

## Evaluation of Nitrogen Levels and Methanol Spraying on the Yield, Yield Components and Catalase Activities of Sugar Beet in Karaj and Moghan Region

Tohid Nooralvandi<sup>1</sup>, Davoud Habibi<sup>1\*</sup>, Dariush Fathollah Taleghani<sup>2</sup>,  
Ali Kashani<sup>1</sup> and Farzad Paknejad<sup>1</sup>

<sup>1</sup>Department of Agronomy and Plant Breeding, Islamic Azad University, Karaj Branch, Alborz, Iran.

<sup>2</sup> Sugar Beet Seed Institute, Research, Education and Extension Organization, Karaj, Iran.

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One of the main problems about cultivation of sugar beet in Moghan region is the growth of aerial organ, while in Karaj the problems is reduction of sugar cutie and yield that are due to high temperature of harvest time and water stress. Present paper was carried out for 1 year using strip split block experiment in the form of complete accidental blocks with 4 times repetition in 2 different regions; irrigation was done in tape form. Experimental factors included 4 levels of N: non-application, 13, 19 and 25 mg/kg and 4 different varieties of sugar beet were experimented: Pars, Ekbatan, Razor and Flores; and the third factor was methanol spraying in 3 levels: non-application, 10 and 20% by volume. In Karaj region, the most yield achieved due to interaction effect of Razor\*non application of methanol (78043 kg/hectare). In Moghan region, the most root yield achieved due to Pars var.\* 20% by volume of methanol (92286 kg/hectare) that was classified in the same statistical group with Flores\* 10% and 20% by volume of methanol and Pars var.\* 10% by volume of methanol (91296, 85725 and 87543 kg/hectare in order). The result of compound analysis indicated that the main effects of region, N, variety and methanol had significant effect on catalase activity amount in 1% level as well as interaction effects of N\*variety, N\* methanol, variety\* methanol and N\*variety\*methanol. According to the results of experiment in two regions, selection of proper variety can be the most crucial factor beside application of balanced level of methanol and management of N fertilizer at optimum level in order to improve the yield.

**Keywords:** Sugar beet, yield of white sugar, root yield, catalase, Karaj, Moghan.

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Sugar beet is the most important primary material in production of sugar in dry and semi dry regions such as Iran (Mohammadian *et al.*, 2001). The process of accumulation of sugar in plant's root in Moghan region is different with other areas where sugar beet is cultivated in spring season, and does not obey the common pattern of sugar enhancement in root from cultivation to harvest time. It follows an ascending route in the middle of summer and then reduces. In this region, leaf

growth is very rapid in the primary stages and reaches to the maximum leaf index soon, but plant cannot maintain its aerial organ, therefore, plant leaves fall suddenly in the middle of summer and then after the passage of this critical period, the secondary growth of aerial organ starts in the plant, i.e. new leaves appear rapidly. Appearance of each leaf in plant needs consumption of root sugar and reduces root sugar percentage (Gohari *et al.*, 1992; Talaghani *et al.*, 2000). In the other hand, cultivation of sugar beet in Karaj has been accompanied with reduction of cultivation area and this issue has led to closure of sugar factories in this region. In the other hand, agronomical

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\* To whom all correspondence should be addressed.

management is not correct in some years and weather is not proper as well. A study that was carried out to consider sugar yield and its components reactions in various cultivation dates in Karaj showed that higher temperature of August and September is improper, because the temperature produces new leaves and causes to use existed root resources (Habibi, 2002). N is the most crucial nutrition that is required for sugar beet that is used more than other elements (Weeden, 2000; Salardiny, 2005). Its amount and consumption manner both affect crops' quality and quantity (Hills *et al.*, 1978). So that, absorption of high amount of N from the soil increases root impurities and reduces achieved sugar amount (Cattanach *et al.*, 1993). Therefore, overuse and unprincipled consumption of sugar beet reduces efficiency of used fertilizer and this is the most important factor of underground water contamination (Hills *et al.*, 1978). As a summary, management of soil N is a key for balancing crops quality and quantity (Hauck, 1984). Therefore, it seems that exact management of N (timing and determining consumption amount) has a key role in material transference pattern (Webb *et al.*, 1997). Carried out studies on different amounts of labelled N indicated that about 50% of used N is absorbed by plant; 20% remains in the soil and 30% becomes unavailable for the plants through washing and denitrification (Draycott, 1993). Proper amount of consumed N depends on various factors such as the rest amount of N in the soil, time and application of N, farmer management level, previous cultivation and details of contract between farmer and company (Blaylock, 1995). Those ways that increase stability of CO<sub>2</sub> in agronomical plants can be proper solutions for enhancement of yield and plants biomass (Nassiri-Mahalati, 2006). In recent studies, application of methanol has spread as a carbon resource for agronomical plants (Benson and Fall, 1994; Downie *et al.*, 2004; Arizona Department of Agriculture, 1993). Methanol is a material that increases CO<sub>2</sub> stability in tri-carbonic plants such as sugar beet. Moreover, this material, as a rich resource of carbon, can compensate some stabled carbon that is wasted by photosynthesis under circumstances that optical breathing of plant is done in huge amount (similar to Moghan region). Methanol is produced by pectin de-methylation in cell walls (Fall and Benson, 1996; Galbally and

Kristine, 2002; Nonomura and Benson, 1992).

One of the other works that is done by methanol in the plant is accelerating the production of sugar and amino acids in comparison with CO<sub>2</sub> inside the plants. The important note is that enhancement of methanol concentration in plant tissue affect efficiency if carbon transformation and related metabolic routes (McGiffen and Manthey, 1996; Remberg *et al.*, 2002; Downie *et al.*, 2004).

