THE FERMENTED LIQUID BIOFERTILIZER USE DERIVED FROM SLAUGHTERHOUSE WASTE IMPROVES MAIZE CROP YIELD †

[EL BIOFERTILIZANTE LÍQUIDO FERMENTADO DE RESIDUOS DE RASTRO MEJORA EL RENDIMIENTO DEL CULTIVO DE MAÍZ]

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SUMMARY

Background: The exclusive application of synthetic or organic fertilizers continues to generate controversy. Evidence indicates that the integrated use of these practices can enhance crop nutrition, reduce the reliance on synthetic fertilizers, and mitigate their polluting impact on soil quality. **Objective:** To evaluate organic and mineral fertilization doses used on hard yellow maize Megahybrid 619 INIA growth and yield using a liquid biofertilizer derived from slaughterhouse waste fermentation. **Methodology:** Using a randomized complete block experimental design with a 4x2 factorial arrangement, four doses of NPK chemical fertilization and biofertilizer application were tested. Mineral fertilization was divided into two parts, while slaughterhouse waste fermented biofertilizer applications were carried out via drench during vegetative growth and between the tasselling and grain filling stages at a 50 $L \cdot ha^{-1}$ product dose. **Results:** Liquid biofertilizer (K1) use positively impacted growth, with a comparable effect on height and leaf area applying fertilization medium dose (F2_K1). The lowest dose of chemical fertilization in combination with the biofertilizer (F1_K1) obtained a significantly higher maize harvest index (+14%) compared to complete fertilization (F3_K1). **Implications:** While the highest fertilization levels did not result in increased yields, it is plausible that under different conditions and with other maize hybrids, significant differences may be observed. **Conclusion:** The application of liquid biofertilizer combined with a reduced dose of mineral fertilization results in a higher harvest index and a yield comparable to that achieved with full mineral fertilization in the hard yellow maize Megahybrid 619 INIA.
Key words: sustainable agriculture; fertilization; soil; bioresource technology.

RESUMEN

Antecedentes: La aplicación exclusiva de fertilizantes sintéticos u orgánicos sigue generando polémica. La evidencia muestra que su aplicación conjunta puede mejorar la nutrición de los cultivos, evitar el uso excesivo de fertilizantes sintéticos y amortiguar su efecto contaminante en el suelo. **Objetivo:** Evaluar el uso de dosis de fertilización orgánica y mineral sobre el crecimiento y rendimiento del maíz amarillo duro Megahíbrido 619 INIA empleando un biofertilizante líquido derivado de la fermentación de residuos de camal. **Metodología:** Mediante un diseño experimental de bloques completos al azar con arreglo factorial 4x2, se ensayaron cuatro dosis de fertilización química NPK y la aplicación del biofertilizante. La fertilización mineral se fraccionó en dos partes, mientras que las aplicaciones del biofertilizante fermentado de residuos de camal se realizaron vía *drench* durante el crecimiento vegetativo y entre las etapas de panojamiento y llenado de grano a una dosis de 50 $L.ha^{-1}$ de producto. **Resultados:** El uso del biofertilizante líquido (K1) tuvo un impacto positivo en el crecimiento, con un efecto equiparable en la altura y área foliar de la planta al aplicar una dosis media de fertilización química (F2_K1). La dosis más baja de fertilización

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química en combinación con el biofertilizante (F1_K1) obtuvo un índice de cosecha estadísticamente superior (+14%) en comparación con el la fertilización completa (F3_K1). **Implicaciones:** Si bien las fertilizaciones más altas no produjeron rendimientos superiores, es posible que en otras condiciones y con otros híbridos de maíz sí se observendiferencias significativas. **Conclusión:** La aplicación del biofertilizante líquido junto con una dosis reducida de fertilización mineral permite obtener un mayor índice de cosecha y rendimientos comparables con el uso de una fertilización mineral completa en el maíz amarillo**.**
Palabras clave: agricultura sostenible; fertilización; suelo; tecnología de recursos biológicos.

INTRODUCTION

Nowadays, fertilization aims to maintain or improve nutrient availability in the soil so plants can grow healthily and faster. Inorganic fertilizers play a crucial role in conventional production models that prevail in global agriculture, to increase the growth and production of crops such as maize. However, inorganic fertilizers intensive use has detrimental effects (Mulyati *et al*., 2021). Long-term application of these fertilizers causes soil degradation and increases its apparent density, preventing water infiltration and absorption, which harms plant growth and development, causing fragility, vulnerability, and risks to the environment, human health, and agroecosystems (Setyowati *et al*., 2022). An example of an inorganic fertilizer is Urea. The 40 % to 90 % of the urea applied to the agricultural soil is lost to the environment causing pollution issues, such as soil acidification, soil hardening, and water contamination, and also, it can affect the farmers' economy due to low-quality products and undesired yields. The nitrogen losses are carried out through three processes; denitrification, volatilization, and leaching, having a negative environmental impact (Barreras-Urbina *et al*., 2018; 2023; Tapia-Hernández *et al*., 2022). Degraded soil fertility cannot be easily restored, even with high application of synthetic fertilizers. Therefore, rational use of this type of fertilizer is necessary (Muktamar *et al*., 2016). Likewise, dependence on non-renewable raw materials is one of the main problems for future generations (Funes-Monzote, 2017). Excessive use of inorganic chemical fertilizers can increase greenhouse gas emissions. Likewise, not taking advantage of solid and liquid waste from the livestock industry may also increase greenhouse gas emissions in the form of methane (CH_4) and carbon dioxide (CO_2). Of all livestock industries, cattle produce more CH_4 than other ruminants, which is the second most prevalent greenhouse gas responsible for global warming (Tani *et al*., 2021).

