



## Morfo-agronomic behavior of pineapple plants 'MD2' with foliar applications of efficient microorganisms

### Comportamiento morfo-agronómico de plantas de piña 'MD2' con aplicaciones foliares de microorganismos eficientes

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**ABSTRACT:** Pineapple (*Ananas comosus* var. *comosus*) is the most economically important species within the Bromeliaceae family. For year, it has been one of main export resources in many countries, especially the cultivar 'MD2', which, due to its soluble solids content, aroma and color, has been preferred in global markets. Although its organoleptic qualities have made it a consumer favorite, the 'MD2' is more susceptible to fungal diseases than the 'Cayenne' and 'Champaka' varieties, and more demanding in terms of agrotechnical management. This study aims to determine the morfo-agronomic aspects of 'MD2' pineapple plant and fruits. Clonal suckers weigh approximately 300 g were used, and the morphological indicators evaluated were survival (%) at 120 and 180 days. Quality indicators analyses of the 'MD2' fruits was also conducted: fresh fruit mass with crown (kg), fresh fruit mass without crown (kg), crown-to fruit mass ratio, soluble solids content (°Brix), acidity content (%), ascorbic acid content (mg of ascorbic acid in 100 ml of juice). The results showed that foliar application of efficient microorganisms increased the survivor and fresh mass of the plants. These increases led to higher fresh fruit mass without affecting the fruits quality.

**Key words:** Fruit quality, growth, soil, fruits, biostimulant.

**RESUMEN:** La piña (*Ananas comosus* var. *comosus*) es la especie económicamente más importante dentro de la familia Bromeliaceae, ha sido por años uno de los recursos económicos de exportación en muchos países, en especial el cultivar 'MD2' que, por su contenido de sólidos solubles, aroma y color ha sido preferido en los mercados mundiales. Aunque sus cualidades organolépticas le han convertido en el favorito del consumidor, la 'MD2' es más susceptible a enfermedades fungosas que la 'Cayena' y la 'Champaka', y más exigente en manejo agrotécnico. El presente trabajo tiene como objetivo determinar los aspectos morfo-agronómicos de las plantas y frutos de piña 'MD2' con las aplicaciones foliares de microorganismos eficientes que permitan mejorar la calidad de las plantas y frutos de piña. Se utilizaron hijos clavel de aproximadamente 300 g y los indicadores morfológicos que se evaluaron fueron: supervivencia (%) a los 120 y 180 días de plantadas y masa fresca de la planta (g) mensual. También se realizaron análisis de indicadores de calidad de los frutos 'MD2': masa fresca del fruto con corona (kg), masa fresca del fruto sin corona (kg), relación masa de corona y masa de fruto, contenido de sólidos solubles (°Brix), contenido de acidez (%) y contenido de ácido ascórbico (mg de ácido ascórbico en 100 ml de jugo). Los resultados demostraron que la aplicación foliar de microorganismos eficientes incrementó la supervivencia y la masa fresca de las plantas. Estos incrementos indujeron una mayor masa fresca del fruto sin incidir en la calidad del mismo.

**Palabras clave:** Calidad del fruto, crecimiento, suelo, frutos, bioestimulante.

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## INTRODUCTION

The pineapple (*Ananas comosus* var. *comosus*) is the most economically important species within the Bromeliaceae family. For years, it has been one of the main export resources in many countries, especially the cultivar Gold "Extra Sweet" 'MD2', which, due to its soluble solids content, aroma, and color, has been preferred and maintained as the number one in world markets. It is marketed as a processed or fresh product because of its high nutritional value, pleasant flavor, industrialization potential, and visual appeal. After banana and mango, it is the most important tropical fruit, with global production in 2022 reaching 29361138 tons (1).

Although the organoleptic qualities of 'MD2' have made it the consumer's favorite, it is more susceptible to fungal diseases than Cayenne and Champaka, and more demanding in agrotechnical management, which increases its production cost. From the perspective of opportunity cost, however, this is compensated by a better price, a condition that justifies its investment (2).

The inadequate use and management of cultivation soils and the intensive application of agrochemicals worldwide have caused severe environmental deterioration processes, reflected in their biological deactivation and loss of productive capacity, which poses a serious risk to society's food security (3).

Management of this crop, beginning with soil preparation, is intensive. Twelve consecutive tasks are carried out, which in some ways eliminate weeds and harmful microorganisms, but also those that benefit and stimulate crop development.

