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INTEGRATED NITROGEN AND BORON FERTILIZATION IMPROVES THE PRODUCTIVITY AND OIL QUALITY OF SUNFLOWER GROWN IN A CALCAREOUS SOIL

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ABSTRACT

Among biotic and abiotic factors, imbalanced plant nutrition is more indispensable for low sunflower productivity. To assess the interaction behavior of nitrogen with boron on sunflower growth, yield and its oil quality in alkaline-calcareous soils, a field experiment was conducted for two consecutive growing seasons of 2011 and 2012. Sunflower hybrid (*Helianthus annuus* 'Hysun-33') was grown on sandy clay loam soil that was amended with diverse boron rates of 0, 2, 4, and 6 kg ha^{-1} under variable nitrogen levels of 50% RN, 75% RN, and 100% RN 'recommended nitrogen'. Extended duration of phenophases like 50% inflorescence emergence, 50% flowering and 50% maturity stages of sunflower was observed with nitrogen and boron application. Plant height, stem and head diameters were increased significantly through individual use of nitrogen and boron nutrients. Concerning sunflower yield and yield related attributes as number of achenes/head, 1000-achene weight and achene yield were significantly improved with 2.0 kg ha^{-1} boron rate with 100% RN. Application of nitrogen and boron considerably declined the achene oil contents. Achene protein contents were found maximum with boron rate of 2.0 kg ha^{-1} under 100% RN nutrition. Significant decline in stearic acid and oleic acid contents while considerable increase in palmitic acid and linoleic acid contents was recorded by individual use of nitrogen and boron supplements. However, boron application in combination with nitrogen could play a vital role to improve the productivity and economic performance of sunflower in calcareous soils.

Keywords:Sunflower, growth, nitrogen-boron, oil contents, calcareous soil

INTRODUCTION

Severe shortage of edible oil in Pakistan has created an endless growing gap between local production and demand. Out of total accessibility of edible oil (2.33 million tons), local development provided 0.61 million tons. In 2013-14, the country invested Rs. 148.63 billion (US$ 1.43billion) for the import of 1.72 million tons edible oil (GOP, 2013-14). Among conventional and non-conventional sources, winter oilseed rape and mustard crops contributed 10-13% of edible oil production, while high contents of glucosinolates and erucic acid limit its oil use as cooking oil regularly. Cottonseed leads to about 70% oil production but mainly grown for fiber intention. Hence, sunflower among oilseed crops with high adaptation capacity places a superior applicant for edible oil making (Ishfaq, 2010; Gul and Kara, 2015).

Inadequacy of mineral nutrients in the soil rhizosphere may lead to reduce the growth and productivity of sunflower. In calcareous soils, nitrogen availability is reduced owing to high temperature and volatilization losses (Khan et al., 2013), and has become a crucial problem for crop yield and quality. These volatilization losses may approach up to 50 percent of the nitrogen fertilizer application in calcareous soils with high pH or low buffering capacity. Proper supply of nitrogen stimulates the shoot growth more rapidly than root growth because of its critical role in proteins and chlorophyll formation (Mahmood et al., 2001; Awais et al., 2015).Nitrogen losses can be minimized by micronutrient application like boron. Addition of boron fixes the nitrogen through binding ENOD2 proteins (Bonilla et al., 1997), which is responsible to provide sufficient oxygen to nitrogenase enzyme for surviving which fixes the nitrogen.In calcareous soils, inadequate boron availability has also been of common scene (Nadian et al., 2010).Boron relates with cell wall structure integrity, cell differentiation, helps in root elongation and shoots growth. Its application improves the growth attributes by enhanced metabolic and photosynthetic activity; however, boost up the diverse plant metabolic pathways liable for cells partitioning and elongation (Hatwaret al., 2003). Increased photosynthesis in the presence of boron helps to activate the synthesis of tryptophan, which is responsible for promoting plant growth and biomass accumulation. Boron being a component of ferrodoxin and electron transport chain also associates with chloroplast which accelerates the photosynthetic efficiency of crop plants (Patil et al., 2008).

