

Physiological quality of *Cucumis melo* seeds under saline stress and application of salicylic acid

ABSTRACT

Aim: The aim of this study was to evaluate the physiological quality of seeds of two varieties of melon (*Cucumis melo*) in response to saline stress and the application of salicylic acid.

Study design: The experimental design was a completely randomized design with five water salinities and five doses of salicylic acid, with four replicates of 50 seeds.

Place and Duration of Study: The experiment was carried out in the Laboratory of Seed Analysis, located in the Center of Agricultural Sciences of the Federal University of Paraíba, Areia-PB, Brazil.

Methodology: The experiment was conducted with two cultivars of melon (Cantalupensis and Eldorado 300). The experimental design was a completely randomized design with five water salinities ($EC_w = 0.0, 1.74, 6.0, 10.26$ and 12.0 dS m^{-1}) and five doses of salicylic acid ($SA = 0.0, 0.29, 1.0, 1.71$ and 2.0 mM), with four replicates of 50 seeds. The following variables have evaluated: percentage of germination, first germination count, percentage of abnormal seedlings, germination speed index, mean germination time, root length, shoot and seedling dry mass.

Results: The use of salicylic acid was not efficient to attenuate the deleterious effects of saline stress on seed germination. Cantalupensis cultivar is more tolerant than the salinity effects of Eldorado 300.

Conclusion: Saline stress negatively influences the germination and vigor of melon seeds.

Keywords: Melon; mitigation; salinity; seed vigor.

1. INTRODUCTION

Melon (*Cucumis melo* L.) is considered one of the main cultivated species in the Northeast region, possessing high economic value and high adaptability to semi-arid conditions. Despite the high adaptation of the melon, in arid and semi-arid climates there are problems on its cultivation, mainly by the use of waters with high salt concentration [1]. One of the ways of mitigating the effects caused by salinity is to achieve adequate water management, as well as to adopt technologies that minimize the deleterious effects of salinity [2].

Salinity affects plant growth at all stages of development, with most crops being more sensitive during the germination phase [3]. In the literature, there are several studies on the effect of salinity on the germination of the melon [3,4,5]. These authors verified that the melon germinates under considerable saline condition, however the increase of saline stress leads to the reduction of the germination rate and the seedlings development.

Therefore, a series of researches has carried out with the purpose of developing techniques that attenuate the effects caused by saline stress. Among these studies, the use of salicylic acid [6,7], a substance synthesized from L-phenylalanine, which, through the action of phenylalanine ammonium lyase (PAL) trans-cinnamic acid, then converted to benzoic acid and finally converted to salicylic acid through the action of the enzyme benzoic acid-2-hydroxylase. Salicylic acid has involved in several plant processes and have reported with biotic and abiotic stress reliever [8]. Therefore, the aim of this work was to evaluate the germination of two varieties of melon (*Cucumis melo* L.) in response to saline stress and the application of salicylic acid.

2. MATERIAL AND METHODS

Local and plant material

34 The experiment was carried out in the Laboratory of Seed Analysis (LSA), located in the Center of
35 Agricultural Sciences of the Federal University of Paraíba (CCA/UFPB), Campus II, Areia, PB, Brazil.

36 The experiment has conducted with two species of melon, a Creole cultivar (cucumber melon
37 Cantalupensis) and the commercial cultivar (Eldorado 300). The cucumber melon seeds has
38 harvested from completely mature fruits produced in the rural area of the municipality of Santa Luzia,
39 PB, Brazil. The seeds have removed and washed in running water and dried on paper towel sheets at
40 room temperature for 7 days. The seeds of the cultivar Eldorado 300 have obtained from the market
41 in the city of Areia, PB.

