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IMPACT OF INTEGRATED ROW SPACING, FERTILIZER APPLICATION METHODS AND SOWING DATES ON BIOETHANOL PRODUCTION IN SORGHUM

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Abstract

In present times the world is facing a severe energy crisis. Therefore, alternative resources of energy have been studied to cope with this ever increasing global issue. The bioethanol from sorghum crop is a safe and environment-friendly energy resource. In this context, a series of field experiments had been conducted in two consecutive years 2016 and 2017 on bioethanol production in sorghum as influenced by row to row distance, fertilization and sowing date at the Department of Agronomy, Sindh Agriculture University, Tandojam. The use of bioethanol through sorghum can reduce the effects of greenhouse gases on the environment. It is also a source of renewable energy in the world. Sorghum is an excellent choice to meet future energy demands. Integrated approaches can maximize the overall benefits of farmers. The randomized complete block design (RCBD) with three replications was used for this study. The sowing was done with combined treatments based on three-row spacing such as 30 cm, 45 cm, and 60 cm, three fertilizer application methods (broadcasting, band application, and fertigation) and three sowing dates (18^{th} April, 03^{rd} May and 17^{th} May) respectively. The statistical analysis of variance for all tested factors was significant at (p< 0.05%) probability level. The results for combined impact of these three factors on all observed traits of study showed that leaves plant^{-1}, nodes plant^{-1}, plant height (cm), stem girth (cm), distance between nodes plant^{-1} (cm), brix (ºBx) and ethanol yield (L ha^{-1}) were affected at highly significant level except tillers plant^{-1} that was non-significant. The maximum bioethanol yield (1725.9 L ha^{-1}) was recovered with 45 cm rowspacing under fertilizer applied through the broadcasting method and sowing date of 17^{th}May. Therefore, these three combined approaches should be applied in sorghum crop for establishing a developed and improved production technology to enhance bioethanol production.

Key words: Sorghum, Bioethanol, Row spacing, Band application, Sowing dates, Climate.

Introduction

Sorghum (*Sorghum bicolor* L.) is a widely adapted crop which is grown in different environments (Mohamed, 2011). In the world, it is 5th major cultivated crop (Umakanth *et al.*, 2012); requires fewer fertilizers (Sher *et al*., 2012) and pesticides (Serna-Saldivar *et al.*, 2012). Fodder sorghum could produce high biomass for several years in relation to ethanol production (Dahlberg *et al.*, 2011). Biomass of sorghum includes especially second generation ethanol for generating electricity (May *et al.*, 2016). The human population must unearth to reduce land competition between production of food and fuel (Wanbin *et al.*, 2013). The increased concentration of carbon dioxide (CO_2) and other greenhouse gases expected to increase the earth's temperature (Patricia *et al.*, 2012).Renewable energy is growing rapidly in the world (15% expected growth rate annually by 2035) for playing a key role to meet future energy demands (Gruenspecht, 2011). Sorghum could play a vital role to meet the increased demand for renewable energy to replace resources of fossil fuel leaned energy (Mathur *et al.*, 2017). Biofuels including a variety of fuels derived from biomass of crops. It covers solids, liquids and gaseous fuels (Demirbas, 2009) and renewable energy as energy from resources naturally replenishes on human timescales like sunlight, wind, precipitation, tidal waves, and high-temperature range (Omar *et al.*, 2014). The biofuel crops are first or second generation biofuel crops (Mohr & Raman, 2013). Biofuel first generation crops are included food crops, while the second generation is lignocellulosic energy crops such as sorghum and other crop residues. Hence, second-generation biofuel crops are observed as a compensated way of increasing debate based on first-generation biofuel crops. So, about 2.5% of the transport fuel of the world produced from biomass (Searchinger & Heimlich, 2015). Sweet sorghum is a particular energy crop with the ability for accumulating sugar @ 10-20% (Houghton, 2005). However, its juice with readily available "free soluble sugars" can be directly fermented into bioethanol (Rao *et al.*, 2010) and ethanol is produced from any sweetened or starch-containing material (Ali *et al.*, 2008). It is known that yeast *Saccharomyces cerevisiae* has everyday use in the world for producing ethanol (Zaldivar *et al.*, 2001; Kaisa *et al*., 2006). Shah (2010) reported that the mutant strain demonstrated a higher yield compared to ethanol @ 7.5% (w/v). He further concluded that this strain could be used in Pakistan for commercial purpose as cheap ethanol production sources.

