

# Effect of rice husk biochar, sugarcane sludge fertilizers, and *Enterobacter asburiae* bacteria on soil nitrogen and phosphorus availability to improve hybrid maize yield

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## ABSTRACT

The study aimed to see what would happen if biochar, sugarcane sludge, and *Enterobacter asburiae* strain were used alone or together to improve the soil's N and P availability and hybrid maize seed yield. An experiment with hybrid maize on alluvial soil (Fluvisols) collected in An Phu, An Giang, was carried out at the net house of the College of Agriculture, Can Tho University. The two-factor factorial experiment included: (i) four formulations of mixed materials and (ii) two levels of bacterial strains. The results showed that adding biochar or sugarcane sludge did not improve soil pH and N, P availability. However, the growth and yield of maize significantly increased when applying biochar or sugarcane press mud fertilizer. The inoculation with *E. asburiae* makes more N and P available in the soil, which helps hybrid maize grow and make more food. Of the three soil factors, available N is the most important in the predictive models for determining plant height ( $R^2 = 0.73$ ) and grain yield ( $R^2 = 0.81$ ). In addition, the combination of available N and P strengthens the interpretation of maize stem diameter ( $R^2 = 0.83$ ). The soil's pH, N, and P availability were not improved by adding rice husk biochar or sugarcane sludge organic fertilizer separately or together. However, when these materials were used, corn growth and yield significantly increased.

## 1. INTRODUCTION

Maize (*Zea mays* L.) is a member of the Poaceae family and is considered a multifunctional cereal crop providing human food, animal feed (poultry and livestock), and raw materials for industries [1]. According to the Council [2], global corn consumption is expected to reach a new peak in the coming years, and corn's use for animal feed is expected to continue to increase. The advantage of chemical fertilizers in increasing crop yields and yields has been demonstrated and documented since the advent of the green revolution [3,4]. Recently, several studies have noted that excessive use of chemical fertilizers to increase yield has proved ineffective and leads to soil deterioration. The degradation had led to reduce the soil fertility, destroy the soil microbiota, imbalance the nutrients, and pollute the environment [5,6]. In contrast, organic fertilizers help improve soil fertility and balance the ecosystem in the soil. Still, crop yields with only organic fertilizers are often lower than with chemical fertilizers alone, precisely. The studies of Ponisio *et al.* [7] and Seufert's *et al.* [8] experimental results showed that organic farming yields were 19.2%–50% lower than conventionally cultivated fields.

Research by Mi *et al.* [9] and Agegnehu *et al.* [10] concluded that using chemical fertilizers combined with organic fertilizers or biochar helps increase crop yield and improves soil fertility. Endophyte/endophytic bacteria are effective in replacing/reducing chemical fertilizers and stabilizing/increasing crop yields. According to Dudeja *et al.* [11] and Khalifa *et al.* [12], endophytic bacteria plants live in the soil and plant tissues and do not cause harm or compete for nutrients with plants. Group of *Bacillus*, *Burkholderia*, *Enterobacter*, and *Pseudomonas* [13], these bacteria stimulate plant growth directly and/or indirectly through various mechanisms such as (a) production of plant hormones and inhibition of ethylene production [14], (b) capable of biological nitrogen fixation [15], (c) dissolution of inorganic P and mineralization of organic P and/or other nutrients, and (d) antagonize pathogens by producing antimicrobial compounds, enzymes, and/or compounds that inhibit pathogenic fungi, enhancing plant disease resistance [16]. Experiments by Rudolph [17] recorded that the rhizosphere bacteria strain was transplanted into corn kernels to increase germination and help the seedlings develop well. According to Vedderweiss [18], maize's root length and mass increased when seeds were inoculated with *Azospirillum* spp. Experiments by Breedts [19] showed a yield increase from 24 to 34% when *Paenibacillus alvei*, *Bacillus safensis*, *Bacillus pumilus*, and *Brevundimonas vesicularis* were inoculated into corn kernels. Studies on using organic fertilizers, biochar, and endophytic bacteria on plants have been studied extensively. However, most of the studies were performed

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alone, and the source of endogenous bacteria used was the *Bacillus*, *Burkholderia*, and *Pseudomonas* group, and very few studies on the *Enterobacter* group. The study's objective is to evaluate the effect of using alone or combined with organic materials (rice husk biochar and sugarcane sludge) and nitrogen-fixing rhizosphere bacteria *Enterobacter asburiae* on soil N, P availability and yield of hybrid maize.