Although most agriculturists prefer using chemical fertilizers for crop management, these are out of their economic reach due to their high price. In contrast, organic fertilizers contain low levels of macronutrients, but sufficient micronutrient amounts that are highly necessary for plant growth (Barus *et al*., 2019), which implies an alternative to reduce the harmful impact of synthetic fertilizers because they are derived from plant residues and livestock manure. Organic fertilizers supply macro and micronutrients necessary for plant growth and development, hormonal action stimulation or antibiosis, and organic waste decomposition. They also help to improve the soil's physical, chemical, and biological activity, prevent erosion, and reduce the soil crack appearance (Khosro and Yousef, 2012, Setyowati *et al*., 2022). However, organic fertilizers cannot satisfy the nutrient demand of intensively grown crops due to their limited availability, low nutrient content, and high labor requirements (Tolera *et al*., 2005). Consequently, higher prices of chemical fertilizers and greater organic waste availability in the field may require the combination of organic fertilizers with inorganic fertilizers (Laekemariam and Gidago, 2012). Integration of both fertilizers increases yield, sustains productivity, and improves soil chemical properties (Oyedeji, 2016). Integrated soil fertility management is a viable approach to overcome soil fertility limitations. Combined fertilization enhances soil carbon storage and reduces emissions from nitrogen fertilizer use (Abbasi and Yousra, 2012, Zhao *et al*., 2009).

Solid and liquid cattle waste availability in feces and urine form at the agricultural level is generally quite high, but it has not been optimally utilized and generally tends to accumulate around the stables (Tani *et al*., 2021). If used, solid and liquid fertilizers could be produced which have different characteristics. For example, a liquid organic fertilizer is said to be superior compared to a solid one, since nutrients are more available when applied to soil and can be absorbed more easily by spraying them directly on the plants (Muktamar *et al*., 2023). Abundant organic producing sources have been reported, such as domestic waste, industry, agricultural production, and livestock, therefore, their quality depends largely on the chosen source (Setyowati *et al*., 2022). A cow can excrete 6 L of urine in 24 hours. If a livestock farmer can have 2 cows on his farm, he can provide 4380 L of urine per year which is equivalent to 65 kg of nitrogen or 136 kg of urea (Vala and Desai, 2021).

On the other hand, conventional management and slaughterhouse wastewater control are limited due to their high costs and difficulties related to the nutrient-using process. For this reason, there are cost-effective methods that remove or recover nutrients from agroindustrial surplus and meat processing waste streams in a sustainable manner (Yetilmezsoy *et al*., 2022). Globally, blood and rumen content are the main slaughterhouse wastes, having around 85 g/kg water content and creating a disposal problem (Roy *et al*., 2013). Nitrogen presence is one of the most problematic components in raw wastewater, due to its effects on the environment, being in the form of ammonia or organic nitrogen (Akpor *et al*., 2014). Waste processing reported methods are not yet viable in developing countries where cattle slaughter is mainly carried out in small and dispersed units. Therefore, the evolution of acceptable processing technologies for slaughterhouse waste is important (Makinde and Sonaiya, 2010). Roy *et al*. (2013, 2016) reported on a fertilizer made of mixing rumen and bovine blood from highly contaminated slaughterhouse waste, that increased eggplant, tomato, and chili pepper growth yield inside a greenhouse. In addition, this type of fertilizer provides micronutrients such as zinc, boron, iron, and copper, which are essential for plant growth (Adediran *et al*., 2005).

In Peru, there are some experiences in cattle waste used as fertilizer. Chávez-Távara and Vásquez-Zorilla (2017) analyzed effluents from a Moyobamba district slaughterhouse, finding high element contents necessary for plants, including 36.39 $mg \cdot L^{-1}$ ammoniacal nitrogen and 17.33 $mg \cdot L^{-1}$ total phosphorus. According to this, it was recommended to collect this effluent in tanks, generating aggregates with rice husk ash (20 %) solutions, plus magnecal (10 %), to obtain foliar fertilizer. Likewise, Enríquez-Espinoza and Soto-Huanca (2017) used the ruminal content present in South American camelids, sheep, and cattle collected from a Huancavelica district slaughterhouse for vermicompost production, testing their quality at different production times Likewise, studies have been carried out using slaughterhouse waste such as horn and hoof flour in maize cultivation, concluding that these wastes are a good nitrogen source for applications in maize (Aguirre-Yato and Alegre-Orihuela, 2015).

These fertilizers have been tested on a variety of crops, especially in maize (*Zea mays L*.) due to their high worldwide production. Annual production is 1137 million megagrams of grain that is grown on 197 million hectares, with an average production of 5.8 $Mg.ha^{-1}$ (Erenstein *et al*., 2022). Likewise, it is interesting to evaluate the effect of these fertilizers on hybrids since they have a greater yield potential compared to common varieties. There is a 99 % use of these hybrids in developed countries, while in developing countries it only reaches 39% (Barus *et al*., 2019). On the other hand, in addition to the grains the other parts of maize have multiple uses, young stems, and leaves are beneficial for animal feed, while old stems and leaves (after harvest) are useful for green compost elaboration (Syofia *et al*., 2014). The livestock by-products have the potential to mitigate potential losses and generate additional value for slaughterhouses that would otherwise discard them as waste or process them inadequately. So this study aims to evaluate the implementation of an organic fertilizer based on slaughterhouse waste with different doses of mineral fertilization on hard yellow maize Megahybrid 619 INIA growth and yield in the north coast soil of Lima, Peru.

MATERIAL AND METHODS

Geographic and environmental characteristics of the studied zone

This research was carried out in 2023, at the Donoso agrarian experimental station of INIA in Huaral (11° 33´ 60´´ S, 77° 13´57´´ W, 133 m.a.s.l.) of the district and province of Huaral in the Lima region. It has slightly inclined physiography (0 – 4 %), a 20.3 °C average annual temperature, and a 23.6 mm per year precipitation rate. There was an 11.9 mm accumulated precipitation, a 79.6 % average relative humidity, and a 21.1 °C average temperature (SENAMHI, 2023) during this research.

