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The Effect of Azotobacter On Growth Indices, Yield and Essence Content of Two Cultivars of Cumin (*Cuminum cyminum* L.) Under Salinity

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ABSTRACT

In order to investigate the effect of azotobacter on growth indices,grain yield and essence content of two cultivars of cumin (*Cuminum cyminum* L.) under saline conditions, an experiment was carried out in a factorial design arranged as randomized complete block with three replications in 2015 at Kashan University, Iran. Two cumin local varieties including Ardestān and Mashhad Ardehal were used. In a rudimentary experiment, the tolerance threshold was at 150 and 250 mM of Sodium Chloride for both varieties. The result showed that salinity and inoculation with bacteria had a significant effect on the percentage and germination rate, root length, plant height, and seed vigor. Increased concentrations up to 250 mM molar sodium chloride resulted in a significant reduction in germination percentage and germination rate, root length, stem height, seed vigor, grain yield and essence yield. Also, the results presented that in both varieties and at all levels of salinity,inoculation with bacteria compared to the control, lead to a significant increase in chlorophyll (a, b), carotenoids, catalase, ascorbate peroxidase, plant height, grain yield and essence yield. Overall, Ardestan variety showed higher values for plant height, Chlorophyll (a, b), the carotenoids, catalase, ascorbate peroxidase and essence content compared to Mashhad Ardehal variety. In general, the result of this experiment can be used to decrease salinity effects by azotobacter inoculation and increase grain yield and essence content of cumin.

Keywords: Azotobacter, Cumin, Essence, Grain yield, Salinity

INTRODUCTION

The cumin which is cultivated in arid and semiarid area; rain fed and irrigated, is considered as the second exporting economical medical plant in Iran (Alizadegan, *et al*., 2011; Haghirsasat, *et al*., 2011). There are many significant properties for cumin such as diuretic, diaphoretic, appetizer stomach strengthener, mucus enhancer, carminative, anti-cancer, anti-diarrhea controlling female discharge, regulating menstruation in young women,

treats diabetes, and increasing lactation (Saeed najad and Rezvanimoghaddam, 2011; Haghirsadat, *et al*., 2011; Zargari, 1994).

A lot of the cumin planting zones are exposed to salinity. Recently, researchers were able to partially decrease the negative effects of salinity in most types of soil by means of transferring salinity resistance genes to plants or inoculating them in combination with growth stimulant bacteria (Arzanesh, *et al*., 2012; Rojas-Topias, *et al*., 2012; Kreps, *et al*., 2002). The prime example of salinity tolerance mechanisms is mainly due to the result of an increase in antioxidant enzymes such as catalase, ascorbate peroxidase, carotenoids, and chlorophyll protection (Mittova *et al*., 2002). Azotobacter not only consolidate nitrogen, but also synthesis the stimulants growth and antibiotics. Azotobacter holds the most active cytochrom oxidase and dismotase systems. According to these properties, these bacteria can perform some utmost roles in plant stimulating growth and optimizing nutrition (Akhter *et al*., 2012; Koocheki *et al*., 2008). Also, they can be beneficial to the plants through multiple actions. In addition, it leads to germination strength and seedling vigor. Overall, it causes the base plant growth. It is proven that plants seem more sensitive to salinity in germination phase. Generally, a significant decrease of germination and growth is presented. Therefore, where, the salt density increases, a fall in growth value is observed, which presents proper indices for the tolerance determination rate to salinity with respect to different plants (Hasheminia *et al*., 2009). In fact, cumin is defined as a modest plant regarding its need for fertilized soil in order to obtain relative resistance to salinity (Kafi, 2002; Tatari, 2004). Nabizadeh Marvdasht *et al*., (2003) reported that as the salinity increased, the cumin seed performance decreased.

