Biostimulant doses x stress conditions on the physiological quality of sunflower seeds

ABSTRACT

Considering the importance of sunflower crop and its versatility in the world market, this study aimed to evaluate the physiological quality of sunflower seeds subjected to different doses of biostimulant under stress conditions. The experiment was carried out at the Federal University of Viçosa, using the cultivar Hélio 250. The studied factors consisted of biostimulant doses (0, 2, 3, and 5 mL kg^{-1}), water retention capacities (40, 60 and 90%), and osmotic potentials (0.0, -0.2 and -0.4 MPa). Seeds were pre-soaked with solutions of 0.009% kinetin, 0.005% indolebutyric acid and 0.005% gibberellic acid for 4 hours and then tested for germination and vigor. The study was divided into three trials: 1. Biostimulant x field capacity; 2. Biostimulant x osmotic potential; both in a completely randomized design (DCR) with factorial scheme 5x3; and 3. Biostimulant x Temperature, in DIC, with 5x2 factorial arrangement and four replications. After collection, data were tested by analysis of variance and regression in the statistical program SISVAR. Under water and osmotic stress conditions, the biostimulant action was maximized in the applied tests, demonstrating superior performance in doses of 3 and 4 mL.

Keywords: Field capacity, hormones, *Helianthus annusus*, Polyethylene glycol

1. INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the four largest vegetable oil producing crops in the world. Its world production in 2014 was 41.34 million tons, with Brazil contributing 158.56 thousand tons of this production [1]. The commercialization of sunflower has a growing demand of 13% per year, which involves the market of oil, animal feed and biofuel production [2].

Although there is a great demand for sunflower cultivation, like many other economically important plants, its production is limited by biotic and abiotic stresses that cause production decrease in traditional cropping regions [3].

One of the alternatives to minimize the negative response of plants to stress and to increase productivity is the use of biostimulants, which favor nutrient absorption and efficiency, abiotic stress tolerance and crop quality [4].

The application of growth regulators in the early stages of plant growth contributes to root development, rapid recovery after exposure to water stress, increases resistance to pests and diseases, and helps plant establishment, enhancing absorption and yield of crops [5]. Other authors have also reported the beneficial effect of biostimulants in cultivated plants such as pea, tomato and corn [6], almond [7] and beans [8].

Given benefits of the application of biostimulant and the response under stress conditions, there is little research on the action of plant hormones on the germination and vigor of sunflower seeds, thus evidencing the need for studies to confirm its action. Thus, the goal of this study was to evaluate the physiological quality of sunflower seeds subjected to different doses of biostimulant and stress conditions.

2. MATERIAL AND METHODS

The experiment was carried out between April and June 2017, at the Laboratory of Seed Analysis of the Department of Plant Science at the Federal University of Viçosa, State of Minas Gerais (20°45'14" latitude, 42°52'53" longitude and 690 m altitude). The cultivar of sunflower used was Helio 250 from the company Heliagro Agricultura e Pecuária Ltda., harvested in 2015.

Seeds were initially analyzed for the moisture content according to [9], where a moisture content of 9% was verified. Subsequently, seeds were pre-soaked for 4 hours in solutions with biostimulant (0.009% kinetin, 0.005% indolebutyric acid and 0.005% gibberellic acid) except for the control, which was pre-soaked only in water. They were then allowed to dry at room temperature for 12 hours.

The study was divided into three trials. The initial trial [Biostimulant (Bioest) x Field Capacity (CC)] was developed in a completely randomized design (DCR) with four replications, in a 5x3 factorial arrangement. The first factor corresponded to five doses of biostimulant: 0; 2; 3; 4 and 5 mL Kg^{-1}. The second factor evaluated three water retention capacities: 40%, 60% and 90%. The field capacity was obtained using a methodology used by [10].