Nonomura and Benson (1992) concluded that methanol spraying on aerial organs of plant enhances plants growth in dry and warm regions. They observed that methanol spraying prevents plants withering in front of sun light. They attributed growth enhancement to methanol role as a carbonic nutrition and cited that it is a carbonic resource for plant nutrition (Fall and Benson, 1996). Under an environmental improper conditions, free radicals are produced. Free radicals of oxygen have a potential that react with most of the cell compounds and damage membrane and other necessary macro molecules such as photosynthesis pigments, proteins, acid nucleic and lipids (Blokhnia *et al.*, 2002). Therefore, its amount should be controlled in the cell. Plants have anti-oxidant system that contain enzyme compounds (superoxide dismutase, catalase, peroxide, glutathione, peroxidase, ascorbate peroxidase and glutathione reductase) and non-enzymatic (ascorbate acid, glutathione, carotenoids and tocopherols). Usually the levels of free radicals keep the cells' oxygen in a balanced level (Al-Aghabary *et al.*, 2004). Today, some of the researchers believe that enhancement of the amount of anti-oxidants can increase the plants tolerance against environmental stresses (Esfandiari *et al.*, 2008; Asada, 1999; Guo *et al.*, 2005). Studies show that oxidant responses depend on sensitivity and resistance of understudied varieties (Alexiva *et al.*, 2003). As hydrogen peroxide has oxidative effects on plants, it is harmful and is removed by the activity of catalase enzyme. These enzymes preserve cell against hydrogen peroxide effects and have important role in enhancement of resistance in front of oxidative stress under natural conditions (Ames *et al.*, 1993). Anti-oxidant system included multi enzymes such as super oxide dismutase, catalase and guaiacol peroxidase. The produced super oxide radicals has

been transformed to hydrogen peroxide by the function of super oxide dismutase and the activities of ascorbate peroxidase, catalase, Guaiacolperoxidase and glutathione peroxide prevent from accumulation of hydrogen peroxide. Therefore, the balance between production of free radicals and their elimination guarantee system survival (Khatun *et al.*, 2008). These enzymes contribute in some required biological processes for growth, development and preservation (Gaetke and Chow, 2003) and preserve living creatures against oxidative damages (Garczarska and Ratajczak, 2000). Catalase is one of the most important enzymes that plays role in cell peroxides analysis (under environmental stresses). Catalase enzyme exists in cell organs such as mitochondria, peroxisome and glioxisoma (Sinclair, 1985). Enhancement of chlorophyll amount can be attributed to methanol oxidation in bushes that have water lack. Because bushes encounter with oxidative stress under water lack conditions. In this circumstances, methanol is oxidized easily by leaf extract and this work is done by catalase enzyme (Nemecek-Marshall *et al.*, 1995).

## MATERIALS AND METHODS

Present paper was carried out for 1 year using strip split block experiment in the form of complete accidental blocks with 4 times repetition in 2 different regions of Motahari engineering research center of Karaj and agricultural research station of Oltan-Moghan; irrigation was done in tape form. Moghan plain is located in north west of Iran, north of Ardebil province between longitudes of 48',22" and 47',35" and latitude of 39',22" and 39', 45". The mean height of plain is 60m from sea level, and mean of annual temperature is about 14.5°C, the average amount of rain fall is about 432mm (Rahnamaian, 1993). Karaj research station is located in north-west of Karaj city between longitudes of 29', 51" and 11',50" and latitude of 35',31" and 36', 12", the height of this region is about 1313m from sea level. The average annual rain fall is about 243 mm. This region has 150-180 dry days and is classified among regions with dry and warm Mediterranean climate with cold and wet winters and dry and warm summers. Corn was cultivated in the under studied sections during previous year without any fertilizer to reduce the

amount of N as much as possible. Each plot included 6 cultivation lines with 12m length and row interval was 40\*50 cm. The depth of cultivation was 2-3 cm and the distance between bushes was about 20 cm on cultivation line. Experimental factors included 4 levels of N fertilizer, 4 varieties of sugar beet and 3 levels of methanol spraying. Each main plot included different treatments of N: non application, 25 (optimal amount), 19 (25% lower than optimal amount) and 13 (50% lower than optimal amount) mg/kg of soil, variety factor contained 4 varieties of Pars, Ekbatan, Rozir and Flores. Six numbers of each variety were cultivated in 12 m length with 40\*50 planting pattern. Subsidiary plots included methanol spraying in 3 levels of 1- control (non-application), 2- 10% by volume of methanol and 3- 20% by volume of methanol. The amount of N was determined before cultivation based on N concentration that remained in the depth of 0-30cm of soil. Fertilizer source was ammonium nitrate that was consumed in 5 parts along with irrigation (the irrigation system was tape form). Fertilizer application steps contained: after weeding, and each of 4 other stages was done 20 days after previous stage. N consumption time was the same for all the treatments and in each step 20% of fertilizer was used. To avoid permeation of N from one plot to another, two lines were remained empty between each two main plots. Before experiment, soil sampling was done from 0-30 and 30-60 cm depth and soil characteristics were determined (Table 1).