Additionally, other research results showed that applying salinity to cumin created significant negative effects on root length, plant length, and seed vigor (Ekhtiari *et al*., 2010). Ghorbanli *et al*., (2012) reported that by increasing sodium chloride density on cumin, proline, catalaise, ascorbate proxidate, and malondialdehyde would increase. Plants affected synchronously by sodium chloride and ascorbate, represented a high increase rate of proline, catalase, ascorbate proxidate in the sodium chloride density. Rezaei *et al*., (2004) reported that applying azotobacter to cumin, caused higher germination rate in saline conditions. Accordingly, the positive effects raised by stimulating the growth of bacteria on the negative effects of salinity decreased upon plant growth. In accordance with the importance of cumin and the effect of soil or water salinity on its growth, yield and essence content; the impact of azetobacter on reducing the negative effects was studied.

MATERIALS AND METHODS

In order to investigate the effect of azotobacter on growth indices, grain yield and essence content of two cultivars of cumin (*Cuminum cyminum* L.) under saline conditions, an experiment was carried out in a factorial design arranged as randomized complete block with three replications in 2015 at Kashan University, Iran. Two cumin local varieties including Ardestān and Mashhad Ardehal were used. First, in a rudimentary randomized completed blocks design experiment with three ,under the condition of in vitro and pot planting, the

levels of the variety tolerance to salinity was determined using six levels (0, 100, 150, 200, 250 and 300 (mM) of sodium chloride. The results showed that 150 and 250 mM were threshold tolerance for the tow varieties. Also, ($7H_2O$) $MgSO_4$ solution was used to keep the azotobacter (Pierson, 1955). Based on the rudimentary experiment, salinity at 150 and 250 mM sodium chloride levels were used. Sterilized seeds were soaked for 24 hours in a solution of magnesium sulfate azotobacter. Hoagland fertigation containing 150 and 250mM sodium was performed every 5 days regularly. Germinated seeds were counted after planting time and every 5 days during 30 days. Harvesting and other characters within 49 days after bacterial treatment, including percentage and rate of germination, root length , plant length, and seed vigor, ascorbate proxidate and catalase enzymes , chlorophyll (a, b), carotenoid, grain yield, essence percentage and yield essence, were measured. The percentage of germination was calculated as equation (1), (Ellis *et al*., 1981):

(1) Germination percentage (GP) = $\frac{n}{N} \times 100$

Where: n= the number of germinated seeds, N= total seeds.

On the other hand, by adding the total numbers of plumul and radical lengths seedling length was calculated and the induced vigor was obtained as equation 2), (Abdul-baki and Anderson, 1973) :

(2) Seed vigor indices (VI) = $\frac{\text{(plant and root) mean seedling length} \times \text{GP}}{100}$

The rate of catalase enzyme action was calculated as equation (3), (Aebi, 1984):

(3) $EA = \frac{[\Delta OD \times (\frac{1000}{A}) \times B]}{EC \times C}$

EA= represents the Enzymes action rate based on unit weight grams (based on revival of one mm H_2O_2/min), ΔOD=based on presented amount by spectrophotometer, A=the spilled amount into Cuvette. B= added amount of phosphate to the sample in order to pulverize, EC= 0.03mM/cm (Catalase enzyme activity coefficient), C= to pulverize tissue weight in grams.

The ascorbate proxidate activity rates was calculated as follow equation (4), (Nakano and Asada, 1981):

(4) $EA = \frac{[\Delta OD \times (\frac{1000}{A}) \times B]}{EC \times C}$

EA= represents the enzymes action rate based on unit weight grams (based on ascorbate proxidate absorbtion /MIN), EC= 0.028 mM/cm (ascorbate proxidate enzyme activity coefficient). (Arnon, 1949) and (Lichtenthaler, 1987)

Chlorophylls a, b and carotenoid in mg /g were calculated as:

$$Cl\ a = \frac{[(12.7 \times OD663) - (2.68 \times OD645)] \times V}{1000 \times W}$$

$$Cl\ b = \frac{[(22.9 \times OD645) - (4.93 \times OD663)] \times V}{1000 \times W}$$

$$Caratonoid = \frac{(100 \times OD470 - 1.82 \times ch\ a - 85.02 \times chl\ b)}{198}$$

Analysis of variance was done by SPSS software. Means were compared Duncan test at 5% probability level. Figures were drown by Excel.

