

INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGI (*Rhizoglyphus intraradices*) AND PLANT GROWTH STIMULATORS IN *Pennisetum purpureum* SCH. CV. CUBA CT-115

Influencia de hongos micorrízicos arbusculares (*Rhizoglyphus intraradices*) y un estimulador del crecimiento vegetal en *Pennisetum purpureum* Sch. cv. Cuba CT-115

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ABSTRACT. With the aimed to evaluate the arbuscular mycorrhizal fungi (AMF) (*R. intraradices*) and FitoMas-E[®] effect in production of biomass *Pennisetum purpureum* Sch. cv. Cuba CT-115, this study was carried R. intraradices strain was reproduced in arbuscular mycorrhizal laboratory and plant growth stimulator FitoMas-E[®] from Research Institute derivatives Sugarcane was used. Five treatments were used: T1 (Control), T2 [Production (50 kg ha⁻¹ of urea)], T3 (*R. intraradices*), T4 (FitoMas-E[®]) and T5 (*R. intraradices* + FitoMas-E[®] + 25 kg ha⁻¹ of urea). A design randomized block with five replications was used. Fungal indicators, leaf contents of N, P and K, fresh and dry biomasses and yield in dry biomass were evaluated. Results showed significant differences between treatments in studies. It was found that treatment with *R. intraradices* and FitoMas-E[®] + 25 kg ha⁻¹ of urea reached values of 5,7 t ha⁻¹. With these products application, biomass production can be increased.

RESUMEN. Este trabajo tuvo como objetivo evaluar el efecto de hongos micorrízicos arbusculares (HMA) (*R. intraradices*) y FitoMas-E[®] en la producción de biomasa de *Pennisetum purpureum* Sch. cv. Cuba CT-115. Se utilizó la cepa eficiente *R. intraradices* se reprodujo en el laboratorio de micorrizas arbusculares del INCA y el estimulador del crecimiento vegetal FitoMas-E[®] proveniente del Instituto de Investigaciones de Derivados de la Caña de Azúcar. Se utilizaron cinco tratamientos: T1 (Testigo Absoluto), T2 [Testigo de Producción (50 kg. ha⁻¹ de urea)], T3 (*R. intraradices*), T4 (FitoMas-E[®]) y T5 (*R. intraradices* + FitoMas-E[®] + 25 kg ha⁻¹ de urea). Se utilizó un diseño de bloques al azar con cinco repeticiones. Se evaluaron indicadores de funcionamiento fúngico, contenidos foliares de N, P y K así como el rendimiento en masa seca. Los resultados mostraron diferencias significativas entre los tratamientos en estudio. Se encontró que el tratamiento con *R. intraradices* y FitoMas-E[®] + 25 kg ha⁻¹ de urea alcanzó valores de 5,7 t ha⁻¹ MS en un corte. Con la aplicación de estos productos se incrementa la producción de biomasa.

Key words: mycorrhiza, grass forage, yield, stimulator

Palabras clave: estimulador, gramínea forrajera, micorriza, rendimiento

INTRODUCTION

One of the problems facing current Cuban livestock is a decrease in the fertility of their soils, due to the loss of organic matter, phosphorus and potassium (1, 2);

Therefore, it is necessary to search for alternatives to increase productivity in these agroecosystems (3, 4).

Pennisetum purpureum, is a grass with favorable characteristics for its use as forage, due to its adequate height, wide and long leaves, acceptable yield and chemical composition (5, 6). In addition, due to its rusticity and plasticity, it adapts to a great diversity of soils (including those of low fertility), as well as adverse climatic conditions (high temperatures and low rainfall) (7).

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The use of microbial inoculants, specifically arbuscular mycorrhizal fungi (AMF); and plant growth promoters should be considered in the design of any agricultural production system; since, besides being inseparable components of agroecosystems, they perform several important functions in their association with plants (8, 9).

