AGRONOMIC ANALYSIS OF TWO MAIZE HYBRIDS UNDER THE INFLUENCE OF A BIOFERTILIZER †

[ANÁLISIS AGRONÓMICO DE DOS HÍBRIDOS DE MAÍZ BAJO LA INFLUENCIA DE UN BIOFERTILIZANTE]

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SUMMARY

Background. The use of synthetic fertilizers has contributed to environmental pollution and the impact of areas for agricultural use, generating the need to look for ecological alternatives. Currently it has been shown that the application of the commercial biofertilizer Azofert® (*Rhizobium leguminosarum*, CF1, 1x109 CFU mL^{-1}) increases the growth, development and yield of some crops, including corn, which is of great importance in the world due to its high consumption. **Objetive.** To evaluate the agronomic response of two corn hybrids (ADV-9789 and Emblema Ultra), under the application of said organic biofertilizer Azofert®. **Methodology.** A completely randomized block experimental design (BCA) with a bi-factorial arrangement was used, applying doses of 4, 8 and 12 mL Kg^{-1} seed and a control treatment. **Results.** It was observed that, in the growth, production and yield variables, the three Azofert® treatments were superior to the control treatment, which assume that the inoculation in seed was efective as a treatment recommendation for this crop. The highest dose (12 mL Kg^{-1} seed) was the one that presented the significantly higher values in both varieties. **Implications.** Azofert® is a biofertilizer whose main use is in legumes however, this opens an area of opportunity to study it in other types of crops. **Conclusions**. The organic biofertilizer Azofert® has a high potential to increase the growth, development and yield of the two corn hybrids studied.

Key words: *Rhizobium leguminosarum*; plant development; production; sustainability.

RESUMEN

Antecedentes. El uso de fertilizantes sintéticos ha contribuido a la contaminación ambiental y al impacto de áreas de uso agrícola, generando la necesidad de buscar alternativas ecológicas. Actualmente se ha demostrado que la aplicación del biofertilizante comercial Azofert® (*Rhizobium leguminosarum*, CF1, 1x109 UFC mL^{-1}) aumenta el crecimiento, desarrollo y rendimiento de algunos cultivos, entre ellos el maíz, el cual es de gran importancia en el mundo por su alto consumo. **Objetivo.** Evaluar la respuesta agronómica de dos híbridos de maíz (ADV-9789 y Emblema Ultra), bajo la aplicación de dicho biofertilizante orgánico Azofert®. **Metodología.** Se utilizó un diseño experimental de bloques completamente al azar (BCA) con arreglo bifactorial, aplicándose dosis de 4, 8 y 12 mL Kg^{-1} de semilla y un tratamiento control. **Resultados.** Se observó que, en las variables crecimiento, producción y rendimiento, los tres tratamientos con Azofert® fueron superiores al tratamiento testigo, lo que supone que la inoculación en semilla fue efectiva como recomendación de tratamiento para este cultivo. La dosis más alta (12

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mL Kg^{-1} de semilla) fue la que presentó valores significativamente mayores en ambas variedades. **Implicaciones.** Azofert® es un biofertilizante cuyo principal uso es en legumbres, sin embargo, esto abre un área de oportunidad para estudiarlo en otro tipo de cultivos. **Conclusiones.** El biofertilizante orgánico Azofert® tiene un alto potencial para incrementar el crecimiento, desarrollo y rendimiento de los dos híbridos de maíz estudiados.
Palabras clave: *Rhizobium leguminosarum*; desarrollo vegetal; producción; sostenibilidad.

INTRODUCTION

Corn (*Zea mays* L.) is an important crop worldwide, it represents the food of 15 to 20% of humanity (Garibaldi *et al.*, 2023). There are more than 200 million hectares in the world dedicated to this crop, which represents 40% of world cereal production, with its greatest representation in the American continent (FAO, 2024).