The tests conducted were: Emergence test (E) - conducted in the seed analysis laboratory, using 50 seeds, for each replication. These were sown at a depth of 2 cm in plastic trays containing washed and sterilized sand. Sand was moistened with an amount of water corresponding to 40, 60 and 90% of the water retention capacity of the substrate. Count of emerged seedlings was performed on the 14th day after sowing. The results were expressed as a percentage [11]. Emergence Speed Index (IVE) determined in conjunction with the emergence test. Seedlings were counted every day until stabilization of the number of seedlings. The emergence speed index of the seedlings was calculated according to [12]. Seedling shoot dry matter (MSPA) - normal seedlings obtained from the emergence test were evaluated. The replications of each treatment were placed in identified paper bags and taken to the forced air oven, maintained at a temperature of 60 ± 5°C until reaching constant mass. After this period, each repetition had the mass determined on a scale accurate to 0.001g, and the mean results expressed in milligrams per seedling [13].

Trial 2 [Bioestimulant (Bioest) x Polyethyleneglycol 6000 (PEG)] was carried out in a completely randomized design, with four replications, in a 5x3 factorial arrangement. The first factor corresponded to five doses of biostimulant: 0; 2; 3; 4 and 5 mL Kg^{-1}. The second factor evaluated three osmotic potentials: 0.0; -0.2 and -0.4 MPa.

The tests carried out were: Determination of the degree of moisture - by the greenhouse method (105°C ± 3°C), for 24 hours with results expressed as a percentage, according to the Rules for Seed Analysis [10]. Germination test (G) - four replicates of 50 seeds were sown on germitest paper rolls moistened with solutions of polyethylene glycol 6000, equivalent to 2.5 times the mass of the non-hydrated paper and kept in biochemical oxygen demand (BOD) under temperature of 25°C. Normal seedlings were counted on the tenth day after the test, and the results were expressed as percentage of normal seedlings, according to [10]. First germination count test (PCG) - performed in conjunction with the germination test, considering the percentage of normal seedlings present on the 4th day after the test setup [13]. Seedling length (CS) - the average length of normal seedlings obtained by sowing four replicates of 10 seeds on germitest paper rolls moistened with polyethylene glycol (PEG) 6000 solutions equivalent to 2.5 times the mass of the non-hydrated paper and maintained in biochemical oxygen demand (BOD) at 25°C for 7 days. Length of root (CPR) and shoot (CPA) of normal seedlings was measured using a millimeter ruler, with results in mm.$seedling^{-1}$ [13]. Dry matter of shoot (MSPA) and root (MSR): after separation, shoot and root were oven-dried at 60 ± 5°C to constant mass; and then weighed. The results were given in mg. $seedling^{-1}$ [13].

Trial 3 was performed in a completely randomized design, with four replications, in a 5x2 factorial arrangement. The first factor corresponded to five doses of biostimulant: 0; 2; 3; 4 and 5 mL Kg^{-1}. The second factor evaluated two temperatures: 10 and 25 ° C. The tests were: Cold test (F), in which the seeds were sown on moistened germtest paper rolls and kept at 10°C for seven days. After this period, the rolls were transferred to a temperature of 20-30 ° C and the evaluations were carried out at 4 and 10 days [14]. Germination test (G) - four replications of 50 seeds were sown on moistened germitest paper rolls with solutions of polyethylene glycol 6000, equivalent to 2.5 times the mass of the non-hydrated paper and kept in biochemical oxygen demand (BOD) under temperature of 25°C.

Normal seedlings were counted on the tenth day after installing the test, and the results were expressed as percentage of normal seedlings, according to [10].

With the obtained data, the interactions Biostimulant x PEG, Biostimulant x Field Capacity and Biostimulant x Temperature were analyzed and when significant ($p < 0.05$), the necessary breakdown was performed. The means were compared by the regression test ($p < 0.05$), with the aid of the software System for Analysis of Variance - SISVAR.