Methanol solvent was prepared as percentage by volume of methanol with 99.9% purity. Methanol spraying was done during 3 stages with 3 different concentrations. Spraying time of one stage was done before reaching to maximum temperature (according to 40-year statistic) and two other stages were done with two weeks interval in the evening (between 17-20 p.m). Moreover, to have homogeneous solvent, different percentages of methanol were calibrated and kept in special sources. Spraying was done with constant pressure. In the final harvest, sampling was done from 4 middle lines with 8 m length (equal to 3.6 m<sup>3</sup>). The number and weight of roots were measured and root dough samples were prepared and their qualitative characteristics were measured by sugar technology laboratory of Sugar Beet Seed Improvement Institute (such as sugar percentage,

obtainable sugar percentage, N and K elements, etc). Sugar amount was measured by Saccharomat instrument based on the spin amount of polarized light (Clover *et al.*, 1998). N and K amounts were measured by flame photometer method and by comparing lithium-wide emission spectrum. To determine harmful N amount blue number method was used and betalyser instrument measured it (Clover *et al.*, 1998). Then Mishra and Fridorich (1972) method was used to measure changing amount of catalase enzyme. To measure these factors, fresh and developed leaves were used and at the end, Paglia and Valentine (1987) method was used to determine enzyme change amount. Leaves samples were transferred to chemistry and biology laboratory of Kharazmi University, and were put in tris-phosphate buffer solvent 0.16 mole (PH= 7.5) and were crushed and homogenized after washing by distilled water. Next, digitonin buffer with the same volume was added, which has a digestive enzyme, to digest membrane and cell walls. Eventually, 0.5 ml of homogenized solvent was divided to evaluate protein by Lowry (1951) method and its protein amount was determined based on mg/ml.

Data were analyzed by SPSS<sub>v.9.2</sub> statistical software and mean comparison was done by Duncan test, at the end graphs were drawn by Excel software.

## RESULTS

First, normalization of data was considered and then Bartlett test was done for all characteristics. According to the results, the yield of white sugar, sugar cutie and harmful N were significant, therefore, variance analysis was done on them; while storage root yield, N and K amounts and catalase enzyme amount were not significant, so Bartlett test was done from compound analysis of data.

### The yield of storage root

According to the table of data compound analysis (Table 2) the main effects of region and N and interaction effects of region\* variety\* N\*methanol and also region\*variety \*methanol had significant effect in 5% level on root yield. Other treatments did not have significant effect on root yield. Based on cutting table about region effect (Table 4) the interaction effects of

variety\*methanol had significant effect in 1% level in both regions. In the other word, there was a significant difference between variety\*methanol levels for Karaj and Moghan regions and the reaction of varieties was different to methanol levels in each of the regions. In Karaj, the most yield obtained under interaction effect of Rozirvar\* non-application of methanol (78043 kg/hectare). Although its yield did not have significant difference with interaction effects of Rozir\*20% by volume of methanol and Rozirvar\* 10% by volume of methanol (75858 and 72515 kg/hectare orderly). Therefore, it was observed that Rozir variety had the most yield in Karaj region, while application and non-application of methanol didn't have any effect on enhancement or reduction of the yield (Figure 1). In Moghan (Figure 2), the most root yield obtained by using Pars variety\*20% by volume of methanol (92286 kg/hectare) that was classified in the same statistical group with Flores variety\*20% and 10% by volume of methanol and also Pars variety\* 10% by volume of methanol (their root yields were 91296, 85725 and 87543 kg/hectare in order). According to the figure, in Moghan region, Pars and Flores react positively to application of methanol and application of methanol has led to enhancement of root yield in comparison with control treatment. The reason of these results can be lower leaf fall of Pars variety in Moghan region, therefore this variety could have more optimal leaf surface and have more root weight. While Ekbatan and Rozir varieties did not react to application of methanol. In addition, yield enhanced under the effect of methanol application in Pars and Flores was more than control treatment, but there was not any significant difference between applying 10% and 20% by volume of methanol. In Pars variety, application of 10 and 20% by volume of methanol had led to 4 and 9% enhancement of root yield in order; and in Flores variety the enhancement amounts of root yield were 11 and 5.2% in order.

### Sugar cutie

Sugar cutie was affected by the main effects of N and region in 1% level in Karaj region and other main and interaction effects did not have any significant impact on this characteristic (Table 3).

Using 13mg/kg of N, non-application and 19 mg/kg were classified in a same statistical class

with 16.49, 1675 and 16.9% that had led to the most amount of sugar cutie. Application of 25mg/kg of N, which was the most amount of applied N in the experiment, stayed at the last statistical group (15.65%) (Figure 3).

Variety treatment showed a different reaction to sugar cutie. The results indicated that Flores and Rozir varieties have led to 17.26 and 17.21% of sugar cutie and were classified in the most statistical class. Ekbatan variety led to 16.21% that was classified in the same statistical group

with two aforementioned varieties. The lowest cutie was attributed to Pars variety (15.11%) tha was classified in lower statistical group in comparison with Rozir and Flores varieties (Figure4).

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Table 1. Soil test

Parameter	Experimental region			Parameter	Karaj	
	Moghan amount	unit	unit		amount	unit
pH	7.82	—		pH	7.84	—
EC	1.54	ds.m <sup>-1</sup>		EC	1.03	ds.m <sup>-1</sup>
P	8.46	mg.kg <sup>-1</sup>		P	10.46	mg.kg <sup>-1</sup>
K	669.64	mg.kg <sup>-1</sup>		K	598.84	mg.kg <sup>-1</sup>
NH <sub>4</sub>	6.3	mg.kg <sup>-1</sup>		NH <sub>4</sub>	5.95	mg.kg <sup>-1</sup>
No <sub>3</sub>	15.82	mg.kg <sup>-1</sup>		No <sub>3</sub>	14.63	mg.kg <sup>-1</sup>
Soil Texture	clay	—		Soil Texture	Clay-loam	—

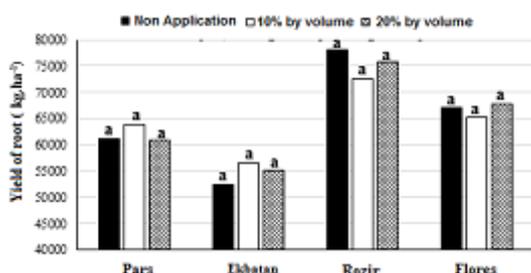


Fig. 1. Mean comparison of interaction effects of region\*variety\* methanol on the yield of *Sugar beet* root in Karaj region

