Scientific Paper

Effect of IHPLUS® on the germination process of *Sorghum bicolor* L. (Moench)

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Abstract

The objective of this study was to evaluate the effect of the bioproduct IHPLUS® and its immersion times, on the germination process of *Sorghum bicolor* L. Moench cv. UDG-110. Ten treatments were studied: control (distilled water); and other nine with application of the bioproduct in three concentrations (2, 4 and 6 %) and three immersion times (2, 4 and 6 h). The seeds were planted on 5-cm diameter Petri dishes, on support of filter paper moisturized with distilled water. The following indicators were evaluated: germination percentage and value, root length and aerial part length, α-amylase activity and content of proteins and reducing sugars. A completely randomized design was used with four replicas per treatment. The results were processed with the statistical package SPSS® version 15.0. An ANOVA was used and Tukey's multiple range test was used for mean comparison. The treatments with IHPUS® showed a positive effect on the germination process. An increase in the germination value was observed in the first 48 h of the essay with the application of the product, as well as higher values in root and aerial part length. There was an increase of the α-amylase activity which was in correspondence with an increase in the concentrations of reducing sugars and total soluble proteins. It is concluded that IHPLUS® is an effective bioproduct to stimulate the germination process of *S. bicolor* L. Moench cv. UDG-110, with potentialities for the development of agroecological agriculture.

Keywords: germination, enzymatic activity, reducing sugars

Introduction

At present, there is a global trend towards sustainable agriculture with the minimum use of chemical products, which destabilize the environment and cause damage to human and animal health. Obtaining new bioproducts that stimulate crop growth and development constitutes an important strategy to develop agroecological management of ecosystems. These products include biopesticides, biostimulants and biofertilizers, for example, efficient microorganisms (Ullah *et al*., 2012).

The use of bioproducts, such as IHPLUS®, is based on the inoculation of mixed cultures of beneficial microorganisms to the soil, and contributes to the development of ecological agriculture, because it is a low-cost technology (Olle, 2015). These products based on microorganisms are traditionally used to stimulate plant germination, growth and development, because they produce many bioactive compounds (Biswas *et al*., 2014; Changas Jr. *et al*., 2015); for the control of diseases present in the soil (Grosu *et al*., 2015); and, more recently, they are successfully used in the reduction of organic pollutants as result of the industrial activity (Khatab *et al*., 2015).

In Cuba, sorghum (*Sorghum bicolor* L. Moench). cv. UDG-110, of free pollination, was introduced and selected at the Central University Marta Abreu of Las Villas at the end of 1990. The period comprised between seeding and germination varies from 3 to 5 days. From planting and until floral differentiation35-45 days pass, stage in which the plant has a height of 45-55 cm. The flowering or anthesis takes from 6 to 8 days; in this phenophase the plant reaches its maximum leaf area, and its height fluctuates between 122 and 142 cm (García-Martín, 1993).

Sorghum is considered a multipurpose crop of importance in world's agricultural economy, and represents a valuable resource for animal and human feeding due to its high nutritional potential. The grain is rich in protein content and micronutrients, and constitutes an option for celiac or intolerant people because of the absence of gluten. In addition, it is an ideal plant for the sustainability of

the agrifood system (Proietti *et al*., 2015). Thus, the objective of this work was to evaluate the effect of the bioproduct IHPLUS® and immersion times on the germination process of *S. bicolor* cv. UDG-110.

Materials and Methods

Plant material. Certified sorghum UDG-110 seeds were used, which were provided by the Pastures and Forages Research Station Indio Hatuey, Matanzas province.

IHPLUS®. The experiments were conducted with lot 31 of liquid inoculant of IHPLUS®, on which a quality test was performed; thus, the total count of the main groups of beneficial microorganisms was found within the established range and there was no pathogen growth.

Treatments. The *S. bicolor* seeds were previously treated with different concentrations of IHPLUS® and different immersion times. Afterwards they were placed on two layers of filter paper, on 5-cm diameter Petri dishes for germination (table 1).

Morphophysiological indicators

Germination test. The germination test was conducted on 5-cm diameter Petri dishes. Four replicas (Petri dishes) were used per treatment, with 25 sorghum seeds each. The seeds were placed on filter paper moisturized with water, in a proportion of three times the weight of the dry substrate (ISTA, 2014). The germination process was evaluated daily during seven days, and the results were expressed in percentage of normal seedlings. The germination trial was developed in a growth chamber at a temperature of 25 ± 2 ºC, with a photoperiod of 16 h day^{-1} (flow of photosynthetic photons: µmol m^{-2} s^{-1}).