42 **Experimental design and treatments**

43 The experimental design was a completely randomized design, with five water salinities ($EC_w = 0.00$,
44 1.74 , 6.00 , 10.26 , 12.00 $dS\ m^{-1}$) and five salicylic acid doses ($SA = 0.0$, 0.29 , 1.0 , 1.71 and 2.0 mM),
45 with four replicates of 50 seeds, totaling in 9 combinations generated through the Central composite
46 design (CCD) suggested by Box and Wilson [9].

47 Saline solutions have prepared using a mixture of the following salts: sodium chloride ($NaCl$), calcium
48 chloride di-hydrate ($CaCl_2 \cdot 2H_2O$) and magnesium chloride hexa-hydrate ($MgCl_2 \cdot 6H_2O$) in a ratio of 7:
49 2: 1. This ratio was equivalent to the amount of salts predominant in the main sources of available
50 water for irrigation in the Northeast region of Brazil. The doses have dimensioned with the use of
51 portable conductivity meter.

52 With regard to salicylic acid, the doses were prepared from the dilution in 200 mL of distilled water
53 and placed in plastic containers containing the seeds. After the eight-hour imbibition period, the seeds
54 have washed with distilled water to remove excess acid.

55 **Variables analyzed**

56 The germination test has carried out in a BOD (Biochemical Oxygen Demand) type germination
57 chamber, regulated at a constant temperature of $25\ ^\circ C$ and photoperiod of 12 hours, with as
58 substratum Germitest® paper sheets moistened with distilled water 2.5 times the weight of dry paper.
59 The germination was evaluated from the 4th to the 8th day after the test, considering the germination of
60 root protrusion and leaf primordia, with results expressed as percentage of normal seedlings [10].

61 The first germination count has performed on the 4th day after the germination test, with the results
62 expressed as percentage of germinated seedlings. The germination rate index has obtained by daily
63 counts of the number of germinated seeds, calculating the results by the formula proposed by
64 Maguire [11]. The average germination time was evaluated from germinated seed count daily, and the
65 results were calculated using the formula proposed by Labouriau [12].

66 After 8 days of the germination test, the percentage of abnormal seedlings has evaluated, considering
67 those that presented some abnormality. It was also evaluated the growth of aerial part, root and
68 seedlings made with the aid of graduated ruler being the results expressed in centimeter. At the end
69 of the experiment, dry mass of the seedlings has obtained by the forced-air circulation oven drying at
70 $65\ ^\circ C$ until reaching the constant weight, and the seedlings have packed in bags of Kraft paper and
71 put to dry in a greenhouse. Subsequently, the seedlings have weighed in a precision analytical
72 balance, with the results expressed in g seedling⁻¹.