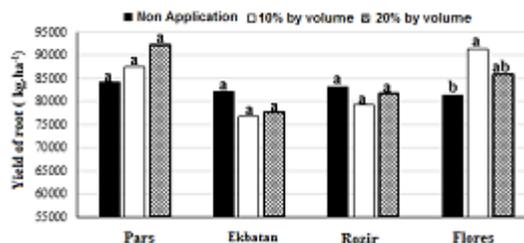


Fig. 2. Mean comparison of interaction effects of region\*variety\* methanol on the yield of *sugar beet* root in Moghan region

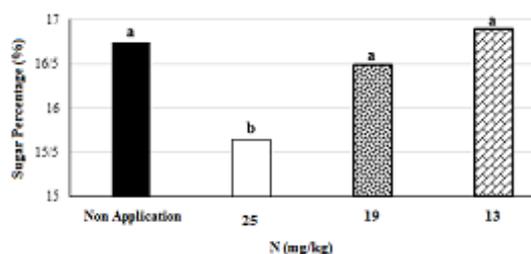


Fig. 3. Mean comparison of the effect of N levels on sugar percentage in Karaj region

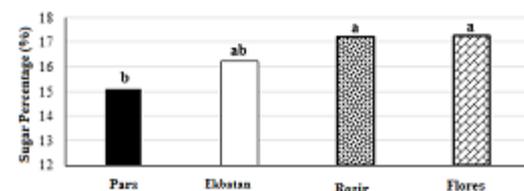


Fig. 4. Mean comparison of varieties effect on sugar cutie in Karaj region

was attributed to Pars variety (15.11%) that was classified in lower statistical group in comparison with Rozir and Flores varieties (Figure 4).

Gohari *et al.*, (1997) claimed that enhancement of N amount can increase root yield, but sugar cutie and achieved sugar reduce. This conclusion was in line with the findings of Gohari (1996) and Emsaki *et al.*, (1998). Emsaki *et al.*, (1998) did not observe any significant difference among 100 and 200 kg.ha<sup>-1</sup> of N.

Most of the carried out studies about the effect of N on quality and quantity of sugar beet root showed reduction of sugar percentage and relative enhancement of root yield, sugar and other impurities of root (Carter and Traveled, 1981; Lee *et al.*, 1987; Bravo *et al.*, 1989; Winter, 1990; Sharifi

and Orazizadeh, 1992; Mohammadkhani, 1992; Ebrahimian, 1994; Gohari, 1994). Consumption of N can reduce root quality by developing aerial organs in comparison with root and reducing sugar percentage (Carter, 1986). Rozbecki and Klinowska (1993) reported that the most sugar yield (7.5 tone sugar) achieved by applying 120 kg of pure N, then enhancement of N amount reduced sugar percentage.

**The yield of white sugar**

The yield of white sugar can be calculated by multiplying root yield by the amount of white sugar (achieved sugar). This factor is the most crucial qualitative and quantitative factor and is considered as a total outcome of root yield, sugar percentage and impurities (Cooke *et al.*, 1993).

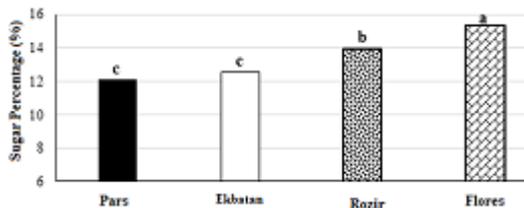


Fig. 5. Mean comparison of the effect of variety on sugar cutie in Moghan region

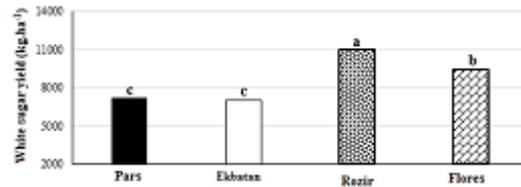


Fig. 6. Mean comparison of the effect of variety on the yield of white sugar in Karaj region

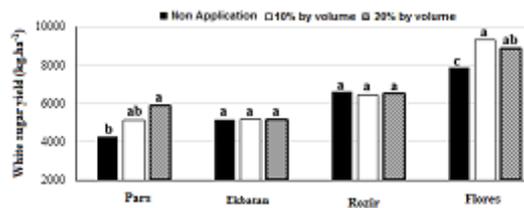


Fig. 7. Mean comparison of interaction effects of variety\* methanol on the yield of white sugar in Moghan region

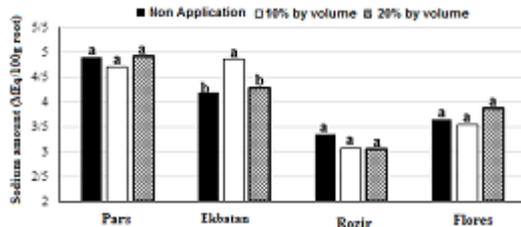


Fig. 8. Mean comparison of interaction effect of variety\* methanol on sodium amount in Karaj region

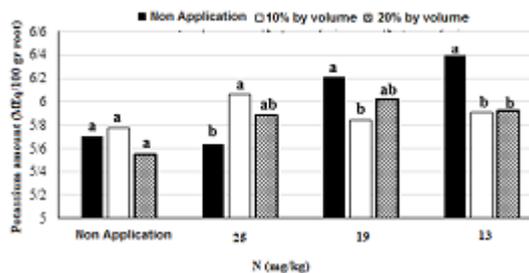


Fig. 9. Mean comparison of the interaction effect of N\* methanol on potassium amount in Moghan region