Evaluation of the germination value (GV). It was determined through the formula proposed by Djavanshir and Pourbeik (1976).

$$GV = \left(\sum_{i=1}^{n} Vedi\right)\left(\frac{Ef}{10N}\right)$$

Where:

Ved: daily emergence rate, calculated as the percentage of accumulated emergence between the number of days since the beginning of the test.

N: frequency or number of Ved that will be calculated during the test.

Ef: percentage of seedling emergence at the end of the test days.

Morphological indicators. The root and aerial part lengths (cm) of the seedlings were evaluated seven days after germination, with the use of graph paper.

Biochemical indicators

Enzymatic α-amylase activity. The enzymatic extract was cold made, by homogenization of the plant material (seedlings seven days after germination), in a buffer solution of sodium citrate pH 5,0 in 1:2 (p/v) ratio. The mixture was centrifuged during 10 minutes at 10 000 rpm and 4 ºC. The supernatant was collected for the determination of the enzymatic activity.

The α-amylase activity was determined as described next. To 0,4 mL of a 1 % starch solution (p/v) in sodium phosphate buffer 20 mmol L^{-1} pH 6,9, 0,1 mL of the enzymatic extract were added and it was left to react during 10 min at 37 ºC. The reaction was stopped with the addition of 3,5-dinitrosalicylic acid. Later the reacting mixture was heated at 100 ºC during 10 minutes and 1, mL

Table 1. Treatments used to evaluate the effect of IHPLUS® on the germination of *S. bicolor.*

Treatment	IHPLUS®, %	Immersion time, h
T1	0	6 (in water)
T2	2	4 (in water) + 2 (IHPLUS®)
T3	2	2 (in water) + 4 (IHPLUS®)
T4	2	6 (IHPLUS®)
T5	4	4 (in water) + 2 (IHPLUS®)
T6	4	2 (in water) + 4 (IHPLUS®)
T7	4	6 (IHPLUS®)
T8	6	4 (in water) + 2 (IHPLUS®)
T9	6	2 (in water) + 4 (IHPLUS®)
T10	6	6 (IHPLUS®)

of distilled water were added. The absorbance was determined at 546 nm. The enzymatic activity was expressed as μmoles×min^{-1} of glucose released per μg of protein at pH 6,9 and 37 ºC.

The enzymatic activity (EA) was calculated through the following formula:

$$EA = \frac{\mu moles}{t} * \frac{VT}{Ve} * fd$$

Where:

T: time of the essay
VT: total volume of the essay (0,5 mL)
Ve: sample volume (0,1 mL)
Fd: Dilution factor of the enzymatic extract

All the spectrophotometric measurements described were performed in a UV/VIS Ultrospec 2000 spectrophotometer (Pharmacia Biotech, Sweden).

Extraction method of the plant material to determine the content of reducing sugars and total soluble proteins. The extraction and quantification of proteins and reducing sugars was conducted in the roots and the aerial part of the *S. bicolor* seedlings, seven days after the beginning of the germination experiment. The plant material was cold macerated with buffer solution of sodium phosphate 50 mmol.L^{-1}, pH 7,0 and in a 1:3 (p/v) ratio. The homogenized product was centrifuged at 10 000 rpm and the supernatant was collected, which was preserved at -20 ºC until the moment of determinations.

Reducing sugars. The content of reducing sugars was determined by the dinitrosalicylic acid method, and D-glucose (Sigma) was used as pattern sugar (Miller, 1959). The absorbance values were read at a wavelength of 456 nm. The concentration was expressed in mg mL^{-1} from the pattern curve.

Total soluble proteins. The protein content was determined colorimetrically through the method described by Lowry *et al.* (1951), using bovine serum albumin (BSA) as pattern. The absorbance values were obtained at 750 nm and the concentrations (mg mL^{-1}) were determined by the pattern curve.

Experimental design. A completely randomized design was used. For the germination essays four replicas (Petri dish) were used per treatment, and in the case of the biochemical analyses they were done in triplicate. Five samples were taken per treatment, while for the evaluation of morphological and physiological parameters 10 were analyzed.

