Scientific Paper

Effect of the inoculation of beneficial microorganisms and Quitomax® on *Cenchrus ciliaris* L., under conditions of agricultural drought

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Abstract

A field trial was conducted, in order to evaluate the effect of the inoculation of two biofertilizers and a biostimulant on agroproductive variables of buffel grass (*Cenchrus ciliaris* L.), under conditions of agricultural drought. For such purpose, the isolate Ho5 (*Bradyrhizobium* sp.), EcoMic® (*Funneliformis mosseae*) and Quitomax® were used. The experimental design was randomized blocks, with nine treatments and eight replicas, and an ANOVA was performed. The differences among means were found through Fisher's LSD. The following variables were evaluated: aerial part dry weight, stem length, spike length, spike dry weight, inflorescence and inoculation efficiency index (IEI). The best treatments in most of the studied variables were: EcoMic®+Quitomax®+Ho5, Ho5+EcoMic® and Ho5+Quitomax®, for showing higher results than the absolute control and other inoculated treatments. With the combination of EcoMic®+Quitomax®+Ho5, there was a high percentage of inflorescence (71 %); which suggests that the inclusion of Quitomax® in such combination should have influenced this variable effectively. It is concluded that the combination of the biofertilizers with Quitomax®, in general, showed a higher effect than the control; although EcoMic®+Quitomax®+Ho5 stood out, due to its superiority with regards to other treatments. On the other hand, the application of each biofertilizer, or of Quitomax®, alone, did not show higher results than the absolute control in most of the variables. To evaluate treatments in which these biopreparations were combined in different pasture grasses and different soil types, as well as the long-term effect of these biopreparations on the plant, is recommended.

Keywords: organic fertilizers, *Bradyrhizobium*, drought.

Introduction

The integrated management of nutrition is among the technologies that increase the yields and nutritional value of biomass in pastures and forages, and in turn contribute to preserve natural resources, because of its potentialities to increase productivity and biomass quality, improve soil fertility and make a rational use of fertilizers (Lambrecht *et al.*, 2016). Nevertheless, the hydric stress caused by drought is the main limiting abiotic factor in crop production (Nakashima and Yamaguchi-Shinozaki, 2013).

One of the options to guarantee acceptable yields in the pasture during the dry season, is the utilization of resistant species to that environmental stress, such as *Cenchrus ciliaris* L. (buffel), which is a pasture grass that is extensively cultivated in arid and semiarid ecosystems in several countries, and is used to increase the productivity of grasslands which have been affected by drought, in addition to overgrazing (Lyons *et al.*, 2013).

On the other hand, soil microorganisms contribute with a wide range of essential services to the sustainability of all ecosystems. They act as the main promoting agents of the nutrient cycle and improve efficiency of nutrient absorption by the plants, among other advantages (Singh *et al.*, 2011).

According to Glick (2016), plant growth promoting bacteria (PGPB) not only promote plant growth directly, but also protect them against a wide range of abiotic stresses, which include drought. Rhizobia, in association with plants, can cause physiological changes that allow to optimize their tolerance to abiotic stresses (Hussain *et al.*, 2014a). Bécquer *et al.* (2017a) obtained promising results with the application of *Bradyrhizobium* sp. and *Trichoderma* in buffel grass, under drought stress conditions.

In turn, González-Cañizares *et al.* (2015) proved that the inoculation of efficient arbuscular mycorrhizal fungi (AMF) species can decrease the fertilizer doses to be applied on pastures, without reducing their yield or their nutritional value. In addition, AMFs form arbuscular structures and large hyphal networks that can transport phosphorus (P) and other nutrients, in order to relieve drought stress in crops (Dick, 2012).

On the other hand, diverse studies suggest the use of biostimulants as substitutes of chemical-origin products, given the beneficial effects they exert on plants (Malerba and Cerana, 2016). According to Falcón-Rodríguez *et al.* (2015), the development of products based on oligosaccharins has potential for their introduction in Cuban and international agriculture, as an alternative of protection against environmental stress, among other advantages.

Due to the above-explained facts, the objective of the work was to evaluate the effect of the inoculation of two biofertilizers elaborated with beneficial microorganisms, and a biostimulant, on the performance of agroproductive variables of *Cenchrus ciliaris* L. (buffel grass), under conditions of agricultural drought.

Materials and Methods

Location of the experiment. The trial was conducted from March to May, 2017, in an experimental plot belonging to the Pastures and Forages Research Station of Sancti Spíritus, located at 21° 53' 00'' North latitude and 79° 21' 25'' West longitude, at an altitude of 40 m.a.s.l.