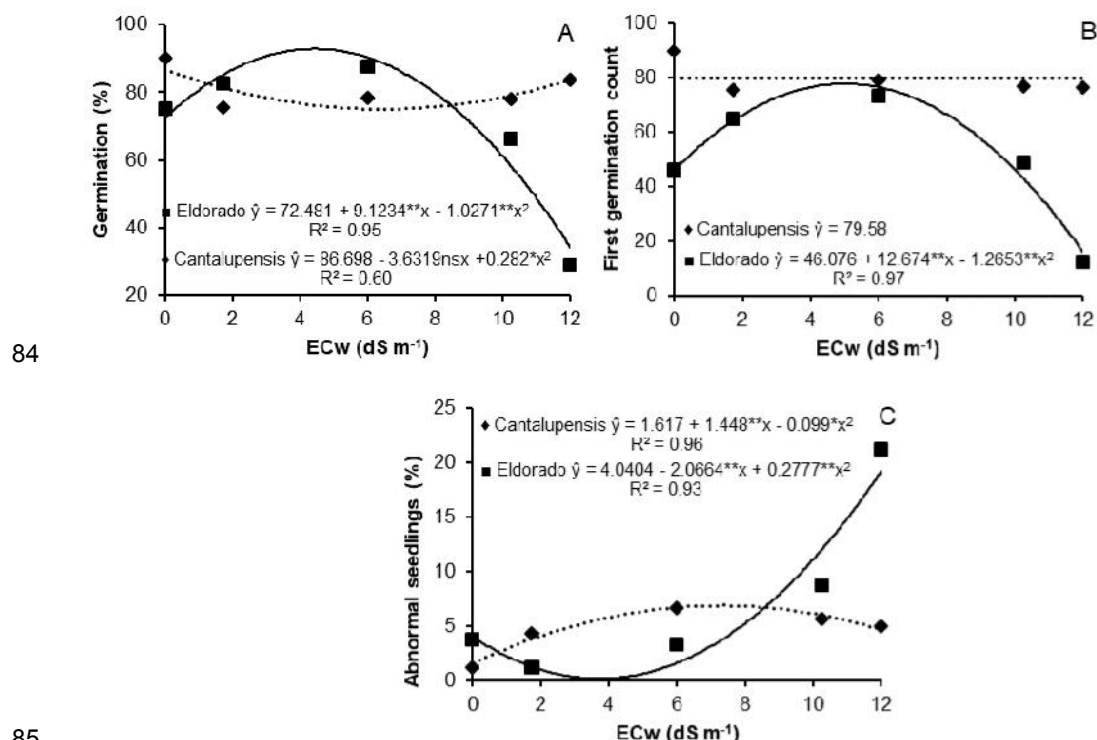
73 **Statistical analysis**

74 Data have submitted to analysis of variance and regression analysis using the SAS University
75 statistical program [13].

76 **3. RESULTS AND DISCUSSION**

77 For the germination percentage, the varieties showed a quadratic behavior, where the cucumber
78 melon (*Cucumis melo* var. Cantalupensis Naud) obtained the highest salinity values of $0.0\ dS\ m^{-1}$,
79 while the Eldorado cultivar showed the best performance in the submitted seeds and salinity of 4.4
80 $dS\ m^{-1}$, occurring from this severe reduction value with increasing salt concentration (Figure 1A).

81 However, when correlating the lowest results, reductions of 3 and 38.4% have observed between the
82 seeds submitted to the lower and higher salinity waters for cucumber melon and to Eldorado cultivar,
83 respectively.



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Figure 1. Percentage of germination (A), first germination count (B) and percentage of abnormal seedlings (C) of two varieties of melon (*Cucumis melo* L.) submitted to different salinities of water.

**** $P < 0.01$; * $P < 0.05$; ^{ns} $P > 0.05$**

91 Salinity tolerance is variable between species and within the same species, being dependent on
92 factors such as cultivar, edaphoclimatic conditions, cultural management, irrigation, intensity and
93 duration of saline stress [14,15,16]. Creole species, such as cucumber melon, have higher resistance
94 to stresses, due to their high genetic variability, promoting plant's ability to adjust osmotically salt
95 stress [17].

96 Seed vigor evaluated by the first germination test showed that the cucumber melon had a higher
97 tolerance to salt stress, with a mean value of 78.5% of germinated seeds on the first day of the
98 germination test (Figure 1B). Eldorado cultivar adjusted to the quadratic effect in response to the
99 different concentrations, with the highest value in the seeds submitted to ECw of 5.0 dS m⁻¹ with
100 77.8% occurring severe reduction with the increase of salinity, reaching losses of 61.8% in the highest
101 ECw.

102 The occurrence of this severe reduction has related to the decrease of water absorption capacity by
103 the seeds, reflecting the number of seeds germinated on the first day of counting. According to Munns
104 and Tester [18], the aggressiveness of the salts is initially installed during the germination process,
105 where the low availability of water promoted by the reduction of the osmotic potential and the high
106 salts contents can severely compromise the germination capacity of the seeds [19].

