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EFFECT OF NITROGEN FERTILIZATION ON JUICE QUALITY AND BIO- ETHANOL PRODUCTION OF SOME SWEET SORGHUM VARIETIES

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ABSTRACT

This study was carried out at Kom Ombo Agricultural Research Station, (latitude of 24o 28′ N and longitude of 32o 57′ E), Aswan Governorate, in 2016 season to study the effect of three nitrogen levels (60, 80 and 100 kg/fad) on juice quality characteristics and ethanol production of four sweet sorghum varieties (Brandes, Honey, Roma and Willey). Stalk juice of sweet sorghum is rich in fermentative sugars. The stalk juice of sweet sorghum was fermented to ethanol using highly ethanol producer strain of ***Saccharomyces cerevisiae*** in batch fermentation process. The experimental design was randomized complete block design with three replications.

The results showed that the increasing of nitrogen fertilization levels from 60 to 100 kg/fad. exhibited a significant increasing in brix, sucrose, reducing sugars, purity, total sugars, alcohol concentration and alcohol efficiency percentages. Applying 100 kg N/fad, gave the highest values of the studied traits.

Sweet sorghum varieties differed in all studied traits except the reducing sugars %. Honey sweet sorghum variety had the highest brix, sucrose, total sugars, purity and scored the maximum alcohol concentration with fermentation efficiency reached to 89.72% of the theoretical value, While Willey sweet sorghum variety gave the lowest values of total sugars and alcohol concentration.

Interaction between factors under study affected significantly in all studied traits except the reducing sugars %. The highest alcohol concentration was recorded in Honey sweet sorghum when fertilized with 100 kg N/fad.

Under conditions of the present work, the results suggest that growing honey sweet sorghum variety fertilized with 100 kg N/fad. produced the best juice quality parameters and alcohol concentration.

Key words: ethanol, fermentation, Nitrogen fertilization, Sweet *Sorghum bicolor*, Varieties.

INTRODUCTION

The sweet sorghum plants {***Sorghum biclor*** (L.) Moench} are a good source for fermentable sugars which can be fermented to bio-ethanol. Ethanol demand is increasing drastically in the present time for getting clean exhaust and fuel sufficiency. The major concerns with using agricultural crops for the production of energy are food and water security. High fermentable sugars in sweet sorghum juice, makes it to be more suitable for plant sweet sorghum for biofuel production in our and similar countries to solve fuel problems such as increasing the octane of gasoline and to reduce greenhouse gases and gasoline imports.

For all crops, the varieties are considered corner stone for production. Many investigators pointed out the important role of sweet sorghum varieties in respect to their influence one yield, quality and bio-ethanol production (**Abo El-Wafa and Abo El-Hamd, 2001**; **Abd El-Razek, and Besheit, 2009**; **EL-Sheikh *et al.*, 2011**; **Prasad and Dhanya, 2011; Rageh *et al.*, 2011; Mekdad and El-Sherif, 2016 and Kering *et al*., 2017).**

Nitrogen is the important element in the superior yield of sweet sorghum crop. Utilization of nitrogen enhanced sugar percentage and ethanol (**Ibrahim *et al.*, 2003; Ismail *et al.*, 2007; Mahmoud *et al.*, 2013; Besheit and Mekdad, 2016; Gajanan and Wani, 2016; Mekdad and El-Sherif, 2016; Harshlata *et al.*, 2018).**

Therefore, the aim of this study was designed to study the effect of nitrogen fertilization on juice quality (technological characters) and bio- ethanol production of some sweet sorghum varieties.

MATERIALS AND METHODS

The present study was conducted at Kom Ombo Agricultural Research Station, (latitude of 24o 28′ N and longitude of 32o 57′ E), Aswan Governorate, in 2016 season to study the effect of three nitrogen levels (60, 80 and 100 kg N/fad.) on juice quality characteristics and ethanol production of four sweet sorghum varieties (Brandes, Honey, Roma and Willey). Stalk juice of sweet sorghum was fermented to ethanol by S***accharomyces cerevisiae*** in batch fermentation process. A factorial experiment conducted in complete randomized block design with three replications was used. Plot size was 21 m2 (1/200 fad.) and consisted of six rows, 7 m long and 50 cm apart and 25 cm between plants.