The row spacing as well as plant populations are two factors that would have a significant effect (Fromme *et al.*, 2012). The research related to crop production systems aimed at establishing an ideal plant population per hectare to increase biomass production in sorghum (May *et al.*, 2016). Therefore, it is mandatory for defining the space in a way that competition between adjacent plant provides the highest yield plant^{-1}. Fertilization methods are crucial in good agricultural practices (Adiaha & Agba, 2016).

The broadcast fertilizer can be incorporated, which increases plant growth and root exposure, especially for the mobility of nutrients like phosphorus and potassium (Clain & Jeff, 2003). Band application of fertilizer on the soil surface or under the crop after planting is a side-dress application (Robert, 2001). Application of fertilizer with water through drip irrigation (fertigation) reduces wastage of both water and fertilizers (Jeelani *et al.*, 2017). The proper sowing date of sorghum is a significant component to get better sorghum (Murthy, 2002). The unexpected losses of yield owing to environmental stress and diseases attacks are the main problems (Sharma *et al.*, 2013). The sowing dates are directly affecting the yield of sweet sorghum (Rao *et al.*, 2013). It is somewhat drought resistant and can be cultivated in marginal land with fewer water supplies (Marta *et al.*, 2014; Olukoya, 2015). Also, excess water usually leads to a decrease in quantity and quality of biomass and production of stem juice (Zhang *et al.*, 2016).

The selection of crop variety, planting geometry, and plant counts are significant sorghum determinants production (Thapa *et al.*, 2017). The diversification and integrated system of farm activities became a crucial tool for the agribusiness stability (Bonaudo *et al.*, 2014; Lemaire *et al.*, 2014). To increase the crop efficiency and production, the alternative planting system may be required to better the soil fertility and to protect the environment (Kiminami *et al.*, 2010).The fertilizer levels and plant spaces significantly affected the crop yields (Cristina *et al.*, 2017).Usually, the planting time depends on the climatic conditions of the area(Jaybhaye *et al.*, 2015). It has become hard to get higher production by availing single technology (Ladha *et al.*, 2009). Therefore, it could be argued that the integrated approaches and the best blend of synchronized technologies can maximize as a full benefit of farmers (Qin *et al.*, 2013).

Thus, this study was aimed to determine the most effective integrated management practices of row spacing, fertilizer application method, and sowing date to increase bioethanol yield of sorghum.

Materials and Methods

The field trials were performed during the year 2016 and 2017 at Students' Experimental Farm, Department of Agronomy, Sindh Agriculture University Tandojam Pakistan. While, ethanol samples were analyzed from an Advanced Laboratory, Department of Chemical Engineering, Mehran University of Engineering and Technology (MUET), Jamshoro, Pakistan. The randomized complete block design (RCBD) was applied for this study in which treatments were thrice replicated by using sorghum genotype Bale II. The net plot size was 5m x 4m = 20 m^2.The disc plough was used to till the land then clods were crushed with clod crusher. Finally, the land was properly leveled. Soil soaking was given followed by two ploughs and leveling. Seed rate was applied @ 50 kg ha^{-1} and drilling were done with a single coulter hand drill. The sowing was done with three row spacing such as; 30 cm, 45 cm and 60 cm,three fertilizer application methods (broadcast, band application and fertigation) @ 113-41-0 kg NPK ha^{-1}. Urea applications were given in two doses, 1st at the time of sowing and 2nd at the time of first irrigation. Phosphorus rate was 41 kg P ha^{-1} as DAP at the time of planting and three sowing dates (18th April, 03rd May and 17th May). There was 4-6 (recommended) irrigation applied. For plant protection, herbicide Primextra Gold 720 SC and hand weeding was applied to control weeds. The insecticide such as Karate (Lambda cyhalothrin) was applied to control stem borer as recommended.