Soil physicochemical characteristics

The physicochemical parameters of the used soil were analyzed before treatments implementation in the Soil, Water and Foliars Analysis Laboratory of the National Institute of Agrarian Innovation (Donoso Agrarian Experimental Station). Obtained results are presented in Table 1.

Experimental design and treatments

A Randomized Complete Block Design (RCBD) with a 4x2 factorial arrangement experimental design was used. Two factors were tested (i) Chemical fertilization dose of N, P_2O_5, and K_2O with four levels (0-0-0, 160-40-60, 200-80-100 and 240-120-140 kg ha^{-1} respectively) and (ii) Slaughterhouse waste fermented biofertilizer application with two levels (with application and without application). The resulting treatments from factors interaction were a total of eight and three repetitions were made per treatment, generating a total of 24 experimental units (Table 2).

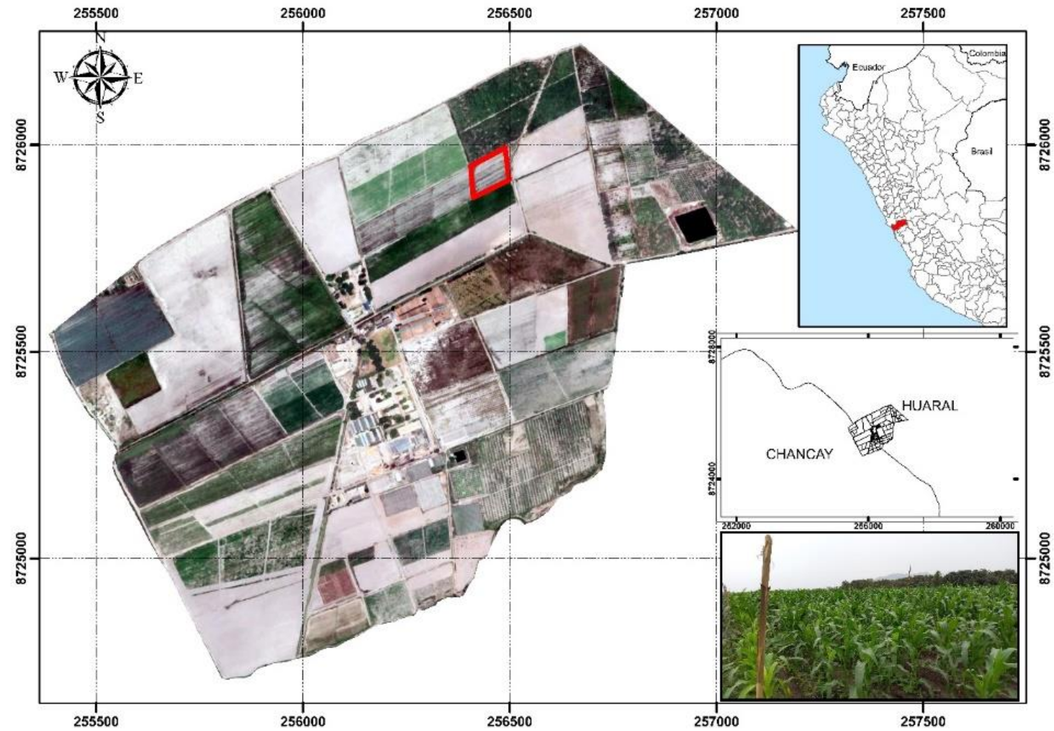

Figure 1. Location of the study area at "Estación Experimental Agraria Donoso" (INIA) - Huaral (authors´ elaboration).

Table 1. Soil physicochemical characterization of the blocks of the experimental plot.

Characteristics	Unit	Blocks		
		1	2	3
Textural class	--	Sandy clay loam	Sandy clay loam	Sandy clay loam
pH (1:5) $_{H2O}$	--	8.2	8.2	8.2
$EC_{(es)}$	$dS·m^{-1}$	1.64	1.64	1.65
O.M.	%	2.4	2.7	2.3
N	%	0.1	0.1	0.1
P available	$mg·kg^{-1}$	7.4	6.8	7.8
K available	$mg·kg^{-1}$	287.44	278.06	259.05
Carbonates	%	10.0	7.5	9.2
CEC	$cmol^{(+)}·kg^{-1}$	9.6	9.6	9.8
Ca^{+2}	$cmol^{(+)}·kg^{-1}$	7.67	7.72	7.97
Mg^{+2}	$cmol^{(+)}·kg^{-1}$	0.93	0.92	0.91
K^{+}	$cmol^{(+)}·kg^{-1}$	0.74	0.74	0.66
Na^{+}	$cmol^{(+)}·kg^{-1}$	0.27	0.25	0.25
Cations sum	$cmol^{(+)}·kg^{-1}$	9.61	9.63	9.79
Base saturation	%	100	100	100

EC: electrical conductivity, OM: organic matter, CEC: cation exchange capacity.

Table 2. Codes and description of the used treatments.

