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Biofertilizer increases the production and yield of sunflower (*Helianthus annuus* L.) oil in soils with adequate water availability

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Abstract

The experiment was carried out with the goal of evaluating the productive aspects of sunflower cultivar Helium 253, in response to the application of cattle biofertilizer and levels of water availability in two types of soils. A completely randomized experimental design was adopted using the factorial 2x2x5 for the 2 types of soils: Entisol and Alfisol, two levels of water availability in the soil (100 and 50% of moisture retention capacity, and five rates of liquid cattle biofertilizer corresponding to 0; 0.75; 1.5; 2.25 and 3 L plant^{-1}, prepared in the proportion of water 1:3. Sunflower plants were cultivated in pots with 30 L volume, individually filled with each type of soil, with daily water supply made based on humidity readings determined in the pots, using a capacitance multi-sensor probe type PR2/6 (Time Domain Reflectometry - TDR). To evaluate the sunflower production, the following variables were measured: internal diameter (IDH) and outer diameter of the head (ODH), number of seeds per plant (NSP), weight of 100 seeds (W100S) and oil content in seeds (OCS) and water consumption per plant (WCP). Among the studied soils, Entisol is the one that presents the best conditions for the production of the sunflower cv. Helium 253, being also the soil where the highest water consumption by plant occurs. For Entisol, it is recommended the rate of cattle biofertilizer estimated in 1.05 L plant^{-1}, and for Alfisol the rate of 3 L plant^{-1}. Regardless of the soil used, the highest production values are obtained when the soil was maintained with 100% of moisture retention capacity.

Keywords: fertilization, irrigation, productivity.

Introduction

Sunflower (*Helianthus annuus* L.) is one of the four largest oilseed crops producing edible vegetable oil in use worldwide. It is successfully cultivated on five continents, in more than 25 million hectares (Oliveira, 2015). However, the increase in production area in recent years is mainly due to the importance of sunflower as a source of raw material for the production of renewable energy (Gatto et al., 2015). One of the most important constraints for plant development in semiarid regions is the water deficit (González et al., 2015). Irrigation is a technique that can overcome the water deficiency of plants and increase productivity, providing better quality products with a good price perspective in the market (Silva et al., 2007). In many areas of agricultural production around the world, inadequate irrigation and mineral fertilizer management are the major causes of soil degradation problems and low agricultural production.

The physical characteristics of the soil, such as aggregation, macro and micro porosity, water retention capacity, content of organic matter, sand, silt and clay influence the volume of water to be applied, even in similar climatic conditions, and may vary in function of the soil type (Maia Filho et al., 2013). The Neossolos (Entisols) and Luvissolos (Alfisols) are the two types of soil that have the greatest predominance in the semiarid region of the state of Paraíba, Brazil, existing the need to carry out studies that can characterize the productive behavior of sunflower under these conditions, so that management strategies can be elaborated in order to obtain high productivity. Associated with irrigation management, organic fertilization is another important factor for cultivation. For example, cattle biofertilizers can provide improvements in the chemical, physical and biological characteristics of the soil (Rivera-Cruz et al., 2008), which can act as an alternative to minimize expenses with synthetic fertilizers, and add greater value to the final product, mainly for small family farmers. There are scarce studies aimed at the use of cattle biofertilizer associated with water supply in the soil as an agricultural alternative for semiarid regions. Thus, this experiment was performed with the objective of evaluating the production of sunflower hybrid Helium 253, in two soil types under different water availability levels and cattle biofertilizer rates.

Table 1. Values of pH, electrical conductivity and cattle biofertilizer composition 45 days after the start of anaerobic fermentation.

pH	EC	Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	Cl^{-}	CO_3^{2-}	HCO_3^{-}	SO_4^{2-}
	dS m^{-1}	......................................$cmol_c$ L^{-1}..							
6.34	1.08	3.71	2.40	3.27	1.69	5.59	0.43	2.03	3.02

EC = electrical conductivity of the biofertilizer.

Table 2. Summary of the variance analysis for the variables internal diameter of the head (IDH), outer diameter of the head (ODH), number of seeds per plant (NSP), weight of 100 seeds (W100S), oil content in the seeds (OCS) and water consumption per plant (WCP) of the sunflower hybrid Helium 253, Catolé do Rocha municipality - Paraíba.