Statistical analysis. The data were processed with the statistical package SPSS®, version 15.0 for Windows®. The data adjustment to normal distribution was determined, through the Kolmogorov goodness of fit test (Sigarroa, 1985). The data fulfilled the variance homogeneity and normal distribution assumptions, and were processed by an ANOVA. Tukey's multiple range test was used for mean comparison.

Results and Discussion

Morphophysiological indicators

Germination of S. bicolor with the application of IHPLUS®

Figure 1 shows the effect of IHPLUS® on the germination percentage of *S. bicolor*, during seven days. At 24 hours, the seeds treated with the bioproduct showed higher response; the highest values were obtained at seven days with the variant 6 %-4 h of immersion, with a germination percentage higher than 80 %.

These results coincide with the ones obtained by Babu and Balasaravanan (2017), who isolated and characterized bacterial strains from the rhizosphere which showed good characteristics as plant growth promoters. The immersion of *Solanum melongena* L. seeds in solutions that contained these bacteria produced an increase of the germination percentage (97 %), compared with that of the control (60 %). Similarly, the immersion of *Solanum lycopersicum* L. seeds in a biopreparation based on halotolerant strains of *Bacillus megaterium* caused an increase of the germination percentage with regards to the control under salinity conditions (Chookietwattana and Maneewan, 2012).

The increase in the germination percentage observed in the treatments with IHPLUS®, during the first days of the experiment, could be related with the entrance into the seeds of growth regulating substances, such as auxins, cytokinins and gibberellins produced by the microorganisms of IHPLUS® during the imbibition process (Khatab *et al*., 2015; Thakur and Parikh, 2015; Damam *et al*., 2016). As stated by Taiz and Zeiger (2013) and Mohite (2013), these compounds stimulate such processes as cell division and elongation, which allow the growth of the different plant structures.

The germination values in the treatments with IHPLUS® were higher than those of the control. The best results were obtained with the treatments 6 %-4 h and 4 %-4 h (fig. 2). The treatments with the bioproduct advanced in 24 h the peak day with regards to the control treatment, whose highest germination value was observed at 48 hours after the beginning of the experiment.

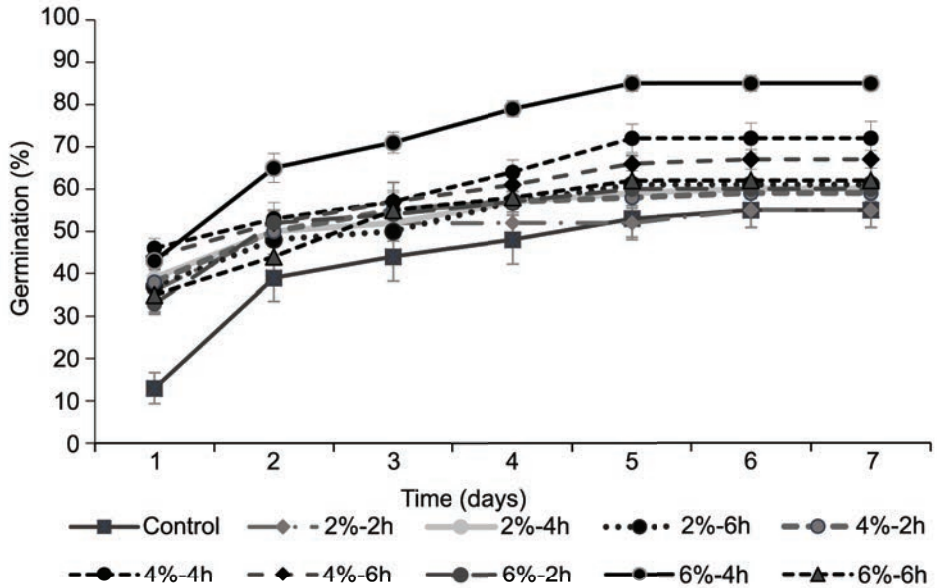
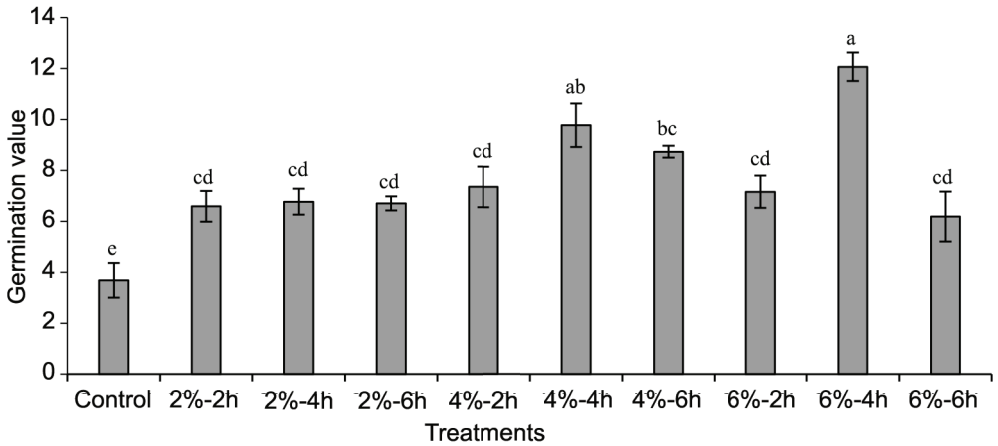