As a concept, effective microorganisms are defined as mixtures of beneficial cultures and natural microorganisms that can be applied as inoculants, thereby increasing the biological diversity of the soil and the plant (4). These include numerous types of microscopic organisms such as bacteria and fungi, which can offer great benefits to agriculture, as they contribute to soil formation and participate in the degradation of organic matter and in the cycles of elements such as carbon, nitrogen, oxygen, and sulfur, among others (4,5).

These microorganisms contribute to soil fertility and are used by living beings in their metabolism. Many of them live around plant roots and influence their growth by helping absorb nutrients and protecting against or preventing attacks from pathogenic microorganisms (6).

Objective of this study is to evaluate the effect of foliar applications of effective microorganisms on the morpho-physiological indicators of pineapple (*Ananas comosus* var. *comosus*) 'MD2' plants and fruits.

## MATERIALS AND METHODS

This study was conducted at the Basic Business Unit (UEB, according its acronyms in Spanish) "Pineapple Production,"

belonging to the Ceballos Agroindustrial Enterprise (21°47'N 78°48'W). The experiment began in 2019 and concluded in 2020. Clavel suckers weighing approximately 300 g, obtained from pineapple plants (*Ananas comosus* var. *comosus*) hybrid 'MD2', were used.

The methodology employed for soil preparation and conditioning was that established by the UEB. The soils are typical red ferrallitic soils over saturated hard limestone, moderately deep, moderately humified, with little erosion, kaolinitic clay, slightly stony, deep, with an effective depth of 30 cm, flat, and with a pH of 5.52 (slightly acidic). Prior to planting, the soil in the experimental area was characterized, and the results are shown in Table 1. Determinations were carried out according to the Soil Analysis Methodology recommended by the National Institute of Soils and Fertilizers (7). Analyses were performed at the Territorial Soil Laboratory of the Ministry of Agriculture (MINAG) in Camagüey Province.

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Throughout the process, the UEB's Technical Instruction for pineapple cultivation, approved by the Company's Technical Advisory Council was followed. Foliar fertilization was applied in the early morning hours (before 09:00) with a mixture of mineral fertilizers totaling 729; 325; 566; 41.92 and 0.65 kg ha<sup>-1</sup> (N,P,K,Mg,Ca) and micronutrients of 6; 2.08; 2.13; 2.07; 0.17 and 0.08 kg ha<sup>-1</sup> (Fe; Mn; Zn; B; Cu; Mo) over the crop cycle. Mineral fertilizer applications were performed with a CLAXON sprayer with a 3200 L capacity and 73 nozzles.

The irrigation system used was sprinkler irrigation with a center-pivot machine (Western, manufactured by TUSA SA), and watering was carried out according to the UEB schedule for the crop's developmental stages.

## Flower induction

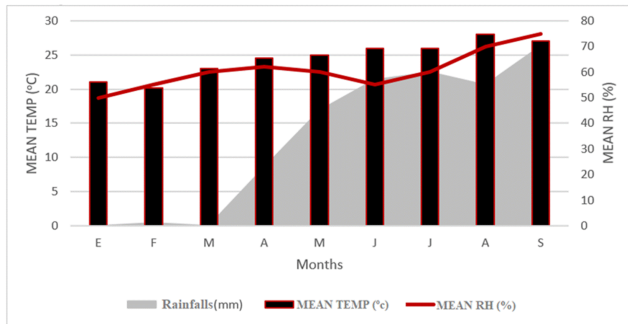
At eight months after planting, artificial flower induction was performed during the early morning hours (04:00-06:00). Each plant received approximately 50 mL of the final applied solution. The inducer solution was prepared for 1 ha and contained a mixture of Ethrel® 480 (4.0 L ha<sup>-1</sup>) + urea (30 kg) and calcium carbonate (CaCO<sub>3</sub>) 2 kg; it was applied using a CLAXON sprayer.

The environmental conditions during the vegetative phase of the experiment are shown in Figure 1 and are considered adequate for normal crop development.