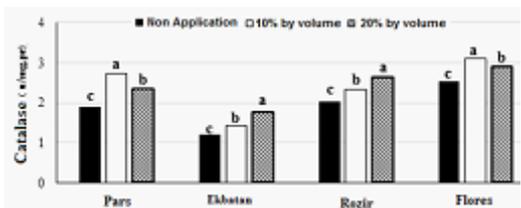


Fig. 10. Mean comparison of the interaction effect of variety\* methanol on catalase activity amount when N is not consumed

Based on the results of variance analysis (Table 3), just the main effect of variety had significant impact on the yield of white sugar in Karaj region; other main and interaction effects did not have significant impact on the yield of white sugar. Regarding Figure 6, Rozir variety was classified in the most statistical group (10976 kg.ha<sup>-1</sup>). After that Flores variety was classified in the next rank with 9397 kg.ha<sup>-1</sup> of white sugar yield. The lowest statistical group was allocated to Ekbatan and Pars varieties (7215 and 7039 kg.ha<sup>-1</sup>).

Figure 7 showed that the interaction effect of Ekbatan and Rozir\* methanol levels was not significant. Pars variety had the most yield of white sugar by applying 20 and 10% by volume of methanol (5879 and 5137 kg.ha<sup>-1</sup>), and the lower statistical group was allocated to non-application of methanol (4276 kg.ha<sup>-1</sup>). In Flores variety the same results obtained, so that application of 10 and 20% by volume of methanol led to the most yield of white sugar (9288 and 8883 kg.ha<sup>-1</sup>) and the lowest yield was related to non-application of methanol (7845 kg.ha<sup>-1</sup>).

Although application of N during growth season reduces the speed of sugar storage and increases the speed of aerial organs growth, total process of sucrose storage in the root is positive generally and sugar yield per unit increases (winter,

1990). An experiment was done to consider the effect of time managing the consumption of N on quality and quantity of sugar beet in Karaj region, the results showed that consumption of 100% N when 4-6 leaves appeared can increase the yield of achieved sugar (Yousefabadi and Mazaheri, 2000).

**Sodium**

Extraction of sugar from root depends on non-sugar materials specifically N, Na and K compounds (Koochaki *et al.* 1993). From technological point of view, the amount of consumed N had significant effect on accumulation of Na and K impurities; and this can be a main factor for reducing sugar percentage and increasing root water percentage (Loilier, 1981). The results of compound analysis (Table 2) showed that the amount of sodium is affected by the main effect of region in 1% level, main effect of variety and interaction effect of region\* variety\* methanol in 5% level. But other main and interaction effects did not have any significant effect on the amount of sodium. By considering Table 4, interaction effect of Karaj region\* Ekbatan variety had significant difference in 5% level; other interaction effects had no significant effect. In Figure 8, it was determined that interaction effect of Karaj region\*Ekbatan variety\*20% by volume of methanol and also Karaj region\* Ekbatan variety\*non-application of methanol had 4.28 and 4.18MEq sodium per 100gr of root dough; therefore, they were classified in lower statistical group than interaction effect of Karaj region\*Ekbatan variety\*10% by volume of methanol. Based on the experiment of Khamim *et al.*, (2003) enhancement of soil N can increase root's sodium, and this was significant in 1% level. Maximum amount of sodium is 6.04 MEq per 100 gr of root dough that was obtained by applying 300 kg.ha<sup>-1</sup> of N. Therefore, according to the results, foreigner varieties have more ability and genetic

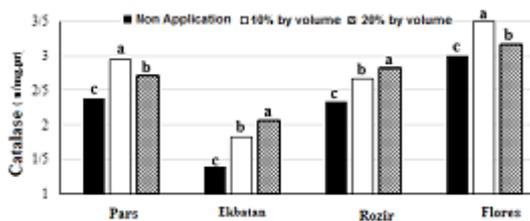


Fig. 11. Mean comparison of the interaction effect of variety\*methanol on catalase activity amount under the treatment of 25mg/kg N

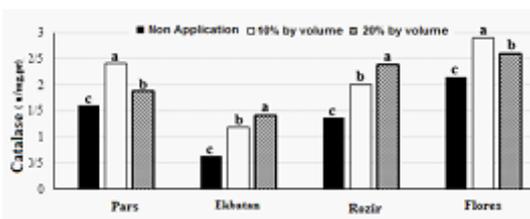


Fig. 12. Mean comparison of the interaction effect of variety\*methanol on catalase activity amount under the treatment of 19 mg/kg N

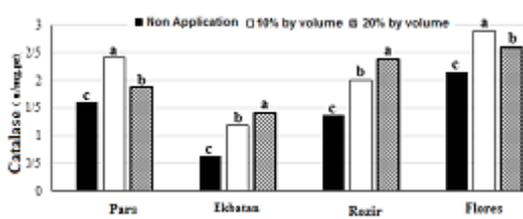


Fig. 13. Mean comparison of the interaction effect of variety\*methanol on catalase activity amount under the treatment of 13 mg/kg N