Since sunflower like other oilseed crops has high nitrogen and boron requirements, while limited information of what's the behavior of sunflower crop against combined nitrogen and boron fertilization particularly in alkaline-calcareous nature soils. Hence, the current field study was aimed to explore the impact of boron on growth, yield and oil quality of sunflower under varying nitrogen levels in calcareous soils of Pakistan.

MATERIALS AND METHODS

Field experiment was conducted at the Research Area of Agronomy, University of Agriculture, Faisalabad (Pakistan) for two growing seasons of 2011 and 2012. The experimental site was situated by 73° 06′ E, 310 26′ N and at altitude of 184.4 m above the sea level under semi-arid climate. The weather features as rainfall, relative humidity, air temperature, sunshine and wind speed for both cropping seasons of sunflower crop were at the experimental site during crop development (Figure 1). The top 30 cm of the soil was analyzed for their chemical and mechanical properties (Table 1).

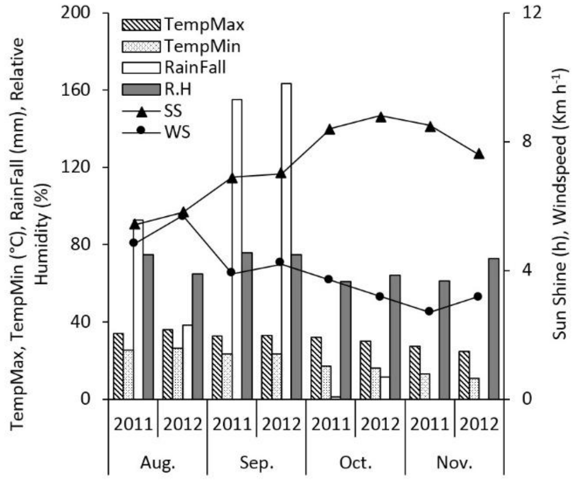

Figure 1. Weather conditions of sunflower crop during the growing seasons of 2011 and 2012

Table 1. Physiochemical analysis of the experimental soil

Determinations	Unit	Value	
		2011	**2012**
Sand	%	58.32	59.15
Silt	%	20.16	18.88
Clay	%	21.52	21.97
pH	--	8.1	7.9
SP	%	35.21	36.58
EC	dS m^{-1}	1.22	1.27
OM	%	0.89	0.81
Total nitrogen	%	0.053	0.061
Available phosphorus	mg kg^{-1}	6.76	6.22
Available potassium	mg kg^{-1}	169	176
Available boron	mg kg^{-1}	0.37	0.41

Experimental treatments and growth conditions

Treatment plan comprised of 50% RN (50% of recommended nitrogen or having 56 kg N ha^{-1}), 75% RN (75% of recommended nitrogen or having 84 kg N ha^{-1}), and 100% RN (100% of recommended nitrogen or having 112 kg N ha^{-1}) and boron rates of 0, 2, 4 and 6 kg ha^{-1} were applied in equal plots. Each plot was 7.0 m long and 3.0 m wide with 4 rows. Hysun-33 sunflower hybrid was used as a test crop and 3 seeds in each well prepared sowing ridge were sown manually on 1st August in 2011 and 7th of August in 2012. Hand dibbling method was used for the placement of seeds at proper depth in the field keeping 75 cm row spacing and 20 cm on-row plant spacing. Only one seedling of sunflower was maintained in each bed by performing thinning at 3-4 leaf stage. Hoeing was done in order to keep the field weed free. Crop was irrigated four times with furrow irrigation during 4-6 leaf stage, inflorescence emergence, flower initiation and grain filling periods for both growth periods. The 57 kg ha^{-1} phosphorus was used as di-ammonium phosphate and 62 kg ha^{-1} potassium was used as triple super phosphate fertilizers. The complete phosphorus and potash while 33% nitrogen as urea (nitrogenous fertilizer) was applied at the time of sowing, while remaining nitrogen was applied into two splits; 33% at 4-6 leaf stage and 33% at flowering stage. Throughout the growing season, boron fertilizer as boric acid (17%) was applied per treatment by mixing into the soil at the time of sowing and at 4-6 leaf stage. Plants were harvested when lower leaves, back of the head and bract leaves turned into yellow and brown, and seeds on head changed to dark and hard. Twenty five plants were harvested manually from central two rows with excluding the effects of side rows and corners of sowing bed. Harvesting was done on 14-18 November of 2011 and 16-23 November of 2012.