**Table 1.** Chemical characteristics of the soil in the experimental area

K <sub>2</sub> O (mg 100 g <sup>-1</sup> S.)	P <sub>2</sub> O <sub>5</sub> (mg 100 g <sup>-1</sup> S)	O.M (%)	Mg (Cmol kg <sup>-1</sup> S)	Ca (Cmol kg <sup>-1</sup> S)	pH (KCl)
28.30	12.0	2.13	2.19	2.75	4.9

Source: Analyses performed at the Territorial Soil Laboratory of the Ministry of Agriculture (MINAG) in Camagüey Province. S: soil



RH: Relative Humidity

**Figure 1.** Histogram of environmental variables during the experiment development before floral induction

For the development of the experiment, two experimental factors were established: the treatments (2) and the different evaluation time points (5), the latter being considered as another factor since it provides the growth dynamics and reveals whether there are differences between treatments and across the different evaluation moments. Fresh mass was evaluated after 60 days and then monthly until 180 days. However, survival was assessed only at 120 and 180 days, corresponding to moments of optimal crop development and before and after intense rainfall during those months.

Factor 1: Treatments. [1] Control (UEB Technology); [2] EM "efficient microorganisms" (20 L ha<sup>-1</sup>)

Factor 2: Evaluation moments after planting. [1] 60 days (initial); [2] 90 days; [3] 120 days; [4] 150 days and [5] 180 days.

The efficient microorganisms inoculum, composed of *Bacillus subtilis* B/23-45-10 Nato (5.4 × 10<sup>4</sup> CFU mL<sup>-1</sup>), *Lactobacillus bulgaricum* B/103-4-1 (3.6 × 10<sup>4</sup> CFU mL<sup>-1</sup>), and *Saccharomyces cerevisiae* L-25-7-12 (22.3 × 10<sup>5</sup> CFU mL<sup>-1</sup>), with a quality certificate issued by ICIDCA, code R-ID-B-Prot-01-01, was acquired at the Labiofam Branch of Sancti Spiritus.

It was applied at a rate of 20 mL per liter using a Mataby backpack sprayer (16 L) on a biweekly basis after the second month of planting, with four foliar applications performed, while plants in the control treatment received water. The single tested dose of EM was recommended by ICIDCA specialists based on previous studies in agricultural crops of interest (8-10).

## Experimental Design

A randomized block design was used, in which each treatment was replicated in three plots measuring 13 m in length by 6.70 m in width (six double-row furrows), planted at a density of 58.600 plants ha<sup>-1</sup>. Each replicate contained 577 plants, for a total of 1.733 plants per treatment.

At each evaluation moment, 20 homogeneous plants per treatment were randomly taken, and the following variables were evaluated:

Survival percentage (%): The initial number of planted plants and its relationship with the plants remaining alive at

the time of the final evaluation. This was performed at 120 and 180 days after planting.

Plant fresh mass (g): Plant fresh mass was quantified after 60 days of planting and every 30 days until 180 days, using a hand scale (Power). When the fruits reached optimal maturity, 10 fruits per treatment were randomly taken, and the following variables were evaluated:

Fresh mass of fruit with crown (kg): The fruit with crown was weighed on a Sartorius technical scale.

Fresh mass of fruit without crown (kg): The fruit without crown was weighed on a Sartorius technical scale.

Crown-to-fruit mass ratio: The ratio between fruit mass and crown mass was calculated for each treatment.

Soluble solids content: This was determined using an ATAGO refractometer calibrated for sucrose solutions. Results were expressed in degrees Brix.

Acidity content (%): Acidity content was determined by acid-base titration of total organic acids in pineapple fruit juice using sodium hydroxide as the titrating agent and phenolphthalein as a color indicator. Results were expressed as acidity percentage referred to grams of citric acid per 100 mL of juice.

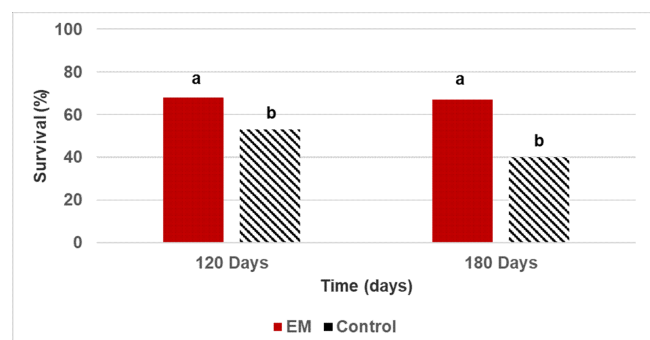
Ascorbic acid content (vitamin C): This was determined by oxidation-reduction titration of ascorbic acid using 2,6-dichlorophenol indophenol as the titrant and oxalic acid as a stabilizing agent. Results were expressed in mg of ascorbic acid per 100 mL of juice.