## 2. MATERIALS AND RESEARCH METHODS

### 2.1. Time and Place of the Experiment

The experiment was carried out from December 2017 to April 2018 at the greenhouse of the Department of Soil Science, College of Agriculture, Can Tho University.

### 2.2. Experimental Materials

#### 2.2.1. Hybrid corn source

Syngenta's NK7328 hybrid corn was used in the experiment. It is a widely utilized variety in Vietnam's corn-growing regions. The NK7328 corn hybrid grows from 100 to 105 days and is about 22 cm long. The seeds are attractive, orange-yellow, hardy, green, and well-resistant to shedding. They are also drought-resistant and hardy. The high and stable yield of 10–12 tons/ha, with few pests and diseases.

#### 2.2.2. Bacteria source

*E. asburiae* strain RS01–04 is an endophytic bacteria in the rhizosphere.

Pot: The experimental pot has a pot diameter of 32 cm and a height of 33 cm.

#### 2.2.3. Fertilizers

The study used urea (46% N), superphosphate (16% P<sub>2</sub>O<sub>5</sub>), and potassium chloride (60% K<sub>2</sub>O) fertilizers. While rice husk biochar (biochar) is produced by pyrolysis method by top lit up draft furnace at 400–600°C, and the organic fertilizer used in the experiment is composted sugarcane sludge. The source of sugarcane filter cake fertilizer is composed of sludge waste and sugarcane carcasses taken from Vi Thanh sugar factory, Hau Giang. The main chemical composition of rice husk biochar and sugarcane sludge organic fertilizer is detailed in Table 1.

#### 2.2.4. Soil

Experimental soil was collected from alluvial soil in the embankment (Fluvisols), specialized in vegetables in Quoc Thai commune, An Phu district, An Giang province, coordinates (X = 10.8984570, Y = 105.0793472). The soil was collected at a depth of 0–20 cm by hand drill at 5 random points in the field and then mixed well into a representative sample for the experiment. Before conducting the experimental arrangement, the physicochemical characteristics of representative soil samples were analyzed. Experimental soil has a clay-meat mechanical composition, with a clay grain grade of 27.8%, Sand of 21.4%, silty clay of 50.8%, and soil content of 1.38 g/cm<sup>3</sup>. Other indicators include pH<sub>H<sub>2</sub>O</sub> (1:2.5) = 6.34, organic matter 1.72%, nitrogen 13.6 mg/kg, and available phosphorus 20.30 mg P<sub>2</sub>O<sub>5</sub>/kg. The soil texture of the experiment is clay loam with 27.8% clay, 21.4% sand, and 50.8% silt, bulk density of 1.38 g/cm<sup>3</sup>. Other indicators include pH<sub>H<sub>2</sub>O</sub> (1:2.5) = 6.34, organic matter 1.72%, nitrogen 13.6 mg/kg, and phosphorus 20.30 mg P<sub>2</sub>O<sub>5</sub>/kg.

## 2.3. Experiment Preparation

### 2.3.1. Soils of experiment

After collection, soil samples were brought to the laboratory to dry naturally, chopped to about 2 cm in size, mixed evenly, and placed in plastic pots weighing 20 kg/pot.

**Table 1:** Nutritional composition of rice husk biochar and sugarcane sludge used in hybrid maize cultivation experiment.

S. No.	Analytical indicators	Biochar rice husks	Sugarcane sludge compost
1	pH <sub>(H<sub>2</sub>O)</sub> (1:2.5)	9.92	7.50
2	Carbon (%C)	49.4	30.0
3	Nitrogen (%N)	0.42	2.50
4	Phosphorus (%P <sub>2</sub> O <sub>5</sub> )	0.98	3.00
5	Potassium (%K <sub>2</sub> O)	0.85	1.00
6	Calcium (%CaO)	0.02	0.05

### 2.3.2. Experimental layout

The experiment was installed in a completely randomized design consisting of two – factors. The first factor was a kind of organic fertilizer (Non-amendment, sugarcane press mud, rice husk biochar, and a mix of organic fertilizer with sugarcane sludge and rice husk biochar), and the other was bacterial strain (With and without bacterial strain). The experiment consisted of eight treatments, with three replicates. The experiment had three pots per treatment resulting in 72 pots in total. The experimental treatments are detailed in Table 2. The general application of chemical fertilizers for all experimental treatments was 150 kg N + 90 kg P<sub>2</sub>O<sub>5</sub> + 80 kg K<sub>2</sub>O/ha [20].