Agriculture has significantly increased its productivity in the last 50 years in the world, reaching 23.7 million tons of food per day (Duque-Acevedo *et al.,* 2020). This increase in production has caused intense pressure on natural resources, thus questioning some aspects of agricultural sustainability. Furthermore, fertilization is excessively used in the world to increase crop yields (Alrbaihat, 2023). In intensive conventional agriculture the soil is poor in nutrients, therefore, producers use fertilizers to increase productivity (Wu and Ge, 2019). The use of N, P and KCl fertilizers has increased in the last 40 years, while nutrient use efficiency -the amount of mineral NPK necessary to produce one kg of crops- has decreased. Therefore currently, the search for alternatives to use synthetic fertilizers is a priority (Rempelos *et al.*, 2021).

It has been shown that the use of synthetic chemicals causes negative effects on the environment, human and animal health, as well as resistance in phytopathogens (Torres-Rodríguez *et al.*, 2021; Dupouy, 2023). In addition, they cause soil erosion, salinity, and accumulation of heavy metals and nitrates (Rahman and Zhang, 2018). According to previously explained, the application of organic products has been in-creasing around the world (Tal, 2018), where biofertilizers could be the alternative that replaces the use of synthetic fertilizers, due to their environmental sustainability and positive effects on the environment. plant development and production (Anju *et al.,* 2023).

Biofertilizers are organic products that contain specific microorganisms; when they are applied to plants, seeds, soil or growing media, and an interaction occurs through signaling cascades that increase the nutrient absorption capacity of plants. This bioproducts help its growth of plants and development in unfavourable conditions, such as extreme temperatures, limited water supply and nutrients, likewise; also biofertilizers can help in response to plant stress, they help to reduce assimilates directed to a non-productive metabolism through the production of beneficial molecules for the plant (Daniel *et al.,* 2022). Also, it has been shown that the application of biofertilizers improves the absorption of nutrients and increases the productivity of crops, helps break dormancy, increases fruit size, improves the development of the root system, increases the activities of photosynthetic tissues and other vegetative tissues improve the vigor and uniformity of plants, regulate flowering and stimulate fruit setting and ripening (Nosheen *et al.,* 2021).

Therefore, the effectiveness of Azofert® biofertilizer was tested, which presents native species of rhizobia with a high degree of effectiveness, purity and biological persistence. Its beneficial effects are mainly due to the function of rhizobia as nitrogen fixers, in addition to the mechanisms of action of plant growth-promoting rhizobacteria [PGPR] (Nápoles-García *et al.,* 2014). Its beneficial effect has been proven in different soils and enhanced with the combination of other biofertilizers (Martínez-Viera, 2010), so its use allows replacing imports of mineral fertilizers and in-creasing income for producers by obtaining better yields.

Due the *Rhizobium* spp. bacteria convert atmospheric N into NH4, which is a way of making it available so that the plant can absorb it and in turn the crops provide carbohydrates for the bacteria to survive (Yzarra-Aguilar *et al.,* 2023), the application of the organic biofertilizer Azofert could increase the growth, development and yield of the ADV 9789 and Emblema Ultra corn hybrids. Based on the above, the present research aims to evaluate the agronomic response of two corn hybrids under the application of the organic biofertilizer Azofert.

MATERIALS AND METHODS

Experimental design

The research was carried out at the experimental campus “La María”, an extension belonging to the State Technical University of Quevedo, located in Quevedo, Los Ríos, Ecuador. A BCA experimental design (Cochran and Cox, 1957) with a 2A × 3B bifactor arrangement was used, where factor A were corn hybrids (ADV 9789 and Emblema Ultra) and factor B, three doses of Azofert® (4, 8 and 12 mL Kg^{-1} seed) in plots of 4 x 2.40 m for each hybrid.

For the implementation of the plots, a spacing of 0.80 m distance between rows and 0.20 m distance between plants was used. Giving a total of 2,560 plants in the trial. For the evaluation of the indicators, 10 randomly chosen plants were marked in each plot that represented the hybrids (treatments). The evaluations were carried out by selecting the plants from the two center rows, to avoid the edge effect and minimize the influence of cross-pollination.

Treatments

The corn hybrids: Advanta 9789 (ADV-9789) and Emblema Ultra, were inoculated by suspending the seeds in the biostimulant Azofert® under three different treatments (T) for each hybrid: 4 (T1), 8 (T2) and 12 (T3) mL Kg^{-1} seed respectively. They were kept suspended under these concentrations for a period of 24 hours, after this time they were removed from the suspension and left to dry before sowing. Each variety had its control treatment (TC) without inoculation.