Treatment	Chemical fertilization	Biofertilization
F0K0	0-0-0 NPK	With biofertilizer (100 $L·ha^{-1}$)
F0K1		Without biofertilizer
F1K0	160-40-60 NPK	With biofertilizer (100 $L·ha^{-1}$)
F1K1		Without biofertilizer
F2K0	200-80-100 NPK	With biofertilizer (100 $L·ha^{-1}$)
F2K1		Without biofertilizer
F3K0	240-120-140 NPK	With biofertilizer (100 $L·ha^{-1}$)
F3K1		Without biofertilizer

Treatments application

Doses of urea, diammonium phosphate, and potassium sulfate were used. 314-87-120, 367-174-200, and 420-261-280 $kg.ha^{-1}$ of fertilizers were applied for the first, second, and third doses respectively. Fertilization was divided into two parts. The first fertilization was carried out 21 days after sowing (das), applying entire doses of phosphorus and half dose of N and K_2O. The second fertilization was applied at 57 dap to complete the entire dose. Slaughterhouse waste-fermented biofertilizer applications were carried out via drench in two phenological stages, during vegetative growth and between the tasselling and grain-filling stages. In each stage, 50 $L \cdot ha^{-1}$ product was applied, divided into three parts, applying at 36, 50, and 64 days for vegetative growth; and 89, 102, and 116 days for tasselling/grain filling. Liquid fertilizer chemical characteristics are shown in Table 3.

Treatments were evaluated in INIA-619 Megahybrid hard yellow maize experimental plots obtained from Vista Florida-Lambayeque Agrarian Experimental Station (INIA) seedbed. There was a total of 24 experimental units. Each one consisted of five 6 m long furrow plots and 0.8 m separation furrows. The experimental unit area was 24 m^2. Two seeds were used per stroke every 0.35 m in all experimental units.

Evaluated variables

Hard yellow maize growth and yield-associated parameters were evaluated. Within the growth variables, height, leaf area, and fresh and dry aerial biomass were measured at 92 days (R2). For yield variables, cob length and diameter, cob weight, grains per cob weight, 100 grains weight, and grain yield ($Mg.ha^{-1}$) at 146 days were measured. Plant height was measured with a metric ruler, from the soil surface to the longest stretched leaf. To determine maize plant leaf area, the leaves of each plant were extracted and spread on a table, where the length from the base to the apex and the maximum width of the leaf was measured. The maize leaf area was estimated using the Montgomery formula (Yu *et al*., 2020):

$$AF = \Sigma (L * Am * 0.75)$$

L = base to apex leaf length
A = leaf maximum width
n = plan number of leaves

Plants aerial tissues were chopped and placed in bags with plant material, previously weighed and labeled, to determine fresh weight. Plant material bags were placed in an oven at 70 °C until a constant weight was obtained to determine dry weight. Regarding yield variables, cob length and diameter, cob weight, grain weight per cob, 100 grains weight, and grain yield ($Mg.ha^{-1}$) at 146 das were measured. The performance ratio was calculated according to the following formula (Díaz-Chuquizuta *et al*., 2022):

$$\text{Yield } (Mg.ha^{-1}) = (FW * DM(\%) * G(\%)) * 8600^{-1}$$

Where FW (kg) is field weight, %DM is calculated dry matter percentage from a grain sample of five freshly harvested cobs (this is an estimated value of five cobs per treatment, taken to 75° C for 48 hours and their subsequent weight. In this test 75 % weight was taken), %G is the obtained grain percentage as the ratio between grain weight and cob weight, FC is the yield per hectare conversion factor (for this trial it was 1.14), and 8600 is a constant used to estimate yield with grain moisture of 14 %.

The harvest index was measured by the following formula (Prakhar *et al*., 2021):

$$\text{Harvest index} = \text{Yield } (Mg.ha^{-1}) * \text{Total biomass yield } (Mg.ha^{-1})^{-1}$$

Considering the total biomass yield as the sum of dry foliar biomass plus cob weight.

Statistical analysis

The results were subjected to Variance Analysis, for means comparison and multiple comparison tests the Duncan test was used, both with a significance level of 5 %, for which statistical software R was used (R Core. Team, 2023).

Table 3. Liquid fertilizer's chemical composition.

Parameter	Value	Parameter	Value	Parameter	Value
N (%)	4.25 - 6.5	$O.M_T$ (%)	6.75 ± 0.45	pH	6.75 - 7.25
P ($mg \cdot L^{-1}$)	5.0 - 124.5	$O.M_{sol}$ ($mg \cdot L^{-1}$)	96.24	EC ($dS \cdot m^{-1}$)	16.42
K (%)	38 - 41	Humic extract (%)	6.44	Zn (ppm)	174
Ca (%)	33.6	Humic acids (%)	0.58	Fe (ppm)	147.8
Mg (%)	2.8	Fulvic acids (%)	5.57	Cu (ppm)	23.4
S (%)	11.4	C:N	1.00 - 5.81	Mo (ppm)	0.1

$O.M_T$: total organic matter, $O.M_{sol}$: soluble organic matter, C:N: carbon to nitrogen ratio, EC: electric conductivity.

RESULTS AND DISCUSSION

Biometric growth variables

To evaluate maize crop growth performance and monitor differentiated fertilization application, parameters of plant height, fresh and dry biomass, and leaf area were evaluated. Obtained results from the chemical fertilizer and biofertilizer applications can be seen in Table 4.

It is observed that a gradual increase in the applied fertilization doses increases maize leaf area by 13.58, 17.20, and 28.46 %, to control (Table 4). Fertilization doses of 240-120-140 kg ha^{-1} of N-P-K (F3) are the ones with the greatest increase. Leaf area increase indicates a greater photosynthetic capacity of the plant, as well as a greater transpiration area, which results in greater nutrient absorption and greater starch production capacity (Marschner, 2012). Greater leaf area formed by the highest fertilization level (F3) supports the greatest plant height and dry weight increase. This is because 240 kg ha^{-1} nitrogen dose (F3), compared to 160 kg ha^{-1} (F1), increases available soil nitrogen and, therefore, greater cell division (Boron and Vissenberg, 2014). It has been reported that nitrogen is the nutrient with the greatest participation in cell division, directly related to cytokinin biosynthesis (Taiz and Zeiger, 2010).