### 2.3.3. Time to apply fertilizer

Organic fertilizer of sugarcane sludge, rice husk biochar, and phosphorus was applied once just before sowing seeds. Nitrogen and potassium fertilizers are used at different stages of the growing season: 1<sup>st</sup> application (7–10 DAS, 3–4 leaves): 1/3 of N and 1/2 amounts of K<sub>2</sub>O; 2<sup>nd</sup> time (20–25 DAS, 7–9 leaves): 1/3 of the amount of N; and the 3<sup>rd</sup> time (40–45 DAS, 5–8 days before flowering): all remaining fertilizer (1/3 N and 1/2 K<sub>2</sub>O).

### 2.3.4. Bacterial preparation

*E. asburiae* strain RS01-04 was preserved and maintained in tryptic soy broth. For experimental use, bacteria were transferred to TSA medium (tryptic soy agar) and incubated at 30°C for 24 h before use. *E. asburiae* bacteria were grown in a 250 mL conical flask containing 150 mL of the medium (liquid) used for isolation (LGI/NFb) until a 1–2 × 10<sup>8</sup> CFU/ml density was obtained.

### 2.3.5. Treatment of seeds and bacterial strains into hybrid corn kernels

Corn kernels are sterilized with 70% ethanol for 3 min and 1% sodium hypochlorite (NaOCl) solution for 10 min. Then, the seeds were washed with demineralized water 3 times. After washing, seeds are incubated for 24 h in the dark for germination. When these seeds germinate, soak the seeds in a suspension containing tested bacteria and adjust the cell density (density 10<sup>8</sup> CFU/ml), incubate at 28 ± 2°C for 3 h, 500 mL of bacterial strain for 0.5 kg of seeds.

### 2.3.6. Sowing seeds

Each pot was sown with 2 seeds. When the plants reached 15 days old, one plant was removed so that only one plant was kept in each pot.

## 2.4. Experimental Method

### 2.4.1. Collecting soil samples at the end of the crop

At the end of the experimental season, when harvesting hybrid corn, the soil was collected individually for each treatment and repeated. After collection, the soil was dried naturally. After that, the dry soils were smashed through a sieve of 2 mm and 0.5 mm for analysis. First

analyze soil chemical parameters, including pH, EC, available nitrogen ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ), and phosphorus.

#### 2.4.2. Record the agronomical indicators

Plant height (cm) and internode diameter (cm) were recorded at the R1 stage (60 DAS). In addition, dry biomass and grain yield were collected at the end of season R6 (110 DAS).

#### 2.4.3. Soil and plant samples analysis

##### 2.4.3.1. Soil sample parameters

Soil pH was extracted with distilled water, extraction ratio of 1:2.5 (soil: water), and determined by pH meter. Soil organic matter (%C) was determined by the method of Walkley and Black. Available nitrogen ( $\text{N-NH}_4^+$  and  $\text{N-NO}_3^-$ ) in the soil was extracted with KCl 2M 1:10 ratio. The concentration of  $\text{N-NO}_3^-$  was determined by a spectrophotometer at 650 nm. A spectrophotometer determined the concentration of  $\text{N-NH}_4^+$  at 540 nm. Easily digestible phosphorus (according to Bray II method), soil extraction with 0.1N HCl + 0.03  $\text{NH}_4\text{F}$ , and water ratio 1:7 then measured on a spectrophotometer at 880 nm.

##### 2.4.3.2. Criteria related to plant samples

Plant height at the R1 stage was measured from the base close to the soil to the first plant branching node. Electronic calipers were used to measure the root internode diameter (cm) and its position near each plant base. Dry biomass (g/plant) was determined by weighing the plants' fresh weight (stems and leaves) and then drying them at 70°C to constant weight to calculate the average dry biomass/plant. Plant biomass was collected at the R6 stage (110 DAS). Corn yield (g/pot) was determined at the time of harvest, collected all the fruits on each plant of each replicate in the treatment, dried naturally, then separated the seeds, weighed the grain weight, and changed to 13% humidity.