3. RESULTS AND DISCUSSION

1° trial - Interaction Bioestimulant x Field capacity

There was no significant effect for seedling emergence (E) (Table 1). For the emergence speed index, it was observed that in field capacity 60 and 90% there was an increase in the index up to the dose 4 mL, beginning to decrease afterwards (Figure 1, Table 2). The increased availability of water associated with the biostimulant may have promoted a stimulus, with the action of gibberellin, in the synthesis of enzymes that degrade nutrient reserves stored in the endosperm, forming simple sugars, amino acids and nucleic acids, which provide food and energy for seedling growth [15]. This process favors cell elongation, which results in the coat rupture and root emergence, accelerating germination with greater uniformity [16]. This rapidity stimulated by the regulators in the emergence is important, because the longer the germination of the seed, the more prone it will be to injuries due to pests or diseases present in the soil, compromising the integrity of the embryo [17].

Table 1. Summary of analysis of variance for emergence percentage (E), emergence speed index (IVE) and dry matter of emerged seedlings (MSPAE) subjected to seed treatment with different doses of biostimulant and field capacities.

SV	DF	QM		
		E (%)	IVE (%)	MSPAE (mg.seedling^{-1})
Bioest	4	9.73 ^{ns}	1.29**	3,19**
CC	2	2.60 ^{ns}	2.73**	26,07**
Bioest*CC	8	8.93 ^{ns}	1.43**	0,86^{ns}
Error	45	5.73	0.15	0,54
C.V. (%)		2.45	3.61	5,11
Mean		97.80	10.89	14,4

^{ns}Non-significant, and *Significant at 5% probability by F-test. Biostimulant (Bioest), field capacity (CC), degrees of freedom (DF), mean square (QM), interaction between biostimulant and field capacity (Bioest*CC).

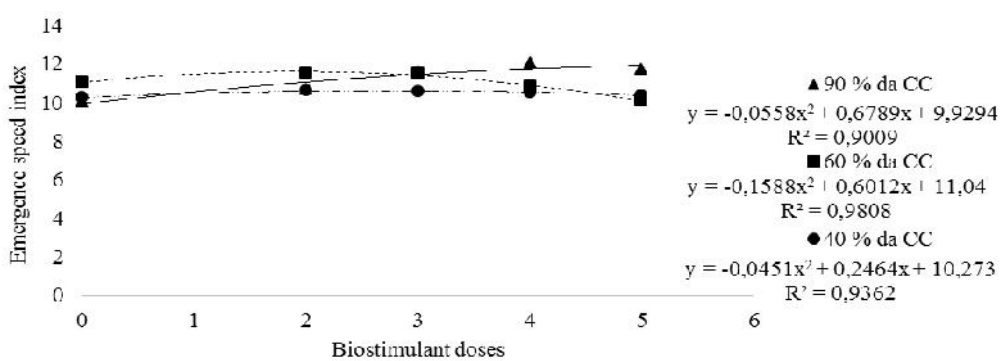

Figure 1. Response of the emergence speed index of seedlings subjected to biostimulant doses and different field capacities.

Table 2. Mean values of the biostimulation x field capacity interaction breakdown for emergence speed index (IVE)

Biostimulant	Field capacity		
	IVE		
	90%	60%	40%
0	10.02 Bb	11.05 Aa	10.26 Ba
2	10.70 Bb	11.52 Aa	10.40 Ba
3	11.64 Aa	11.54 Aa	10.50 Ba
4	12.02 Aa	10.83 Bab	10.57 Ba
5	11.55 Aa	10.09 Bb	10.64 Ba

Mean values followed by different letters, uppercases in the same row, and lowercases in the same column are significantly different by Tukey's test at 5% probability.