Arbuscular mycorrhizal fungi (AMF) establish symbiosis with most of the higher plants. Among its main functions are: to favor the absorption surface of the plants as well as to increase the uptake of mineral nutrients (10), improve the tolerance of plants against biotic (pathogenic) and abiotic stress (salinity, drought) (11, 12), therefore play a fundamental role in the remediation of areas contaminated with heavy metals (13).

On the other hand, FitoMas-E[®], anti-stress product with natural substances of the plant metabolism, is known to stimulate the growth and development of plants, from germination to fructification, reducing damages by salinity, drought, excess humidity and phytotoxicity^A. Taking into account this background, the objective of this research was to evaluate the influence of the AMF strain *Rhizoglyphus intraradices* and FitoMas-E[®] on the biomass production of *Pennisetum purpureum* cv. Cuba CT-115.

MATERIALS AND METHODS

General experimental conditions

The study was carried out in the “La Unión” farm of the Genetic Livestock Company Valle del Perú, located in San José de las Lajas, Mayabeque province, in a Brown Ochril Carbonated soil (14) and some of its properties are shown in Table I.

A random block design with five replicates was used. The preparation of soil consisted of plowing and crossing, with alternating passes of average grade of 4 500 kg. The plots were carried out with animal traction; each consisting of eight rows,

an area of 6x5 m² with a separation of one meter between parcels. Stakes (20-25 cm) of forage grass *Pennisetum purpureum* Schumach cv. Cuba CT-115. The planting was done by cutting to a depth 20 cm and irrigation was applied during the first week to guarantee the soil moisture. Five treatments were used: T1 (Absolute control), T2 (Mineral fertilizer (50 kg ha⁻¹ urea)), T3 (*R. intraradices*), T4 (FitoMas-E[®]) and T5 (*R. intraradices* + FitoMas-E[®] + 25 kg ha⁻¹ of urea). The plantation was carried out in May (2012 and 2013), coinciding with the rainy season and the control of the weeds was done manually.

The fungal species *Rhizoglyphus intraradices* (N.C. Schenck & G.S. Sm.), (INCAM-11) was used, which was reproduced in the Arbuscular mycorrhizal laboratory of the National Institute of Agricultural Sciences (INCA). Its inoculation was done by the technique of coating the seeds at the time of planting (17). Certified inoculum was used with a purity of 20 spores g of soil-1 on average.

The FitoMas-E[®], from the Cuban Sugar Cane Derivatives Research Institute (ICIDCA, according to its acronyms in Spanish), was applied at 15 and 30 days after planting at a dose of 2 mL L⁻¹ with a manual backpack of 16 liters of capacity.

DETERMINATIONS MADE

Cultivation sampling was carried out at 45 and 90 days after planting (DAP) in the early hours of the morning. 10 frames of 1 m² were distributed at random, which constituted the experimental unit. The edge effect was taken into account and the cutting was performed with a machete at a height of 5 cm.

^A Montano, R. *FitoMas-E[®], bionutriente derivado de la industria azucarera. Composición, mecanismo de acción y evidencia experimental*. Ed. Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar (ICDCA), 2008, La Habana, 35 p.

Table I. Chemical characteristics of the soil corresponding to the experimental area at depth of 0-20 cm

Variables	pH	OM (%)	P (mg*kg ⁻¹)	Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	Sum of bases	Spores AMF/50g
	7,2	1,02	256,43	10,26	3,7	0,42	0,09	14,47	12

Chemical determinations: pH to H₂O, Potentiometer; Organic matter (OM), Walkley Black (15); Phosphorus (P), Oniani; Cations, Ca²⁺, Mg²⁺, Na⁺ and K⁺, Maslova's method; Spores of AMF, Gerdemann and Nicholson (16)

FUNGAL PARAMETERS

In order to estimate the frequency and colonization intensity (CI), samples of the roots of the culture were washed with plenty of water and placed in an oven at 70 °C until constant weight was obtained. Subsequently they were stained according to the methodology described by Phillips and Hayman (18). For the calculation of the indicators, the methodology was used to evaluate the fungal occupation of each intercept (19).

