digester include *Ruminococcus* sp., *Thiomargarita* sp., *Clostridium* sp. *Anaerobacter* sp., *Bacillus* sp., *Sporobacterium* sp., *Saccharofermentans* sp., *Oscillibacter* sp., *Sporobacter* sp., and *Enterobacter* sp. Liaquat *et al.* (2017) also reported abundance of *Bacillus, Clostridium*, and *Enterobacter* spp. in an anaerobic digester of wastewater when producing biogas.

Effect of biogas sludge

The dose of biogas sludge significantly increased the plant height, total fresh weight (8, 12, and 16 WAA), total dry weight (12 and 16 WAA), uptake (N and P), and crop growth rate of upland rice at 8-12 WAA. However, it did not have a significant effect on the content (N and P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAA). An increase in plant height, total dry weight, uptake of nitrogen and phosphorus, and also crop growth rate of upland rice in an Ultisol with a higher dose of biogas sludge of 630 ml/polybag was seen at the end of this study (16 WAA). In contrast, the total fresh weight increased along with the higher dose of biogas sludge of 315 ml/polybag and then decreased at the dose of 630 ml/polybag. This result is due to the chemical characteristics of the biogas sludge such as pH (7.41), total N (0.051%), available P (0.013%), organic C (0.14%), total K (0.18%), and the biological characteristics such as total nitrogen-fixing bacteria (29.4×10^5 CFU ml^{-1}) and total phosphate solubilizing bacteria (7.0×10^4 CFU ml^{-1}) (Tab. 2). The organic C content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could increase the uptake of nitrogen and phosphorus in upland rice with an increasing dose of biogas sludge of 630 ml/polybag (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the plant height, biomass, and crop growth rate of the upland rice. A similar result was reported by Mustamu and Triyanto (2020), who determined the macro and micronutrients from the biogas sludge and the population of nitrogen-fixing and phosphate solubilizing bacteria of 480×10^4 and 42×10^4 CFU ml^{-1}, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that the total phosphate solubilizing bacteria (1.6 to 2.5 CFU ml^{-1}) was significantly higher than nitrogen-fixing bacteria (0.5-1.4 CFU ml^{-1}), showing a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported an increase in concentrations of NH_4^+-N from 45% to 80% in the anaerobic waste.

Interaction of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice in Ultisols at 12-16 WAA but did not have a significant effect on the other parameters in this study. The interaction of B1 with biogas sludge at the dose of 630 ml/polybag (B1S3) showed the highest crop growth rate of

upland rice compared to other interactions and was 5.76 times greater compared to the control. This was caused by the application of biogas sludge that could have increased soil organic matter and the total population of beneficial bacteria. Likewise, the biogas sludge contained organic C (0.14%), total nitrogen-fixing bacteria (29.4×10^5 CFU ml^{-1}), and total phosphate solubilizing bacteria (7.0×10^4 CFU ml^{-1}) (Tab. 2) that could improve soil quality and increase the CGR. This result is supported by Urra *et al.* (2019) who found that the application of sewage sludge in the long term significantly increases the organic matter contents in the soil, causing a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of the organic matter. Bhardwaj *et al.* (2014) and Carvajal-Muñoz and Carmona-Garcia (2012) showed that the application of a biofertilizer had advantages for the plants such as availability of nutrients balanced for plant health. It also stimulates nutrient mobilization that can increase soil biological activity and nutrient availability for microorganisms to encourage the growth of beneficial microorganisms, increasing the soil organic matter content and, therefore, the cation exchange capacity. Siswanti and Lestari (2019) indicated that the interaction of biogas sludge + biofertilizer (36 ml + 10 L ha^{-1}) significantly increased the plant height, number of leaves, and capsaicin content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

The isolates N3, P7, N3+P7 from the biogas sludge significantly increased the uptake of nitrogen (20.77%) and crop growth rate (2.81 times higher than the control) of upland rice in Ultisols with the highest increase found with the P7 isolate. The dose of biogas sludge significantly increased plant height (14.81%), total dry weight (50.55%), uptake of nitrogen (54.11%) and phosphorus (65.20%), and also crop growth rate (129.44%) of upland rice in Ultisols with the highest increase at a dose of 630 ml/polybag. Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice by 41.81% with the highest increase at the dose of 315 ml/polybag. The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice in Ultisols 5.76 times with the highest increase found with B1S3.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

All authors formulated the overarching research goals and aims, provided the study materials, and developed the methodology. NEM analyzed and interpreted the study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in the field, and collected the data. ZN and I verified the overall reproducibility of the results and the other research outputs. All authors conducted the critical review of the manuscript.

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