RESULTS AND DISCUSSION

The results of variance analysis showed that salinity and inoculation with bacteria had significant effects on the germination rate and percentage (Table 1). Salinity of 250 mM led to the most decrease in germination (84% compared to the control) (Table 2). Germination percentage in salinity of 150 mM and the control treatments were not significantly differente. The increase of salinity to 250 mM, significantly decreased germination rate (Table 2). So, inoculation with Azotobacter led to effective germination rate increases compared to control (Table 3). Germination rate increase with bacteria inoculation (37% compared to the control) (Table 2). Ekhtiari *et al*., (2010) also reported that cumin germination rate decreased in salinity conditions. The salinity effect can be adjusted according to plant genotype and varieties (Salami *et al*, 2006). The results showed that when salinity increased to higher than threshold, the nutrition absorption disorder and also nitrogen absorption would decrease; In another study the cumin germination rate and percentage decreased with salinity (Salami *et al.*, 2006). Inoculation with azetobacter leads to significant increase in germination percentage. Although germination percentage decreased by salinity, inoculating by active bacteria would decreased the effect of salinity. It is potentially proven that bacteria are able to produce and change some plant hormones such as giberellin which plays an important role in germination (Hilhorst and Toorop, 1997).

Salinity, inoculating with bacteria and varieties had significant effects on plant and root length (Table 1). Treatment with 150 mM sodium chloride had the highest root length and the increase was 15.6 percent over the control (Table 2). Increasing sodium chloride concentration to 250 mM led to a significant decrease in root length (Table 2). The presence of bacteria increased the root length up to 26.5% compared to no- inoculation treatment. Plant length variation among treatments was similar to root length (Table 2). Generally under high salinity stress, plant length will reduce. Also, inoculation with bacteria compared to the control treatment led increase of to plant length by 22.8 percent (Table 2). Plant length and root length are the most effective and important characters in germination process under salinity stress. Root is in direct contact with soil; therefore, salinity is considered as an obstacle to the root and plant length growth, because of reduction in water absorption. As the osmosis pressure gets higher, the osmosis potential lessens and therefore, less water feed the seeds (Jamil *et al*., 2005). Gilck *et al*. (1998) stated that Rhyzobacteria have the power of production of ACC di-aminase cause an increase in the root length through a reduction of ethylene levels.

Seed vigor was affected by salinity, variety ,bacteria, salinity × variety, and salinity × bacteria. (Table 1). Increase of sodium chloride to 250 mM, led to significantly decrease of seed vigor (Table 2). The control and 150 mM salinity treatment with inoculation by bacteria had significantly more seed vigor (Table 2).

Table 1. Analysis of variance of physiological and biochemical traits, growth characteristics and seed yield.