Planting took place in the 1st week of May and harvest was done after about four months from sowing (dough stage). Nitrogen was applied as urea (46 % N) in three equal doses, one third after thinning directly, the second and third ones were after 30 and 45 days from planting date. The other agronomic practices were done as recommended for growing sweet sorghum crop.

Soil chemical and mechanical analysis of the experimental site showed that the upper 30-cm of the soil was clay loam containing 57, 13 and 190 ppm N, P, K, respectively, with pH of 7.6.

At harvest time a random sample of twenty millable stalks from each plot was taken, stripped and crushed, the extracted juice was strained through a wire screen, then through a fine mesh screen to remove the suspended matters. The samples of juice were prepared to determine the studied traits.

Fermentation process

Stalks juice was transferred without any treatment into 500 ml Erlenmeyer flasks with a final working volume of 200 ml juice in each flask and autoclaved at 110 ºC for 15 min then used as fermentation medium. Yeast strain used in this study was ***Saccharomyces cerevisiae*** (EL-Tayeeb) which kindly obtained from EL-Hawamdia Chemical Factories, Egyptian Sugar and Integrated Industries Company (ESIIC), Giza, Egypt. The inoculum was prepared by transferring one loopfull of 48 hours’ yeast culture grown on a slant of YMPGA medium of the following composition: 3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 10 g/L glucose and 20 g/L agarand dispensed in 50 ml sterilized YMPG broth aliquots in 250 ml Erlenmeyer flask. After incubation on a rotary shaker (150 rpm) at 30ºC for 48 hours, it was transferred at the rate of 10% (v/v) to the fermentation medium. The initial concentration of inoculum was maintained at 2×107 cells/ml. **(Wickerham, 1951).**

The cultures were incubated on a rotary shaker (150 rpm) at 30 °C for 5 hours under aerobic conditions. Fermentation flasks were tightly sealed using parafilm and the fermentation process was completed under anaerobic conditions for 48 hours,as the CO2 evolved during fermentation expels all the air from these flasks. Ethanol was measured at the end of fermentation period.

Recorded data:

1. **Brix percentage (Total soluble solids, TSS %):** was determined using Brix Hydrometer according **to A.O.A.C. (2005).**
2. **Sucrose percentage:** was determined using Saccharemeter according to **A.O.A.C. (2005).**
3. **Purity percentage:** It was calculated according to the following formula of **Singh and Singh (1998)**.

$$\text{Juice purity percentage} = \frac{\text{sucrose percentage}}{\text{brix percentage}} \times 100$$

4. **Reducing sugars percentage:** It was determined using Fehling method according to **A.O.A.C. (2005).**
5. **Total sugars percentage:** It was determined by using phenol-sulphuric acid method according to **Herbert *et al.* (1977).**
6. **Alcohol concentration:** It was estimated by bichromate method as described by **Zohri and Mostafa (2000)**
7. **Alcohol efficiency:** Alcohol efficiency is expressed as g sugar utilized/100 g initial sugar according to **Roukas (1996).**

The collected data were statistically analyzed according to the method described by **Snedecor and Cochran (1981)**. Treatment means were compared using LSD at 5% level of difference as outlined by **Steel *et al.* (1997).**

RESULTS AND DISCUSSION

1.Brix percentage

Data in the **Table (1)** cleared that significant difference among the examined varieties in brix percentage. From the data it is clear that

Honey sweet sorghum variety had the highest brix percentage (18.51 %) followed by Brandes variety (18.14 %), while the lowest brix percentage (17.71 %) was recorded in Roma variety. Differences among varieties in brix percentage may be due to differences in growth and their reaction to the surrounding environmental conditions prevailing during maturity development of soluble solids in sweet sorghum plants. The effective role of sweet sorghum varieties on brix percentage has been reported by **Abo El-Wafa and Abo El-Hamd (2001); Abd El-Razek and Besheit (2009)** and **EL-Sheikh *et al.* (2011).** They found that TSS % significantly
different between the studied varieties.