Soil preparation

Two steps of harrowing and plowing were carried out in both directions on the land, in order to leave the land well mulched, facilitating the germination and root development of the crop. Subsequently, the experimental plots were divided according to the established field sketch.

Control of weeds, pests and diseases

For weed control, a pre-emergent chemical control of systemic herbicide, blue band slightly toxic Task 80 WP (Triazines), was applied at doses of 1.7 – 3.0 kg ha^{-1}, then manual controls were carried out at 20, 40 and 60 days, during the vegetative cycle.

In the case of pests and diseases, a chemical control, blue band (200 mL ha^{-1} of Expander® (alfacypermethrin + teflubenzuron) was applied to control the fall armyworm (*Spodoptera frugiperda*) after 15 days, and after 25 days, 330 mL ha^{-1} of Crysconazole (Propiconazole), blue tape systemic fungicide was applied to control diseases such as Leaf blight (*Helminthosporium maydis*), with a CP3 hand sprayer and a 110° flat fan nozzle.

Variables evaluated

Growth and performance indicators were determined according to the methodology of Torres-Rodríguez *et al.* (2018) Therefore, for the growth variables like height of the plant (cm) at 35 days after sowing (DAS), diameter of the stem (cm) at 55 DAS and length and width of leaf (cm) were evaluated at 65 DAS. On the other hand, for the performance indicators, the diameter and length of cob (cm), number of rows of grain on the cob, weight of cob (g), weight of 1000 grains (g), weight of cob (g) and yield (Kg ha^{-1}), at 120 DAS in a dry state and with a moisture between 14 – 18% of humidity of the grains.

Statistic analysis

The data referring to the agronomic and physicochemical variables were evaluated by analysis of variance (ANOVA) and Tukey's test to verify the differences between the means ($p \leq 0.05$), using the Statistical Analysis software (SAS, 2002).

RESULTS

It has been shown that biofertilizers increase plant growth and yield between 10 until 40% (Nosheen *et al.*, 2021) and can improve the absorption of macro-nutrients and this effect is related to the stimulation of nitrogen metabolism (Calvo *et al.*, 2014). Starting from these premises, the following results are presented, which agree with these authors.

Effect of Azofert® on the growth of ADV-9789 and Emblema Ultra hybrids

The analysis of variance between both varieties showed statistical differences ($p \leq 0.05$). The best results were presented in T3 of the Emblema Ultra hybrid, causing increases of 7.65% (55 DAS) and 1.3% (105 DAS) in the height variable; For the stem diameter variable, there was an increase of 4.74% 55 DAS only (Table 1).

In the case of the leaf area variables, the application of Azofert® had an impact by increasing the length and width of the leaf in all concentrations (8, 10 and 12 mL Kg^{-1} seed), which was possible to notice mainly in T1 Emblema Ultra (Table 2), being 0.62 – 1.5% and 1.78 – 4.79% respectively.

Table 1. Effect of the application of Azofert® in three different concentrations on the plant growth of Emblema Ultra hybrid corn at 55 and 105 DAS.

Treatments	Height (cm)		Diameter (cm)	
	55 DAS	105 DAS	55 DADS	105 DAS
TC-E	174.5 c	195.22 a	15.81 b	18.26 a
T1-E	182.28 b	189.06 b	16.16 ab	17.81 a
T2-E	179.33 bc	194.73 a	15.91 b	18.18 a
T3-E	187.86 a	197.77 a	16.56 a	17.88 a

† E: Emblema Ultra; TC (control treatment); T1-E: 4 mL Kg^{-1} seed; T2-E: 8 mL Kg^{-1} seed; T3-E: 12 mL Kg^{-1} seed. (DAS) days after sowing. Means with a different letter in each column indicate a statistical difference (Tuckey $p \leq 0.05$).

Table 2. Effect of the application of Azofert® in three different concentrations on the leaf length and width of *Zea mays* L. variety Emblema Ultra.