Potassium plays a crucial role in the proper development of maize. It helps to maintain an ionic balance in cells and activates around 60 enzymes, that are essential during water stress conditions (Sharma *et al.*, 2013; Iqbal *et al.*, 2015). These conditions are prevalent in the study area due to the presence of saline soils, high carbonate content, fine texture, and low precipitation (refer to Table 1). Therefore, it makes sense that the increasing application of 140 kg.ha^{-1} of potassium (F3) in these conditions leads to better growth parameters such as dry weight and leaf area. Likewise, the results of phosphorus levels increase in greater absorption, since it is a nutrient that moves by soil solution diffusion and it is essential to increase the doses to facilitate its mobility and absorption. In this sense, treatment with 120 kg ha^{-1}(F3) has a greater effect than 80 kg ha^{-1}(F2) and 40 kg ha^{-1}(F1) phosphorus treatments. On the other hand, no significant differences have been observed between applying the biofertilizer and not applying it in height, fresh weight, dry weight, and leaf area variables.

Regarding fertilization doses combined effects and biofertilizer application (Table 5), it is observed that at fertilization levels F0 (without fertilizer), F2 (200-80-100), and F3 (240-120- 140), plant height increases by 7.30 %, 6.12 % and 1.16 %, respectively, when applying 100 L·ha^{-1} of biofertilizer, divided into 6 applications, via drench. However, regarding plants' leaf area and dry weight, the biofertilizer applying effect with mineral fertilization is only significant when fertilizing with the highest level of 240-120-140 kg·ha^{-1}(F3).

Biofertilizers' positive effect is due to the high contribution of Fe and Zn in chelated form since humic acids and soluble organic matter effects are present in biofertilizers (Table 3). It has been observed that after overcoming N, P, and K deficiencies with a high level of fertilization (F3), as shown in the simple effects of each level, it is possible to increase leaf area, plant height, and dry matter, complementing nutrition with a chelated source of micronutrients such as the used biofertilizer. In alkaline soils, such as the ones used in the study, the high pH and carbonate contents (Table 1) make the application of mineral micronutrients less efficient. When these elements interact with the soil, they tend to form insoluble complexes, and only a small percentage will be utilized by the plant (Saleem *et al*., 2023). Therefore, applying them in chelated form allows for better utilization, as they form complexes with organic compounds, preventing fixation and enabling the plant to assimilate them (Zanin *et al*., 2019). Biofertilizer chemical analysis at a 100 L·ha^{-1} application dose provides 174 ppm Zn and 147.8 ppm of Fe, complemented by 5.57 % of fulvic acids. However, if adequate levels of N, P, and K are not applied there is no growth with the application of biofertilizer because the limiting elements have not been corrected.

On the other hand, Ayoola and Makinde (2009) conducted experiments where the application of an organic fertilizer enriched with nitrogen made from municipal waste and cow manure, presented plant heights comparable to the application of inorganic fertilizers. The highest plant height and leaf area values were reported with organic fertilizers. Likewise, Al-Suhaibani *et al*. (2021), point out a comparable effect on plant height, resulting from the chemical fertilizer application in its highest doses (208.8 – 219.9 cm), and biofertilizer inoculation (204 cm).

It is important to note that height increases were accompanied by biomass increases, which could confer greater physical resistance and possibly greater tolerance to biotic and abiotic stresses. Nitrogen availability, solar radiation, and optimal humidity levels favor plant photosynthetic rate increase. This translates into the various plant organ's ability to synthesize carbohydrates. This process enables vegetative growth during phenological phases, having a direct impact on crop overall yield (Maddonni and Otegui, 2006). Biofertilizers improve nitrogen assimilation because they present 0.1 ppm of molybdenum, an important nutrient for nitrate reductase activity and amino acid formation (Marschner, 2012).

Table 4. Main fertilization dose (F) and biofertilizer application (K) effects on maize growth.

Treatment	Height (cm)	Fresh weight ($g \cdot pl^{-1}$)	Dry weight ($g \cdot pl^{-1}$)	Leaf area (cm^2)
Fertilizer dose (F)				
F0	190.29 ±12.6 **b**	443.5 ±72.0 **c**	106.73 ±15.3 **c**	5798.34 ±1507.9 **c**
F1	198.53 ±16.7 **b**	496.62 ±80.9 **b**	112.69 ±18.1 **bc**	6585.59 ±1011.1 **bc**
F2	213.74 ±13.1 **a**	505.05 ±78.6 **b**	116.77 ±13.1 **b**	6795.86 ±1094.8 **ab**
F3	211.31 ±15.2 **a**	606.23 ±87.7 **a**	135.16 ±21.3 **a**	7448.63 ±1591.9 **a**
Biofertilizer application (K)				
K0	201.13 ±16.3 **a**	505.94 ±83.7 **a**	116.55 ±19.4 **a**	6466.09 ±1427.3 **a**
K1	205.8 ±17.8 **a**	519.76 ±111.6 **a**	119.12 ±20.7 **a**	6848.12 ±1419.7 **a**
Fertilizer Dose (F)	***	***	***	**
Biofertilizer (K)	**ns**	**ns**	**ns**	**ns**
FxK	*	**ns**	**	**ns**

The means with the same letter within the same column do not differ significantly ($p \leq 0.05$, ANOVA and Duncan's test, equivalent to a simultaneous confidence level of 95 %); ± Standard deviation. * Statistically significant, *** Highly significant, n.s. Not significant

Table 5. Differences between the characteristics of the growth of maize plants associated with the effect of treatments.