Results showed that increasing in nitrogen levels up to 100 kg/fad. was significantly increased the values of brix %. This result may be due to the fact that nitrogen increases the vegetative growth of plants and consequently resulted in more metabolites required for the formation of soluble solids. These results are in accordance with those obtained by **Ismail *et al.* (2007); Mahmoud *et al.* (2013)** and **Harshlata *et al.* (2018).** They found that brix percentage in juice exhibited significant affected due to levels of nitrogen.

Data also showed that brix percentage was significantly affected by the interaction between the two studied factor. The varieties did not behave the same under the three nitrogen levels. The increasing in brix percentage of Brandes variety was about 1.3 times of that obtained with Honey variety due to increasing the nitrogen rate from 60 to 100 Kg/fad.

Table (1): Effect of nitrogen fertilization levels on brix percentage of some sweet sorghum varieties.

Nitrogen level (kg N/fed)	Varieties				Mean
	Honey	Brandes	Roma	Willey	
60	17.99	17.54	17.26	17.46	17.56
80	18.58	18.16	17.69	17.90	18.08
100	18.95	18.72	18.18	18.42	18.57
Mean	18.51	18.14	17.71	17.93	
LSD at 0.05 levels of significance					
Varieties (A)					0.36
Nitrogen levels (B)					0.31
Interaction A x B					0.63

2.Sucrose percentage

Data in **Table (2)** showed that the evaluated sweet sorghum varieties varied significantly in sucrose percentage. Honey sweet sorghum variety recorded higher sucrose percentage than other varieties (9.98 %). This result may be due variation in genetic structure among varieties. Also there are difference between varieties in this trait as reported by **Abo El-Wafa and Abo El-Hamd (2001); Rageh *et al.* (2011)** and **Mekdad and El-Sherif (2016)** they noticed that varieties significantly differed in sucrose percentage.

Sucrose percentage was significantly influenced by the application of N. levels. Sucrose percentage increased positively as N. level was increased up to 100 kg /fad. This result may be due to the highest nitrogen dose was appropriate and required to feed them and to give the maximum sucrose. These results are in line with that shown by **Ibrahim *et al.* (2003); Ismail *et al.* (2007)** and **Besheit and Mekdad (2016).** They found that as nitrogen increasing from 80 to100 and 120 kg/fed sucrose percentage were gradually and significantly increased.

The interaction between varieties and nitrogen levels had a significant effect on sucrose percentage. In general, the maximum values of sucrose percentage were obtained from fertilization all varieties with 100 Kg/fad, but the effect of N. levels was not the same was the studied varieties. Increasing in sucrose percentage in Brandes variety was about double of that obtained in Honey variety due to increasing the nitrogen rate from 80 to 100 Kg/fad.

Table (2): Effect of nitrogen fertilization levels on sucrose percentage of some sweet sorghum varieties.

Nitrogen level (kg N/fed)	Varieties				Mean
	Honey	Brandes	Roma	Willey	
60	9.34	8.83	8.47	7.93	8.64
80	10.10	9.45	9.05	8.55	9.29
100	10.51	10.25	9.59	9.10	9.86
Mean	9.98	9.51	9.04	8.53	
LSD at 0.05 levels of significance					
Varieties (A)					0.51
Nitrogen levels (B)					0.45
Interaction A x B					0.92

3. Reducing sugars percentage

Data presented in **Table (3)** showed that the differences in reducing sugars percentage among tested varieties, and the interaction between varieties and applied nitrogen levels were insignificant. This probably due to the fact that the tested varieties have the same ability to synthesis and storage reducing sugars percentage or utilize nitrogen.