Plant material. Cenchrus ciliaris L. cv. Formidable (buffel) plants were evaluated, from a germplasm bank of the Research Station Sancti Spíritus.

Rhizobium isolate, preparation of the inoculant and inoculation. The isolate Ho5, belonging to the genus *Bradyrhizobium* sp. (Bécquer *et al.*, 2016), was applied. Such isolate grew on solid yeast-mannitol medium, and was resuspended in liquid medium until achieving a cell concentration of 10^6–10^8 CFU/mL. For inoculating the plants, the inoculant was diluted in a 1:10 ratio in tap water. The inoculation was done after the establishment cutting, with an inoculant that had a cell concentration of 10^7–10^8 CFU/mL. A 5-L backpack sprayer was used, whose content was sprayed on the newly-cut plants, so that when the spray was regulated each plant received approximately 120 mL of the liquid inoculant.

Arbuscular mycorrhizal fungal strain and inoculation. The product EcoMic®, elaborated from *Funneliformis mosseae*, supplied by the National Institute of Agricultural Sciences (INCA, for its initials in Spanish), was used. The inoculation was made at a concentration of 30 spores/g, which was applied on the newly-cut plants, without being mixed with water, at a rate of 15,7 g/plant, with a dose equivalent to 157,0 kg/ha. This dose, which is higher than the one applied in practice (50 g/ha), was used to guarantee the survival of the highest number of mycorrhizal spores in the soil, as the trial was conducted under hydric stress conditions. This treatment received 960 mL of water (120 mL/plant), after inoculation, to homogenize with the other treatments the hydric conditions of the plants at the moment of inoculation.

Preparation and application of the Quitomax® solution. A stock solution of 1 % Quitomax® was prepared, which was diluted in distilled water until obtaining a concentration of 0,1 g/L (Terry-Alfonso *et al.*, 2017). The application of this product was made with a 5-L backpack sprayer, at a rate of 205 mL (200 mg/ha). Each plant was uniformly sprayed and 755 mL of water were applied later. Water was applied to the combined treatments in sufficient quantity to homogenize the hydric conditions of the plants at the moment of inoculation.

Plant management. The experiment was conducted in a previously established area, aimed at the production of buffel grass seeds. An establishment cutting was done in the plots before inoculation, and no irrigation was applied during the experimental period. Harvest took place three months after the cutting.

Evaluation of the climate variables

The collection of temperature, rainfall, relative humidity and wind data, as well as their analysis in order to determine the agricultural drought status, was carried out by the Meteorological Station Sancti Spíritus (CMP, 2017).

The rainfall in the study zone was very scarce during the 3 months of analysis (table 1). In March no rainfall was reported, while in the other months it was very low. The accumulated rainfall of April stands out, which was 45,6 mm, below the historical average.

In correspondence with the positive anomalies of the air temperature, in the period from March to May, 2017, relative humidity values below the historical averages were recorded, which, along with the rainfall scarcity, typical of the season, must have generated higher hydric stress in the crops.

Table 1. Rainfall and relative humidity of the study zone.

Variable	March, 2017	April, 2017	May, 2017
Historical monthly rainfall, mm	38,2	51,8	253,4
Real accumulated rainfall, mm	0	44,6	81,6
Average relative humidity per month, %	70,0	70,0	69,3
Average relative humidity, historical values, %	76,0	74,7	76,0

Source: Meteorological Station Sancti Spíritus (CMP, 2017) Cuba

Determination of the agricultural drought status

The agricultural drought status was determined through the Aridity index or Agricultural Drought index (IE) (Solano *et al.*, 2004), which was used to test whether the trial was conducted under hydric stress conditions:

IE = ETR / ETP

where:

ETR – Real estimated evapotranspiration, dependent on the soil humidity status; ETP – Estimated potential evapotranspiration, dependent on the atmospheric conditions. When ETR = ETP, the water supply is adequate. When ETR<ETP, there is water insufficiency.

Basic agrochemical composition of the soil

The soil of the experimental area was identified as carbonated loose Brown, according to Hernández-Jiménez *et al.* (2015). It showed a macronutrient content very low in phosphorus and potassium, just like organic matter (table 2).

Treatments and experimental design. A randomized block experimental design was used, with nine treatments, three replicas and eight repetitions per replica (table 3).