Treatment	Height (cm)	Fresh weight ($g.pl^{-1}$)	Dry weight ($g.pl^{-1}$)	Leaf area (cm^2)
Fertilizer dose x Biofertilizer application (F:K)				
F0K0	183.59 ± 10.0 **e**	431.14 ± 72.5 **d**	102.65 ± 14.9 **d**	5297.97 ± 1844.1 **c**
F0K1	196.99 ± 11.8 **cd**	455.85 ± 73.6 **cd**	110.82 ± 15.4 **cd**	6298.71 ± 931.7 **bc**
F1K0	203.46 ± 14.9 **bcd**	520.27 ± 69.4 **bc**	120.24 ± 18.6 **bc**	6777.05 ± 893.0 **bc**
F1K1	193.61 ± 17.7 **de**	472.98 ± 88.6 **cd**	105.13 ± 14.9 **d**	6394.14 ± 1136.9 **bc**
F2K0	207.4 ± 13.8 **bc**	502.95 ± 47.3 **cd**	116.95 ± 11.4 **bcd**	6619.82 ± 1122.2 **ab**
F2K1	220.09 ± 9.2 **a**	507.15 ± 104.3 **cd**	116.59 ± 15.2 **bcd**	6971.89 ± 1103.5 **ab**
F3K0	210.09 ± 13.0 **bc**	569.4 ± 85.4 **ab**	126.36 ± 24.6 **b**	7169.54 ± 1108.3 **ab**
F3K1	212.52 ± 17.9 **ab**	643.06 ± 77.4 **a**	143.97 ± 13.5 **a**	7727.73 ± 1995.3 **a**

The means with the same letter within the same column do not differ significantly ($p \leq 0.05$, ANOVA and Duncan's test, equivalent to a simultaneous confidence level of 95%); ± Standard deviation.

Organic fertilization is an important practice in maize nutritional management, especially in arid and semi-arid conditions, where low precipitation, high temperatures, soil alkaline pH, and intensive cultivation systems are the predominant factors that usually lead to progressive degradation of soil fertility. Likewise, maize is a crop that can adapt to marginal abiotic conditions such as water deficit. However, reducing nitrogen contribution and increasing the efficiency of its use are key aspects of its production. Biofertilizers application applied outside the rainy season can reduce production costs, increasing nutrient use efficiency (Sukanteri *et al*., 2020). It is worth mentioning that the evaluation for at least two campaigns can give us a greater scope of biofertilizers' positive effects (Ayoola and Makinde, 2009, Al-Suhaibani *et al*., 2021).

Yield variables

Statistical analysis results regarding cob quality are presented in Table 6. These results showed that the cob's length improves significantly when applying fertilization with N-P-K, being able to increase the cob by more than 3 cm. However, there are no significant differences as the fertilization doses increase. Regarding the liquid biofertilizer, there is a significant improvement in the cob's length when it is applied to maize. For the diameter, inorganic fertilization has also a significant influence, especially in the intermediate fertilization with 200-80-100 (F2) of N-P-K that produced 4.84 cm average diameters. However, in this variable, there are no significant differences related to liquid biofertilizer application. Finally, the cob's weight had similar behavior to the cob´s length, resulting from N-P-K application, key for the cob's weight gain, obtaining 244-253 g weight per cob average values. Similarly, liquid fertilizer application significantly improves cob´s weight.

These results are similar to those obtained by Fauziah *et al*. (2022) for cob's length and diameter variables with increasing doses of fertilization together with the application of organic fertilizers, reporting 21.31 cm cob´s length and values close to 5 cm diameter for a combination of inorganic fertilization with a granular organic fertilizer. Both variables are related to the cob's weight, therefore, the combined application of synthetic fertilizers with organic fertilizers (liquid or solid) increases its weight as reported by Laekemariam and Gidago (2012) with a 280.72 g weight for the combined treatment of N-P-K and 5 $Mg.ha^{-1}$ of compost. Similarly, Sara *et al*. (2023) reported a significant increase in cob´s weight when combining inorganic fertilization with a liquid biofertilizer.

Regarding grain quality, Table 6 shows that grains per cob weight improve significantly when applying fertilization with N-P-K, increasing its average weight by more than 60 g at least. However, there are no significant differences as the fertilization doses increase. Concerning liquid biofertilizer, there is a significant grain per cob weight gain when applied to the crop. When analyzing 100 grains weight, inorganic fertilization also turned out to be key, although there seems to be no effect when the dose is increased. However, there are no significant differences related to liquid biofertilizer application. Grain filling is very important to achieve high yields, so a necessary amount of nutrients is needed for adequate nutrient translocation to grain (Ning *et al*., 2013), given that maize grain's weight may increase significantly when fertilization is accompanied by organic fertilizers application. Obtained Results for 100 grains weight almost double those found by Babaji *et al*. (2014) for its treatment with N-P-K and 10 $Mg·ha^{-1}$ of sheep manure and are much higher than the 26.89 g reported by Budiastuti *et al*. (2023) for 12 $Mg.ha^{-1}$ treatment of composted material.

Yield per hectare encompasses the aforementioned variables, in addition to being the standard when weighing crop productivity. Statistical analysis results are shown in Table 6. These results point out that crop yield increases significantly when applying fertilization with N-P-K, with a 2.5 $Mg·ha^{-1}$ average increase. However, there are no significant differences as the fertilization dose increases, so for this trial conditions, it would only be necessary to apply the N-P-K dose of 160-40-60 (F1) to achieve statistically similar yields to the highest dose evaluated 240-120-140 (F3). In the same way, when applying liquid biofertilizer, there is almost one megagram per hectare yield increase, so its application is highly recommended. Many factors intervene in performance, which is why scientific publications reported quite broad values. However, comparable works regarding liquid biofertilizers use such as those of Díaz-Chuquizuta *et al*. (2022) reported 6.95 $Mg·ha^{-1}$ values with bovine manure hydrolyzate use, as do Maintang *et al*. (2021) who obtained 8.22 $Mg·ha^{-1}$ with a combined application of N-P-K and a liquid biofertilizer. García-Gonzales *et al*. (2020) evaluated liquid fertilizer application to the soil (drench) with 4.28 $Mg·ha^{-1}$ yields in tropical conditions. A 6.10 $Mg·ha^{-1}$ yield was even achieved only with weekly liquid fertilizer application as reported by Sutharsan and Rajendran (2016). There is much other research that evaluates N-P-K application with solid organic fertilizers, finding that combined application can help reduce spending on synthetic fertilizers and reduce production costs (Farfán and Perales, 2021; Fauziah *et al*., 2022; Laekemariam and Gidago, 2012).