Reducing sugars percentage was significantly influenced by nitrogen levels,the reducing sugars improved with raising nitrogen levels from 60 up to 100 kg/fad. This result may be due to higher nitrogen dose was appropriate and required to feed them and to give the maximum reducing sugars percentage. These results are in accordance with those obtained by **Besheit and Mekdad (2016).** They found that the effect of nitrogen fertilizer was significant on reducing sugar percentage.

Table (3): Effect of nitrogen fertilization levels on reducing sugars percentage of some sweet sorghum varieties.

Nitrogen level (kg N/fed)	Varieties				Mean
	Honey	Brandes	Roma	Willey	
60	4.280	4.210	3.670	3.750	3.978
80	4.380	4.300	3.770	3.900	4.088
100	4.690	4.500	4.000	4.110	4.325
Mean	4.450	4.337	3.813	3.920	
LSD at 0.05 levels of significance					
Varieties (A)					NS
Nitrogen levels (B)					0.292
Interaction A x B					NS

4. Purity percentage

Results in **Table (4)** showed that a significant variation was observed in purity percentage among the tested varieties. The results pointed out that Honey variety characterized with the best purity percentage followed by Brandes variety. The superiority of Honey variety in purity % may be due to higher sucrose percentage (Table 2). These results are in line with those obtained by **Rageh *et al.* (2011)** and **Besheit and Mekdad (2016).** They noticed that a significant difference in purity percentage was found among the tested varieties.

Results showed that purity percentage increased significantly by increasing nitrogen levels up to 100 kg/fad. Meantime, there was no significant difference in purity percentage of sweet sorghum plants when they received 80 and 100 kg N/fad. These results are in line with those recorded by **Ibrahim *et al.* (2003); Ismail *et al.* (2007)** and **Mekdad and El-Sherif (2016).** They found that that the effect of nitrogen fertilizer was significant on purity percentage.

Concerning the interaction effect, it could be noted that the effect of the interaction between varieties and nitrogen fertilization levels was significant. This means that the varieties did not behave the same at the different N Levels, purity percentage of Brandes and Willey varieties were significantly increased by increasing N levels from 60 to 100 Kg/fad but this was not in the case with the other two varieties. In general, the highest purity % was recorded with Honey variety, when it was received 100 kg N/fad.

Table (4): Effect of nitrogen fertilization levels on purity percentage of some sweet sorghum varieties.

Nitrogen level (kg N/fed)	Varieties				Mean
	Honey	Brandes	Roma	Willey	
60	51.97	50.33	49.03	45.32	49.16
80	54.25	52.07	51.11	47.70	51.28
100	55.47	54.69	52.69	49.31	53.04
Mean	53. 90	52.36	50.94	47.44	
LSD at 0.05 levels of significance					
Varieties (A)					2.07
Nitrogen levels (B)					1.97
Interaction A x B					3.93

5. Total sugars percentage

Data in **Table (5)** indicated that total sugars percentage was significantly affected by the two studied factors and their interaction. Differences among the studied varieties were significant in total sugars percentage. The Honey variety surpassed Brandes, Roma and Willey by 0.46, 1.51 and 2.05%, respectively.

This result may reflect the capacity of Honey variety to produce more survival sugars compared to other varieties. This result is in agreement with that obtained by **Besheit and Mekdad (2016) and Kering *et al.* (2017).** They found there were variety differences in total sugars content.

Data also showed that total sugars percentage was significantly increased by increasing nitrogen levels up to 100 kg /fad. This result may be due to the highest nitrogen level was appropriate and required to feed them and to give the maximum total sugars. Similar results were reported by **Besheit and Mekdad (2016)** who found that a gradual increase in total sugars as nitrogen rate increase to reach its maximum value corresponding to 120 kg/fad.