Average Square							
SV	DF	IDH	ODH	NSP	W100S	OCS	WCP
Biofertilizer (B)	4	1269^{**}	2182^{**}	19511^{ns}	3.58^{*}	56.22^{**}	173^{**}
Soils (S)	1	15152^{**}	23253^{**}	801108^{**}	34.65^{**}	219.88^{**}	25741^{**}
Levels (L)	1	78214^{**}	96032^{**}	124479^{**}	2.16^{ns}	0.53^{ns}	25357^{**}
Int. B x S	4	881^{**}	1565^{ns}	188532^{**}	1.81^{ns}	183.14^{**}	1047^{**}
Int. B x L	4	3579^{**}	4473^{**}	441378^{**}	1.76^{ns}	106.20^{**}	498^{**}
Int. S x L	1	550^{ns}	121^{ns}	1782^{ns}	11.44^{**}	3.10^{ns}	876^{**}
Int. B x S x L	4	749^{**}	1336^{ns}	368169^{**}	1.99^{ns}	133.51^{**}	972^{**}
Residue	152	47.18	175.18	10653	0.35	0.70	0.23
CV (%)		6.79	11.98	13.48	10.18	2.08	0.69

SV, sources of variation; Int., Interaction; DF, degree of freedom; *, ** and ^{ns} significant at 5%. and at 1% probability and not significant by the F test; CV, coefficient of variation

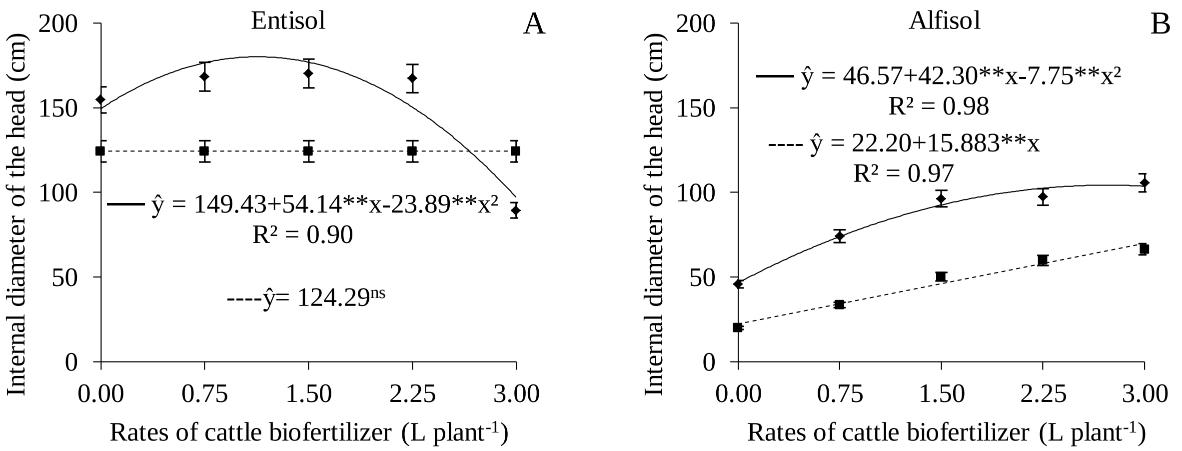

Fig 1. Internal diameter of the head of sunflower Helium 253 cultivated in Entisol (A) and Alfisol (B), according to rates of cattle biofertilizer, with 100% (—) and 50% (---) of moisture retention capacity (MRC).