## 2.5. Data Processing

The collected and analyzed data are processed and calculated using the Microsoft Excel 2013 program. R-studio v1.4 statistical software analyzes the ANOVA and compares the statistics. Compare the mean difference of treatments by Duncan test with 5% test level ( $P < 0.05$ ).

**Table 2:** List of treatments in the experiment of growing hybrid corn.

Treatments	Sugarcane sludge (Ton/ha)	Biochar	Bacteria ( $\times 10^8$ CFU/ml)	Symbols
Treatment 1	-	-	-	Control
Treatment 2	5	-	-	SS5-B0 -No RS01
Treatment 3	-	10	-	SS0-B10 -No RS01
Treatment 4	5	10	-	SS5-B10 -No RS01
Treatment 5	-	-	x	SS0-B0 -RS01
Treatment 6	5	-	x	SS5-B0 -RS01
Treatment 7	-	10	x	SS0-B10 -RS01
Treatment 8	5	10	x	SS5-B10 -RS01

RS01 is *Enterobacter asburiae* strain RS01-04.

## 3. RESULTS AND DISCUSSION

### 3.1. The Effect of Soil Amendment Materials of Organic Fertilizer of Sugarcane Sludge, Biochar, and Bacteria on pH, Available N, and P

The results presented in Table 3 showed no difference in soil pH value between treatment with biochar, sugarcane sludge, and *E. asburiae* bacteria. However, there was a significant difference ( $P < 0.001$ ) in the available N and P content in the soil between the treatments with soil amendment organic materials and *E. asburiae* rhizobacteria strain RS01-04 corn kernels before sowing. In addition, the interaction analysis results showed a significant interaction between the soil amendment material and the *E. asburiae* strain to enhance the amount of accessible N and P in the soil before planting.

Using organic fertilizers in combination with common bacteria help to increase the available N and P in the soil because (i) the organic materials themselves are a source of carbon for the soil, a food source for microorganisms, and a source of food for microorganisms. The addition of organic matter altered the physicochemical properties of the soil and strengthened symbiotic connections with the rhizosphere [21] and microorganisms in the soil [22]. It helps to convert organic materials into available nutrients for plants; (ii) *E. asburiae* bacteria caused by endogenous bacteria can participate in various biological processes such as dissolving insoluble P into soluble P form. It was done through acidification, iron chelation, metabolism [23,24], and fixing  $\text{N}_2$  from the atmosphere into ammonium cations ( $\text{NH}_4^+$ ) [25]. The research of Bhattacharya [26], Anzuay [27], Alfaro-Espinoza and Ullrich [28], and Ludueña [29] also had a similar record.

### 3.2. Effect of Organic Fertilizer with Sugarcane Sludge, Biochar, and *E. asburiae* on the Growth and Yield of Hybrid Corn Kernels

The results of Table 4 show a statistically significant difference between the treatments of factor A (bacteria strain) and factor B (soil improvement materials). The injection of bacteria into maize

**Table 3:** Effect of soil amendment materials and bacteria on pH, available content (N, P) in soil.

Experimental factor		pH <sub>H2O</sub>	N <sub>available</sub> ( $\text{mgNH}_4^+$ - $\text{N+NO}_3^-$ - $\text{N/kg}$ )	P <sub>available</sub> (mg/kg)
F (A)	F (B)	(1:2.5)		
No strains of bacteria	SS0-B0	6.8±0.08	17.7±0.59 <sup>f</sup>	22.4±0.34 <sup>d</sup>
	SS5-B0	6.9±0.20	23.5±0.49 <sup>d</sup>	27.6±2.22 <sup>c</sup>
	SS0-B10	6.7±0.09	26.7±0.42 <sup>c</sup>	26.9±0.18 <sup>c</sup>
Bacterial strain	SS5-B10	6.9±0.09	21.9±1.01 <sup>e</sup>	23.9±0.21 <sup>d</sup>
	SS0-B0	6.8±0.18	28.4±0.14 <sup>b</sup>	32.8±0.70 <sup>a</sup>
	SS5-B0	7.0±0.10	25.5±0.70 <sup>c</sup>	30.0±0.96 <sup>b</sup>
	SS0-B10	6.9±0.15	26.1±0.14 <sup>c</sup>	29.7±1.05 <sup>b</sup>
	SS5-B10	6.9±0.06	31.3±1.20 <sup>a</sup>	34.0±1.26 <sup>a</sup>
	F (A)	ns	***	***
F (B)	ns	***	ns	
F (A×B)	ns	***	***	
CV (%)		1.83	2.72	3.78

The symbol "\*\*\*\*" has a significant difference of 0.1%; "ns" is not statistically different. Data in the same column followed by the same letter are not significantly different. However, the data in the same column followed by different letters are statistically significant when comparing Duncan with  $P=0.05$ .