Measurements

Sunflower phenology traits as days to 50% IES (inflorescence emergence stage) was determined by counting the days from the day of sowing up to the day when inflorescence of five plants just visible among youngest leaves of ten tagged plants. Likewise, 50% FS (flowering stage) and 50% MS (maturity stage) were determined by calculating the days from sowing up to the day when five of ten tagged sunflower plants exhibited completely opened flowers and achieved full maturity, respectively. Ten plants were selected at random from each plot and their heights were measured in cm with measuring tape, and then averaged. At final harvesting, vernier caliper was used to determine stem diameter of ten randomly selected plants at base, middle and top of each stem and then averaged. The diameter of ten randomly selected heads was measured with the help of a measuring tape and average diameter in cm was computed. After threshing, the heads of ten randomly selected plants were separated and accounted for total achenes in these heads and then averaged per head. From the seed lot of every plot five samples, each of 1000-achenes was randomly selected and then recorded their weight and mean 1000-achene weight was computed. Achene yield was calculated per hectare using the yields of each plot. Percent oil contents in seeds were determined by means of Soxhlet extraction method (AOAC, 1990) as "% Oil = Wt. of flask + oil - Wt. of flask /Wt. of flask + seed - Wt. of flask × 100". Nitrogen contents in seeds were determined as consistent with Kjeldhal method (Bremner, 1964) by "% Crude protein = (V_1-V_2) N/ 100W × 14 × 6.25 × 100". Fatty acid profile (stearic, palmitic, oleic, and linoleic acids) in sunflower oil was determined by gas liquid chromatography (GLC) as said by Martin, (1979).

Statistical analysis

Proposed field study was carried out under factorial arrangement with RCB design to randomize the different nitrogen and boron application rates with twelve treatments in triplicate run. Experimental results on different traits were analyzed using Fisher's ANOVA technique (Steel et al., 1997), and treatments means were compared using LSD test at 5% probability level.

RESULTS

Combined analysis of nitrogen and boron variance for various sunflower yield and yield components showing nitrogen and boron effects were significant ($p \leq 0.05$) (Table 2).Fertilization with 100% RN nutrition significantly enhanced the days to 50% inflorescence emergence, 50% flowering and 50% maturity stages than 50% RN and 75% RN levels during both years of study (Table 3). Boron application exhibited considerable response in terms of prolonged growth stages of sunflower, as soil application of boron with 4.0 kg ha^{-1} showed maximum days to 50% inflorescence emergence and50% flowering stages irrespective to the control during both years (Table 3). Increased days for 50% maturity stage was recorded when plants treated with boron rate of 2.0 kg ha^{-1} during 2011, while in next year boron rate of 4.0 kg ha^{-1} remained best to attain maximum days for 50% maturity. Within the boron application rates, 6.0 kg ha^{-1} not responded well to increase the duration of 50% inflorescence emergence, 50% flowering and 50% maturity stages (Table 3).Interaction effect between nitrogen and boron for phenological growth stages of sunflower was found non-significant during both of the years.