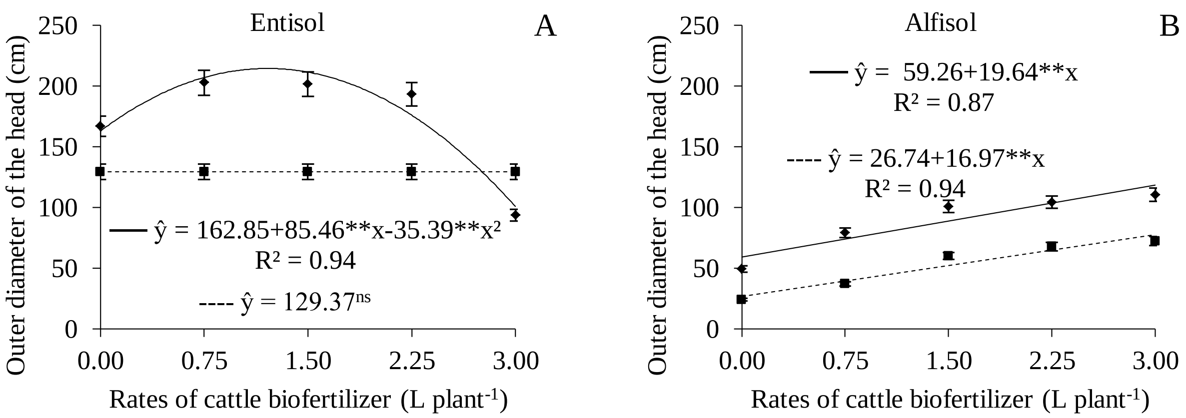

Fig 2. Outer diameter of the head of sunflower Helium 253 cultivated in Entisol (A) and Alfisol (B), according to rates of cattle biofertilizer, with 100% (—) and 50% (---) of moisture retention capacity (MRC).

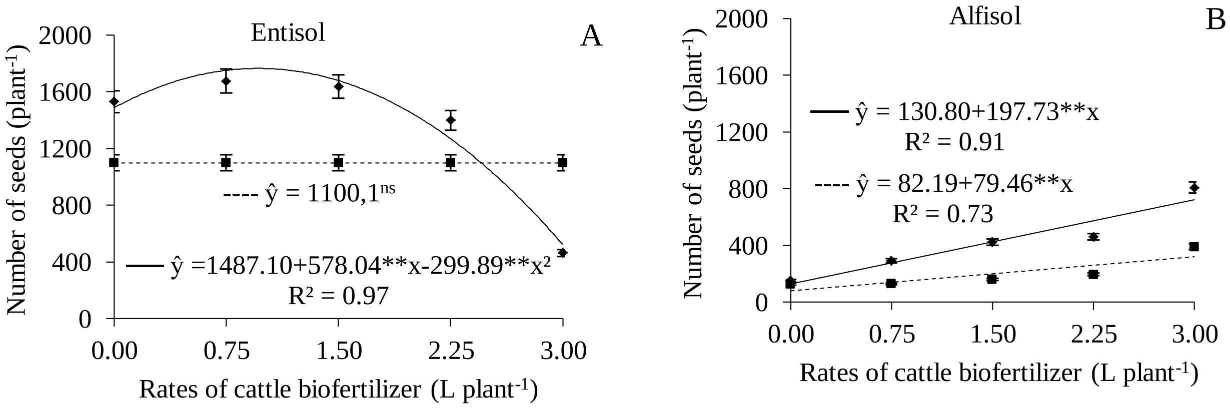

Fig 3. Number of seeds per plant of sunflower Helium 253 cultivated in Entisol (A) and Alfisol (B), according to the rates of cattle biofertilizer, with 100% (—) and 50% (---) of moisture retention capacity (MRC).

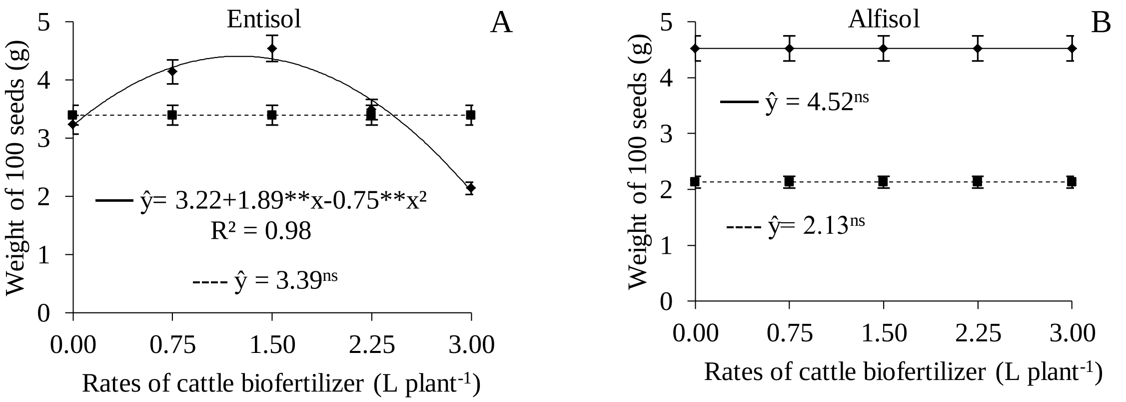

Fig 4. Weight of 100 seeds of sunflower Helium 253 cultivated in Entisol (A) and Alfisol (B), according to rates of cattle biofertilizer, with 100% (—) and 50% (---) of moisture retention capacity (MRC).