**Table 4:** Effect of organic fertilizer with sugarcane sludge, biochar, and *Enterobacter asburiae* on growth and grain yield.

Experimental factor		Grain yield	Biomass (stem+leaf)	Height plant	Diameter stem
F (A)	F (B)	(g/plant)	(g/plant)	(cm)	(mm)
No strains of bacteria	SS0-B0	73.0±2.61 <sup>f</sup>	38.2±0.68 <sup>c</sup>	140.3±4.73 <sup>d</sup>	210.0±10.00 <sup>d</sup>
	SS5-B0	98.8±2.31 <sup>de</sup>	48.0±1.07 <sup>b</sup>	155.0±7.55 <sup>bc</sup>	240.0±20.00 <sup>c</sup>
	SS0-B10	109.1±7.38 <sup>bc</sup>	57.1±5.87 <sup>a</sup>	164.7±6.51 <sup>ab</sup>	276.7±15.28 <sup>b</sup>
	SS5-B10	92.8±2.21 <sup>e</sup>	47.6±6.36 <sup>b</sup>	149.7±4.51 <sup>cd</sup>	244.3±6.03 <sup>c</sup>
Bacterial strain	SS0-B0	103.6±4.85 <sup>cd</sup>	46.8±1.56 <sup>b</sup>	161.7±5.86 <sup>ab</sup>	258.3±8.02 <sup>bc</sup>
	SS5-B0	110.9±2.68 <sup>bc</sup>	53.4±4.78 <sup>ab</sup>	159.7±7.02 <sup>b</sup>	245.3±5.69 <sup>c</sup>
	SS0-B10	115.9±3.47 <sup>b</sup>	56.1±5.25 <sup>a</sup>	163.3±3.51 <sup>ab</sup>	273.3±5.77 <sup>b</sup>
	SS5-B10	132.7±4.65 <sup>a</sup>	56.1±3.96 <sup>a</sup>	171.7±2.08 <sup>a</sup>	309.7±9.50 <sup>a</sup>
	F (A)	***	**	***	***
	F (B)	***	***	**	***
	F (AxB)	***	ns	**	***
	CV (%)	3.93	8.44	3.47	4.32

The symbol “\*\*\*” has a significant difference of 0.1%; “\*\*” is 1% significant difference, and “ns” is not statistically different. Data in the same column followed by the same letter are not significantly different. However, the data in the same column followed by different letters are statistically significant when comparing Duncan with  $P=0.05$ .

**Table 5:** Multivariable regression equation to evaluate the correlation between soil properties and growth and maize yield.

Parameter	A multivariable linear regression equation	Value p	F <sub>test</sub>	R <sup>2</sup> <sub>value</sub>
Plant height	PL = 139.92–5.54×pH+2.10×N <sub>avai</sub> +0.125×P <sub>avai</sub>	pH: 0.5377 N <sub>avai</sub> : 0.0048*** P <sub>avai</sub> : 0.8552	17.8	0.73
Diameter stem	DS = 117.95–0.12×pH+10.05 ×N <sub>avai</sub> –3.97×P <sub>avai</sub>	pH: 0.9953 N <sub>avai</sub> : 1.49e-06 *** P <sub>avai</sub> : 0.0167*	33.44	0.83
Plant biomass	PB = 36.44–1.57×pH+1.76×N <sub>avai</sub> –0.69×P <sub>avai</sub>	pH: 0.857 N <sub>avai</sub> : 0.013 ** P <sub>avai</sub> : 0.308	5.52	0.45
Seed yield	Yield=–72.56+11.46×pH+3.3×N <sub>avai</sub> +0.55×P <sub>avai</sub>	pH: 0.35201 N <sub>avai</sub> : 0.00155 *** P <sub>avai</sub> : 0.55943	29.07	0.81

seed showed significant differences in stem and leaf biomass, plant height, internode diameter, and maize yield. Similarly, adding organic materials helped increase the yield, plant biomass (stems, leaves), plant height, and internode diameter of corn plants. Again, the difference was statistically significant with no fertilizer.