Evaluated variables

Aerial part dry weight (APDW, g/m^2), stem length (SL, cm), spike length (EL, cm), spike dry weight (EDW, mg), inflorescence (Infl., %). In the sampling to determine the APDW, a 0,25-m^2 frame was used.

The inoculation efficiency index (IEI, %) was determined, according to the formula proposed by Santillana *et al.* (2012): IEI: [(Inoculated treatment – Absolute control)/Absolute control] x 100.

This index, in spite of being conceived for biofertilizers, was also applied to Quitomax® in this experiment, under the assumption of constituting a bioactive product, with which several treatments were inoculated, alone or combined with microorganisms.

Table 2. Basic characteristics of the soil of the experimental site.

Soil type	P_2O_5, mg/100g	K_2O, mg/100g	OM,%	pH (KCl)
Carbonated loose Brown	2,63	6,0	1,51	5,9

Table 3. Experimental treatments.

No.	Treatment
1	EcoMic®
2	Quitomax®
3	Ho5
4	Ho5+EcoMic®
5	Ho5+Quitomax®
6	EcoMic®+Quitomax®
7	EcoMic®+Quitomax®+Ho5
8	Absolute control
9	Fertilized control

Statistical analysis. For the statistical processing of the data an ANOVA was performed, after testing normality through the Kolmogorov-Smirnov test and variance homogeneity by Levene's test. The percentage data were transformed by *arcsin*√x to guarantee the fulfillment of the assumptions. The differences among means were determined through Fisher's LSD. The statistical program StatGraphics Centurion XV was used.

Results and Discussion

Status of agricultural drought. Table 4 shows the values of the soil humidity content (SHC), at the beginning and the end of each month, as well as the estimated values of potential evapotranspiration (ETP) and real evapotranspiration (EPR), runoff and category of agricultural drought (IE), for the Pastures and Forages Research Station, based on the data of the pluviometer placed in the periphery of the area under study. It was observed that the humidity loss of the soil was stressed in April and decreased in May. The ETR of the crops was poor, as response to the hydric stress to which they were subject.

March in the study area ended with a Very Critical IE, it was Insufficient in April, and was stressed in May, reaching the category of C (Critical). Under these conditions the crop is not capable to survive for a long time, unless it is drought resistant or it is under irrigation, for which it is inferred that the crop was subject to high hydric stress (Solano *et al.*, 2004).

Stem length. The SL (table 5) showed that the fertilized control showed higher values with regards to the absolute control and to the treatments inoculated with EcoMic®, Ho5 and EcoMic®+Quitomax®. Nevertheless, EcoMic®+Quitomax®+Ho5 equaled its values to those of the fertilized control, to Quitomax®, Ho5+ EcoMic® and was higher than the absolute control, as well as Ho5, Ho5+Quitomax® y EcoMic®+Quitomax®.

From this result it is inferred that there was a synergic effect between the biofertilizers that were applied and Quitomax®, where the isolate Ho5, due to is characteristic as catalase producer (Bécquer *et*

Table 4. Main indicators of the water balance for the crops, and category of agricultural drought (IE).

Month	SHC Initial, %	SHC Final, %	ETP	ETR	IE
March, 2017	12,4	6,9	5,99	0,11	VC
April, 2017	6,9	8,2	4,86	2,21	I
May, 2017	8,2	38,5	16,4	11,2	C

VC- Very Critical I- Insufficient C- Critical

SHC: Soil humidity content, ETP: Potential evapotranspiration, EPR: Real evapotranspiration and IE: Category of agricultural drought

Table 5. Stem and spike length.

Treatment	SL, cm	EL, cm
EcoMic®	43,2 ^{bcd}	7,0 ^{bc}
Quitomax®	52,8 ^{abcd}	6,2 ^{c}
Ho5	38,2 ^{d}	6,6 ^{bc}
Ho5+EcoMic ®	53,0 ^{abc}	7,5 ^{ab}
Ho5+Quitomax®	39,9 ^{cd}	7,8 ^{ab}
EcoMic ®+Quitomax®	37,8 ^{de}	7,0 ^{bc}
EcoMic ®+Quitomax®+Ho5	55,3 ^{ab}	7,7 ^{ab}
Absolute control	39,7 ^{cd}	6,4 ^{bc}
Fertilized control	65,3 ^{a}	8,5 ^{a}
Standard error ±	5,05	0,45

Different letters in the same row differ at p<0,05 *al*., 2017a), influenced favorably plant tolerance to hydric stress. According to Hussain *et al.* (2014b), the capacity of rhizobia to produce catalase and exopolysaccharides constitutes not only a drought tolerance factor, but a reliable attribute to select efficient isolates to be applied to crops. In addition, inoculated AMFs have sufficient potential to increase the efficiency of nutrient absorption by plants (Yang *et al*., 2014).