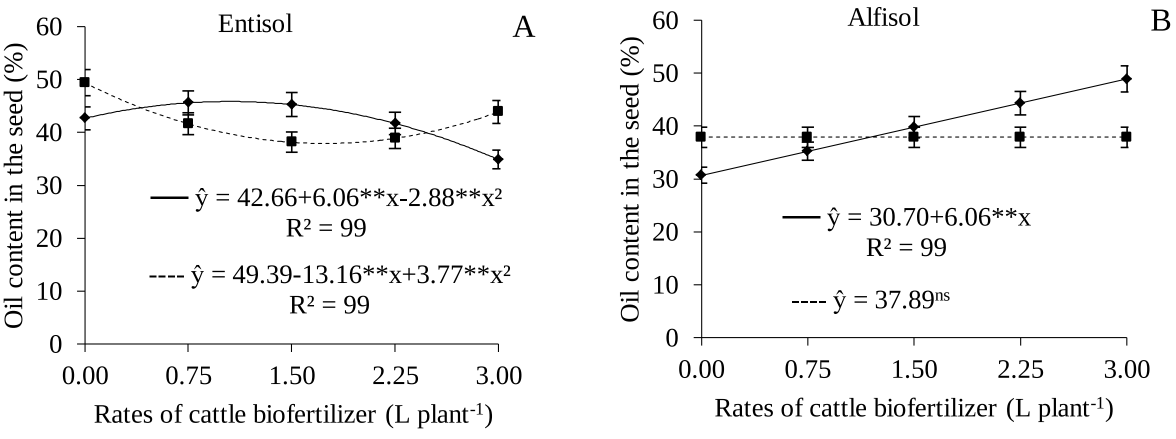

Fig 5. Oil content in the seeds of sunflower Helium 253 cultivated in Entisol and Alvisol (B), according to rates of cattle biofertilizer, with 100% (—) and 50% (---) of moisture retention capacity (MRC).

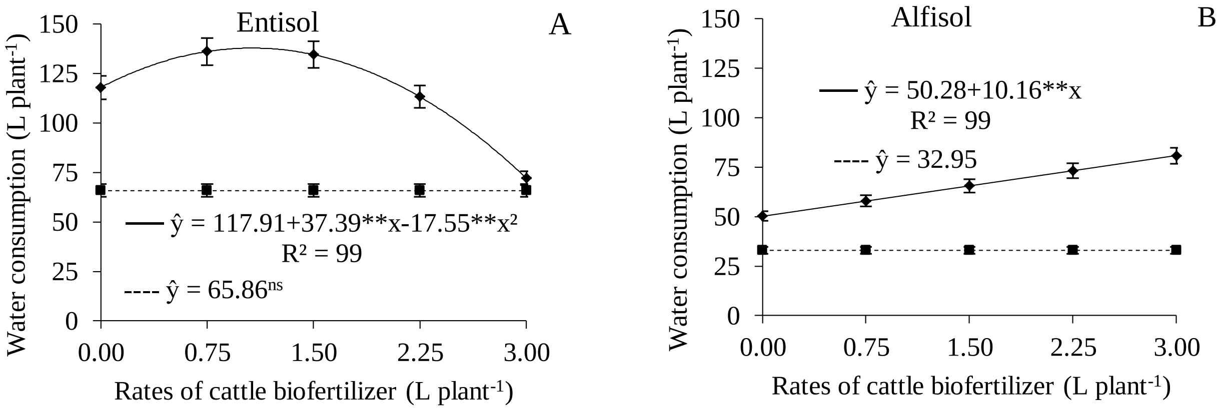

Fig 6. Water consumption per plant of sunflower Helium 253 cultivated in Entisol (A) and Alfisol (B), according to rates of cattle biofertilizer, with 100% (—) and 50% (---) of moisture retention capacity (MRC).