Foliar nutrient concentration: a sample of 250 g was taken and the contents of nitrogen (N), phosphorus (P) and potassium (K) foliar (90 DAP) (20) were determined. **Yield:** dry mass percentage (DM) was determined according to the following formula:

$$\text{DM (\%)} = \left[\frac{\text{DM of sample (g)}}{\text{Fresh mass of the sample (g)}} \right] \times 100$$

The yield of DM was estimated from the yield of MV and the percentage of DM, by the following formula:

$$\text{DM (t ha}^{-1}\text{)} = \left[\frac{\text{MV (kg plot}^{-1}\text{)} \times \text{DM (\%)} \right] / 100 \times f$$

Where: f = factor to convert DM yield from kg plot⁻¹ to t ha⁻¹ (0, 48 for plots of 21m²).

STATISTICAL ANALYSIS

Statistical package STATGRAPHICS for Windows (21) was used. All the characters fulfilled the assumptions of normality and homogeneity of variance by which a simple classification ANOVA analysis was performed. For media discrimination, the Duncan procedure (22) with a significance of 5 % was used in cases where ANOVA was significant.

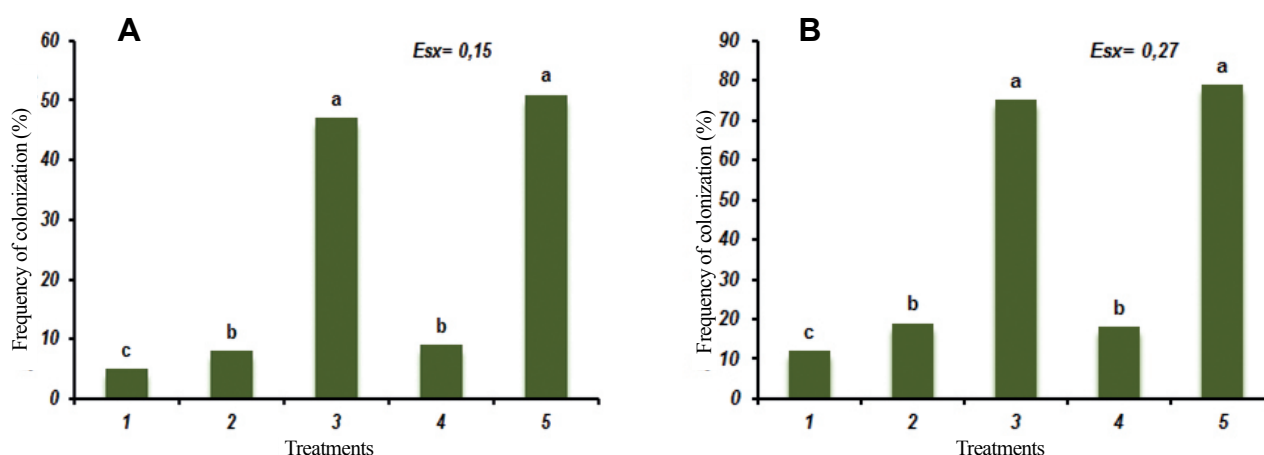
RESULTS AND DISCUSSION

Figure 1 (A and B) shows the frequency behavior of mycorrhizal colonization in *P. purpureum* for the two samples performed. It was found that at 45 DAP treatments T3 (*R. intraradices*) and T5 (*R. intraradices* + FitoMas-E® + 25 kg ha⁻¹ of urea) reached colonization frequency values of 48 and 50 % respectively and exceeded rest of the treatments under study. The treatments T2 and T4 showed a similar behavior and exceeded the control treatment which only obtained 5 %. The values obtained by treatments T3 and T5 were higher when compared to those reported in *P. clandestinum* with 41 % of colonization frequency in loamy soils (23).