Although higher results than the absolute control were observed from the combined treatment with the three biopreparations, when applying Quitomax® alone, similar values to the absolute control and also to the fertilized control, as well as to the other inoculated treatments, with or without Quitomax®, were obtained. In spite of these contradictory values, the beneficial effect this biostimulant could have had on such variable is not discarded, because there are antecedents in other crops, such as the ones reported by Terry-Alfonso *et al.* (2017), about the superiority of Quitomax® in the growth of the tomato stem, when different doses were applied to it and its effectiveness on the stem length in potato (Morales-Guevara *et al*., 2015), as well as this last variable in beans (Morales-Guevara *et al*., 2016). Nevertheless, future essays must be conducted in pasture grasses to further study this aspect.

Spike length. Table 5 shows that EcoMic®+Quitomax®+Ho5, Ho5+ EcoMic® and Ho5+Quitomax®, were higher than the application of Quitomax® alone, and likewise, they shared common superscripts with the fertilized treatment, absolute control, and the other inoculated treatments. These results indicate a discreet influence of the combination of these two biofertilizers and Quitomax® on spike formation.

However, Quitomax® did not show any effectiveness on spike length, when applied alone, which contradicts the report by Chibu *et al.* (2002), regarding the fact that the quitosane polymer, as well as its lower-size derivatives, are considered plant growth and development regulator, by stimulating root and vegetative growth of several species.

Aerial part dry weight (APDW), and inoculation efficiency index based on the APDW (IEIAPDW). Table 6 shows that the fertilized treatments showed higher values than the other treatments, while Ho5, Ho5+EcoMic®, Ho5+Quitomax®, EcoMic®+Quitomax® and EcoMic®+Quitomax®+Ho5 were higher than the absolute control; but in turn shared common superscripts with EcoMic® and Quitomax®. In the IEIAPDW the higher efficiency of the inoculation was observed in Ho5+EcoMic®, Ho5+Quitomax® and EcoMic®+Quitomax®, where the last one was higher than the others (43,3 %).

The lower results that were obtained in the absolute control are in agreement with the results referred by Emami-Bistgani *et al.* (2017), regarding that the decrease of dry matter is not a surprising response to drought stress in plants.

In the APDW, the higher effect of the treatments in which the AMF strain, the isolate Ho5 and Quitomax® were combined in different ways, was observed, when comparing them with the absolute control, although it was the only variable where the simple inoculation of Ho5 also exerted a higher effect than the control. This same isolate previously showed high efficiency, when being applied to corn (Bécquer *et al*., 2017b) and to Bermuda grass Tifton 85 (Bécquer *et al*., 2018), under conditions of agricultural drought.

There are interrelations among microorganisms in ecosystems, and their multifunctionality in the agricultural systems is expressed according to biotic factors, as well as to the edaphoclimatic factors (Salinas-Ventura and Soriano-Bernilla, 2014). The possible higher effect of the isolate Ho5 which, like many rhizobacteria, has the potential of inhibiting ethylene production in plants during drought stress (Ali *et al*., 2014), is not obviated; it allows the root system to be developed without the typical inhibition of this compound and propitiates higher nutrient absorption by the plant. Although these results also suggest that AMFs, in association with the other biopreparations, favored to a certain extent a better hydric status of the host plants. Díaz-Franco and Garza-Cano (2006) found that the inoculation with *Glomus intraradices* increased the production of biomass and other agroproductive variables in three buffel grass genotypes.

Spike dry weight (EDW) and inoculation efficiency index based on the EDW (IEIEDW). Table 6 shows that the fertilized control was only higher than the absolute control, Quitomax® and Ho5; while it showed similar values to the other inoculated treatments. However, EcoMic®+Quitomax®+Ho5 showed similar values to those of the fertilized control, EcoMic®+Quitomax®, Ho5+Quitomax®, Ho5+EcoMic® and EcoMic®. Nevertheless, it was higher than the absolute control, Ho5, and Quitomax®. The combination of biofertilizers and Quitomax®, just like EcoMic®, were the only inoculated treatments that turned out to be higher than the

Table 6. Values of aerial part dry weight, spike dry weight and inoculation efficiency indexes.