## Statistical treatment of results

The statistical treatment of the results was carried out using the "STATISTIC 8.0" software from StatSoft (2007). Parametric analyses (ANOVA, Tukey test, P ≤ 0.05) were performed after checking normal distribution (Kolmogorov-Smirnov, P ≤ 0.05) and homogeneity of variances (Levene, P ≤ 0.05).

## RESULTS AND DISCUSSION

Figure 2 shows plant survival at 120 and 180 days after planting.



Means with different letters indicate significance (ANOVA and Tukey test, P ≤ 0.05). Data were transformed according to  $y' = 2 \arcsin (y/100)^{0.5}$ . Each data point represents the mean for n = 20. SE = 7.15

**Figure 2.** Survival of 'MD2' pineapple plants at 120 and 180 days after planting under field conditions

The results show that in both the 120- and 180-day evaluations, plants treated with foliar applications of efficient microorganisms (EM) reached the highest and most significant values when compared to control plants. During this period, frequent favorable climatic conditions increased the presence of fungal diseases (Figure 1), among them increased rainfall (60 mm), higher temperature (23 °C) and high relative humidity (60 %). This caused the greatest plant losses to be associated with the presence of *Phytophthora* spp. in both treatments, all combined with inadequate soil preparation, since the drainage required for this crop was not available. However, it should be noted that in the control plants, losses reached 60 % at 180 days. In just 60 days (120-180 days), 17 % of the plants were lost in this treatment, while those treated with EM maintained the same survival values.

Among the main effects of EM, it is recognized that they protect the plant against pathogens and carry out atmospheric nitrogen fixation. Nitrogen is one of the main plant nutrients, being a limiting factor in plant growth and, consequently, in agricultural ecosystems (4,11-13).

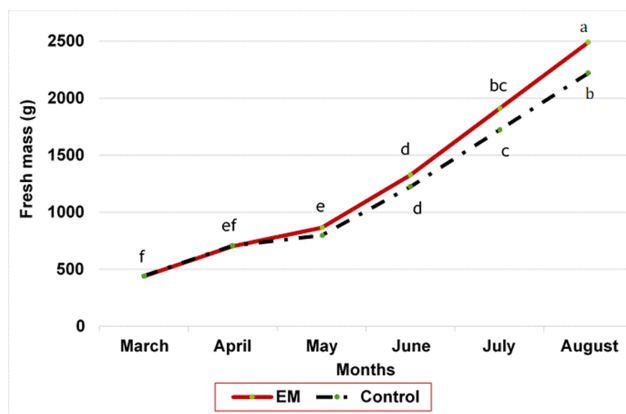
Biological nitrogen fixation allows, through the bacterial nitrogenase complex, the conversion of atmospheric nitrogen into nitrates assimilable by plants. Regardless of whether the nitrogen fixation mode is symbiotic or not, several studies have shown that adding EM to crops significantly increases the amount of available nitrogen, as well as plant yields (5,14,15).

The results achieved with the foliar application of EM on plant survival are encouraging, but it is also important to know how they influence the vegetative growth of 'MD2' pineapple plants.

Figure 3 shows the behavior of plant fresh mass in the vegetative growth stage and before flowering induction.

The figure shows that at eight months of age (August), the treatment with foliar application of EM (Effective Microorganisms) reached the highest and most significant values compared to the control treatment and to all previous evaluations conducted before that date. These values exceeded the control by 300 g (12 %). On the other hand, the UEB treatment in August did not differ from the evaluation carried out in July for the EM treatment.

However, both treatments did not show significant differences between them in the evaluations conducted from March to July. No significant differences were observed between March and April, nor between April and May, when plants increased by only about 500 g. After this date, the increase was more pronounced. It is recognized that the application of EM accelerates crop growth as a result of the gradual increase in microbial population density and,



Means with different letters indicate significance (ANOVA and Tukey's test,  $p \leq 0.05$ ). Each value represents the mean for  $n = 20$ . SE = 120

Figure 3. Behavior of plant fresh mass (g) during the vegetative growth stage

consequently, microbiological activity near the root system (14,16-18). This may explain why the EM treatment reached the highest values in plant fresh mass.