107 For the percentage of abnormal seedlings, obtained results to the studied varieties have adjusted to
108 the quadratic effect, being the Eldorado cultivar more significantly affected by the concentration of 6.0
109 dS m⁻¹, reaching the largest number of abnormal seedlings with 19.2% in the highest ECw (Figure
110 1C). The effects of the salts present in both soil and irrigation water can be manifested by reducing

the osmotic potential and by the toxic action of some elements, especially Na^+ and Cl^- , which promote physiological disturbances to the plant [19]

The rate of germination was also influenced by salinity, where the cucumber melon was more tolerant to salinity, with an average value of 5.26 (Figure 2A). However, seeds of Eldorado cultivar were less vigorous, with the values adjusting to the quadratic model, where the increase of the salinity reduced the germination process speed from the ECw of 3.5 dS m^{-1} .

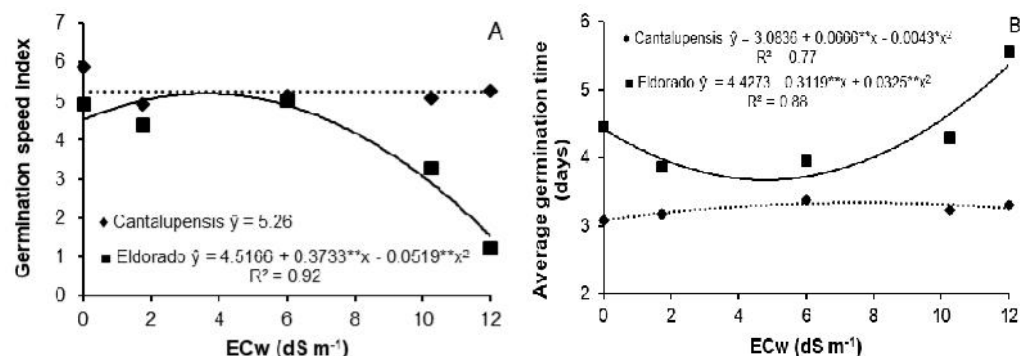


Figure 2.

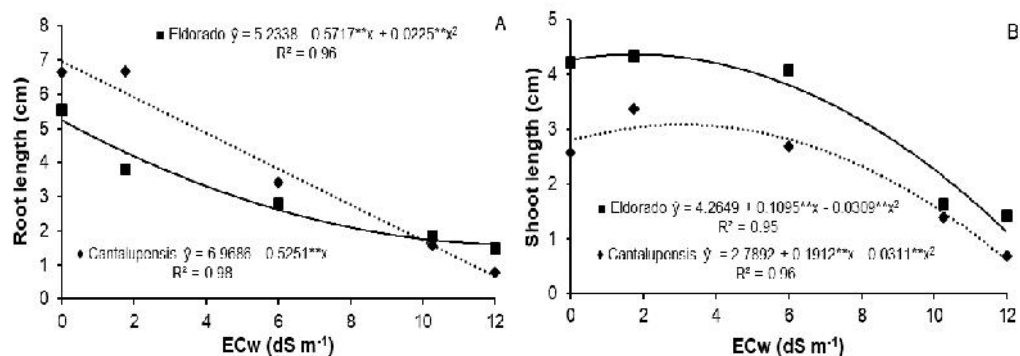
Germination speed index (A) and average germination time (B) of two varieties of melon (*Cucumis melo* L.) submitted to different salinities of water.

**** $P < 0.01$; * $P < 0.05$**

The occurrence of this behavior is mainly due to the water restriction imposed by the high salt content, resulting in the retardation of germination. The excess of salts affects the germination of seeds in function of promoting changes in the kinetics of water absorption [20]; followed by the reduction of hydrolysis of reserves and the ability to translocate them to the embryonic axis [21], resulting in reduced germination speed and seeds.

Mean germination time values for both cultivars have adjusted to the quadratic effect, and the cucumber melon presented a higher germination speed when compared to the Eldorado cultivar, independent of the ECw to which it was submitted (Figure 2B). This behavior demonstrates that the seeds of cucumber melon are more vigorous, resulting in a faster germination process. During germination, proteins contained in the cotyledons are degraded and consumed almost entirely, resulting in protrusion of the radicle [22]. Therefore, the bigger the germination speed the bigger the reserve capacity and most vigorous the seedling will be [23].