Results

According to the summary of the variance analysis (Table 2), it was verified that, except for the outer diameter of the head and the weight of 100 seeds per plant, there was a significant effect by the F test ($p < 0.01$) of the triple interaction composed by the factors: available water levels, soil types and rates of cattle biofertilizer. It can be observed in the Fig. 1A that there was a significant quadratic response ($p<0.01$) for the internal diameter of the head as a function of the rates of cattle biofertilizer applied in Entisol, the highest value being 180.11 cm, obtained with the estimated rate of 1.13 L plant^{-1}, decreasing with the increase of the rate, in conditions of 100% MCR. When under conditions of 50% MCR in Entisol, there was no significant effect of the rates of cattle biofertilizer on the internal diameter of the head, with an average value of 124.29 cm. The plants submitted to the highest volume of water available in the soil (100% MCR) presented superiority of 44.91% in relation to those cultivated with 50% MCR. The regression analysis for the rates of cattle biofertilizer applied in Alfisol, referring to the internal diameter of the head, can be visualized in Fig. 1B, where a quadratic effect is verified in the plants cultivated with 100% MCR, with a maximum value of 104.27 cm for the estimated rate of 2.72 L plant^{-1}. For the regression regarding the internal diameter of the head in Alfisol with 50% MCR, there is an increase of 4.76 cm for each unitary increase of the rate cattle biofertilizer, reaching a maximum value of 69.86 cm with the rate of 3 L plant^{-1}. It is observed superiority of 49.25% of the plants submitted to 100% MCR in comparison to the same treatments with 50% MCR. When comparing the two soils, a superiority of 72.73 and 77.91% of the internal diameter of the head was observed in the plants in Entisol, in comparison to the plants cultivated in Alfisol with 100 and 50% MCR, respectively. The sunflower hybrid Helium 253 responded similarly as for the increase in internal and outer diameter of the head in function of the rates of cattle biofertilizer applied in Entisol, maintained at 100% MCR. It was verified, through regression analysis, that the mathematical model that best fit the data for the outer diameter of the head was quadratic, with significant effect ($p<0.01$), obtaining the maximum value of 214.29 cm at the estimated rate of 1.20 L plant^{-1}, decreasing with application of rates above the estimated (Fig. 2A). The outer diameter of plants cultivated in Entisol submitted to 50% MCR did not present a significant response for the unfolding of the interaction cattle biofertilizer x moisture retention capacity x soils, presenting a mean value of 129.37 cm. The plants cultivated without water deficit (100% MCR) were superior in 86.51% to the same treatments submitted to 50% MCR. In Fig. 2B, the regressions for the factor dose of cattle biofertilizer applied in Alfisol can be visualized, showing a linear increase in the outer diameter of the head as the rates of cattle biofertilizer in the soil were increased, varying from 5.89 to 5.09 cm per unitary increase of the rates, reaching the maximum values of 118.2 and 77.67 cm, when the plants were submitted to 100 and 50% MCR in the soil, respectively. Statistically, it was found that the quadratic equation was also the one that best fit the number of seeds per plant data, in the plots cultivated in Entisol kept at 100% MCR of soil (Fig. 3A), obtaining the maximum number of seeds equal to 1765.0, achieved with the cattle biofertilizer rate estimated at 0.96 L plant^{-1}. When in conditions of 50% MCR, no significant effect of the interaction was observed, with an average of 1100 seeds (plant^{-1}). In Fig. 3B, the regression for the factor rate of cattle biofertilizer can be visualized, with an increasing linear tendency of the number of seeds per plant as the rates of cattle biofertilizer increased in the soil maintained at 100% MCR, with an increase of 57.88% of seeds for each unitary increase of the percentage of rate cattle biofertilizer in Alfisol, reaching a maximum value of 709.6 seeds with the highest input rate. Concerning the regression analysis of the number of seeds (plant^{-1}) in relation to 50% MCR in the soil, there was an increasing linear trend, with the maximum number of seeds of 319.71, obtained with the highest rate of the applied input. Statistically, it was found that the quadratic equation was the one that best fit the data weight of a hundred seeds in the plants that were submitted to 100% MCR in Entisol, with the highest value resulting in 4.54 g (Fig. 4A). In the unfolding of the interaction between soil types, available water levels and rates of cattle biofertilizer, there was no significant effect of the weight of a hundred seeds in the plants cultivated in Entisol submitted to 50% MCR, with a mean of 3.39 g; as for Alfisol, there was no significant effect regardless of the level of available water in the soil, presenting an average of 4.52 and 2.13 g in the plants cultivated with 100 and 50% of MCR, respectively (Fig.