Ethanol sampling procedure: *Saccharomyces cerevisiae* yeast of HUUM of M/S Xinjiang Shengli Biotechnology Co., Ltd. grown at popular yeast medium with minimum constituents composition as used by Rajoka *et al.*, (2005) followed by the fermentation process. The chemicals were of analytical grade and obtained from Department of Chemical Engineering, MUET, Jamshoro, Pakistan.

Inoculum preparation: For preparing inoculum, yeast medium was used with composition of peptone (as nutrient) 0.5%, yeast extract 1%, *Saccharomyces cerevisiae* 5%, NaCl 2%, Glucose 2%, distilled water 89.5% and pH 5.5. All components were weighed in digital balance model AV-65 (Adventure OHAUS, USA) and mixed one by one in conical flask covering it by muslin cloth plugged with cotton lid and also covered by aluminum foil in laminar flow cabinet with the sterilized environment. The media pH was adjusted with 2 ml NaCl, and volume was made @100 ml/flask by adding distilled water. The flasks were autoclaved in electric pressure steam sterilizer model 50X&75X (American company, America) to sterilize nutrient media at 121°C temperature for 2 hrs and after cooling in room temperature, kept in rotary shaker model TS-40XY (ADVANTEC) at 300 rpm for 24 hrs.

Fermentation media preparation: The fermentation media was prepared with component composition of sorghum juice (sugar) 15%, $(NH_4)_2SO_4$ (Ammonium Sulphate) 0.5%, peptone 0.1%, $MgSO_4$ (Magnesium Sulphate) 0.1%, inoculum 10% and distilled water 74.3%.The sterilized samples were collected on slides with a loop by adding 1% saline and observed visible development of *Saccharomyces cerevisiae* under a compound microscope. The fermentation media preparation was carried out; all above components were weighed and mixed in conical flasks then covered with muslin cloth having a cotton lid, and aluminum foil in the laminar cabinet then kept in a rotary shaker for 24 hrs, at 300 rpm. After fermentation, the samples were centrifuged (H-103 N Series KOKUSAN) at 4000 rpm for5 minutes under the temperature inside centrifuge at 25°C. For ethanol recovery, fermented samples were further taken to process through distillation with Soxhlet apparatus, Japan at 80°C temperature. The ethanol % was determined at 25°C through the distilled sample volume by portable density meter method model DMA 35 (Anton Paar).

Soil status of experimental field: The physical and chemical properties of soil (0-30 cm depth) was tested, and state of the experimental soil was found clay loam in texture, moderately saline (pH 8.0-8.5), organic matter (OM) was 0.72-0.73%, deficient in nitrogen (0.036%), low in phosphorus (0.7-1.8 ppm) and medium in potassium (96-191 ppm).

Statistical analysis

The data was statistically analysed using Statistix 8.1 (Statistix, 2006). However, the LSD test was applied to compare the superiority of the treatment.