**Table 2.** Compound analysis of understudied characteristics

Catalase	Potassium	Sodium	Mean squares ( MS)		df	S.O.V
			Root dry material (%)	Root yield		
2.302**	20.768**	69.753**	36.63 <sup>ns</sup>	117.07*	1	Location (L)
0.0002	0.039	0.365	18.85	11.03	6	Error L
1.082**	0.077 <sup>ns</sup>	0.116 <sup>ns</sup>	4.51 <sup>ns</sup>	5.7*	3	N
0.00001 <sup>ns</sup>	0.12 <sup>ns</sup>	0.054 <sup>ns</sup>	5.51 <sup>ns</sup>	0.37 <sup>ns</sup>	3	L×N
0.00003	0.015	0.196	7.52	1.71	18	Error N
1.91**	0.090 <sup>ns</sup>	2.2*	139.76*	9 <sup>ns</sup>	3	Variety (V)
0.00004 <sup>ns</sup>	0.752**	0.182 <sup>ns</sup>	7.19 <sup>ns</sup>	7.406*	3	L×V
0.0003	0.049	0.245	9.94	1.59	18	Error V
0.02**	0.026 <sup>ns</sup>	0.023 <sup>ns</sup>	3.59 <sup>ns</sup>	0.37 <sup>ns</sup>	9	N×V
0.000002 <sup>ns</sup>	0.018 <sup>ns</sup>	0.079 <sup>ns</sup>	3.13 <sup>ns</sup>	0.74 <sup>ns</sup>	9	L×N×V
0.0004	0.012	0.079	3.67	0.73	54	Error N×V
0.685**	0.002 <sup>ns</sup>	0.015 <sup>ns</sup>	3.49 <sup>ns</sup>	0.132 <sup>ns</sup>	2	Methanol (M)
2.635 <sup>ns</sup>	0.013 <sup>ns</sup>	0.021 <sup>ns</sup>	10.65**	0.043 <sup>ns</sup>	2	L×M
0.0001	0.012	0.51	2.16	0.212	12	Error M
0.022**	0.02 <sup>ns</sup>	0.034 <sup>ns</sup>	6.3*	0.965*	6	N×M
0.00004 <sup>ns</sup>	0.03**	0.027 <sup>ns</sup>	1.05 <sup>ns</sup>	0.120 <sup>ns</sup>	6	L×N×M
0.047**	0.027*	0.020 <sup>ns</sup>	1.37 <sup>ns</sup>	0.385 <sup>ns</sup>	6	V×M
0.000006 <sup>ns</sup>	0.005 <sup>ns</sup>	0.052*	0.603 <sup>ns</sup>	0.707*	6	L×V×M
0.013**	0.008 <sup>ns</sup>	0.019 <sup>ns</sup>	2.4 <sup>ns</sup>	0.227 <sup>ns</sup>	18	N×V×M
0.000004 <sup>ns</sup>	0.012 <sup>ns</sup>	0.019 <sup>ns</sup>	1.12 <sup>ns</sup>	0.25 <sup>ns</sup>	18	L×N×V×M
0.0005	0.01	0.23	1.634	0.294	180	Total Error
8.57	14.63	14.3	11.87	9.34		C.V. (%)

ns, \* and \*\* means non-significant, significant in 5% and 1% level

**Table 3.** Variance analysis of characteristics in Karaj and Moghan regions

Sugar Percentage (Moghan)	White Sugar Yield (Moghan)	Mean squares ( MS)		df	S.O.V
		Sugar Percentage (Karaj)	White Sugar Yield (Karaj)		
12.031 <sup>ns</sup>	4.673 <sup>ns</sup>	2.287 <sup>ns</sup>	79.39**	3	Repetition (R)
6.782 <sup>ns</sup>	9.573 <sup>ns</sup>	15.012**	1.531 <sup>ns</sup>	3	N
6.878	2.488	1.62	4.373	9	Error N
103.02**	134.596**	49.6**	170.046**	3	Variety (V)
7.298	7.03	6.62	170.046	9	Error V
2.353 <sup>ns</sup>	2.92 <sup>ns</sup>	1.976 <sup>ns</sup>	1.849 <sup>ns</sup>	9	N×V
2.693	2.89	2.367	2.815	27	Error N×V
5.61**	7.6*	0.731 <sup>ns</sup>	0.057 <sup>ns</sup>	2	Methanol (M)
2.49	3.167	3.129	1.804	6	Error M
0.613 <sup>ns</sup>	3.88*	0.783 <sup>ns</sup>	1.524 <sup>ns</sup>	6	V×M
0.808 <sup>ns</sup>	2.61 <sup>ns</sup>	1.36 <sup>ns</sup>	1.875 <sup>ns</sup>	6	N×M
0.893 <sup>ns</sup>	0.689 <sup>ns</sup>	1.325 <sup>ns</sup>	0.895 <sup>ns</sup>	18	N×V×M
0.924	1.615	1.362	1.365	90	Total Error
7.12	19.98	7.09	13.49		C.V. (%)

ns, \* and \*\* means non-significant, significant in 5% and 1% level

potential in accumulation of sugar and also have lower amount of harmful elements.

**Potassium**

The results of compound analysis (Table 2) showed that the main effect of region and interaction effect of region\* variety and region\*N\* methanol had significant effect on potassium amount in 1% level; and interaction effect of variety\*methanol was significant in 5% level; other main and interaction effects were not significant on root potassium. As interaction effect of region \*N \*methanol was significant, therefore, after considering the cutting table of interaction effects it was determined that just interaction effect of Moghan region\*50% lower than optimal level of N was significant in 1% level and other were not significant (Table 5). The interaction effect of Moghan region\* 50% lower than optimal level of

N\* non application of methanol led to the most amount of potassium and was categorized in the most statistical group. Potassium amount of this treatment was 6.396 MEq per 100gr root dough; while, interaction effects of Moghan region\* 50 % lower than optimal level of N\* 20% by volume of methanol and also Moghan region\* 50% lower than optimal level of N\*20% by volume of methanol had 5.921 and 5.91 MEq per 100 gr root dough. In fact, here, application of methanol in Flores variety and Moghan region reduced potassium amount of root dough. Although there was not any significant difference between 10 and 20% by volume of methanol and they were categorized in the same statistical group. This indicated that this variety and the lowest level of methanol can lead to the minimum amount of potassium (Figure 9).