4 A and B). The regression analyses for the variable oil content in the seeds in relation to the levels of cattle biofertilizer applied in Entisol, independently of the level of available water in the soil, showed that the best adjustments were represented by a quadratic curve (Fig. 5A). It was observed that the increase in the rate resulted in an increase in the oil content of the seeds, where the maximum increase (41.30%) was estimated at 1.05 L plant^{-1} of the cattle biofertilizer under the conditions of 100% MCR. From the maximum point there is a tendency to reduce the oil content of the seeds. When the plants were submitted to 50% MCR, there was a decrease in function of the estimated rate of 1.74 L plant^{-1}, and from this on, an increase, reaching the value of oil in the seeds equal to 43.43%, referring to the rate of cattle biofertilizer of 3.0 L plant^{-1}. For the oil content of sunflower seeds in function of the levels of cattle biofertilizer applied in Alfisol, when the plants were cultivated with 100% MCR, it is possible to observe an increasing linear tendency of the percentage of oil in the seeds. However, for treatments with 50% MCR, no statistical difference was observed, with a mean oil content in the seeds of 37.89% (Fig. 5B). From the regression analysis, it was verified that in Entisol the water consumption by plants reached the maximum value of 137.79 L, when under conditions of 100% MCR, at the cattle biofertilizer rate estimated at 1.06 L plant^{-1}, and decrease with the increased rates. For the plants submitted to water deficit (50% MCR), water consumption data did not fit any mathematical model, with a mean of 65.86 L plant^{-1} (Fig. 6A). For the water consumption of the plants cultivated in Alfisol, there was an increase of 3.77% for each unitary increase of the cattle biofertilizer, in plants cultivated with 100% MCR (Fig. 6B), that is, for each 1% of biofertilizer added to the soil, it is estimated by the regression equation that the plant increased 3.05 L of water in its consumption during the cycle. While in water deficit conditions (50% MCR), the water consumption by the plants was adjusted to the quadratic model, reaching a maximum consumption of 34.66 L corresponding to the rate 3.0 L plant^{-1}.

Discussion

The fact that the highest values referring to the variables of production are observed when the plants are cultivated in Entisol in relation to Alfisol may have occurred due to the 68.42% superiority of potassium content present in Entisol. Potassium acts on the regulation of water demand parameters of plants, since it is the main solute involved in water absorption by the cell, maintenance of turgescence pressure and cell stretching, and also in the regulation of stomatal opening (González et al., 2015). Low potassium, as can be observed in Alfisol, increases the susceptibility of plants to water deficit, which may have contributed to the fact that the plants cultivated in this soil have anticipated the end of the phenological vegetative stage; without, however, having completed the full development of the photosynthetic apparatus, limiting the ability of the plant to intercept and use light. This situation converges to a condition in which less developed plants have smaller amount of accumulated photoassimilates and, therefore, less capacity to translocate them to the grains in the filling phase, reflecting in significant reductions in production (Mengel and Kirkby, 2001; Taiz and Zeiger, 2013). Other studies have also verified that the application of potassium sulphate significantly improves growth, seed yield, rate of photosynthesis, transpiration, stomatal conductance, water use efficiency, leaf turgor and larger aerial part of the sunflower (Mengel and Kirkby, 2001; Akram et al., 2009), which reinforces the idea exposed in this discussion. In addition to the higher potassium content, Entisol also showed higher amounts of calcium, magnesium, sodium, phosphorus and organic matter, which justifies the higher values of production of sunflower observed in this type of soil. Regardless of the type of soil studied, it is possible to observe, through the variables analyzed, that the plants cultivated with 100% MCR presented higher values of production, suggesting that the sunflower cultivar Helium 253 is sensitive to the water deficit it was submitted (50 % MCR). This occurs when the plant feels the need to close the stomata to avoid greater losses of cellular turgor through transpiration, which causes significant inhibition of photosynthesis, negatively affecting physiological and biochemical processes, such as respiration, translocation, carbohydrates and nutrient absorption metabolism, which reduce plant growth (Heidari and Karami, 2014). Other studies in the literature have also reported a decrease in the production of several sunflower cultivars when submitted to water deficit, and gains when water availability in the soil is high at values close to 100% AW (Silva et al., 2007; Maia Filho et al., 2013). The application of rates of cattle biofertilizer up to the estimated value of 1.05 L plant^{-1} increased the production of the plants cultivated in Entisol, decreasing as the rates increased. Possibly, this tendency occurred due to a nutritional imbalance caused by the excess of nutrients supplied with the rates above the estimated, especially since the Entisol used in the experiment had better fertility conditions compared to Alfisol. This way, the linear trend of sunflower production is also justified in function of the rates of cattle biofertilizer in Alfisol, because in addition to the biofertilizer providing nutrients to the plants, it can improve the physical attributes of the soil, such as porosity and the relationship of micro and macropores (Castro et al., 2006) as well as influence the amount of bacteria and fungi that participate in the nutrient cycling process, and maintain the balance of microbial flora (Shen et al., 2013). Concerning the higher water consumption by the plant in Entisol, when under conditions of 100% MCR, this occurred due to water being the main constituent of vegetal tissues, and may correspond up to 95% of the total weight of the green mass, being responsible for the maintenance of leaf turgidity (Floss, 2006). Therefore, the larger the plant surface exposed to solar radiation, the greater its transpiration and consequently the water consumption for the maintenance of cellular turgidity and plant temperature (Taiz and Zeiger, 2013).