A variable that helps to determine the proposed treatment's effectiveness is the harvest index since it reflects plant investment in developing reproductive parts (grains). Liu *et al*. (2020) stated that for maize varieties with yields less than 15 $Mg.ha^{-1}$, analyzing the HI is crucial to evaluate the behavior of maize in different environmental conditions and with different varieties. Hütsch and Schubert (2017) suggest that there are primarily two ways to improve the HI: one is to enhance the transport of leaves to the grain, and the other is to reduce vegetative growth without decreasing yield. For both cases, it is essential to use phytohormones and organic compounds to improve these processes, which is in line with the use of organic biofertilizers to enhance the HI. This is because the organic compounds in the chemical composition of this biofertilizer (Table 3) enhance the translocation of photosynthates in the grain-filling stage (Abdo *et al*., 2022). Additionally, the high content of micronutrients in this liquid fertilizer also plays a significant role in grain filling and yield increase (Chinipardaz *et al*., 2022).

Figure 2 and Table 7 allow us to observe in greater detail the importance of the harvest index analysis. It can be seen that the treatment that obtained the highest yields is F3_K1, however, it has the lowest harvest index, which reflects that a large part of the nutrients provided developed aerial biomass without achieving effective translocation to the grains. On the other hand, treatment F1_K1 has similar yields to F3_K1 but with significantly higher harvest rates. Likewise, the statistical analysis indicated a positive interaction between N-P-K fertilization doses and biofertilizer applications.

The behavior of the HI observed in this study is similar to that reported by Abdo *et al*. (2022) who obtained the highest HI (45.60 %) in their treatment with 25 % N-P-K fertilization together with the application of biofertilizers, while the lowest HI was with 100 % N-P-K fertilization with the application of biofertilizers (25.81 %). Likewise, the harvest indices obtained are comparable to that reported by Ion *et al*. (2015) for different maize hybrids, more specifically, Mahmood *et al*. (2017) determined harvest indices for different treatments, including the joint application of N-P-K with different solid organic fertilizers, reporting index values of 0.56 - 0.57.

Table 6. Main effects of fertilization dose (F) and biofertilizer application (K) on the evaluated cobs, grain characteristics, and maize yield.

Treatment	Cob's length (cm)	Cob´s diameter (cm)	Cob´s weight (g)	Grain weight ($g \cdot cob^{-1}$)	100 grain weight (g)	Yield ($Mg \cdot ha^{-1}$)	Harvest index
			Fertilizer dose (F)				
F0	16.19 ± 1.6 **b**	4.44 ± 0.3 **c**	163.78 ± 40.3 **b**	128.78 ± 32.2 **b**	36.71 ± 5.3 **b**	5.72 ± 0.7 **b**	0.43 ± 0.04 **c**
F1	19.49 ± 1.8 **a**	4.71 ± 0.2 **b**	244.00 ± 38.8 **a**	195.91 ± 32.9 **a**	40.92 ± 4.3 **a**	9.12 ± 0.8 **a**	0.53 ± 0.04 **a**
F2	20.01 ± 1.4 **a**	4.84 ± 0.2 **a**	259.76 ± 31.2 **a**	209.15 ± 24.1 **a**	41.10 ± 4.7 **a**	9.20 ± 0.4 **a**	0.53 ± 0.01 **a**
F3	19.47 ± 1.1 **a**	4.80 ± 0.2 **ab**	252.92 ± 31.1 **a**	202.74 ± 27.0 **a**	41.98 ± 3.1 **a**	9.58 ± 0.8 **a**	0.50 ± 0.03 **b**
			Biofertilizer application (K)				
K0 Without biofertilizer	18.43 ± 2.2 **b**	4.67 ± 0.3 **a**	220.83 ± 51.0 **b**	176.17 ± 42.1 **b**	39.84 ± 5.0 **a**	8.01 ± 1.8 **b**	0.49 ± 0.05 **b**
K1 With biofertilizer	19.13 ±2.0 **a**	4.72 ± 0.3 **a**	238.82 ± 52.6 **a**	191.62 ± 43.8 **a**	40.50 ± 4.7 **a**	8.80 ± 1.6 **a**	0.51 ± 0.05 **a**
Fertilizer dose (F)	***	***	***	***	***	***	***
Biofertilizer (K)	*	n.s.	*	*	n.s.	**	**
FxK	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*

The means with the same letter within the same column do not differ significantly ($p \leq 0.05$, ANOVA and Duncan's test, equivalent to a simultaneous confidence level of 95%); ± Standard deviation. * Statistically significant, *** Highly significant, n.s. Not significant

Table 7. Maize yield parameters associated with the treatments.