The interaction analysis showed an interaction between the injection of bacteria and the addition of organic soil amendments to the increase in corn kernel yield, plant height, and stem internode diameter. Furthermore, it shows that adding soil improvement materials combined with bacterial strains for corn kernels has helped the plant grow better and fully absorb nutrients, thereby helping to increase yield.

The studies of Agegnehu *et al.* (2016) [10] noted that the soil physicochemical and biological properties of the soil were significantly improved when organic chalk was supplied, and the fertilizer use efficiency increased [30]. Improved nutrient uptake increased root internode diameter, dry biomass, and corn kernel yield [15,16]. Research by Baldani [31] noted that adding plant endophytic bacteria *Herbaspirillum seropedicae* to corn kernels grown in greenhouses increased yield by 49–82% compared to chemical nitrogen fertilizers.

### 3.3. Univariate Regression Model of Soil Properties on the Growth and Yield of Hybrid Maize Seeds

The univariate regression analysis shows no relationship between soil pH with plant height, root internode diameter, biomass, and maize yield. In contrast, the content of available nitrogen and phosphorus in the body was strongly correlated with the following growth and yield characteristics:

#### 3.3.1. Plant height

The results in Figure 1 show a positive and very close correlation between available nitrogen, available phosphorus in the soil, and tree height with the coefficient of determination  $R^2 = 0.72$ , respectively.  $P < 0.001$  and  $R^2 = 0.72$ ,  $P < 0.001$  and  $R^2 = 0.5821$ ;  $P < 0.001$ , respectively. It shows that available nutrients (N, P) greatly influence plant height.

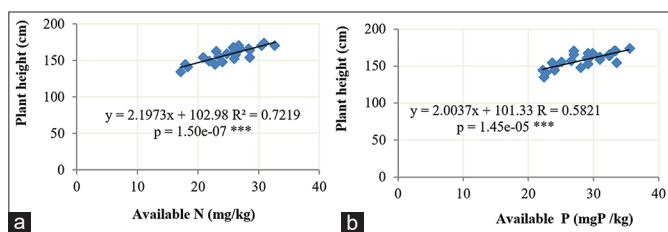
#### 3.3.2. Root internode diameter

Root diameter has a positive, very close correlation with the content of available nitrogen ( $R^2 = 0.78$ ;  $P < 0.001$ ), and available phosphorus ( $R^2 = 0.46$ ;  $P < 0.001$ ). Furthermore, the results of the correlation analysis showed that as the root internode diameter function increased as the content of N, available P in the soil increased [Figure 2].

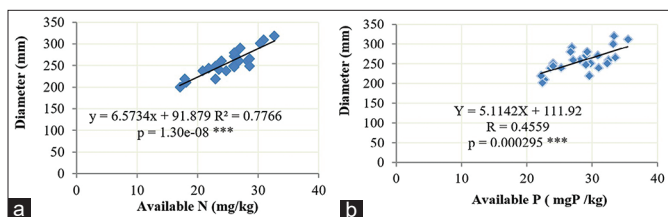
#### 3.3.3. Crop biomass

The results in Figure 3 show a positive, tight correlation between available N and tree biomass ( $R^2 = 0.42$ ;  $P < 0.001$ ). It is similarly

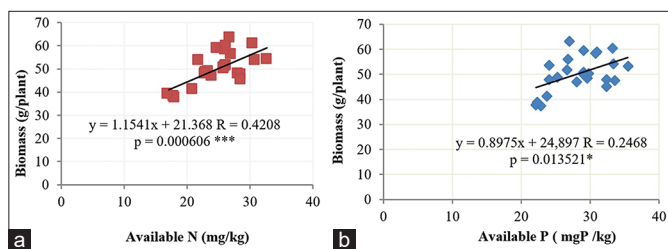




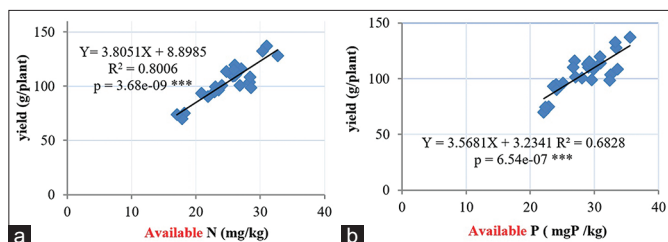
**Figure 1:** Regression between usable nitrogen (a) and usable phosphorus (b) to the height of hybrid maize NK 7328.