Here it can be mentioned that when Pars and Ekbatan varieties are used, methanol should not be used to achieve the minimum amount of root potassium; while application of methanol in Rozir and Flores reduces potassium amount in comparison with non-application of methanol. The reason of such reaction is hormone and biochemical characteristics. Most of the carried out studies about the effect of N on quality and

**Table 4.** Cutting of the effect of methanol based on interaction effect of region\* variety (R\*V\*M)

Sodium	Root yield	df	S.O.V
0.009 <sup>ns</sup>	0.13 <sup>ns</sup>	2	L <sub>1</sub> V <sub>1</sub>
0.099*	0.299 <sup>ns</sup>	2	L <sub>1</sub> V <sub>2</sub>
0.022 <sup>ns</sup>	0.361 <sup>ns</sup>	2	L <sub>1</sub> V <sub>3</sub>
0.024 <sup>ns</sup>	0.124 <sup>ns</sup>	2	L <sub>1</sub> V <sub>4</sub>
0.048 <sup>ns</sup>	0.746 <sup>ns</sup>	2	L <sub>2</sub> V <sub>1</sub>
0.015 <sup>ns</sup>	0.415 <sup>ns</sup>	2	L <sub>2</sub> V <sub>2</sub>
0.026 <sup>ns</sup>	0.13 <sup>ns</sup>	2	L <sub>2</sub> V <sub>3</sub>
0.008*	1.247*	2	L <sub>2</sub> V <sub>4</sub>

ns, \* and \*\* means non-significant, significant in 5% and 1% level

**Table 5.** Cutting of the effect of methanol based on interaction effect of region\* N (R\*N\*M)

Potassium	df	S.O.V
0.012 <sup>ns</sup>	2	L <sub>1</sub> N <sub>1</sub>
0.003 <sup>ns</sup>	2	L <sub>1</sub> N <sub>2</sub>
0.029 <sup>ns</sup>	2	L <sub>1</sub> N <sub>3</sub>
0.005 <sup>ns</sup>	2	L <sub>1</sub> N <sub>4</sub>
0.008 <sup>ns</sup>	2	L <sub>2</sub> N <sub>1</sub>
0.03 <sup>ns</sup>	2	L <sub>2</sub> N <sub>2</sub>
0.028 <sup>ns</sup>	2	L <sub>2</sub> N <sub>3</sub>
0.049**	2	L <sub>2</sub> N <sub>4</sub>

ns, \* and \*\* means non-significant, significant in 5% and 1% level

**Table 6.** Cutting of the effect of methanol based on interaction effect of N\* variety (N\*V\*M)

Catalase	df	S.O.V
0.052**	2	N <sub>1</sub> V <sub>1</sub>
0.057**	2	N <sub>1</sub> V <sub>2</sub>
0.026**	2	N <sub>1</sub> V <sub>3</sub>
0.014**	2	N <sub>1</sub> V <sub>4</sub>
0.017**	2	N <sub>2</sub> V <sub>1</sub>
0.06**	2	N <sub>2</sub> V <sub>2</sub>
0.014**	2	N <sub>2</sub> V <sub>3</sub>
0.009**	2	N <sub>2</sub> V <sub>4</sub>
0.06**	2	N <sub>3</sub> V <sub>1</sub>
0.257**	2	N <sub>3</sub> V <sub>2</sub>
0.119**	2	N <sub>3</sub> V <sub>3</sub>
0.033**	2	N <sub>3</sub> V <sub>4</sub>
0.158**	2	N <sub>4</sub> V <sub>1</sub>
0.051**	2	N <sub>4</sub> V <sub>2</sub>
0.011**	2	N <sub>4</sub> V <sub>3</sub>
0.042**	2	N <sub>4</sub> V <sub>4</sub>

ns, \* and \*\* means non-significant, significant in 5% and 1% level

quantity of sugar beet root showed reduction of sugar percentage and relative enhancement of root yield, sugar and other impurities of root (Carter and Traveler, 1981; Lee *et al.*, 1987; Bravo *et al.*, 1989; Winter, 1990; Sharifi and Orazizadeh, 1992; Mohammadkhani, 1992; Ebrahimian, 1994; Gohari, 1994).

#### **Catalase enzyme**

Studies show that oxidant response depend on sensitivity and resistance of varieties (Alexiva *et al.*, 2003). As hydrogen peroxide has oxidative effects on plants, it is harmful and is removed by the activity of catalase enzyme. These enzymes preserve cell against hydrogen peroxide effects and have important role in enhancement of resistance in front of oxidative stress under natural conditions (Ames *et al.*, 1993). The results of compound analysis, which are offered in Table 2, indicate that main effect of region, N, variety and methanol and also interaction effects of N\*variety, N\*methanol and N\*variety\*methanol had significant effect on the activity amount of catalase enzyme in 1% level, but other effects were not significant. In Figure 10, the effect of methanol\*non application of N\* variety were considered and classified separately. According to this figure, in Pars variety, 10% by volume of methanol was classified in the higher rank (2.74), then 20% by volume of methanol stayed at the next rank (2.347) and non-application of methanol was categorized in the lowest rank (1.888). Interaction effects of methanol\*Ekbatan variety showed that 20% by volume of methanol has led to the most activity of catalase (1.775). Then 10% by volume of methanol was classified in the next statistical group (1.426), and the lowest group was allocated to non-application of methanol (1.202). The interaction effects of methanol\* Rozir variety indicated that 20% by volume of methanol had led to 2.636 activity amount and was classified in the first statistical group, then application of 10% by volume of methanol was classified in the second group (2.337) and the lowest group was allocated to non-application of methanol (2.03). The interaction effect of methanol\* Pars variety indicated that when N is not consumed, 20% by volume of methanol was classified in group a (3.102), after that 10% by volume and non-application of methanol were grouped in the next classes (2.903 and 2.546).