Treatment	Cob's length (cm)	Cob´s diameter (cm)	Cob´s weight (g)	Grain weight ($g \cdot cob^{-1}$)	100 grain weight(g)	Yield ($Mg \cdot ha^{-1}$)	Harvest index
			Fertilizer dose x Biofertilizer application (F:K)				
F0K0	15.88 ± 1.7 **c**	4.46 ± 0.3 **c**	158.67 ± 39.8 **c**	124.98 ± 32.4 **d**	36.85 ± 6.8 **b**	5.13 ± 0.6 **d**	0.41 ± 0.05 **e**
F0K1	16.52 ± 1.4 **c**	4.42 ± 0.2 **c**	169.26 ± 41.5 **c**	132.85 ± 32.7 **d**	36.55 ± 3.4 **b**	6.31 ± 0.2 **c**	0.45 ± 0.02 **d**
F1K0	18.95 ± 1.9 **b**	4.67 ± 0.2 **b**	234.14 ± 39.5 **b**	187.74 ± 33.6 **c**	40.50 ± 4.3 **a**	8.75 ± 1.0 **b**	0.51 ± 0.03 **bc**
F1K1	20.03 ± 1.4 **ab**	4.75 ± 0.2 **ab**	253.87 ± 36.7 **ab**	204.08 ± 31.1 **abc**	41.34 ± 4.3 **a**	9.49 ± 0.4 **ab**	0.56 ± 0.03 **a**
F2K0	20.25 ± 1.0 **a**	4.84 ± 0.2 **ab**	257.32 ± 20.8 **ab**	206.57 ± 15.7 **abc**	40.84 ± 3.5 **a**	8.98 ± 0.1 **ab**	0.52 ± 0.01 **bc**
F2K1	19.81 ± 1.6 **ab**	4.83 ± 0,2 **ab**	261.88 ± 38.6 **ab**	211.39 ± 29.9 **ab**	41.32 ± 5.7 **a**	9.41 ± 0.6 **ab**	0.53 ± 0.02 **b**
F3K0	18.92 ± 1.0 **b**	4.73 ± 0.2 **ab**	239.28 ± 32.6 **ab**	190.4 ± 27.1 **bc**	41.42 ± 3.5 **a**	9.17 ± 0.6 **ab**	0.50 ± 0.03 **bc**
F3K1	19.98 ± 0.9 **ab**	4.86 ± 0.2 **a**	265.64 ± 24.1 **a**	214.25 ± 22.1 **a**	42.51 ± 2.5 **a**	9.99 ± 0.8 **a**	0.49 ± 0.04 **c**

The means with the same letter within the same column do not differ significantly ($p \leq 0.05$, ANOVA and Duncan's test, equivalent to a simultaneous confidence level of 95%); ± Standard deviation.

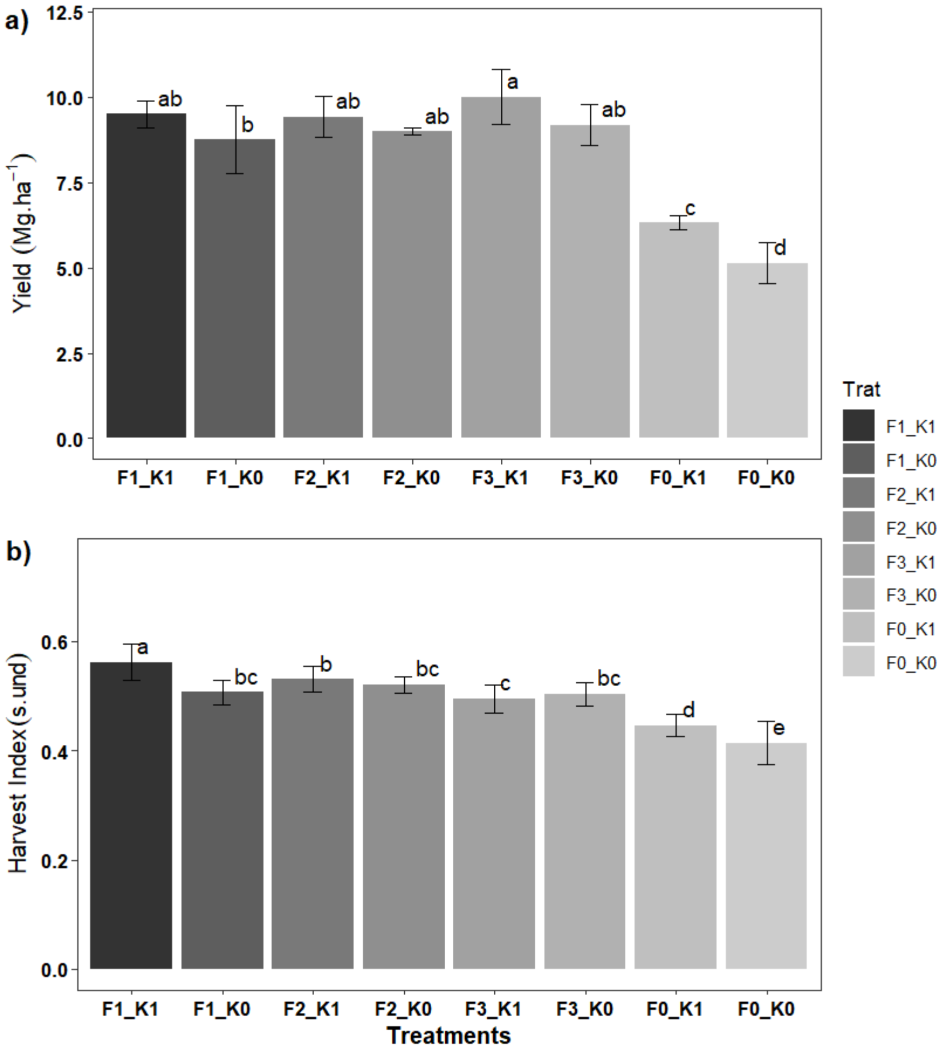

Figure 2: a) Fertilizer dose x Biofertilizer application on yield; b) hard yellow maize harvest index. Points with the same letter indicate that there was no significant difference between treatments (Duncan p = 0.05). Error bars indicate standard deviation.

CONCLUSIONS

The combined fertilization of 160-40-60 of N-P-K together with a liquid biofertilizer used, a slaughterhouse waste fermentation product, can have a positive impact on maize growth, harvest index, and yield of hard yellow maize Megahybrid 619 INIA.

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