For shoot dry matter of emerged seedlings (MSPAE) (Figures 2 and 3), there was a significant effect of the isolated factors. The higher water availability implied in the maximum absorption of water and consequently higher weight of the dry mass. Similar results were reported by [9], with the EMBRAPA 122/V-2000 sunflower, in which they concluded that under conditions of water availability of 80 to 100% water retention capacity, the cultivars showed better performance than when subjected to 60% field capacity

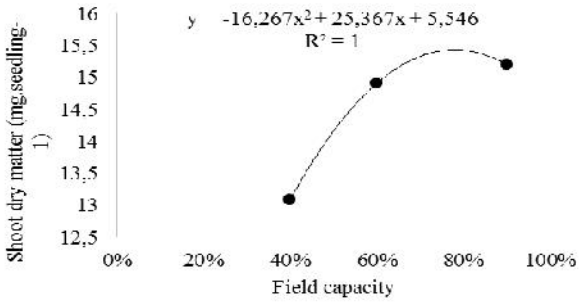

Figure 2. Response of the shoot dry matter of emerged seedlings subjected to different field capacities.

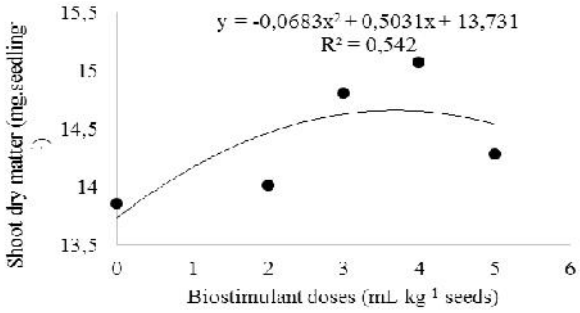

Figure 3. Response of the shoot dry matter of emerged seedlings subjected to different doses of biostimulant.

For the doses of biostimulant, the MSPAE (Figure 3) presented increasing behavior up to the dose of 4 mL. Thereafter, a decline of 5 mL was found, representing a 9% drop in MSPAE in relation to the maximum concentration applied. Thus, up to the 4 mL dose, the biostimulant promoted better seedling performance. [18] also noticed that the treatment of soybean seeds with biostimulants favored the shoot dry matter of the plant.

2° Trial– Interaction Bioestimulant x Polyethylene glycol 6000

There was a significant effect of the Biostimulant (Bioest) x Polyethylene glycol 6000 (Peg) interaction, for germination (G), first germination count (PCG), shoot length (CPA), root length (CPR) and dry matter of root (MSR) and shoot dry matter (MSPA) (Table 3).

Table 3. Summary of analysis of variance for the germination percentage (G), first germination count (PCG), seedling shoot length (CPA), primary root length (CPR), shoot dry matter (MSPA), root dry matter (MSR), subjected to the treatment of seeds with different doses of biostimulant and osmotic potential.

SV	DF	QM					
		G (%)	PCG (%)	CPA (mm.seedl^{-1})	CPR (mm. seedl^{-1})	MSPA (mg. seedl^{-1})	MSR (mg. seedl^{-1})
Bioest	4	1068.4**	331.4**	116.7*	3015.2**	1.3^{ns}	1.5^{ns}
Peg	2	6247.6**	32415.2**	18592.9**	5055.1**	246.6**	77.1**
Bioest*Peg	8	318.7**	337.4**	101.1*	1473.2**	2.9^{ns}	2.4**
Error	45	46.0	35.0	34.7	132.6	1.2	0.8
C.V. (%)		9.7	14.9	12.9	12.4	12.7	16.0
Mean		69.7	39.7	49.6	92.6	8.6	5.6

^{ns}Non significant, *Significant at 5% and ** Significant at 1% probability by F-test. Seedling (seedl), Biostimulant (Bioest), polyethylene glycol (Peg), Interaction between Bioestimulant and polyethylene glycol (Bioest x Peg).