Results and Discussion

The statistical analysis of variance for row spacing, fertilizer application methods, and sowing dates were significantly affected at (p< 0.05%) probability level. These combined effects showed that leaves plant^{-1}, nodes plant^{-1}, plant height (cm), stem girth (cm), the distance between nodes plant^{-1} (cm), brix (°Bx) and ethanol yield (L ha^{-1}) were highly significant. While the statistical analysis of variance showed that tillers plant^{-1} was non-significant. The integrated approach influenced almost on all traits and indicated that the maximum tillers plant^{-1} (8.73) was recorded under 30 cm row spacing with fertigation and sowing date of 17th May. The higher leavesplant^{-1} (20.03) was observed with 30 cm row spacing under broadcast and sowing date of 17th May. The maximum nodes plant^{-1} (14.43) was recorded with 60 cm row spacing under broadcast and sowing date of 18th April. The higher plant height (247.5 cm) was gained with 30 cm row spacing under broadcast method of fertilizer applied and sowing date of 17th May (Table 1; Fig. 1).

The maximum stem girth (4.33 cm) and distance between nodes plant^{-1} (15.99 cm) were recorded with 60 cm row spacing under broadcast and sowing date of 18th April. The higher brix (20.7 °Bx) was obtained with 30 cm row spacing under band application and sowing date of 18th April. While the maximum ethanol yields (1725.9 L ha^{-1}) was recovered with 45 cm row spacing under broadcast and sowing date of 17thMay (Table 2; Figs. 2, 3 and 4).

Table 1. Effects of row spacing, fertilizer application methods and sowing dates on tillers plant^{-1}, leaves plant^{-1}, nodes plant^{-1} and plant height (cm) in sorghum.

Row spacing	Fertilizer application methods	Sowing dates	Tillers plant^{-1}	Leaves plant^{-1}	Nodes plant^{-1} (cm)	Plant height (cm)
		18th April	2.60	17.47 b-e	12.63 bc	203.57 cd
30 cm	Broadcast	03rd May	2.60	19.43 ab	12.73 bc	233.56 ab
		17th May	2.60	20.03 a	13.33 ab	247.50 a
		18th April	2.10	17.73 bcd	11.77 c-h	201.42 cde
45 cm	Broadcast	03rd May	2.47	18.43 abc	13.30 b	194.54 def
		17th May	2.27	16.83 c-g	11.13 f-I	207.36 cd
		18th April	2.63	17.83 bcd	14.43 a	219.93 bc
60 cm	Broadcast	03rd May	2.73	14.43 hi	10.73 hi	156.31ij
		17th May	2.73	15.53 e-I	11.33 e-h	216.83 bc
		18th April	2.57	15.20 f-I	10.87 ghi	213.90 bcd
30 cm	Band	03rd May	1.80	12.07 j	10.17i	121.13 k
	application	17th May	3.17	17.67 b-e	11.93 c-g	172.99 ghi
		18th April	2.33	13.97 ij	11.10 fi	149.04 j
45 cm	Band	03rd May	2.83	15.70 d-I	11.33 e-h	150.82 j
	application	17th May	2.00	12.13 j	10.10 I	127.37 k
		18th April	2.30	15.07 f-i	11.80 c-h	193.69 d-g
60 cm	Band	03rd May	2.07	16.63 c-g	12.03 c-f	200.73 cde
	application	17th May	2.70	17.63 b-e	12.50bcd	203.57 cd
		18th April	2.73	16.20 d-h	11.47 d-h	156.05 ij
30 cm	Fertigation	03rd May	2.70	17.50 b-e	12.33 b-e	181.04 e-h
		17th May	8.73	16.67 c-g	12.10 c-f	205.97 cd
		18th April	2.50	17.47 b-e	12.33 b-e	178.80 fgh
45 cm	Fertigation	03rd May	2.70	15.90 d-i	11.77 c-h	216.03 bc
		17th May	2.33	14.77 ghi	11.77 c-h	164.00 hij
		18th April	2.43	16.67 c-g	17.07 c-f	206.49 cd
60 cm	Fertigation	03rd May	2.50	17.00 c-f	12.27 b-e	177.49 fgh
		17th May	2.47	17.07 c-f	11.93 c-g	204.90 cd
S.E.			1.6698	1.0876	0.5513	10.511
LSD (%)			-	2.1823	1.1062	21.092