The results revealed that total sugars percentage was significantly affected by the interactions between sweet sorghum varieties and nitrogen fertilization levels. This means that the sweet sorghum varieties did not behave the same at the different nitrogen dose, total sugars of Honey and Brandes varieties were significantly increased by increasing N levels from 60 to 100 Kg/fad but this was not the case with the other two varieties. In general, the highest values of total sugars were recorded with Honey variety when receiving 100 kg N/fad.

Table (5): Effect of nitrogen fertilization levels on total sugars percentage of some sweet sorghum varieties.

Nitrogen level (kg N/fed)	Varieties				Mean
	Honey	Brandes	Roma	Willey	
60	14.49	13.95	13.07	12.59	13.53
80	15.06	14.55	13.46	12.84	13.98
100	15.40	15.07	13.87	13.36	14.43
Mean	14.98	14.52	13.47	12.93	
LSD at 0.05 levels of significance					
Varieties (A)					0.40
Nitrogen levels (B)					0.34
Interaction A x B					0.89

6. Alcohol concentration

Data in **Table (6)** pointed out that alcohol concentration percentage was significantly affected by the tested varieties. Honey sweet sorghum variety produced the best ethanol concentration (6.868 %) with 14.98 % initial total sugar concentration, without any significant variance with Brandes variety. It can be noticed that the variance among Brandes, Roma and Willey varieties was insignificant. The differences among varieties in ethanol concentration depend on the interaction between varieties and environmental factors during growth, sucrose formation and storage periods. This result is in agreement with that obtained by **Abd El-Razek and Besheit (2009)** and **Besheit and Mekdad (2016).** They obtained that significant variations in alcohol concentration among the tested varieties.

Results showed that ethanol concentration was statistically and positively influenced by the nitrogen levels. It was found that raising nitrogen levels from 60 to 80 and 100 kg/fad resulted in increasing in ethanol concentration 0.441 and 0.946 % respectively. The obtained result is in agreement with those reported by **Ibrahim *et al.* (2003) and Gajanan and Wani (2016).** They found that for obtaining the highest ethanol from sweet sorghum should be fertilized with 90 kg N /ha. **Harshlata *et al.* (2018)** reported that ethanol concentration was significantly influenced by various nitrogen rates under study.

Table (6): Effect of nitrogen fertilization levels on alcohol concentration percentage of some sweet sorghum varieties.

Nitrogen level (kg /fed)	Varieties				Mean
	Honey	Brandes	Roma	Willey	
60	6.170	5.883	6.003	5.790	5.962
80	6.933	6.480	6.230	5.967	6.403
100	7.500	7.300	6.500	6.333	6.908
Mean	6.868	6.554	6.244	6.030	
LSD at 0.05 levels of significance					
Varieties (A)					0.513
Nitrogen levels (B)					.0428
Interaction A x B					0.997

Also, data showed that ethanol concentration was significantly affected by the interaction between the two st factors. Tested varieties did not behave the same at the different N levels. Ethanol concentration of Honey and Brandes varieties was significantly increased by increasing N rates from 60 to 100 Kg/fad, but this was not the case with the other two varieties. Generally, the highest ethanol concentration recorded with Honey variety when it was received 100 kg N/fad.

7. Alcohol efficiency

Data presented in **Table (7)** revealed that alcohol efficiency percentage was significantly affected by varieties. Concerning Willey juice fermentation, the produced ethanol value was 91.27 % of their theoretical value. While Brandes variety gave the lowest value (88.33 %) of their theoretical value. This superiority of Willey Sweet sorghum variety may be due to genetically causes and/or high values of juice production. Also the difference between varieties in this trait was previously recorded by **Prasad and Dhanya (2011).** They found that varieties significantly differed in alcohol efficiency percentage.

Also, data indicated that alcohol efficiency percentage was significantly affected by increasing the levels of N. fertilization. Application of 100 kg N/fad surpassed by 7.22 and 3.93 % in alcohol efficiency more than application of 60 and 80 kg/fad, respectively. This increasing may be attributed to the important role of nitrogen in building up, and support photosynthesis process, which result in increasing total sugars in the plant juice. These findings are in line with those obtained by **Harshlata *et al.* (2018).** They recorded that the potential ethanol of sweet sorghum was significantly influenced by different levels of nitrogen.