Figure 1. Germination percentage of S. bicolor seeds, treated with different concentrations of IHPLUS® and immersion times.



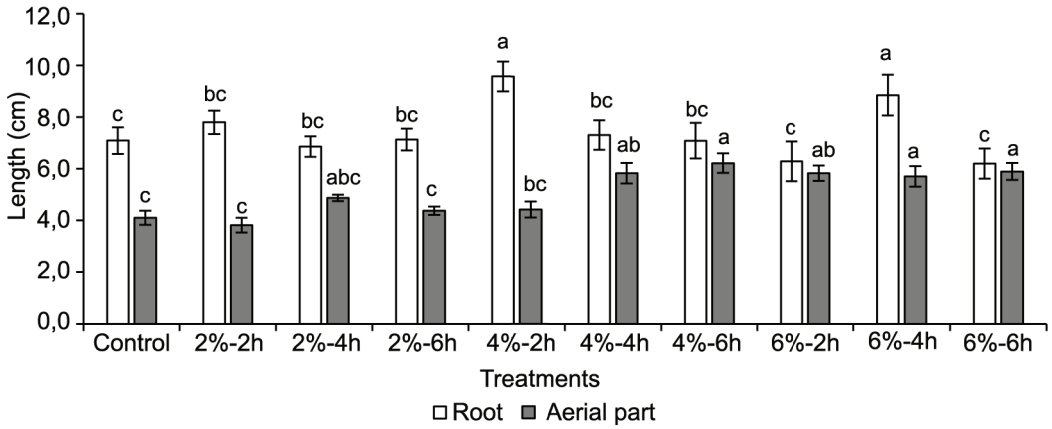
Different letters indicate statistical differences among the treatments according to Tukey's test ($p \leq 0,05$).
Figure 2. Germination value in *S. bicolor* seeds treated with IHPLUS®.

These results coincide with the ones obtained by Higa (1991), who reported an increase in the germination rate with the application of efficient microorganisms, whose composition mainly included acid lactic bacteria, photosynthetic bacteria, yeasts and actinomycetes which are commonly present in the soil. A similar effect on the germination vigor was shown in seeds from tomato (*Solanum lycopersicum* L.) and corn (*Zea mays* L.) with the application of natural products based on fungi and bacteria, respectively, present in the rhizosphere (Mahadevamurthy *et al*., 2016).

Seedling length

The application of IHPLUS® in different concentrations and immersion times stimulated the growth of roots and aerial parts of the *S. bicolor* seedlings (fig. 3). In the case of roots, the highest values were obtained with the treatments 4 %-2 h and 6 %-4 h; while for the aerial part the best results were achieved with the concentrations 4 %-4 and 6 h and with all the 6-% treatments.

The differences observed between the treatments with IHPLUS® and the control can be associated with the hormonal balance that is



Different letters indicate significant differences among treatments for the same organ, according to Tukey's test ($p \leq 0,05$).

Figure 3. Root and aerial part length of *S. bicolor* seedlings.

established inside the seeds, among the different endogenous and exogenous growth regulators. The concentration of auxins and the interaction between them and other growth regulators have a fundamental role in the physiological response of plants (Lambrecht *et al*., 2000).