Table 2. Effects of row spacing, fertilizer application methods and sowing dates on stem girth (cm), distance between nodes plant^{-1}(cm), brix (ºBx) and ethanol (L ha^{-1}) yield in sorghum

Row spacing	Fertilizer application methods	Sowing dates	Stem girth (cm)	Distance between nodes plant^{-1} (cm)	Brix (ºBx) (cm)	Ethanol yield (L ha^{-1})
30 cm	Broadcast	18th April	3.14 i-l	13.18 ij	19.83 abc	1292.00 bcd
		03rd May	3.67 b-I	13.99 fgh	15.53 e	1378.70 b
		17th May	4.06 a-d	14.99 bcd	12.41 gh	1554.80 a
45 cm	Broadcast	18th April	3.54 d-j	14.28 d-g	15.18 e	945.00 f-j
		03rd May	4.26 ab	14.89 bcd	17.42 d	669.60 k
		17th May	3.44 e-j	14.44 c-f	19.34 bc	1725.90 a
60 cm	Broadcast	18th April	4.33 a	15.99 a	11.91 h	882.90 hij
		03rd May	3.62 c-j	14.15 e-h	13.67 fg	1033.50 e-h
		17th May	3.59 d-j	15.17 b	15.19 e	961.50 f-i
30 cm	Band application	18th April	3.16 i-l	15.17 b	20.70 a	820.70 ijk
		03rd May	2.67 l	11.84 k	20.26 ab	1199.60 cde
		17th May	3.57 d-j	14.48 b-f	14.90 ef	915.90 g-j
45 cm	Band application	18th April	3.03 jkl	14.44 c-f	19.29 bc	823.00 ijk
		03rd May	3.25 g-l	15.03 bc	14.82 ef	666.90 k
		17th May	2.83 kl	13.11 ij	20.27 ab	778.80 jk
60 cm	Band application	18th April	3.82 a-g	14.56 b-f	15.06 e	660.90 k
		03rd May	4.02 a-e	13.63 ghi	20.29 ab	862.80 hij
		17th May	4.18 abc	14.05 e-h	19.53 abc	945.80 f-j
30 cm	Fertigation	18th April	3.77 a-h	12.70 j	20.49 ab	1036.60 e-h
		03rd May	4.07 a-d	14.48 b-f	14.87 ef	1118.30 def
		17th May	4.07 a-d	13.11ij	19.53 bc	1556.60 a
45 cm	Fertigation	18th April	3.99 a-e	14.12 e-h	15.61 e	1084.10 efg
		03rd May	3.52 d-j	14.74 b-e	13.17 gh	1062.60 efg
		17th May	3.22 h-l	14.14 e-h	15.36 e	806.90 ijk
60 cm	Fertigation	18th April	3.82 a-g	13.97 fgh	17.02 d	1332.70 bc
		03rd May	3.58 d-j	13.53 hi	18.89 c	1556.60 a
		17th May	3.87 a-f	14.15 e-h	18.71 c	1035.00 e-h
S.E.			0.2947	0.3601	0.6357	86.646
LSD (%)			0.5914	0.7226	1.2756	173.87