According to root length, aerial part and seedlings, it has verified that the increase of the salinity influenced negatively the development of the two cultivars of the melon (Figure 3). For root length, linear decreases have observed for Eldorado cultivar, ranging from 5.54 to 1.49 cm in the salinity levels of 0.0 to 12.0 dS m^{-1} , with a decrease of 73.1% between the lowest and the highest ECw. For cucumber melons, obtained values have adjusted to the quadratic effect, ranging from 6.67 to 0.77 cm in the ECw from 1.74 to 12.0 dS m^{-1} , decreasing 88.5% (Figure 3A).



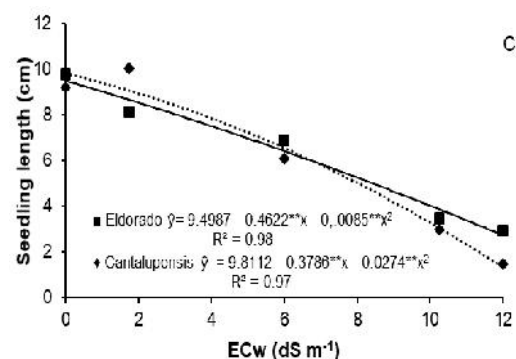


Figure 3. Root (A), shoot (B) and seedling (C) lengths of two varieties of melon (*Cucumis melo* L.), submitted to different salinities of water.

**** $P < 0.01$**

According to the length of the shoot, a quadratic effect has observed for salinity of the water, independent of the varieties (Figure 3B). For the Eldorado cultivar, values varying between 4.34 and 1.43 cm have observed in the salinities of 1.74 to 12.0 dS m⁻¹, with a reduction of approximately 67.1% between the highest and lowest salinity (Figure 3B). While for cucumber melon, it has observed that the values ranged from 3.37 to 0.69 cm in saline levels of 1.74 to 12.0 dS m⁻¹, with a total decrease of 79.6% between the lowest and highest level of water salinity (Figure 3B).

The root and shoot length were inhibited by the high concentrations of salts in the water, being observed a bigger reduction of the aerial part due to the increase of the salinity. Fageria et al. [24] emphasized in short and long-term experiments the aerial part more sensitive to salinity than the root system.

For seedlings length, the quadratic effect of different saline levels has observed in both varieties (Figure 3C). For cucumber melon values ranging from 10.05 to 1.47 cm have obtained in the ECw from 1.74 to 12.0 dS m⁻¹, reducing 85.3% (Figure 3C). For the Eldorado cultivar, values ranging from 9.20 to 2.92 cm have recorded in salinities from 0.0 to 12.0 dS m⁻¹, with a total reduction of 68.3% (Figure 3C).

In the presence of saline levels, the inhibition of growth related to the toxicity caused by the salts interfering in the cellular metabolite processes, decreasing or hindering plants growth [25]. In other studies, high concentrations of salts resulted in a reduction in vigorous seedling production, inhibiting initial growth [3,4].

The application of salicylic acid showed no differences for the aforementioned variables, however, their mean values have showed on Table 1.

Table 1. Germination (G), first germination count (FGC), germination speed index (GSI), mean germination time (AGT) and abnormal seedlings (AS) of two varieties of melon (*Cucumis melo* L.) submitted at different doses of salicylic acid.