Table (7): Effect of nitrogen fertilization levels on alcohol efficiency percentage of some sweet sorghum varieties.

Nitrogen level (kg N/fed)	Varieties				Mean
	Honey	Brandes	Roma	Willey	
60	83.33	82.52	89.88	89.99	86.41
80	90.09	87.16	90.58	90.95	89.70
100	95.31	94.79	91.70	92.76	93.63
Mean	89.72	88.33	90.72	91.27	
LSD at 0.05 levels of significance					
Varieties (A)					3.02
Nitrogen levels (B)					2.71
Interaction A x B					4.71

Alcohol efficiency was significantly affected by the interaction between the two factors studied. The tested varieties did not behave the same at the different nitrogen levels. Alcohol efficiency of Honey and Brandes varieties was significantly increased by increasing N levels from 60 to 100 Kg/fad but this was not the case with the other two varieties. In general, the best alcohol efficiency recorded with Honey variety, when it was received 100 kg N/fad.

CONCLUSION AND RECOMMENDATION

Under conditions of the present work, the results suggest that growing honey sweet sorghum variety fertilized with 100 kg N/fad. produced the best juice quality parameters and alcohol concentration.

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تأثير التسميد النيتروجينى على جودة العصير و انتاج الايثانول الحيوى لبعض اصناف الذرة الرفيعة السكرية

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اجريت هذه الدراسة فى محطة البحوث الزراعية بكوم امبو(دائرة عرض 24,28 شمال و خط طول 57,32 شرق)، محافظة اسوان فى الموسم الزراعى 2016 لدراسة تأثير ثلاثة مستويات من التسميد النيتروجينى 100 , 80 , 60 (كجم نيتروجين للفدان)على صفات الجودة و انتاج الايثانول الحيوى لأربعة أصناف من الذرة الرفيعة السكرية(هانى ,براندس , روما ، ويلى)حيث تم انتاج الايثانول الحيوى عن طريق تخمير عصيرسيقان الذرة السكرية باستخدام الخميرة. ***Saccharomyces cerevisiae*** (وتم أستخدام تصميم قطاعات كاملة العشوائية, مع ثلاث مكررات.

أوضحت النتائج أن زيادة مستويات التسميد النيتروجينى من 60 الى 100 كيلو جرام للفدان أدت الى زيادة معنوية فى النسب المئوية لكلاً من المواد الصلبة الذائبة الكلية(البركس) و السكروز ,و السكريات الكلية, السكريات المختزلة , تركيز الكحول و كفاءة انتاج الكحول , و قد ادى التسميد بمعدل 100 كجم نيتروجين للفدان الى الحصول على أعلى القيم للصفات محل الدراسة.

اظهرت الدراسة وجود فروق معنوية بين الاصناف المختبرة فى كافة الصفات محل الدراسة ماعدا النسبة المئوية للسكريات المختزلة(الاحادية .)تفوق الصنف هانى فى محتواه من المواد الصلبة الذائبة الكلية البركس) ,السكروز ,السكريات الكلية والنقاوة و تركيز الكحول .فى حين تفوق الصنف ويلى فى صفة كفاءة إنتاج الكحول.

ادى التفاعل بين عاملى الدراسة الى تأثير معنوى على صفات جودة العصير (ماعدا النسبة المئوية للسكريات المختزلة)، و كلاًمن تركيز و كفاءة الكحول .فيما اعطى الصنف هانى اعلى تركيز للكحول عند تسميده باستخدام 100 كجم نيتروجين للفدان.

فى مثل ظروف هذا العمل البحثى وللحصول على افضل صفات جودة وأعلى تركيز من الكحول. يوصى الباحثون بزراعة الصنف هانى مع تسميده باستخدام 100 كجم نيتروجين للفدان.