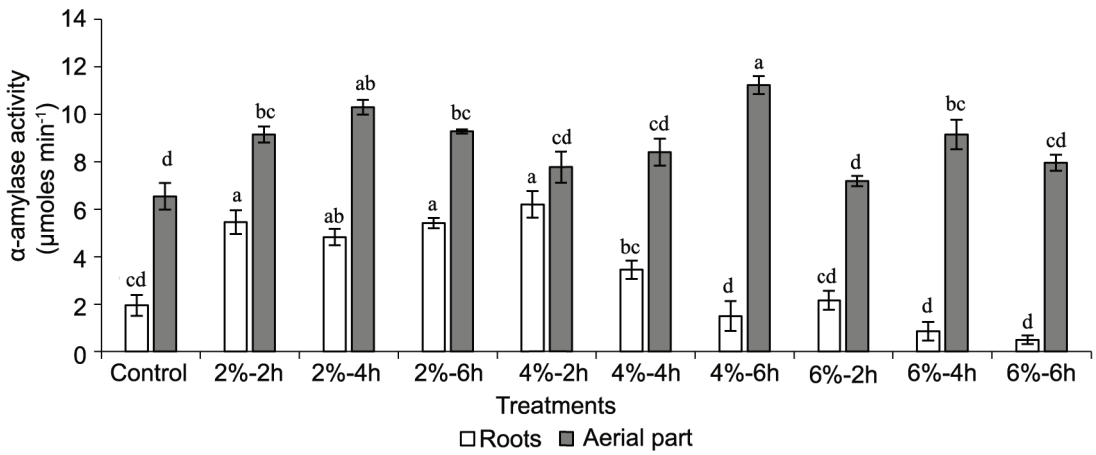
These results coincide with the ones obtained by different authors, who studied the effect of diverse biopreparations based on microorganisms isolated from the rhizosphere, on the process of germination and growth of seedlings of such species as *Medicago sativa* L. (Carrillo-Castañeda *et al*., 2002), *Solanum lycopersicum* L. (Agrawal and Agrawal, 2013), *Oryza sativa* L. (Jamil *et al*., 2014), *Cicer arietinum* L. (Biswas *et al*., 2014) and *Cerasus sachalinensis* Kom. (Qin *et al*., 2016).

Biochemical indicators

A-amylase activity

The α-amylase activity in the roots and aerial part of sorghum seedlings, from the seeds treated with different IHPLUS® concentrations and immersion times, is shown in figure 4. In the case of the roots, the highest values corresponded to the treatments at 2 % and 4 %-2 h; while for the other treatments and the control no significant differences were observed. For the aerial part, with IHPLUS® at 2 %, 4 %-6 h and 4 %-4 h higher values than those of the control were obtained.

The increase in the α-amylase activity in some treatments with IHPLUS® could be related to the presence of gibberellins in the evaluated



Different letters indicate significant differences according to Tukey's test ($P \leq 0,05$).

Figure 4. α-amylase activity in the roots and aerial part of *S. bicolor* seedlings.

product and their absorption by the seeds during the imbibition process, or with the induction of the genetic expression of this enzyme by components of the applied product. The increase in the levels of this phytohormone in plant tissues could have stimulated the expression of α-amylase.

Similar results in the increase of the activity of this enzyme were also reported by Mohd Din *et al.* (2014) and Soares *et al.* (2014) in rice (*Oryza sativa* L.) seeds treated with biofertilizers based on rhizobacteria. The values obtained by these authors, likewise, proved that the amylolytic activity is influenced by several factors, such as plant genotype, composition and concentration of microorganisms present in the natural product, and its physiological moment.

Reducing sugars

In the root as well as the aerial part of the seeds treated with IHPLUS®, an increase was observed in the content of reducing sugars compared with the respective controls (fig. 5). In the roots there was a remarkable increase in the treatment 4 %-6 h of the product, which was higher than the other treatments with application of IHPLUS®. The best results in the aerial part were observed in the treatments with 2 % of the product.