Salicylic acid (mM)	G ----- (%) -----	FGC	GSI	AGT (days)	AS B
<i>Cucumis melo</i> cv. Eldorado 300					
0.00	78.75	61.25	4.36	4.33	6.25
0.29	81.25	68.75	4.31	3.83	4.38
1.00	66.25	46.25	3.90	4.54	8.33
1.71	67.50	45.00	3.38	4.34	5.00
2.00	88.75	78.75	5.08	3.94	3.75
<i>Cucumis melo</i> var. Cantalupensis					

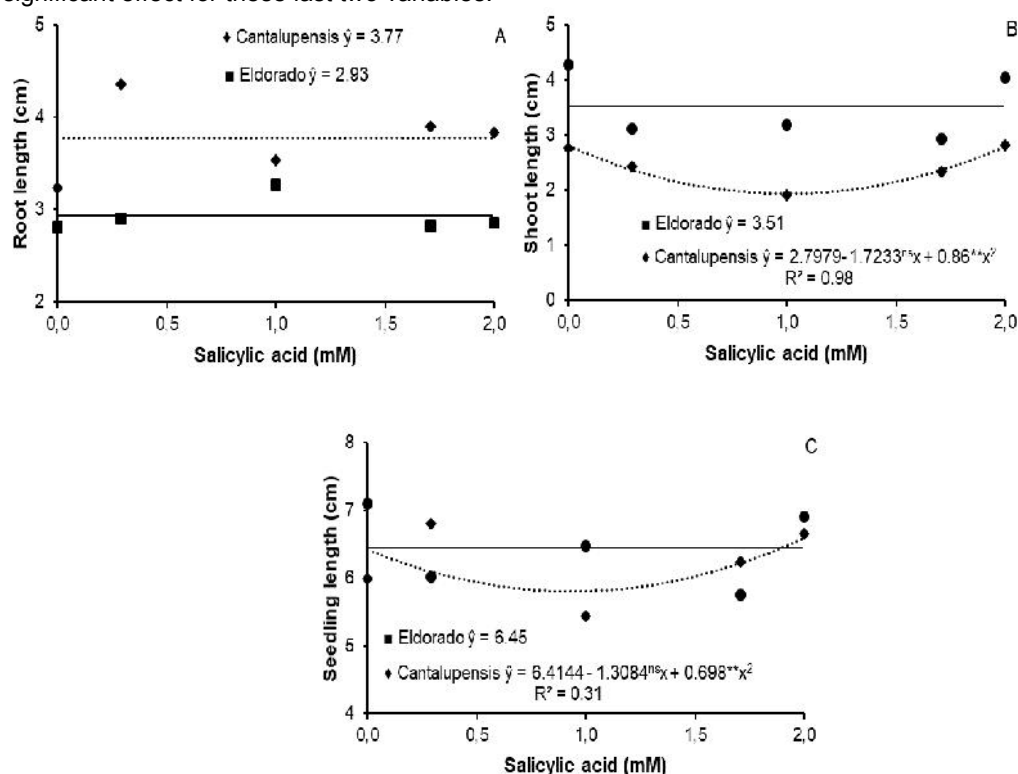
0.00	90.00	90.00	5.88	3.08	1.25
0.29	75.63	75.63	4.90	3.17	4.38
1.00	78.33	79.17	5.15	3.37	6.67
1.71	78.13	76.88	5.09	3.23	5.63
2.00	83.75	76.25	5.27	3.31	5.00

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171 For germination of cv. Eldorado, it has observed that the application of 2.0 mM salicylic acid resulted
 172 in the highest average (88.75%), and the same has observed for the first germination count (78.75%)
 173 and the germination speed index (5.08). Opposite behavior has observed for cucumber melon, where
 174 the highest means for the variables mentioned above have observed in the control treatment (0 mM
 175 SA). For the mean germination time, number of abnormal seedlings and dry matter of seedlings, the
 176 highest averages for both cultivars have observed in the application of 1 mM of salicylic acid, with
 177 reduction occurring with increasing acid dose.

178 Thus, the application of salicylic acid above 1.0 mM can cause negative effects for these variables, in
 179 relation to the two analyzed variables. The exogenous application of salicylic acid does not always
 180 attenuate the deleterious effects of salinity and may vary according to the species, dose studied, form
 181 of application, among other factors [26]. This could explain the fact that this acid did not show a
 182 significant effect on the aforementioned variables.