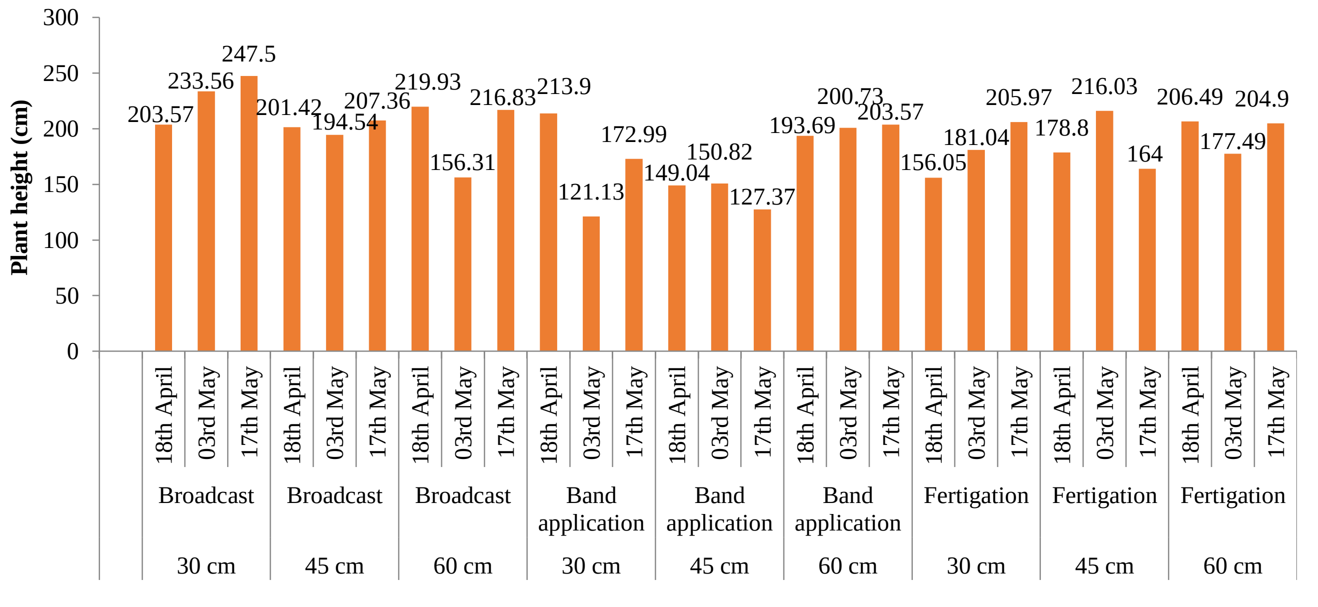

Fig. 1. Plant height (cm) as affected by row spacing, fertilizer application methods and sowing dates.