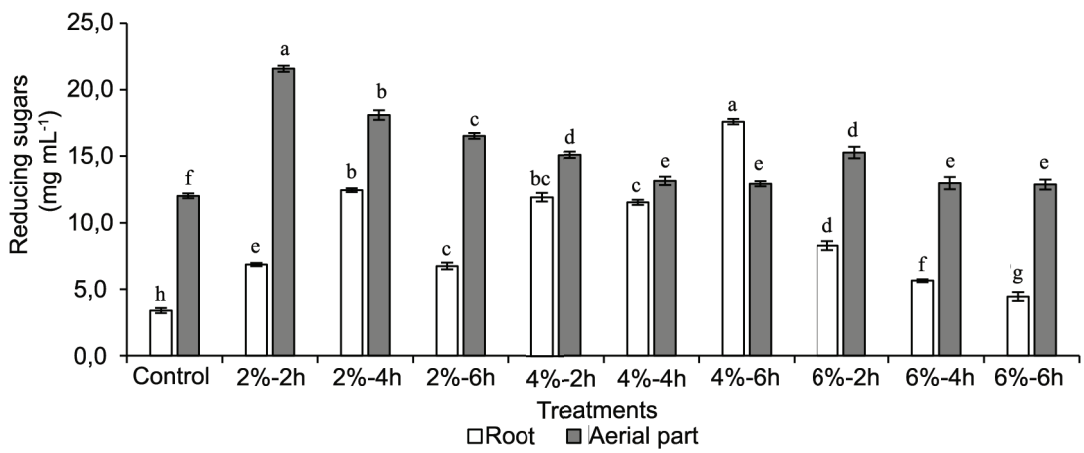
The increase in the reducing sugar content could be related to the increase of amylolytic activity in plant tissues. In this regard, Morais *et al.* (2016), in similar studies conducted in corn (*Zea mays* L.), also observed changes in such activity in the seeds treated with bacterial solutions, which is essential for starch degradation and production of respiratory substrates, which is essential for the embryo growth and development.

Total soluble proteins

The application of IHPLUS® increased the content of total soluble proteins in the roots and aerial parts of the seedlings. The treatments with the doses of 4 and 6 % showed higher values with regards to the other treatments and the control (fig. 6). These results suggest an increase in protein metabolism, which could be related to a higher availability of reducing sugars, such as glucose; these sugars, besides being used in obtaining metabolic energy during cell respiration, constitute carbon skeletons for the synthesis of amino acids and proteins. On the other hand, the product application could cause an increase in the expression of enzymes related to the translation process.

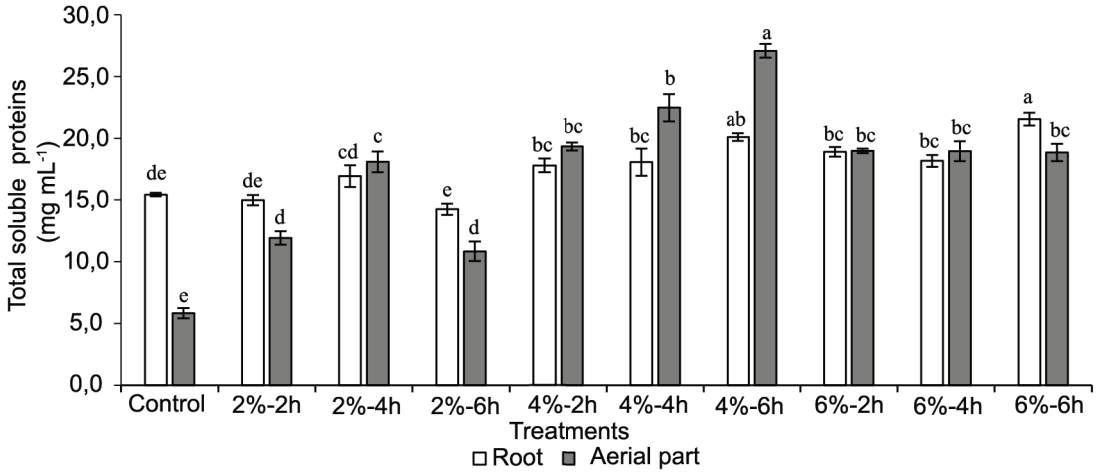
Conclusions

The application of IHPLUS® to *S. bicolor* cv. UDG-110 seeds had a positive effect on the germination process, by increasing the germination percentage, value and growth of vegetative organs, which indicates the presence of bioactive compounds that stimulate it. The results regarding the morphohysiological indicators were related to the biochemical response, because the content of reducing sugars and soluble proteins increased, as a result of an increase of the α-amylase activity or other amylolytic and protein metabolism enzymes. IHPLUS® proved to be an effective bioproduct to stimulate the germination process of sorghum, with potentialities to contribute to the development of the current agroecological agriculture.



Different letters indicate significant differences according to Tukey's test ($p \leq 0,05$).

Figure 5. Content of reducing sugars in the root and the aerial part of *S. bicolor* cv. UDG-110 seedlings.



Different letters indicate significant differences according to Tukey's test ($p \leq 0,05$).

Figure 6. Content of total soluble proteins in the root and aerial part of *S. bicolor* cv. UDG-110 seedlings.

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