183 The application of salicylic acid did not significantly affect the root length of any variable for both
 184 studied varieties (Figure 4). However, for aerial and seedling length, the cucumber melon showed an
 185 effect, decreasing to 1.0 mM and increasing from this molarity. The cv. Eldorado did not show
 186 significant effect for these last two variables.



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Figure 4. Root (A), shoot (B) and seedlings (C) lengths of two varieties of melon (*Cucumis melo* L.) submitted to different doses of salicylic acid.

**** $P < 0.01$; $^{ns} P > 0.05$**

Salicylic acid plays a role in several stages of plant growth, with action in expansion, division and cell death, and maintain the balance between growth and age. The exogenous application of this acid induces the expression of genes that encode stress-related proteins [27], which favors the acclimatization of plants to stressful environments.

For dry mass of cucumber melon seedlings submitted to different salinities of the water, they reached a maximum increase of $13.5 \text{ mg plant}^{-1}$ under the concentration of 6.0 dS m^{-1} , occurring reduction in the accumulation of biomass in increase of salinity (Figure 5). This decrease in dry mass has associated with a possible reduction in seed vigor when submitted to higher salt concentrations, where excess salts may have affected dry matter transfer from the reserve tissues to the embryonic axis [28].

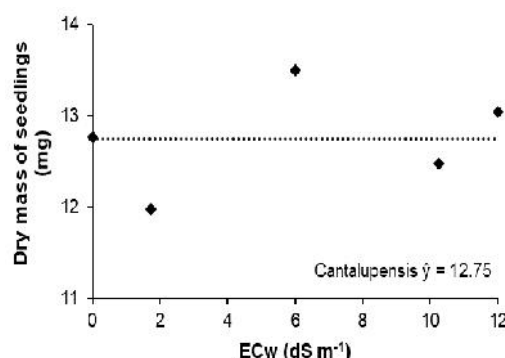


Figure 5. Dry mass of cucumber melon seedlings (*Cucumis melo* cv. Cantalupensis Naud), submitted to different salinities of water.

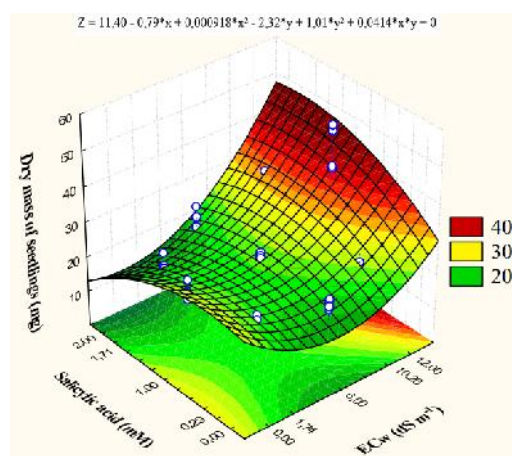


Figure 6. Dry mass of melon seedlings (*Cucumis melo* L. cv. Eldorado), submitted to different salinities of water and doses of salicylic acid.

For dry mass of melon seedlings cv. Eldorado interaction has observed between the factors, and has applied the best result identifying when ECw of 12.0 and 1.0 mM SA. Salicylic acid plays an important role in several plant functions, among them, its action as seed germination, increase in photosynthetic rate, glycolysis, besides regulating cell expansion and division [29]. This acid can promote action of mitigating the deleterious effects of salt stress [30]. This fact has associated to the accumulation of biomass with the increase of doses for both evaluated factors.

4. CONCLUSION

- The stress negatively influences germination and seed vigor of *Cucumis melo*;
- The use of salicylic acid was not efficient to attenuate the deleterious effects of salt stress in the physiological quality of *C. melo* seeds;
- Cucumber melon seeds (*C. melo* var. Cantalupensis) are more vigorous, resulting in a bigger tolerance to the effects of salinity.

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