For germination and first count (Figures 4 and 5, Table 4), there was an upward trend up to a maximum of 4 and 3 mL, respectively, decreasing after these doses, thus, at potentials -0.2 and -0.4, the dose of 5 mL showed less efficiency for the response of these variables. The stress condition provided by these two potentials may have contributed to the 5 mL dose starting to cause seed toxicity, impairing vigor and germination, since the hormones can promote or inhibit changes in the plant, for this to occur, there must be sufficient quantity in the appropriate cells for desired physiological effect [19]. It was also observed that in conditions without application of PEG, there was greater uniformity of G and GPC at all doses studied

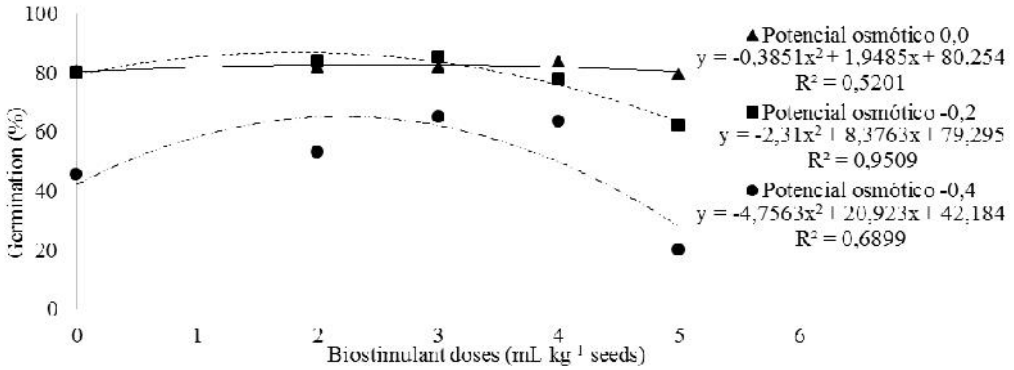

Figure 4. Response of germination of seedlings subjected to biostimulant doses and different osmotic potentials.

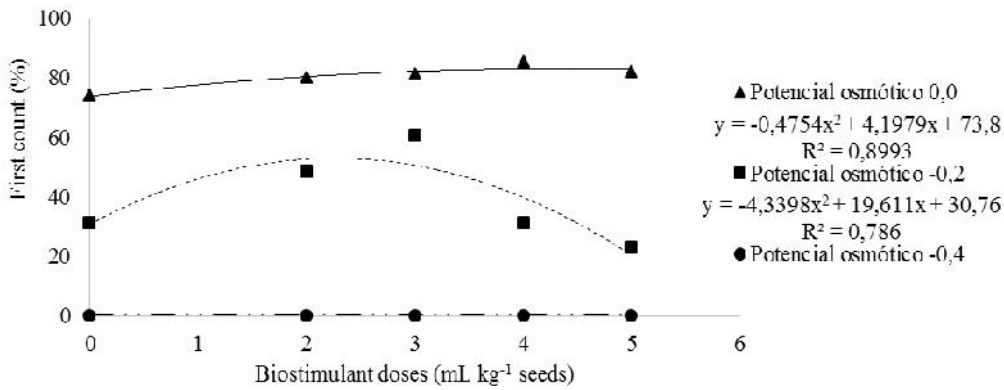

Figure 5. Response of the first count of seedlings subjected to doses of biostimulant and different osmotic potentials.

Table 4. Mean values of the biostimulation x polyethylene glycol interaction breakdown for germination (G), first germination count (PCG)

Bioest	Polyethylene glycol								
	G			PCG			MSR		
	0	-0.2	-0.4	0	-0.2	-0.4	0	-0.2	-0.4
0	80 Aa	80 Ba	45 Bb	74 Aa	31 Bc	0 Ca	7 Aa	5.72 Bb	2.42 Cb
2	82 Aa	84 Aa	53 Bab	80 Aa	48 Bb	0 Ca	7 Aa	6.47 Aab	2.42 Bb
3	82 Aa	85 Aa	65 Ba	81 Aa	60 Ba	0 Ca	6 Aa	6.67 Aab	3.57 Bab
4	83 Aa	78 Aa	63 Ba	85 Aa	31 Bc	0 Ca	6 Aa	6.90 Aab	4.05 Bab
5	75 Aa	62 Bb	20 Cc	82 Aa	23 Bc	0 Ca	6 Aa	7.63 Aa	4.45 Ca