Materials and Methods

Localization, experimental procedure, treatments and plant material

The experiment was carried out from September 2012 to March 2013, in a greenhouse at the Center for Human and Agrarian Sciences, of the Paraíba State University, Campus IV, Catolé do Rocha municipality, Paraíba state, Brazil, located through the geographical coordinates: latitude

6°20’38” South, longitude 37°44’48” West at an altitude of 275 m.

The soils used in the experiment were classified as Neossolo Flúvico and Luvissolo Háplico (Santos et al., 2006), and as Entisol and Luvisol (USDA, 2014), from the municipalities of Catolé do Rocha and Brejo dos Santos in the state of Paraíba, Brazil.

The samples of these soils were collected in a layer 0-0.20 m deep, air dried, screen sieved with a 2.0 mm mesh opening, and later characterized as to the chemical and physical attributes according to the methods adopted by Embrapa (1997), with the following results: Entisol: sand = 820 g kg^{-1}; silt = 125 g kg^{-1}; clay = 55 g kg^{-1}; field capacity = 22.82% of the volume; permanent wilting point = 6.54% by volume; available water = 16.28% by volume; pH (H_2O) = 7.44; Ca^{2+} = 4.44 $cmol_c$ kg^{-1}; Mg^{2+} = 2.81 $cmol_c$ kg^{-1}; Na^+ = 0.26 $cmol_c$ kg^{-1}; K^+ = 0.57 $cmol_c$ kg^{-1}; H^+ = 0,0 $cmol_c$ kg^{-1}; Al^{3+} = 0 $cmol_c$ kg^{-1}; O.M = 6.9 g kg^{-1}; P = 53.3 mg kg^{-1}; Alfisol: sand = 655 g kg^{-1}; silte = 228 g kg^{-1}; clay = 117 g kg^{-1}; field capacity = 19.60% of the volume; permanent wilting point = 5.70% by volume; available water = 13.06% by volume g kg^{-1}; pH (H_2O) = 7.81; Ca^{2+} = 3.97 $cmol_c$ kg^{-1}; Mg^{2+} = 2.45 $cmol_c$ kg^{-1}; Na^+ = 0.15 $cmol_c$ kg^{-1}; K^+ = 0.18 $cmol_c$ kg^{-1}; H^+ = 0.06 $cmol_c$ kg^{-1}; Al^{3+} = 0 $cmol_c$ kg^{-1}; O.M = 4.5 g kg^{-1}; P = 14.3 mg kg^{-1}. For this experiment, the hybrid Helium 253 sunflower was chosen, although it is still little studied, the results obtained so far makes us believe that it is a plant with potential to be cultivated in semiarid regions.