		MS													
S.O.V	df	Germination percent	Germination rate	Root length	Shoot length	Vigor	Carotenoid	Ascorbate peroxidase	Catalase	Chlorophyll a	Chlorophyll b	Chlorophyll b	Essential oil content	Grain yeild	Essence yeild
Replication	2	17.44	0.03	0.49	0.56	0.541	0.04	0.004	1.63	0.003	0. 003	0. 003	0./004	48.47**	0.03**
Salinity	2	20447.86**	1971.10**	22.83 **	63.91 **	217.55**	7.63 **	11.59 **	428.93 **	2.43 **	0.166 **	0.166 **	0.004 ns	1243.71**	3.09**
Cultivar	1	30.25 ns	1.99 ns	2.15*	29.16**	23.51**	1.47 **	0.72 **	20.56 **	0.21 **	0.045 **	0.045 **	1.013 **	8.84ns	0.06 **
Salinity× Cultivar	2	1.08 ns	79.70**	0.22 ns	5.56**	5.07**	0.04 ns	0.002 ns	4.64 **	0.003 ns	0.006 ns	0.006 ns	0.021 ns	13. 91**	0.026 **
Bacteria	1	1406.25 **	205.92**	41.39**	39.69**	54.1**	3.05 **	3.38**	124.58**	0.65 **	0.114 **	0.114 **	0.364 **	674.96**	1.08 **
Salinity× Bacteria	2	7.58 ns	61.02**	1.15 ns	1.44 ns	6.57**	0.01 ns	0.01 ns	3.37 *	0.002 ns	0.002 ns	0.002 ns	0.00 ns	252.757**	0.32**
Cultivar× Bacteria	1	2.25 ns	7.47 ns	0.25 ns	0.001ns	0.18 ns	0.00 ns	0.03 ns	1.17 ns	0.001 ns	0.007 ns	0.007 ns	0.006 ns	11.07 ns	0.002ns
Salinity× Cultivar× Bacteria	2	5.25 ns	12.84 ns	0.66 ns	0.01 ns	0.002 ns	0.01 ns	0.05 ns	0.35 ns	0.008 ns	0.002 ns	0.002 ns	0.001 ns	27.77**	0.012 **
Error	22	29.14	4.78	0.44	0.49	0.517	0.02	0.03	0.81	0.009	0.004	0.004	0.017	3.11	0.0012
C.V.(%)	-	8.83	14.56	9.23	8.65	12.66	4.37	5.97	5.42	5.93	8.07	8.07	7.43	17.2	18.8

ns, * and **: Non-significant and significant at 5% and 1% levels of probability, respectively.

Table 2. Mean comparisons of physiological and biochemical traits, growth characteristics and grain yield

Salt treatment	Germination (%)	Germination rate(no. /day)	Root height (mm)	Shoot length (mm)	Seed vigor (mm)	Carotenoid (mg/gFW)	Ascorbate (mg/FW)	Catalase (mg/FW)	Chlorophyll a (mg/gFW)	Chlorophyll b (mg/gFW)	Essential oil (%)	Grain yield gr. per plant^{-1}	Essentce yield per plant
Control	86.58 a	26.27a	7.21 b	9.78 a	8.49 a	2.56 c	1.63 c	10.86 c	2.04 a	0.90 a	1.71 a	21.64 a	1.10 a
150 mM	83.3 a	17.72 b	8.54 a	9.17 a	7.76 a	3.49 b	3.07 b	16.23 b	1.63 b	0.78 b	1.75 a	7.07 b	0.39 b
150 mM	13.50 b	1.07 c	5.78 c	5.49 b	0.78 b	4.15 a	3.51 a	22.80 a	1.14 c	0.67 c	1.79 a	2.03 c	0.11 c
With Bacteria	68.19 a	17.41a	8.3 a	9.2 a	6.9 a	3.69 a	3.04 a	18.49 a	1.74 a	0.84 a	1.85 a	14.58 a	0.71 a
No Bacteria	54.09 b	12.63 b	6.1 b	7.1 b	4.45 b	3.11 b	2.43 b	14.00 b	1.47 b	0.73 b	1.65 b	5.92 b	0.36 b

Means followed by the same letters in each column are not significantly different at 5% level, according to Duncan's multiple Range test.

The, reduce seed vigor in saline conditions have been reported in many studies (Fazeli-Kakhki *et al*., 2015; Aflaki *et al*., 2017).

Variance analysis showed that salinity, variety and bacteria had significant effects on chlorophylls a and b and carotenoid, (Table1). Increased salinity caused significant increase carotenoid, where the most and the least amounts belonged to 250 mM salinity and control treatments, respectively. Also, inoculating with bacteria significantly increased carotenoid content (Table 2). Chlorophyll a content decrease significantly by increasing of salinity (Table 2). Chlorophylls a and b contents in Ardestan variety compared to Mashad Ardehal variety was higher (Figure 1). Moreover, inoculating with bacteria compared to control increased the Chlorophylls a and b contents (Table 2). Javadi Poor *et al*. (2013) stated that by increased salinity in some variety of safflower; decreased carotenoid content. Rezaei *et al*. (2004) reported that the amount of chlorophylls a and b in cotton under the stress with different salinity levels, significantly decreased.