Bioest	CPR			CPA		
	0	-0.2	-0.4	0	-0.2	-0.4
0	73.25 Ba	35.42 Cc	93.00 Ab	79.00 Aa	35.86 Bb	10.25 Ca
2	81.07 Ba	78.47 Bb	103.2 Ab	79.15 Aa	43.32 Bab	13.42 Ca
3	83.40 Ba	102.15 ABa	110.62 Aab	79.37 Aa	50.30 Ba	16.97 Ca
4	83.05 Ba	118.47 Aa	111.0 Aab	76.7 Aab	51.30 Ba	17.65 Ca
5	66.32 Ba	122.00 Aa	127.92 Aa	66.6 Ab	47.00 Bab	17.50 Ca

Mean values followed by different letters, uppercases in the same row, and lowercases in the same column are significantly different by Tukey's test at 5% probability.

In the osmotic potential -0.4 of the first germination count (Figure 5), a mean of 0 was observed at all doses, due to the lower availability of water for the seed. The interference on vigor may be the response to high viscosity caused by the mixture of polyethylene glycol, whose general formula is $HOCH_2(OCH_2CH_2)_nOH$, and water that moistens the substrate, limiting the availability of oxygen to the seeds [20]. This action has a negative effect on sunflower germination processes that depend on the presence of water and oxygen for the proper functioning of plant metabolism [21].

For shoot length (CPA, Figure 6), a rising curve was observed in all osmotic potentials up to the 4 mL dose when there was a reduction in biostimulant efficiency in most of the potentials studied. This may possibly be justified by phytotoxicity of the embryo, which at doses of 5 mL maximizes negative effects of abiotic stress, by decreasing the efficiency of the plant hormones used [17]. Thus, the beneficial action of the biostimulant in the stress situation caused by PEG is noted. [22], concluded that applications of hormones directly on seeds promoted the emergence of vigorous seedlings with longer length.

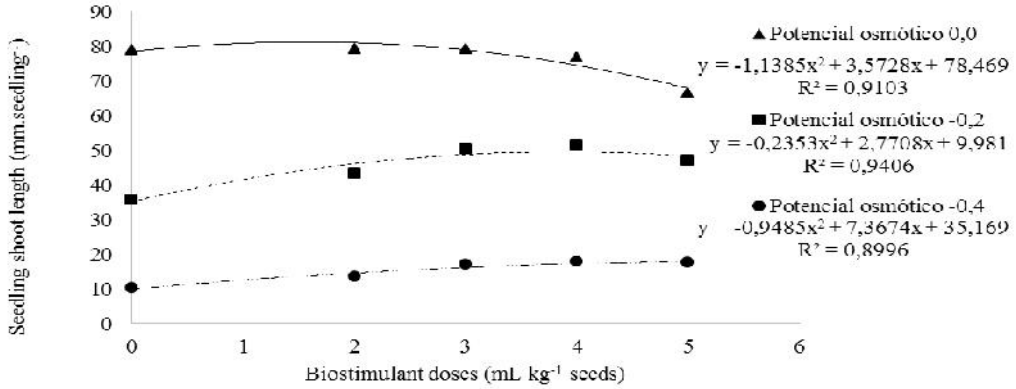

Figure 6. Shoot length of seedlings subjected to doses of biostimulant and different osmotic potentials.