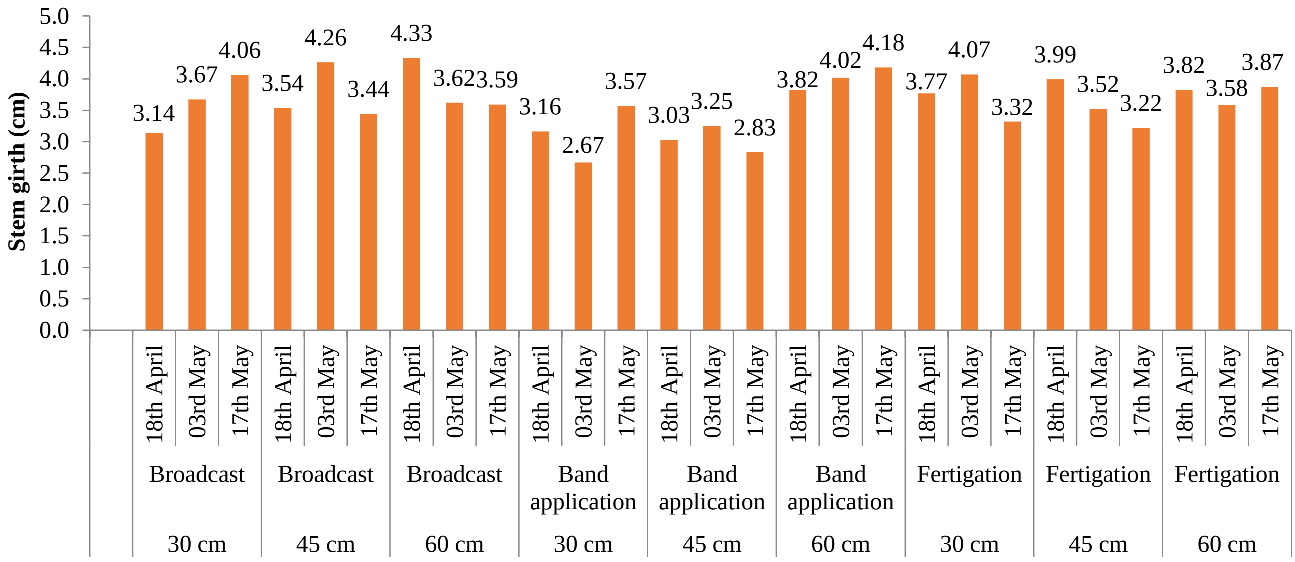

Fig. 2. Stem girth (cm) as affected by row spacing, fertilizer application methods and sowing dates.

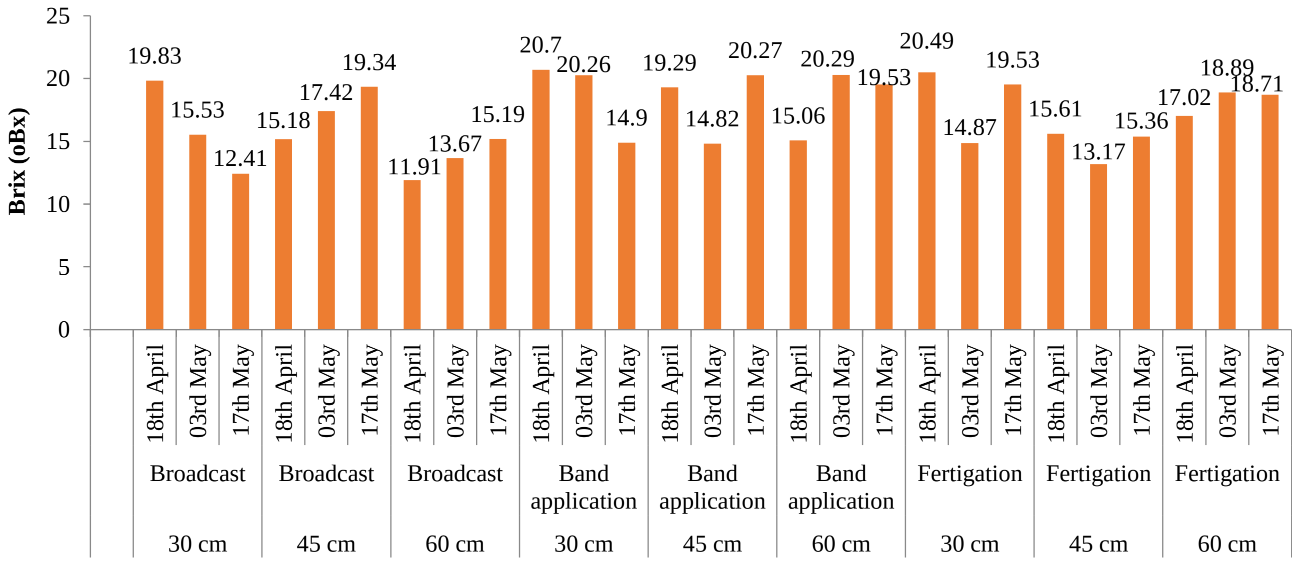

Fig. 3. Brix (°Bx) as affected by row spacing, fertilizer application methods and sowing dates.