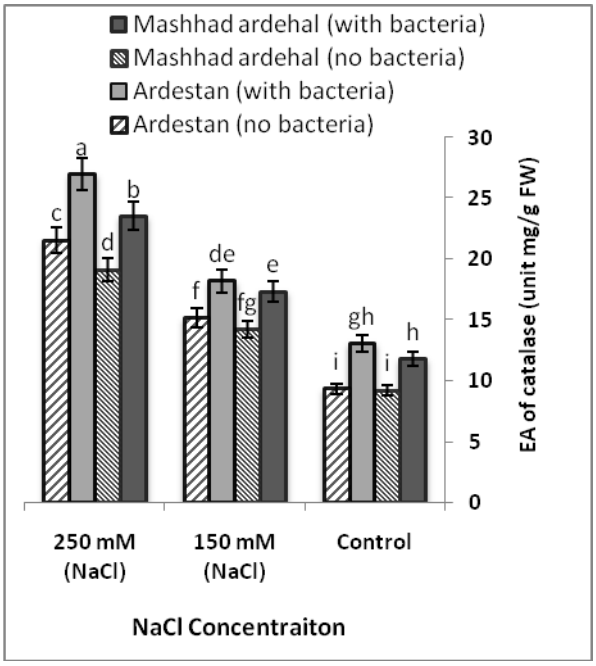

Figure1.Interaction effects of salinity, cultivar and bacterial inoculation

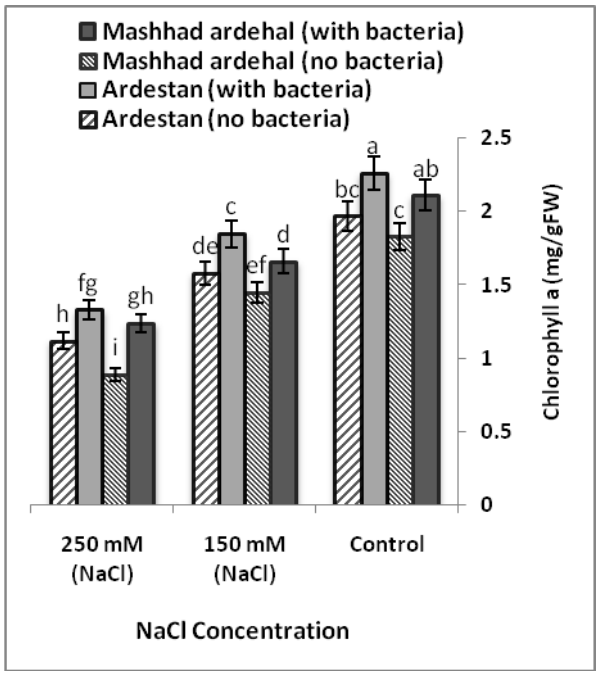

Figure 2.Interaction effects of salinity, cultivar and bacterial on chlorophyll a inoculation on catalase

