Evaluation of Biofertilizer Action in *Pennisetum purpureum*

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ABSTRACT

Research was done on El Renacer Farm, at the 26 de Julio CCS (cooperative of credits and services) in the municipality of Camagüey, to evaluate the effect of biofertilizers on *Pennisetum purpureum* using a randomized block design with five treatments and five repetitions (liquid humus + phosphorine, liquid humus + azotobacter, liquid humus + phosphorine, liquid humus + phosphorine + azotobacter + essential elements, and a control treatment). Both azotobacter and phosphorine were applied to a 2 kg/ha dose in all the combinations, 1.5 l/ha of liquid humus, and 2 l/ha of essential elements, in the rainy and dry seasons. Samples were taken from the soil, and chemical analysis was performed to water for irrigation, according to the current standards. Evaluation was made of the number of stems per sapling, plant height, stem thickness, and yields. The best yields in the dry (20 t/ha) and rainy (23.6 t/ha) seasons were achieved with the liquid humus + phosphorine + azotobacter + essential elements.

KEY WORDS:/ Fertilization, grass, biofertilizers, phosphorine, azotobacter, humus.

INTRODUCCION

Today, more than 60% of the world population lives in cities. This leads to excessive exploitation of natural resources and a skyrocketing increase in the demand of resources, in addition to an increase in atmospheric and water pollution, broader soil pollution, erosion, deforestation, and an alarming generation of hazardous solid residues.

Agriculture worldwide is a fundamental activity for human sustenance. Several factors have led to the deterioration of the already scarce resources, and increased difficulties to renovate them. The soil, as the basis of most resources and production is within a complex, heterogeneous, and

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fragile framework, evidencing high susceptibility to erosion and low natural fertility. This scenario has influenced crop yields, labor productivity, and the feasibility of sustainable productive systems. The recovery and maintenance of soil fertility on a sustainable basis, is a very relevant factor to the development of agricultural production worldwide (Rueda Puente *et al.*, 2015).

The goal of nutrient management strategies is to accomplish satisfactory production levels with efficiency, cost-effectiveness, and sustainability. Worldwide, there is consensus over the lack of long-term sustainability of chemical-based agriculture, and that the inclusion of organic fertilizers, green fertilizers, and biofertilizers will contribute to sustainable production of foods, soil biodiversity, and prevention of environmental pollution (Aguirre Medina *et al.*, 2009).

Biofertilizers are an alternative to replace mineral fertilizers totally or partially with a more viable option for many countries: plant-interacting bacteria. Today, biofertilizer development is more focused on the use of plant-growth promoting bacteria (Ferlini, 2008).

Cuban agriculture is today implementing radical and unavoidable changes, mainly assumed due to economic reasons, such as the lack of capital and imported inputs to foster development according to the paradigm of the green revolution. In other words, these changes have not been implemented to preserve the environment or use sustainable technologies based on scientific grounds; instead, they have been put into practice due to the need to produce foods from the available natural, material, and human resources. Several agronomical, social, and economic studies have demonstrated the existence of huge opportunities for large-scale development of sustainable agricultural systems that combine technical and economic feasibility, ecological sustainability, and social approval (Funes, 2009).

These issues should be addressed consciously (CITMA, 2010), considering the above, and knowing the conditions of many of the agricultural companies in terms of resources and human working conditions, in addition to the climate changes being operated. The latter is an undeniable phenomenon, which has been demonstrated by strong visual evidence, like the melting of glaciers, increased air temperature, distortions in the precipitation patterns, and the rise of sea level, which are global, regional, and local trends.

The scientific base of sustainable agriculture is the adequate use of local resources and synergy of processes in the agro-ecosystem, which favor local innovation and dialog among farmers. Sustainable agriculture is based on managing production systems (farms) through practices that encourage complexity (agro-forestry, forest-grazing, and multicropping), which embrace more efficient biological control and organic nutrition. (Vázquez & Funes, 2014).

The use of biofertilizers in agriculture allows for the obtainment of healthier products for human and animal consumption, contributes to improvements in the physical conditions of soils, and reduces the levels of mineral fertilization and other environmentally harmful chemicals. These are applied to seeds, soils, and leaves, creating the proper medium for balanced edaphic flora (Soil Institute, 2010).

The area suggested for this project has been affected by erosion, soil degradation, low forestation, irregular topographic conditions and a diversified agricultural population, which is suitable for the implementation of an integrated system of measures with visible and foreseeable impacts in the short, mid, and long run, along with its progressive increment. Another factor to consider is the structural strength and determination to assume this task. Accordingly, the aim of this work was to evaluate the effect of biofertilizers on *Pennisetum purpureum*, as part of integrated fertilization management.