As for the root length (CPR) (Figure 7), the maximal effect of the hormones kinetin, gibberellin and indolebutyric acid occurred under stress conditions promoted by PEG, which contained low water availability. This result was due to water stress, which often negatively interferes with stem growth and leaf expansion, but increases root elongation [15]. This, associated with the action of the doses of biostimulant, favored the development of the root system, resulting in a larger length in this variable. Other authors have obtained similar results, where the application of plant stimulants favors root growth, exhibiting rapid recovery after exposure to water stress [23], [24] [12].

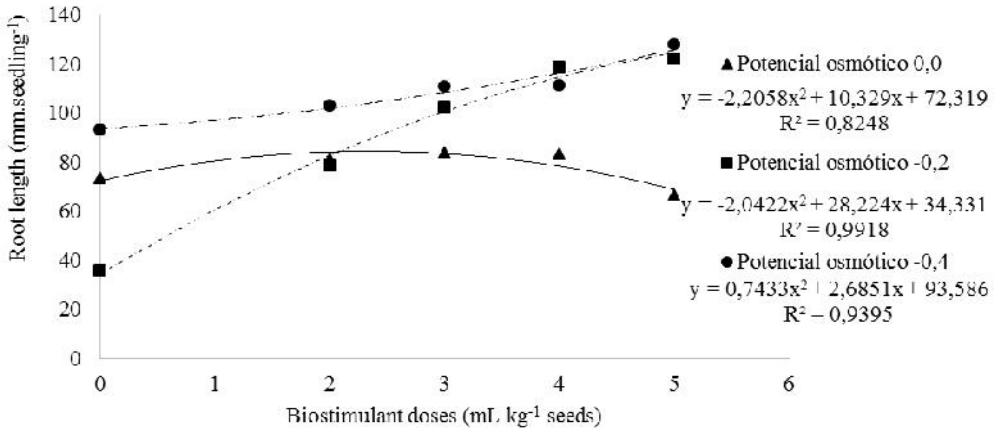

Figure 7. Root length of seedlings subjected to doses of biostimulant and different osmotic potentials.

For the root dry matter (MSPR) (Figure 8), there was an increase in the production at all doses of the biostimulant when subjected to stress with PEG, which did not occur in the osmotic potential 0. This confirms the previously presented results for CPR, and may have been due to the presence of plant hormones contained in the biostimulant that provides the capacity to stimulate root growth, increasing the assimilation of water and nutrients, thus favoring the balance of seedling metabolism [15]. [20] observed that biostimulant doses promoted higher root growth in corn plants subjected to different osmotic potentials, and this result is pertinent, since plants with a well-developed root system show better performance when exposed to osmotic stress.

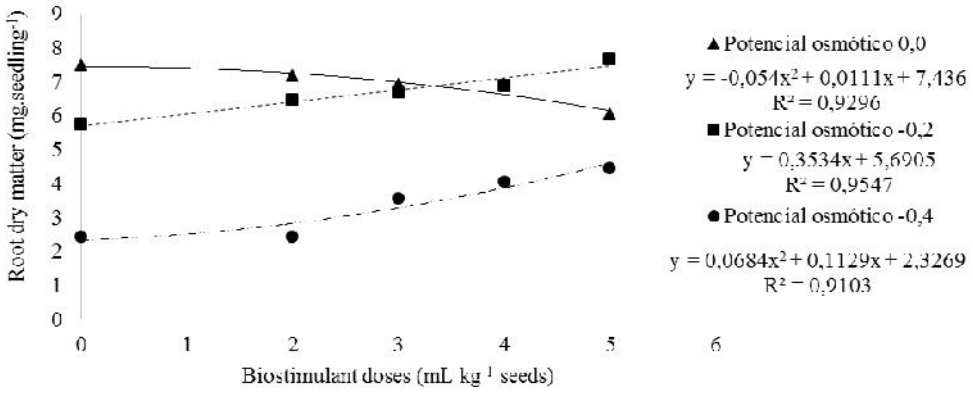

Figure 8. Root dry matter of seedlings subjected to doses of biostimulant and different osmotic potentials.