**Figure 2:** Regression between available nitrogen (a) and available phosphorus (b) to internode diameter of hybrid maize NK 7328.



**Figure 3:** Regression between usable nitrogen (a) and usable phosphorus (b) on the biomass of hybrid maize NK 7328.



**Figure 4:** Regression between usable nitrogen (a) and usable phosphorus (b) on the yield of hybrid maize NK 7328.

with the available P content ( $R^2 = 0.25$ ;  $P < 0.05$ ). It shows that the source of available nutrients (N, P) in the soil greatly influences the growth of hybrid maize when the content of available nutrients in the soil increases, especially N, P will help plants increase height, root diameter, and biomass.

### 3.3.4. Corn kernel yield

The results of correlation analysis [Figure 4] showed that there is a strong, positive correlation between grain yield and available N ( $R^2 = 0.80$ ;  $P < 0.001$ ) and between grain yield with available P ( $R^2 = 0.68$ ;  $P < 0.001$ ). It may indicate that grain yield is influenced by the soil's available N and P content.

### 3.4. Multi-Linear Regression Model to Evaluate the Influence of Soil Characteristics on the Growth and Yield of Hybrid Maize Seeds

The multivariable regression analysis assists in evaluating, selecting, and determining the factor's contribution level (pH, available N and P) affecting the plant growth (plant height, root internode diameter, and biomass) and hybrid maize seed yield. The simple linear regression analysis assists in evaluating the correlation between productivity and individual values. Multivariate regression analysis allows it to determine the extent of contribution, much, minor, and none of each factor (pH, usable nitrogen, and available phosphorus) to the change of the dependent variable (energy productivity, plant growth). Multivariate regression analysis helps to evaluate the overall factors of soil characteristics affecting the growth and yield of crops with variables with coefficients  $R^2$  and P value. The closer  $R^2$  is to 1, the more significant the model is.

Conversely, the closer the  $P_{\text{value}}$  to 0, the weaker the model significance. More specifically, if it is between 0.5 and 1, it is a good model, and  $<0.5$  is a bad model. P value represents the level of significance. If  $P_{\text{value}} < 0.05$  is low in significant (\*),  $P_{\text{value}} < 0.01$  is high in significant (\*\*); and  $P_{\text{value}} < 0.001$  is very high significant (\*\*\*)

Equation (1) – (4) of Table 5 shows the soil's three pH factors, available nitrogen, and phosphorus. Soil pH factor was not correlated with growth (plant height, root growth path, and plant biomass) and seed yield. However, between available nitrogen and plant height, root diameter, plant biomass, and yield, there is a very close linear correlation with the coefficient of determination  $R^2$  of the equations ranging from 0.45 to 0.83; The F value ranges from 5.52 to 33.44; P-value ranges from  $0.05 < P < 0.001$ . In contrast, there was no correlation between available phosphorus and plant height, biomass, and yield. However, the available phosphorus has a linear relationship with the original diameter through the coefficient of determination  $R^2 = 0.83$ ;  $P_{\text{value}} < 0.05$ .

### 4. CONCLUSION

The inoculation of *E. asburiae* strain RS01-04 to corn kernels before sowing did not change soil pH, significantly improving the available N and P sources, which was statistically significant compared with no bacteria strains for maize. Plant height, root diameter, plant biomass, and grain yield were significantly higher in the treatment with bacterial strains, statistically different from those without bacteria strains. There was an interaction between adding rice husk biochar or sugarcane sludge organic fertilizer alone or combined with the injection of corn kernel bacteria. Multivariable regression showed that nitrogen availability was critical in predicting plant height ( $R^2 = 0.73$ ) and grain yield ( $R^2 = 0.81$ ). Furthermore, the combination of available nitrogen and phosphorus increases the explanatory power ( $R^2 = 0.83$ ) of the stem diameter of the corn plant.

Adding rice husk biochar or sugarcane sludge organic fertilizer alone or in combination did not improve soil pH, N, and P availability in the soil. However, there was a significant increase in the growth and yield of corn when applying these materials. It was due to the interaction between the organic materials and the bacteria inoculated into the corn plant.

### 5. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in

drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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## 7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## 8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

## 9. DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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