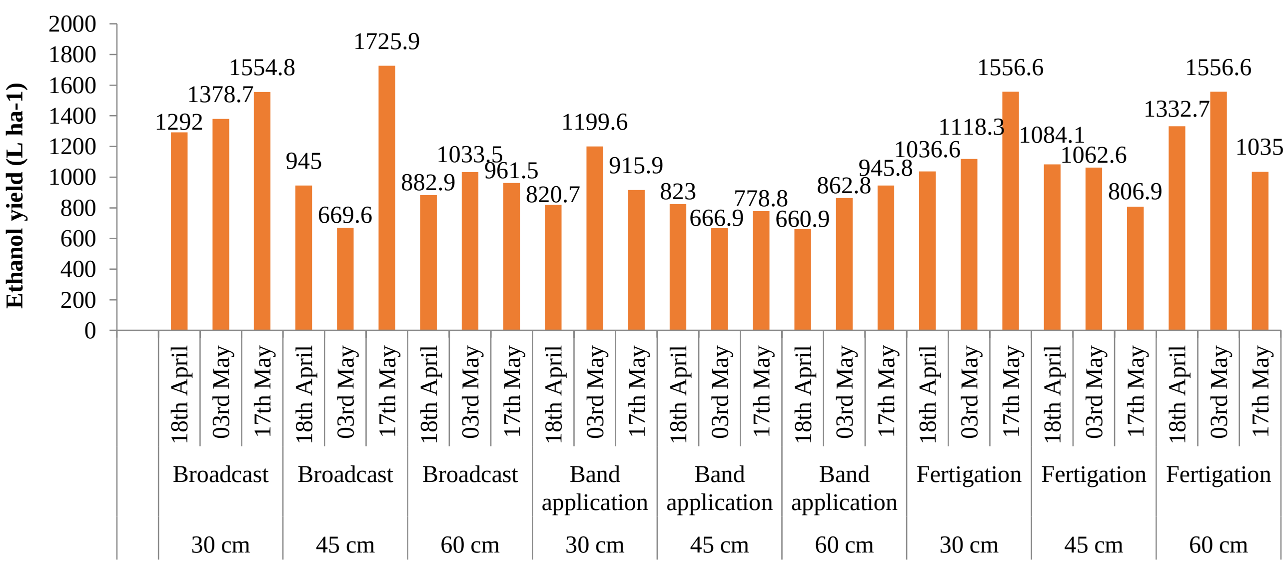

Fig. 4. Ethanol yield (L ha^{-1}) as affected by row spacing, fertilizer application methods and sowing dates.

This integrated impact of row spacing, fertilizer application methods and sowing dates was found positive on almost all traits except tillers plant^{-1}. The results of this study confined with those of May *et al.,* (2016) that the row spacing also led to changes in the final plant stand. Fertilizer application methods were applied which included broadcast, band, and fertigation because the correct placement can generally improve the efficiency of plant nutrients, thereby encouraging the highest yield (Robert, 2001). The optimum sowing date of sorghum is one of the key components for better sorghum grain yields. The climatic change has a significant impact on crop productivity; consequently, have a major role in any change in the global climate on crop yield and productivity (Murthy, 2002). A similar impact is in close agreement as reported by Qin *et al.,* (2013). However, tillers plant^{-1} was non-significant in the present study. In sorghum, tillers numbers per plant can be from zero to about four fertile tillers depend on conditions of growing and genotype (Hammer *et al.,* 1993). It is well known that the tiller of each plant is negatively correlated with plant density. In the case of low plant densities, the higher number of tillers per plant is owing to lesser competition for light, water, and nutrition between plants. These findings are very consistent with previous studies about sorghum (Lafarge & Hammer, 2002; Buah&Mwinkaara, 2009) and obtained learning from study will have advantage for promoting sorghum with preferred characteristic of biofuel (Muhammad *et al.,* 2018).

Conclusion

The innovative findings of present research work could be concluded that the integrated approach of the best compatible technology of sorghum could maximize the benefits of bio-fuel users. This combined study on row spacing, fertilizer application methods, and sowing dates had highly significant effects of ethanol production. The maximum ethanol yield was recovered with 45 cm row spacing under fertilizer applied through the broadcasting method and sowing date of 17th May. The study unearthed a new research area that has a significant impact on sorghum planting establishment to develop enhanced ethanol production to overcome the global energy crisis through the safest source of bio-fuel.

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