The response of this indicator to uninoculated treatments (T1, T2 and T4) could be related to the presence of resident AMF structures in the soil at the time of planting the crop. Some studies have shown that the efficiency of AMF inoculation to promote plant growth depends on its ability to compete with native AMFs (24). This is related to the strain infectivity, its ability to produce external hyphae, the velocity of the hypha to colonize the roots and its ability to maintain colonization levels in a competitive condition (23).

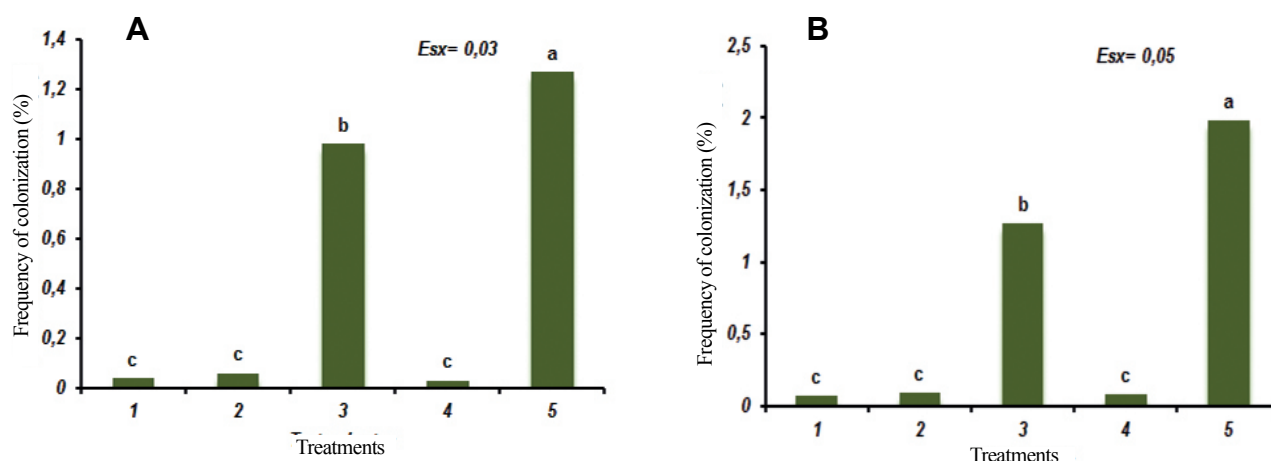
At 90 DAP (Figure 1B) a similar behavior was observed among the treatments studied, with T3 and T5 treatments with higher values (78 and 80 %, respectively).

As for the mycorrhizal colonization intensity, it was found that at 45 DAP (Figure 2A),



1: Absolute control; 2: Production indicator (50 kg ha⁻¹ of urea); 3: *R. intraradices*; 4: FitoMas-E®; 5: *R. intraradices* + FitoMas-E® + 25kg ha⁻¹ of urea

Figure 1. Behavior of the frequency of mycorrhizal colonization in *P. purpureum* evaluated at 45 (A) and 90 DAP (B)



1: Absolute control; 2: Production indicator (50kg ha⁻¹ of urea); 3: *R. intraradices*; 4: FitoMas-E®; 5: *R. intraradices* + FitoMas-E® + 25kg ha⁻¹ of urea

Figure 2. Behavior of the mycorrhizal colonization intensity in *P. purpureum* evaluated at 45 (A) and 90 DAP (B)