With respect to shoot dry matter (MSPA) (Figure 9), there was only effect for the PEG factor, with progressive increase in potentials -0.2 and -0.4, the latter being the maximum response point of this characteristic. This result is the inverse of the CPA and can be explained because the cells have signaling molecules, which under stress conditions are activated by distension, causing in some situations changes in the seedling volume as a stress defense mechanism to which it was subjected [15].

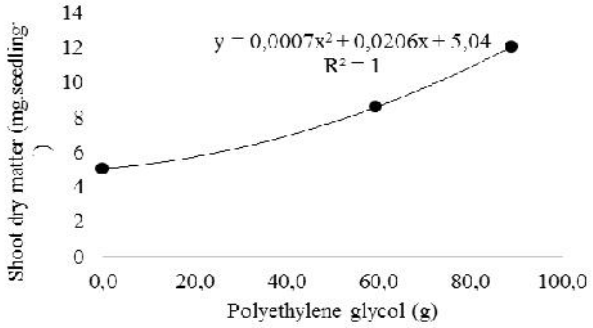

Figure 9. Shoot dry matter of seedlings subjected to doses of biostimulant and different osmotic potentials.

3° Trial –Biostimulant x Temperature Interaction

The number of normal seedlings (PN) (Table 5) was significantly influenced by temperature. It is observed a higher presence of normal seedlings at lower temperature (Figure 10). This response can be explained by the maximization of the genotype expression under adverse situation associated with the biostimulant, promoting good results in the number of PN. Thus, there may have been a positive stimulus of the hormones used in the seeds exposed to the low temperature, thus increasing the stress tolerance and consequently improving the potential of the crop [25].

Table 5. Summary of analysis of variance for normal seedlings subjected to seed treatment with different doses of biostimulant and temperatures.

SV	DF	QM
		PN
Bioest.	4	77.77^{ns}
Temp.	1	855.62^{*}
Bioest*Temp.	4	71.87^{ns}
Error	30	59.36

C.V. (%)	8,94
Mean	86.17

[ns]Non-significant and [*]Significant at 5% probability by F-test.

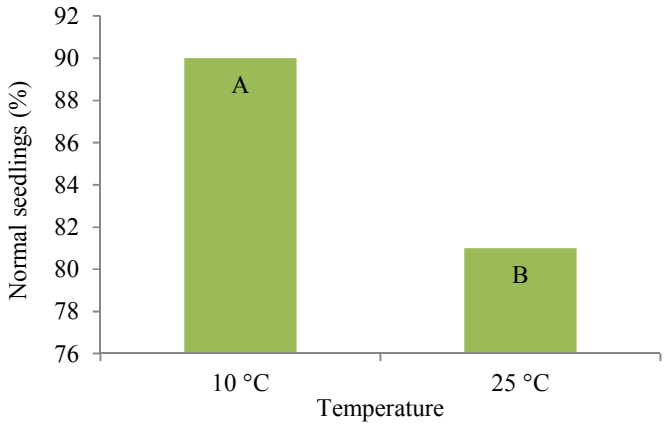

Figure 10. Normal seedlings subjected to biostimulant doses and temperatures.

Despite the absence of normal seedlings at the first count, no fungal infection was observed in the seeds, which probably contributed to the high percentage of normal seedlings in the second count. After changing the temperature, the plants germinated obtaining good results. In a study by [8] with application of biostimulant on corn and beans, the authors concluded that under stress conditions, biostimulants can maximize their effects, since they are constituted by hormones that can be beneficial in plant defense and in the growth and development of plants.

4. CONCLUSION

The use of biostimulant at 3 and 4 mL increases most early growth traits of the seedlings under the experimental conditions of the present study, both when exposed to different water retention capacities and at different osmotic potentials.

The biostimulant maximizes the germination potential of sunflower seeds subjected to low temperatures.

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