The interaction effects of methanol\* variety\* 25 mg/kg of N is shown in Figure 11. As it is determined, Pars variety and 10% by volume of methanol had the most catalase activity (2.952), application of 20% by volume of methanol stayed at the next class (2.703) and non-application of methanol stayed at the lowest rank (2.388). The interaction effect of methanol\* 25mg/kg of N\* Ekbatan variety showed that application of 20% by volume of methanol has led to the most activity and classified in class a. After that application of 10% by volume of methanol stayed in the next class (1.817) and the lowest group was allocated to non-application of methanol (1.388). The interaction effect of methanol\* Rozir variety\* 25mg/kg of N indicated that application of 20% by volume of methanol had led to the most activity (2.825), after that application of 10% by volume of methanol was classified in the next statistical group (2.661) and the lowest group was allocated to non-application of methanol (2.332). In Flores variety, application of 20% by volume of methanol was classified in group a (3.505), and next group was related to 10% by volume of methanol and non-application of methanol (3.168 and 2.997).

Interaction effect of methanol\*19mg/kg N\* Pars variety showed that application of 10% by volume of methanol stayed at the highest level (2.41), then application of 20% by volume of methanol was grouped in the next class (1.878) and non-application of methanol was grouped in the lowest group (1.612). Interaction effect of methanol\*19mg/kg N\* Ekbatan variety showed that application of 20% by volume of methanol stayed at the highest level (1.411), then application of 10% by volume of methanol was grouped in the next class (1.182) and non-application of methanol was grouped in the lowest group (0.642). Interaction effect of methanol\*19mg/kg N\* Rozir variety indicated that application of 20% by volume of methanol stayed at the highest level (2.386), then application of 10% by volume of methanol was grouped in the next class (2.005) and non-application of methanol was grouped in the lowest group (1.375). And finally, Interaction effect of methanol\*19mg/kg N\* Flores variety indicated that application of 10% by volume of methanol stayed at the highest level (2.897), then application of 20% by volume of methanol was grouped in the next class (2.596) and non-application of methanol

was grouped in the lowest group (2.155) (Figure 12).

Interaction effect of methanol\*13 mg/kg N\* Pars variety showed that application of 10% by volume of methanol stayed at the highest level (1.872), then application of 20% by volume of methanol was grouped in the next class (1.617) and non-application of methanol was grouped in the lowest group (1.008). Interaction effect of methanol\*13 mg/kg N\* Ekbatan variety showed that that application of 20% by volume of methanol stayed at the highest level (0.978), then application of 10% by volume of methanol was grouped in the next class (0.953) and non-application of methanol was grouped in the lowest group (0.701). Interaction effect of methanol\*13mg/kg N\* Rozir variety indicated that that application of 20% by volume of methanol stayed at the highest level (1.641), then application of 10% by volume of methanol was grouped in the next class (1.567) and non-application of methanol was grouped in the lowest group (1.387). And finally, Interaction effect of methanol\*13 mg/kg N\* Flores variety indicated that that application of 10% by volume of methanol stayed at the highest level (2.515), then application of 20% by volume of methanol was grouped in the next class (2.203) and non-application of methanol was grouped in the lowest group (1.81) (Figure 13).

Enhancement of chlorophyll amount can related to methanol oxidation in bushes with water lack. Because bushes encounter with oxidative stress under water lack condition. In such circumstances, methanol is oxidized to formaldehyde by leaf extract and this work is done by catalase enzyme (Nemecek-Marshall *et al.*, 1995).

## DISCUSSION

By considering the result it can be inferred that using proper foreign varieties and methanol spray in Moghan region, Flores variety has proper white sugar yield to be used in industry. While, other varieties react positively to the applied managements, but their reactions were not significant. It seems that selection of proper variety is the most crucial factor to achieve maximum yield qualitatively and quantitatively, and this can play dramatic role in enhancement of crop production

in both regions. The importance of N exact management is proved in production of sugar beet. Over use of water and fertilizer can lead to washing the nitrate N and contamination of underground water in one hand, and prevent from sugar crystallization in the other hand (Behera and Panda, 2009). Lee *et al.*, (1987) studied the effect of cultivation time and N fertilizer on yield components of sugar beet, they found that root yield is affected by N fertilizer significantly. Enhancement of N fertilizer increases the yield of storage root. It seems that according results, when the soil N amount is 25 mg/kg, application of more amounts is not preferable; because, enhancement of impurities amount such as sodium and potassium reduces sugar cutie, although they are effective in enhancement of store root yield. The result of other study on improving N consumption in Iran climate showed that if the nitrate amount of soil in the depth of 0-30cm during weeding stage (about 30-40 days after cultivation) is 25 mg/kg, using more N fertilizer will not be necessary to produce 85 tone/hectare root (Noshad and Niromand-Jahromi, 2010).

An important note is that interpretation of the effect of methanol on plant's growth and yield is difficult in filed studies, because interpretation of plant reaction to methanol treatments depend on some variables such as spraying time, the amount of absorbed methanol, tissue morphology and accumulation of methanol in plant tissue (Hemming *et al.*, 1995). Totally, according to the results, selection of proper variety can be introduced as the most important factor in management of field, after that exact management of N consumption and selection of the best methanol level can improve quality and quantity of varieties, encourage farmers to cultivate the plants and prosper factories that are located in both regions and do not have efficiency or work with lower capacity than their potential.

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