MATERIALS AND METHODS

This study was conducted on the El Renacer Farm, at 26 de Julio CCS, on Circunvalación, south of the city of Camagüey, Cuba. The area is located on 21º 20´ 20" north latitude and 78º 54´ 00" west longitude, 100 meters above sea level, according to the cartographic chart of Camagüey, scale of 1:25 000. The average annual precipitation values are 1 390.9 mm, 79% relative humidity, mean annual temperature of 24.8 ºC, and average annual wind velocity of 12.0 km h^{-1}.

El Renacer is mainly engaged in livestock raising, in a 10.8 ha area, distributed as follows: 3.0 ha of grassland; 3.8 ha of forage; 1.0 ha of various crops; 1.0 ha of fruit trees; and 2.0 ha of infrastructure. The farm has 4 employees (two women and two men) whose educational level is 12 grade.

First, a diagnostic of the experimental area was made to determine the behavior of key elements like soil, water, and crops; then various combinations of biofertilizers were applied to *Pennisetum purpureum* cv. CT 169.

Crop tilling and phytosanitary care were performed according to the agronomical recommendations of Padilla & Ayala (2006).

Evaluation of biofertilizers on *Pennisetum purpureum* cv CT-169 was made in the rainy and dry seasons.

Before planting, 30 t/ha of cattle manure were spread on the experimental area. The biofertilizers were applied every 7 days, following the cuttings of reshoots in the foliar area (active leaves).

The planting distance was 0.90 m x 0.60 m. The bud scions measured 25-30 cm; each had 3-5 buds per stem. The seeds used were 90 days old,and they were sown 15-20 cm deep. Later, they were covered with a soil layer of 3-5 cm, using a hoe.

The establishment cutting was made 70 days after planting; trimming was performed every 45 and 35 days in the dry and rainy seasons, respectively. Overhead irrigation was applied according to the vegetative development; the commonly found local weeds, pests, and diseases were completely controlled.

A randomized block design with 5 treatments and 4 repetitions was conducted. In twenty 3 m^2 plots totaling 72 m^2, with 1 m^2 area for calculation (Table 1).

Table 1: Treatments

Treatments	Bioorganic alternatives	Dose
1	Control (C)	-
2	Liquid humus + phosphorine	1.5 l/ha + 2 kg/ha
3	liquid humus + azotobacter	1.5 l/ha + 2 kg/ha
4	Liquid humus + phosphorine + azotobacter	1.5 l/ha + 2 kg/ha + 2 kg/ha
5	Liquid humus + phosphorine + azotobacter + essential	1.5 l/ha + 2 kg/ha + 2 kg/ha +2

elements	l/ha

The response variables selected were,

- Stem thickness: A gauge caliper was used to measure the diameter of all the plants inside the 1 m^2 quadrant.
- Plant height: A ruler was used to measure the stem from the base to the top foliar area of all the plants inside the 1 m^2 quadrant.
- Number of stems per sapling: All the stems from the saplings within the 1 m^2 quadrant were counted.
- Yield: The crops harvested at 5-10 cm from the soil within the 1 m^2 quadrant in every plot, were weighed (kg) Yields were determined according to the dry matter through a 0.33 coefficient.
- Statistical analysis: SPSS, version 21.1, for Windows (2012) was performed; the multiple range test of Duncan (1955) was applied in cases of significant differences between the means ($p \leq 0.05$).
- An economic evaluation was conducted, which comprised indicators expenses and production value in terms of revenue generation in relation to the control.

RESULTS AND DISCUSSION

The experimental area had only one type of soil: brown without carbonates, and adequate overall physical, chemical, and morphological characteristics for forage production, native and introduced grass, crop vegetables, green vegetables, and beans, depending on the effective depth and proper crop rotation.

All the farm soil is productive (category II), according to the Soil Institute (1975), with potential yields of 50-70% for the crops evaluated.

A well on the farm supplies ground water, with a static level of 7.2 m, and a dynamic level of 12.0 m; the distance to the source is 100 m. The water pump meets the demands of a low-intensity stationary overhead irrigation system.

Table 2 shows the chemical characterization of water for irrigation. Based on analysis of water quality for irrigation, the local water has slight or moderate restrictions (mid category).