Treatments

The treatments were arranged in a completely randomized design with 2x2x5 factorial, referring to two levels of available water (100 and 50% of moisture retention capacity (MRC)), two soils (Entisol and Alfisol) and five rates of cattle biofertilizer (0, 0.75, 1.5, 2.25 and 3.00 L $plant^{-1}$), totaling 20 treatments with nine replications and 180 experimental plots. Each experimental unit consisted of a plastic pot with capacity for 30 liters and a sunflower (*Helianthus annuus* L.) plant from the hybrid Helium 253.

Establishment and management of the experiment

The sowing was performed at a depth of 5 cm, using seven seeds per pot, distributed equidistantly. The seedling emergence began on the fourth day after sowing (DAS), at the ninth DAS the first thinning was made, leaving three plants per pot and the second pruning at 15 DAS, leaving only one plant, the most vigorous one. The biofertilizer rates were applied three times in 20 days, starting after the second pruning.

Soil water content was monitored daily by TDR probe model PR2/6 (Time Domain Reflectometry) in the treatments of 100 and 50% of moisture retention capacity (MRC) in four depth intervals: 0-10; 10-20; 20-30 and 30-40 cm; from the readings made with the probe model PR 2 for each depth, the values were put in a spreadsheet that accounted for the water content of each layer (four layers of 10 cm each), making a balance of water content along the existing soil profile as determined in accordance with the following equation proposed by Barbosa et al. (2016):

$$MCR = (CC–CA)/100) \times V \quad (1)$$

Where: MCR = Moisture retention capacity in cm; CC = Moisture at field capacity (dry weight basis); CA = Current capacity of soil and V = Soil volume.

Based on the probe readings a water balance was performed, which recorded the consumption of water by such treatments. Irrigation of the pots was done daily by hand with the aid of a graduated cylinder. At 90 DAS the irrigation was suspended, based on the criterion of the physiological maturity of the grains (Silva et al., 2007); on this same period, all the plants were in the phenological stage R9 (head inclined down, with back and bracts with a color between yellow and brown).

Preparation of cattle biofertilizer

The cattle biofertilizer was obtained through anaerobic fermentation, where 70 kg of fresh cattle manure was mixed, from animals raised in the semi-intensive system, with 100 L of slightly saline water - CE = 0.8 dS m^{-1}, 5 kg of crystal sugar and 5 L of milk were added to accelerate the metabolism of bacteria, and adding 2 kg of leaves and branches of the leguminous plant beans-macassar (*Vigna unguiculata* (L.) Walp), in the plastic biodigester with capacity for 200 liters, kept tightly closed for 45 days. During the fermentation the methane gas produced was released through a duct connected to the upper base and its end submerged in water (Santos, 1992). As a result of being applied in liquid form, composition analysis was performed following common procedures for water sample for irrigation, according to the data in table 1, congenerous suggestion of Cavalcante et al. (2010).

Traits measured

To evaluate the sunflower production, the following variables were measured: internal diameter (IDH) and outer diameter of the head (ODH), number of seeds per plant (NSP), weight of 100 seeds (W100S) and oil content in seeds (OCS) . The oil extraction from the sunflower seeds was carried out by the Soxhlet method, using the petroleum ether as a solvent and then calculating the results as a percentage (dry basis) according to Thomaz et al. (2012). It was also evaluated the water consumption per plant (WCP), obtained by quantifying the water volume applied in each treatment, during the period comprising the phenological stage VE (cotyledons emergence) until the physiological maturation phase R9.

Statistical analysis

Data were submitted to variance analysis, F test, regression for the quantitative factors and Tukey test for the qualitative data using the statistical program Sisvar® 5.3 (Ferreira, 2011).

Conclusion

Among the studied soils, under semiarid conditions, Entisol is the one that presents the best conditions for the production of sunflower Helium 253, being also the soil where the highest water consumption by plant occurs. For Entisol, it can be recommended the rate of cattle biofertilizer estimated in 1.05 L $plant^{-1}$, and for Alfisol, the rate of 3 L plant^{-1} can be recommended. Regardless of the soil used, the highest production values are obtained when the soil is irrigated with 100% MCR.

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