Treatments	Length (cm)	Width (cm)
TC- E	90.88 a	8.97 c
T1-E	92.25 a	9.40 a
T2-E	91.89 a	9.24 ab
T3-E	91.45 a	9.13 bc

† E: Ultra Emblem; TC (control treatment); T1-E: 4 mL Kg^{-1} seed; T2-E: 8 mL Kg^{-1} seed; T3-E: 12 mL Kg^{-1} seed. Means with a different letter in each column indicate a statistical difference (Tuckey p≤0.05).

Effect of Azofert® on the production and performance indicators of ADV-9789 and Emblema Ultra hybrids

On the other hand, Azofert® treatments increased production variables such as: length (cm) and diameter of cob (cm), number of rows of cob, weight of cobs (g), weight of 1000 grains (g) and cob weight (g) in the two corn hybrids (ADV-9789 and Emblema Ultra), showing significant increases compared to TC. For this reason, it should be noted that the maximum dose of Azofert® (12 mL Kg^{-1} seed) was related to the best results in the variables studied with the hybrid ADV-9789 (Table 3 and 4). Regarding the length of the cob (cm), no significant changes were observed by the corn varieties.

For the variable number of rows, the Azofert® treatments in the two hybrids (ADV-9789 and Emblema Ultra), results were superior to TC, indicating an increase of 7.86 to 11.8% (Table 3). Behaving better Emblema Ultra in its T2 (8 mL Kg^{-1} seed).

In ear weight, the results of T3 were superior to the control in both hybrids, with increases of 109 and 110%. With respect to the stubble, the values obtained were 12.1 g in the ADV-9789 hybrid (12 mL Kg^{-1} seed) followed by 12.32 g of Emblema Ultra (8 mL Kg^{-1} seed), which represents an increase of 143% for both (Table 4).

Finally, the application of Azofert® in the two corn hybrids increased the crop yield by 3.83 – 45.31% compared to the control treatment (Table 4). The best results in terms of this parameter were obtained with the highest doses of the biofertilizer (8 and 12 mL Kg^{-1} seed) and the lowest results with the TC (without application of the biofertilizer), achieving an increase in the yield in ADV- 9789 (T3) by 125% and Emblema Ultra (T2) by 109%.

DISCUSSION

The use of biofertilizers in plants of agricultural interest occurs with the aim of promoting the growth, production and yield of crops, as has been studied in this research.

Table 3. Effect of the application of Azofert® in three different concentrations on the number of rows of *Zea mays* L. in the ADV-9786 and Emblema Ultra hybrids.

Treatments	Number of rows	
	ADV-9789	Emblema Ultra
TC	12.71 d	14.17 bc
T1	13.96 bc	14.07 bc
T2	13.71 c	15.14 a
T3	14.21 bc	14.32 b

† CT (control treatment); T1: 4 mL Kg^{-1} seed; T2: 8 mL Kg^{-1} seed; T3: 12 mL Kg^{-1} seed. Means with a different letter in each column indicate a statistical difference (Tuckey p≤0.05).

Table 4. Effect of the application of Azofert® in three different concentrations on production and yield indicators of *Zea mays* L., variety ADV-9789 and Emblema Ultra.

	Cob (g)		Stubble (g)		1000 grains (g)		Yield (Kg ha^{-1})	
Treatments	ADV-9789	Emblema Ultra	ADV-9789	Emblema Ultra	ADV-9789	Emblema Ultra	ADV-9789	Emblema Ultra
TC	161.25 c	163.57 bc	8.46 f	8.6 f	212.25 c	160 e	8515.3 c	7343.5 e
T1	169.25 b	165.5 bc	11.03 c	11.78 b	225.25 b	163.5 de	9963.0 b	7624.8 de
T2	167.71 b	169.14 b	10.03 d	12.32 a	226.75 b	162.25 e	10593.5 a	8077.8 cd
T3	177.07 a	178.5 a	12.10 ab	9.32 e	238.75 a	167.75 d	10671.5 a	7812.3 de

† ADV-9789: Advanta 9789; TC (control treatment); T1: 4 mL Kg^{-1} seed; T2: 8 mL Kg^{-1} seed; T3: 12 mL Kg^{-1} seed. Means with a different letter in each column indicate a statistical difference (Tuckey p≤0.05).