Table 2: Characterization of water for irrigation on El Renacer Farm

Source	pH (H_2O)	EC ms/cm	Ca^{2+} mmol/L	Mg^{2+} mmol/L	Na^{+1} mmol/L	K^{+1} mmol/L	HCO_3^- mmol/L	CO_3^{-2} mmol/L	Cl^- mmol/L
Well	7.03	0.95	1.72	3.45	3.5	0.04	3.75	0	5.0

Based on the Evaluation of Irrigation Water Standard and the properties of the soils (National Soil and Fertilizer Management, 1980), this water can be used for agricultural irrigation, provided the following requisites are met:

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Implementing programed irrigation

- Proper draining system.
- Subsoiling treatment (It depends on soil depth and if the water table does not have an influence on the surface).
- Soil leveling or depressing for planting.
- Application of organic matter in improvement doses.
- Implementation of irrigation at sunset or evening, preferably.

One of the reasons to conduct this study was the little use of biofertilizers, and to demonstrate their importance in crop nutrition, as one of the actions included in the plan for sustainable land management practices.

Table 3 shows the results of chemical tests to the farm's soil (mid-range), considering the statistical parameters of these soils (National Soil and Fertilizer Management, 1981). The analysis of assimilable phosphorus and potassium values showed an increasing trend, which may be caused by the high extraction and exploitation that crops make of these elements, which should be taken into account in relation to farm management. PH, content of organic matter, Ca, and Mg were favored by the application of compost, EC was within the permissible parameters.

Table 3: Chemical characterization of the soil

Depth. cm	pH (KCl)	P_2O_5	K_2O	MO	Ca++	Mg++	Na+	K+	CIC	Ca/Mg	C E
0-20 *	5.5	8.90	16.67	3.36	23.65	7.95	0.30	0.37	33.6	2.97	0.15
0-20 **	5.8	3.35	6.67	3.64	29.64	8.98	0.55	0.16	29.2	3.30	0.18

Note: * Initial sample collection, ** Final sample collection.

Table 4 shows that the number of stems per sapling of *Pennisetum purpureum* had the highest significant differences in the treatments based on liquid humus plus phosphorine and azotobacter, and the liquid humus and phosphorine plus azotobacter and essential elements, which were different from the other treatments, except for the control. This response may be caused by the assimilation of products with the highest nutritional composition. It favored plant growth, evidencing the effect of these auxin-rich stimulants, cytokines, and humic compounds capable of intervening in the physiological processes of plants.

Table 4: Number of stems per sapling

Treatments	Dry	Rainy
1- Control	13.2 e	19.3 e
2- LH + Ph	14.3 d	20.8 d
3- LH + A	17.3 c	22.2 c
4- LH + Ph + A	21.5 b	25.0 b
5- LH + Ph + A + EE	23.7 a	26.8 a
Es_x	0.40	0.23

Note: Values with unequal letters in the same column differ from p≤0.05 (Duncan, 1955).

LH (liquid humus), Ph (phosphorine), A (azotobacter), EE (essential elements).

Table 5 shows the significant differences observed in the height of *Pennisetum purpureum* using the treatment consisting of liquid humus plus phosphorine and *azotobacter*, and the liquid humus and phosphorine plus *azotobacter and* essential elements, which were different from the other treatments, except for the control. This response may be caused by the assimilation of products with the highest nutritional composition. It favored plant growth, evidencing the effect of these auxin-rich stimulants, cytokines, and humic compounds capable of intervening in the physiological processes of plants.

This behavior in foliar development was produced due to the contribution of amino acids, which are not only a nutrient, but according to Simbaña (2011), are a regulating growth factor due to its fast absorption and transportation throughout the aerial parts of the plant. Moreover, they are easily metabolized, play a nutritional role, and have a growth regulating and catalyzing function, influencing on key enzymatic mechanisms, as well as in flower pollination improvements, resistance to water stress and droughts, and as carriers of microelements.

Table 5: Plant height (cm)

Treatments	Dry	Rainy
1.- Control	103.0 e	111.6 e
2.- LH + Ph	113.5 c	113.9d
3.- LH + A	114.9c	116.4c
4.- LH + Ph + A	126.5 b	129.0 b
5.- LH + Ph + A + EE	132.1 a	135.3 a
Es_x	0.49	0.55

Note: Values with unequal letters in the same column differ from p≤0.05 (Duncan, 1955).
LH (liquid humus), Ph (phosphorine), A (azotobacter), EE (essential elements).

Variable behavior of the species evaluated in this paper corroborated the reports made by several authors, such as Abdel Rahman, and Seidhom & Leopold (2010), who referred to selection of species as a strategy to minimize the effects of water absence and other limiting factors on plant growth. As a result, plants may develop a broad range tolerance mechanisms (metabolic, physiological through morphological ones), which will allow plants adapt to a wide variety of edaphoclimatic and management conditions.