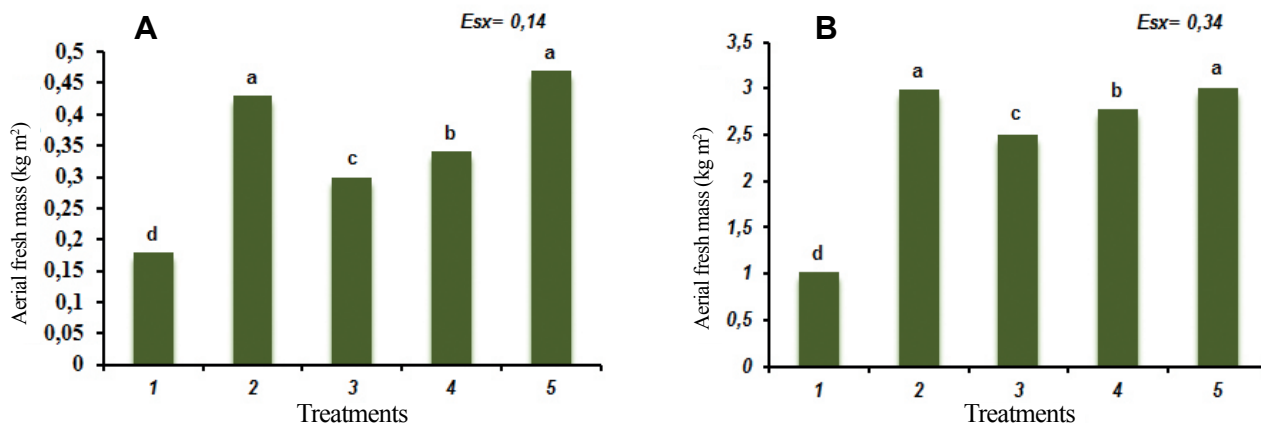
the T5 treatment reached higher values with values of 1,28 %, while in decreasing order, T3 differed with values of 0,98 %. For the rest of the treatments the response was similar without finding any differences among them. At 90 DAP (Figure 2B), the same behavior was maintained and the T5 treatment was emphasized with values of 1,98 %. The response found in this treatment proves that the species *R. intraradices* and FitoMas-E® stimulated the activity of the symbiont and in turn demonstrates the effectiveness of AMF in the presence of low availability of nutrients.

These values of mycorrhizal colonization intensity were lower when compared to those obtained when inoculating different AMF species in *Brachiaria decumbens*, where at 78 days after sowing, values of 2,5 % were reached in the treatment inoculated with *Glomus* Spp (24). On the other hand, these results were also lower than those reported in a study with the same host plant but with evaluation periods of 60, 90 and 120 days after inoculations (25). In that study, the authors demonstrated that after 100 days, there is a tendency for the intensity of colonization to decrease.

Another distinctive element that determines the efficiency of mycorrhizal symbiosis and its relation to the growth and development of plants are the climatic conditions. In our country pastures increase the production of biomass in the rainy season not only by the effect of rainfall, but also by the increase of temperature and humidity; which determines a direct relation between the mycorrhizal structures and the growth of the pastures, being this time the most favorable for the establishment of the fungus (4).