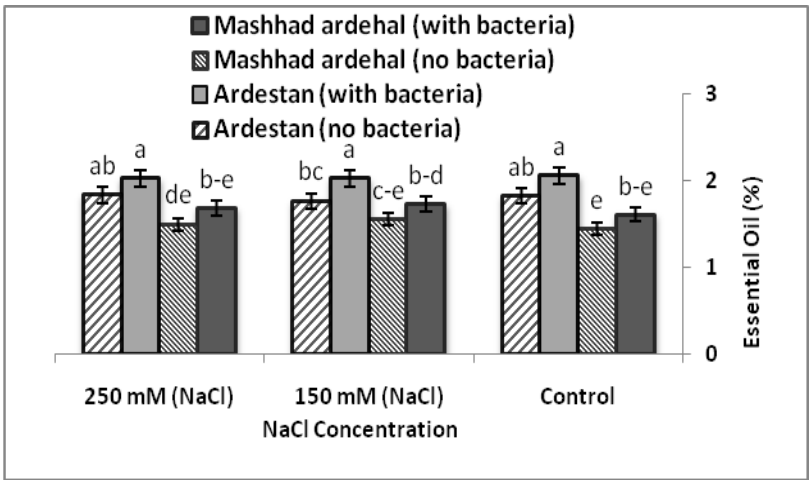

Figure3.Interaction effects of salinity, cultivar and bacterial inoculation on Essence percentage

Catalase and ascorbate proxidase were affected by salinity, variety, and bacteria (Table 1). Salinity compared to control treatment led to increase of catalase enzyme's activity. The most catalase enzyme activity was related to 250 mM sodium chloride treatment (Table 2). Applying bacteria significantly increaed catalase enzyme intense activity (Table 2). Increasing of sodium chloride concentration in the presence of bacteria extended catalase enzyme activity (Figure 2). Increasing salinity, increased ascorbate proxidase enzyme activity, so that the 250 mM sodium chloride and control treatments had the highest and lowest contents, respectively. Ascorbate proxidase enzyme activity was affected by varieties (Table 1). Ascorbate proxidase enzyme content was 10% more in Ardestan varities compared to Mashhad Ardehal. Also, inoculating with bacteria compared to control treatments significantly increased ascorbate proxidase activity (Table 2). Kohler *et al*. (2009) reported that in lettuce seedling (*Lactuca sativa* L.) inoculation with pseudomonas bacteria at the normal condition, decreases the rate of catalase enzyme activity but in stress condition, enzyme activity rate increased significantly. Ghorbanli *et al*. (2012) showed that increase of salinity in cumin increased the rate of ascorbate proxidase activity.

Grain yield was affected by salinity and inoculating with bacteria (Table 1). Varietiy had no significant effect on grain yield (Table 1). Inoculating with bacteria compared to control increased grain yield by 59.39 percent (Table 2). Zabihi *et al*. (2009) observed that by increasing of salinity, yield and yield components were decreased; and with pre-treatment by bacteria these trait will increase. In salvia (*Salvia officinalis* L.), inoculation with azotobacter and azospirillum, increased plant height (Saeed najad and Rezvanimoghaddam, 2010). Stimulating growth bacteria by producing indole acetic acid, giberellin, led to root length increased, root absorption level, and number of root hairs, and nutrition absorption and finally causesd plant health improvement under stress condition (Egamberdieva and Kucharova, 2009).

The essence yield and essence percentage were significantly affected by varieties and inoculating with bacteria (Table 1). However, salinity had no effect on exposed the essence percentage. The variety of Ardestan in comparison to Mashad Ardehal had more essence percentage (Figure 3). Inoculatiion with bacteria increased essence content by10.8 percent (Table 2). Also inoculating with bacteria increased essence yield (Table 2). The observed results showed that bio- fertilizers application increased biological function, grain yield, and essence yield in cumin (Saeed najad and Rezvanimoghaddam, 2010). Bacteria application helps to increase nutrition absorption rate and growth rate, resulting in cumin's increased yield (Saeed najad and Rezvani moghaddam, 2010).

CONCLUSION

The results of this study showed that increasing of salinity decreases the physiological characteristic, contents of chlorophylls a and b, grain yield and essence yield. However, the essence percentage did not affected by salinity. By increasing salinity the amount of carotenoid, catalase, and ascorbate proxidase increased significantly. However, increasing of salinity leads to a reduction in seed germination. It is observed that Ardestan variety tolerates salinity condition more than Mashad Ardehal. In salinity condition inoculating seeds with azotobacter, decreased the negative effects on physiological characteristic. Generally, it is suggested to use azotobacter as bio-fertilizers to decrease the salinity effects on germination, grain yield, and essence yield of cumin.

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