Table 6 shows stem thickness. A significant difference is observed in the liquid humus plus phosphorine and *azotobacter* and liquid humus plus phosphorine and *azotobacter* plus essential elements. These were the most significant ones without differences between themselves, except for the rest of the treatments. This behavior may be due to the fact that the concentration of different organic and chemical compounds in small doses cause an increase in the photosynthetic capacity of the plant, tissue development, and cell multiplication; thus significantly increasing stem thickness in the periods evaluated.

Table 6: Stem thickness (cm)

Treatments	Dry	Rainy
1.- Control	1.73 d	1.80 d
2.- LH + Ph	2.22 c	2.62 c
3.- LH + A	2.45 b	2.65 b
4.- LH + Ph + A	2.60 b	2.65 b
5.- LH + Ph + A + EE	2.74 a	2.87 a
Es_x	0.69	0.47

Note: Values with unequal letters in the same column differ from p≤0.05 (Duncan, 1955).
LH (liquid humus), Ph (phosphorine), A (azotobacter), EE (essential elements).

These results corroborate the reports made by López & Montejo (2012), who referred to the stimulating capacity of these bioproducts, which are rich in hormones, phytohormones, humic acids, auxins, cytokinins, and minerals that enhance crop yields. These products are easily absorbed through foliar fertilization, which uses the nutrient entry mechanisms of the stomas. Then the nutritional substances are taken through the xylem and phloem, allowing the development of important physiological processes like photosynthesis and breathing. It also facilitates energy catalytic functions that can increase yields.

The results observed in the different treatments in relation to yields of dry matter per hectare are represented in table 7. The best treatment was No. 5 in either season, with 20.0 and 23.6 t/ha in terms of dry matter, respectively. No significant differences were observed between the treatments in relation to 4 and 5 in the rainy season, though there were differences in the remaining treatments for the two seasons.

Table 7: Yields of dry matter (t/ha)

Treatments	Dry	Rainy
1.- Control	14.3 d	18.5 d
2.- LH + Ph	15.1 d	20.4 c
3.- LH + A	16.2 c	21.8 b
4.- LH + Ph + A	18.0 b	23.1 a
5.- LH + Ph + A + EE	20.0 a	23.6 a
Es_x	0.26	0.36

Note: Values with unequal letters in the same column differ from p≤0.05 (Duncan, 1955).
LH (liquid humus), Ph (phosphorine), A (azotobacter), EE (essential elements).

The results were similar to the ones reported by López & Montejo (2012) with the application of good ecological practices, and the inclusion of organic enhancers. Crop yields were 30% higher in pastures, beans, crop vegetables, green vegetables, and fruit.

In that sense, Troetsch, & Santamaría, (2009) commented that *Pennisetum purpureum* had increased the yields of dry matter, of which 32% corresponds to leaves. The dry matter of the plant reached 20%, whereas the dry matter from leaves and stems may be higher or lower, depending on plant development and management practices.

Most research aimed to improve plant response, has relied on the use of native bacteria capable of fixing nitrogen in grains and forages. Under certain circumstances, the amount of nitrogen fixed by these microorganisms may be significant. Several trials have demonstrated that biofertilizers produce highly significant root enlargement during the initial stages of plants (Ramírez Elias *et al.*, 2014), which may lead to a better response during grass establishment, as has been shown before.

Table 8 shows the economic benefits after assessment, considering the costs of each treatment. The highest revenues were reported in Treatments 4 and 5, in relation to the other treatments, which shows the effectiveness of these products on crop nutrition and production, linked to farm sustainability.

Table 8: Economy effect

Indicators	T1	T2	T3	T4	T5
Liquid humus	-	30.00	30.00	30.00	30.00
Phosphorine	-	46.4	46.4	46.4	46.4
Azotobacter	-	-	50.4	50.4	50.4
Essential elements	-	-	-	-	12.48
Soil preparation	300.00	300.00	300.00	300.00	300.00
Agamic seeds	300.00	300.00	300.00	300.00	300.00
Color insect traps	32.0	32.0	32.0	32.0	32.0
Labor force	254.00	254.00	254.00	254.00	254.00
Other expenses	104.80	137.81	138.21	142.81	144.06
Total expenses	990.00	1 099.85	1 104.25	1 154.89	1 168.56
Production value	5 184.48	5 357.29	5 616.52	5 875.74	6 048.56
Revenue	4 194.48	4 257.41	4 512.27	**4 720.85**	**4 880.04**

CONCLUSIONS

The use of biofertilizers in *Pennisetum purpureum* is a viable alternative. The treatment based on liquid humus plus phosphorine, azotobacter, and essential elements was the most effective, with the highest yield increases and positive economic effects for the farm.

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