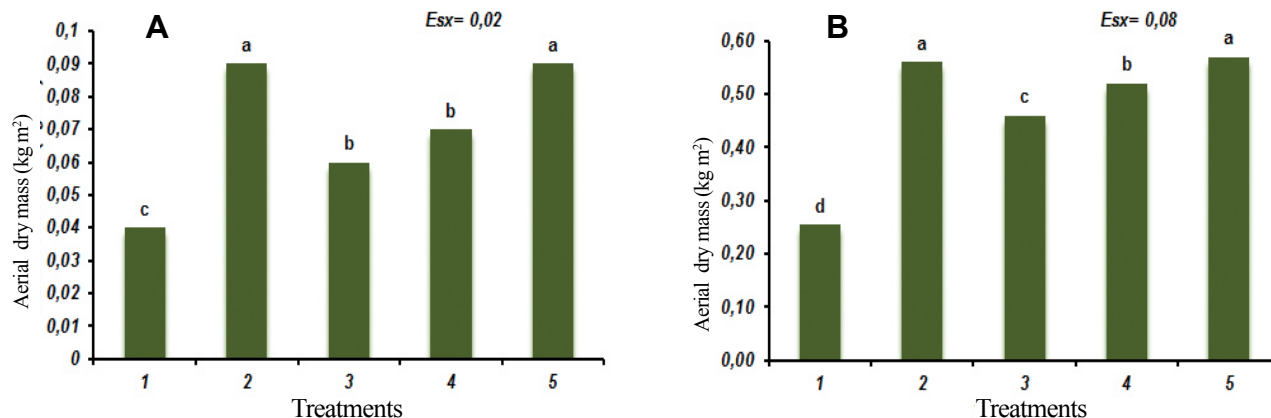
For the fresh biomass it was observed that in both samples, treatments T2 (control of production) and T5 (*R. intraradices* + FitoMas-E® + 25 kg ha⁻¹ of urea) reached higher values without differing among them but of the rest of the variants under study. In the first sampling these values were 0,45 kg m⁻² and at 90 days they were increased to 3 kg m⁻². In both samples, it was verified that the simple inoculation of *R. intraradices* and FitoMas-E® (T3 and T4) stimulated the production of fresh biomass when higher values were found than in T1 treatment (absolute control), which allows to verify the efficiency of this species of AMF and FitoMas-E® in the culture of *P. purpureum* cv. CT-115 (Figure 3A and B).

The effect of the treatments for dry biomass at 45 and 90 days is shown in Figure 4 (A and B) and the treatments T2 (production control) and T5 (*R. intraradices* + FitoMas-E® + 25 kg ha⁻¹ of urea) reached values of 0,09 and 0,57 kg m⁻² respectively. The simple inoculated treatments (AMF and FitoMas-E®) showed a positive response and, in turn, differed from the absolute control treatment, which reached values of 0,04 (45 DAP) and 0,26 kg m⁻² (90 DAP). The benefit reported by the use of mycorrhizal associations in plant growth and development indicators, particularly in tropical soils, has been confirmed by numerous studies (9, 23, 25). In this sense, increases in growth and development indicators were obtained by evaluating the effect of inoculation of the *Glomus cubense* (Y. Rodr. & Dalpé) strain (syn. *G. hoi-like*) on *Brachiaria decumbens* and *Panicum maximun* (26).



1: Absolute control; 2: Production indicator (50kg ha⁻¹ of urea); 3: *R. intraradices*; 4: FitoMas-E®; 5: *R. intraradices* + FitoMas-E® + 25 kg ha⁻¹ of urea

Figure 3. Influence of treatments on fresh air mass (MFA) for *P. purpureum* evaluated at 45 (A) and 90 DAP (B)



1: Absolute control; 2: Production indicator (50kg ha⁻¹ of urea); 3: *R. intraradices*; 4: FitoMas-E®; 5: *R. intraradices* + FitoMas-E® + 25kg ha⁻¹ of urea

Figure 4. Influence of treatments on aerial dry mass (MSA) for *P. purpureum* evaluated at 45 (A) and 90 DAP (B)

The results related to the concentrations of nitrogen, phosphorus and potassium foliar in the crop corresponding to the second sampling, are described in Table II. It was verified that no differences were found among the treatments for the contents of phosphorus and foliar potassium. Considering that one of the main benefits of mycorrhizal symbiosis is its role in the absorption of phosphorus (10), when analyzing the results, it was possible to verify that the initial content of this macroelement in the soil was high (Table I); which could have been related to previous applications of mineral fertilizers in these areas and therefore allows to justify the non-existence of significant differences among treatments.

As for potassium, although studies related to the role of AMF in the transport of this element to plants are scarce, the response found for this macroelement could be related to its greater mobility in soil solution.