Effect of Azofert® on the growth of hybrids ADV-9789 and Emblema Ultra

At the same time, the stem diameter is related to the effect of biofertilizers on the physiological processes of the plants and with absorption of nutrients by the root system (Aguilar *et al.,* 2019). For their part, Márquez-Cruz et al. (2022) did not find significant results when applying different strains of Rhizobium spp. in corn crop. However,
Prieto et al. (2021) indicated that the effects of *Rhizobium leguminosarum* isolates can vary from one experimental site to another in a peas crop, generating variation in performance indicators.

Another aspect to highlight in the present investigation was the increase in the pho-tosynthetic area with the application of Azofert® (Table 2), which translates into greater nutrient processing and increased productivity, another important factor to consider in this experiment was the leaf area, where the findings demonstrated that the application of Azofert® in the corn crop promotes an increase in the photosynthetic area (Table 2), which translates into greater production of nutrients for the plant and therefore greater productivity (Calero-Hurtado *et al.*, 2018). This result agrees with the findings of Pramanik et al. (2019) who investigated the effect of the K-solubilizing strain, *Bacillus pseudomycoides*, finding that its application increased the leaf area in tea plants in soil treated with mica waste.

Effect of Azofert® on the production and performance indicators of ADV-9789 and Emblema Ultra hybrids

Several authors have studied the response of plants with the application of biofertilizers. For example, Estrada *et al.* (2017) found that the application of Azofert® increased the length and width of the pod in a bean crop, showing significant differences compared with the control treatment, which supports the result obtained, even though the values were not very high.

Another factor of great relevance is the number of rows in the corn crop. The data obtained in this work were very interesting as they were higher than those reported by Torres-Rodríguez et al. (2018) with 13.10 rows, demonstrated in both works that the application of Azofert® favors this essential indicator in a corn crop, which, according to previous research, shows that the increase in the nutrient absorption surface by the application of biofertilizers improves some morphophysiological characteristics of the plants, such as number of rows and number of grains per row (Martínez-Reyes *et al.,* 2018).

On the other hand, González (2023) in their study demonstrated that the application of microrganisms in a maiz crop increase biomass. This is because the microorganisms introduced into the soil, by establishing themselves in the rhizosphere or in the internal tissues of the plants, increase the availability of nutrients. In addition, some of these microorganisms act as phytostimulants by producing auxins, which promote root growth facts of great importance considering the increase results that were obtained in the present investigation (Santoyo et al., 2021).

The stubble is a variable with added value, since it has been proven to be used in the preparation of diets for animal feeding, as a soil conditioner and as a source of renewable energy (Ramírez-Contreras *et al.,* 2015). In the present investigation there was a greater increase with the biofertilization of the two hybrids.

In the data obtained for weight of 1000 corn grains, a favorable increase was observed with biofertilization, which coincides with Kesell et al. (2022) in a bean crop. This is because nitrogen-fixing bacteria play an important role supplying nitrogen to the plant and strengthening the growth of the crop. In addition, the biofertilizers synthesize phytohormones that promote morphological and physiological changes in the plant, resistance to stress and biocontrol, which generate better use of water, nutrients (Beltrán, 2022).

The findings in the present study showed that Azofert® increases crop yield, which coincides with Torres-Rodríguez et al. (2018) reporting an increase in yield with the application of the same biofertilizer. One of the most important functions of Azofert® is symbiotic nitrogen fixation, which it was effective in both legumes and non-legume crops, allowing an increase in plant development and yield (Nosheen *et al.,* 2021).

Overall, the findings derived from the present investigation demonstrated a favorable effect for the different variables measured, which coincides with similar investigations such as that carried out by Din et al. (2019) who recorded morphological changes of the plant when applying *Aspergillus niger* to propous it as a biofertilizer.

CONCLUSIONS

For the parameters measured in the present study, the highest values were obtained from the highest dose of Azofert® (12 mL Kg^{-1} seed), which suggests the use of this product as a favorable and sustainable alternative in the field of agriculture, seeking greater yield at a low cost and the partial replacement of synthetic fertilizers, mainly nitrogenous and phosphated.

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Compliance with ethical standards The study did not require human participation, therefore it does not require committee approval.

Data availability. Data is available upon reasonable request with the corresponding author (jessica.ca@lerdo.tecnm.mx).

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