Treatment	APDW, g/m^2	IEIAPDW, %	EDW, mg	IEIEDW, %
EcoMic®	61,6 ^{bc}	26,8	170,2 ^{ab}	77,1
Quitomax®	61,7 ^{bc}	25,4	85,4 ^{d}	-11,1
Ho5	64,6 ^{b}	31,3	99,03 ^{cd}	3,05
Ho5+ EcoMic ®	68,6 ^{b}	39,4	136,9 ^{abc}	42,5
Ho5+Quitomax®	69,2 ^{b}	40,7	162,6 ^{abc}	69,2
EcoMic ®+Quitomax®	70,5 ^{b}	43,3	126,1 ^{abc}	31,2
EcoMic ®+Quitomax®+Ho5	66,8 ^{b}	35,8	198,8 ^{a}	106,9
Absolute control	49,2 ^{c}		96,1 ^{cd}	
Fertilized control	93,2 ^{a}		187,6 ^{a}	
Standard error	4,71***		25,4*	

APDW: Aerial part dry weight, EDW: spike dry weight, IEIAPDW: Inoculation efficiency index based on the APDW: IEIEDW: Inoculation efficiency index of the EDW.
Different letters in the same column differ for p<0,0001 and p<0,05

absolute control. This result proved the efficiency of inoculation, with high values in EcoMic®, Ho5+Quitomax® and EcoMic®+Quitomax®+Ho5.

In these last two treatments, the isolate Ho5 was present, which comes from arid ecosystems of the Holguín province, Cuba (Bécquer *et al*., 2016). According to Timmusk *et al.* (2014), the isolation of plant growth promoting bacteria in stressing ecosystems, such as arid or desert ecosystems, can provide inoculants that stimulate crop development in environments derived from climate change, which coincides with the stressing environmental conditions present in this experiment. However, this isolate, when applied alone, was not effective for spike growth, which indicates that its usefulness for such variable was due to a synergic interaction with EcoMic® and Quitomax®.

On the other hand, as a higher effect on the isolate application of EcoMic® was observed, when compared with that of Quitomax® and Ho5, it is logical to think that the highest bearing in the combined treatment fell mainly on EcoMic®. It is known that AMFs increase absorption and translocation of nutrients, from the morphological and physiological modifications produced in the roots of the host plant, which increase contact surface with the soil and their capacity to have access to those elements that are less available for plants (Kavanová *et al*., 2006).

Inflorescence. The results shown in table 7 indicate that the fertilized control was higher than all the treatments. However, Ho5+EcoMic®, as well as EcoMic®+Quitomax®+Ho5, showed higher values than the absolute control, but only the latter combination was higher than the other inoculated treatments.

With it high inflorescence percentage was observed, which suggests that the inclusion of Quitomax® influenced effectively this variable, because, according to Ohta *et al.* (2004), this product can reduce the flowering period and improve plant flowering and fructification. Nevertheless, it was observed that the application of Quitomax® alone was not sufficient to obtain higher results, which was also observed in some of the above-mentioned variables. Terry-Alfonso *et al.* (2017) also obtained lower values in the tomato inflorescence when applying 0,1 g/L of Quitomax®, equal concentration as the one used in this experiment.

In recent years a synergic effect of this biostimulant with biological nitrogen-fixing microorganisms that are used as biofertilizers of several crops was proven (Corbera-Gorotiza and Nápoles-García, 2013), which could explain the higher results of the combination of biofertilizers which were used in the trial with Quitomax®.

It is concluded that the combination of the biofertilizers with Quitomax®, in general, showed a higher effect than the control, although EcoMic®+Quitomax®+Ho5 stood out, due to its superiority with regards to other treatments. On the other hand, the application of each biofertilizer, or Quitomax®, alone, did not show higher results than the absolute control in most variables. To evaluate the treatments in which these biopreparations were combined in different pasture grasses and on dif-

Table 7. Inflorescence percentage.

	Transformed data arcsin√P	Original data, %
EcoMic®	0,71 ᵈ	20,0
Quitomax®	0,65 ᵈ	21,0
Ho5	0,59 ᵈ	15,8
Ho5+ EcoMic®	1,36 ᶜ	39,0
Ho5+Quitomax®	0,45 ᵈ	13,0
EcoMic®+Quitomax®	0,42 ᵈ	12,2
EcoMic®+Quitomax®+Ho5	2,25 ᵇ	71,0
Absolute control	0,50 ᵈ	14,6
Fertilized control	2,99 ᵃ	95,0
Standard error ±	0,22	
Significance	p<0,0001	

ferent soil types, as well as the long-term effect of such biopreparations on the plant, is recommended.

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