As regards the nitrogen content, it was possible to verify the existence of significant differences between the treatments studied, being the treatments T2 (control of production) and T5 (*R. intraradices* + FitoMas-E® + 25 kg ha⁻¹ of urea) with higher values. The T3 and T4 treatments (*R. intraradices* and FitoMas-E®, respectively) did not differ from each other, but in turn surpassed the absolute control (T1). The response obtained for this indicator could be related to the characteristics of the crop,

because *P. purpureum* is a plant that requires high nitrogen content to increase its growth and development and therefore favor the biomass production. Nitrogen is available in the soil in different forms, but the most prevalent are nitrate, ammonium and amino acids; which in turn make up the main reserve of this element in the soil. Studies have shown that it is possible to transfer 42 % of the total nitrogen required by tomato plants through *Funneliformis mosseae* hyphae (27).

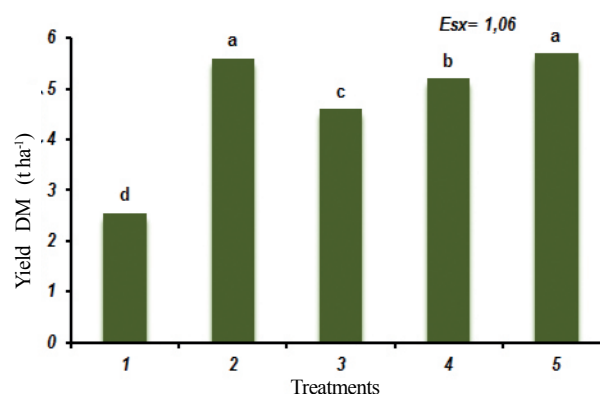
Table II. Nitrogen, phosphorus and potassium content in *P. purpureum* at 90 DAP

Treatments	Foliar nitrogen (g kg ⁻¹)	Foliar Phosphorus (g kg ⁻¹)	Foliar Potassium (g kg ⁻¹)
Absolute control	152,3 d	33,5	30
Control of production (25kg ha ⁻¹ urea)	258,9 a	33,9	29
<i>R. intraradices</i>	221,6 b	33,6	26
FitoMas-E®	218,6 c	33,4	26
<i>R. intraradices</i> + FitoMas-E®+ 25kg ha ⁻¹ urea	259,3 a	33,9	28
Esx	0,46*	0,73 n.s.	0,51 n.s.

1: Absolute control; 2: Production indicator (50kg ha⁻¹ urea); 3: *R. intraradices*; 4: FitoMas-E®; 5: *R. intraradices* + FitoMas-E® + 25 kg ha⁻¹ of urea

Regarding the performance of the dry mass yield (t ha⁻¹) of the cut performed at 90 DAP, it was found that the treatments T2 and T5 reached higher values (5,6 and 5,7 t ha⁻¹ DM, respectively), if compared with the rest of the evaluated variants. The treatments with a simple application of the *R. intraradices* and FitoMas-E® species differed from each other, reaching T4 values higher when compared to T3, with values of 5,2 t ha⁻¹ DM and surpassing T1 treatment which reached values of 2,55 t ha⁻¹ DM (Figure 5).

These results were lower when compared to those obtained when evaluating the behavior of three different species of the genus *Pennisetum* for semi-arid conditions. In this case yields of 22 t ha⁻¹ DM were obtained in four cuts on average, which makes it possible to indicate that the cut frequency is one of the factors that have the greatest influence on the growth and quality of the pastures, because the age Causes changes in its morphology and chemical constituents. To the extent that the age is prolonged, without reaching the point where the quality of the grass is affected, yields decrease (28).



Means with equal letters for each column did not differ significantly (p <0.05)

Figure 5. Influence of treatments on yield in DM for *P. purpureum* at 90 DAP

CONCLUSIONS

Reduction of mineral fertilizer (urea) at 25 kg ha⁻¹, inoculation of *R. intraradices* and application of FitoMas-E® stimulates biomass production in established pasture systems during the rainy season; however, it is necessary to develop new studies for the dry period.

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