In pineapple, plant fresh mass is the most important indicator used by producers to decide when to induce flowering in order to obtain fruits with an average weight above 2.5 kg, as recommended for the hybrid 'MD2' (16-18). A correlation has been established between plant weight at the time of induction and fruit weight at harvest. Some authors state that for 'MD2' pineapple, with plant weights of 2.0 to 2.5 kg, fruit weights above 2.0 kg can be expected, which makes them of optimal quality and high value for marketing in the fresh fruit market (2).

Table 2 shows the results of the variables related to fruit and crown mass. These evaluations were carried out 150 days after artificial flower induction (harvest time).

Regarding fruit mass with crown, the best results were obtained in the EM treatment, with significant differences compared to the Control. Both treatments produced fruits of good caliber, since they exceeded 1.7 kg in fruit mass. Internationally, fruits from the Control treatment (1.7-2.0 kg) are classified as caliber 7, while those from the EM treatment (2.1-2.5 kg) are caliber 6; these calibers achieve good market prices (2).

For fruit mass without crown, a similar behavior was observed. The best value was obtained in the EM treatment, with significant differences compared to the UEB treatment. Meanwhile, crown mass showed a completely different behavior, with no significant differences between treatments. The crown-to-fruit mass ratio also showed no significant differences among treatments.

Table 2. Effect of treatments on variables related to fruit and crown mass

	Fruit mass with crown (kg)	Fruit mass without crown (kg)	Crown mass (kg)	Crown mass/Fruit mass ratio
EM	2.15 (a)	1.93 (a)	0.22 (a)	0.10 (a)
Control	1.72 (b)	1.47 (b)	0.25 (a)	0.14 (a)

For each variable in the rows, different letters indicate significant differences according to Student's t-test and Tukey's test ( $p \leq 0.05$ ). Each value represents the mean for  $n = 10$ . EM = Effective Microorganisms. UEB = Basic Business Unit

EM treatments had a highly significant effect on the final size of harvested fruit, likely due to better nutrient availability achieved through solubilization and enhanced accessibility when EM are applied (14). These results are attributed to plant development prior to induction, since a strong correlation has been established between plant mass and final fruit mass (19,20). Plant weights above 2.3 kg before induction (Figure 2) are associated with fruit masses close to 2.0 kg, as obtained in the EM treatments.

Crown mass tends to increase when plants suffer nitrogen deficiency, indicating that more energy is allocated to vegetative rather than reproductive development. In other words, more metabolic resources are invested in preparing the crown to become a future plant than in fruit development. This behavior was not observed in the results of this experiment.

Table 3 presents the chemical-physical indicators of the fruits according to treatments.

**Table 3.** Effect of treatments on the bromatological indicators of the fruits

	Contenido de sólidos solubles totales (°Brix)	Contenido de acidez (%)	Contenido de ácido ascórbico (mg 100 ml de jugo)
EM	14.23 (a)	0.79 (a)	79.61 (a)
Control	14.10 (a)	0.77 (a)	72.18 (a)

For each variable in the rows, different letters indicate significant differences according to Student's t-test and Tukey's test ( $P \leq 0.05$ ). Each value represents the mean for  $n = 10$ . EM = Effective Microorganisms; UEB = Basic Business Unit

The contents of soluble solids and acidity are within suitable values for commercialization, with all treatments showing more than 12 °Brix and above 0.74 % acidity. The ascorbic acid content shows acceptable values (22). It can be observed that for all variables analyzed, no significant differences were found among treatments.

It is recognized that bromatological variables are closely related to potassium supply, as well as to the micronutrient nutrition provided to the plant during the growth and subsequent fruiting phases (21). In this case, since no differences existed among the experimental treatments in terms of chemical fertilization applied, no differences were observed in the bromatological characteristics evaluated in the fruits (22).

Plant mass at the time of induction and fruit mass at harvest are referred to as the harvest index, which is correlated with specific environmental conditions. This harvest index shows a range of 0.5 under tropical conditions and 1.0 under cold conditions (19).

The results have shown that the EM treatment surpasses the Control in terms of vegetative development of the plants. In the first month after EM application, growth was not substantial. However, after this period, growth became notable.

## CONCLUSION

The foliar application of Effective Microorganisms (EM) increased plant survival and fresh mass. These increments induced a greater fresh mass of the fruit without affecting its organoleptic quality.

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