Increased height for sunflower plants was exhibited with adding more nitrogen nutrition in contrast to medium and lowest rate of nitrogen nutrition during both years (Table 4).Taller plants were recorded with 100% RN than 75% RN and 50% RN levels. The shortest plants were noted in those plots that were treated with least nitrogen nutrition of 50% RN in both years of experimentation. Improvement in getting taller plants was also exhibited with soil application of boron. In 2011, the maximum enhancement in getting taller plants was recorded with both 2.0 and 4.0 kg ha^{-1} boron rates, while in 2012, all boron application rates like 2.0, 4.0 and 6.0 kg ha^{-1} were statistically not varied among each other in providing maximum plant height over control (Table 4). Effect of nitrogen and boron nutrition except their interaction on stem diameter was significant in both years. Thick stems were observed with 100% RN level, while lowest level for nitrogen nutrition of 50% RN revealed poor performance regarding stem thickness of treated plants during both years. The diameter of stems declined gradually in the order of 100% RN > 75% RN > 50% RN (Table 4). In both years, the maximum stem diameter was obtained in those plots which were amended with 4.0 kg ha^{-1} boron nutrition followed by 2.0 kg ha^{-1} compared to that of the control (Table 4).Interactive effect between different nitrogen and boron nutrition levels regarding head diameter continued significant only in 2012 year of study. In 2011, 100% RN nutrition substantially improved the width of treated heads than 75% RN and 50% RN levels. Soil application of boron with 2.0 and 4.0 kg ha^{-1} ensured useful to achieve extraordinary size of sunflower heads (Table 4). In 2012, boron fertilization at 4.0 kg ha^{-1} provided wider heads with 100% RN and 75% RN nutrition. There were no differences for head diameter between 6.0 kg ha^{-1} boron application rate and control (no boron application). Application of boron with 2.0 and 4.0 kg ha^{-1} produced statistically similar head diameter as well with 50% RN (Table 4).

Table 2. Statistical significance from ANOVA of sunflower phenology, yield components and oil quality

Source of variance	d.f.	Mean squares 50% IES	50% FS	50% MS	Plant height	Stem diameter
Reps (R)	2	42.87	328.51	128.43	4011.88	0.40
Year (Yr)	1	51.68**	40.50**	80.22**	1079.96**	0.58**
Nitrogen (N)	2	260.04 **	388.01**	317.72**	2942.70**	3.66**
Boron (B)	3	34.19**	44.16**	35.87**	432.75**	0.19**
Yr × N	2	0.85	1.63	0.05	2.65	2.978E-32
Yr × B	3	1.64	0.093	1.22	9.91	3.512E-32
N × B	6	2.39	3.07	0.87	47.02	0.008*
Yr × N × B	6	0.53	0.16	1.38	24.18	7.703E-32
Error	46	1.29	2.31	1.60	22.40	0.003
		Head diameter	**Achenes/head**	**1000-achene weight**	**Achene yield**	**Achene oil content**
Reps (R)	2	66.15	52973	25.59	15584	117.76
Year (Yr)	1	38.24**	42861**	158.81**	230367**	36.09**
Nitrogen (N)	2	496.17**	961965**	2426.86**	9657575**	505.11**
Boron (B)	3	14.67**	30949**	109.44**	417473**	25.69**
Yr × N	2	0.17	26	0.81	654	0.84
Yr × B	3	0.16	22	0.18	2402	3.96
N × B	6	0.92*	678**	1.41*	9404**	0.66
Yr × N × B	6	0.14	188	1.70*	3592*	0.38
Error	46	0.34	163	0.60	1198	2.12
		Achene protein content	**Stearic acid**	**Palmitic acid**	**Oleic acid**	**Linoleic acid**
Reps (R)	2	8.15	0.54	3.66	2.55	169.55
Year (Yr)	1	12.20**	1.43**	1.47**	39.99**	393.92**
Nitrogen (N)	2	242.98**	7.63**	35.02**	6.66**	251.21**
Boron (B)	3	7.02**	0.13**	0.42**	0.72**	11.138**
Yr × N	2	0.03	0.002	0.006	0.03	0.37
Yr × B	3	0.10	0.001	0.003	0.04*	0.32
N × B	6	0.17*	0.006	0.012	0.03	0.36
Yr × N × B	6	0.09	0.002	0.001	0.003	0.37
Error	46	0.07	0.005	0.019	0.013	0.41

*, ** = significance at $p \leq 0.05$ and $p \leq 0.01$, respectively; df = degree of freedom; 50% IES = 50% inflorescence emergence stage; 50% FS = 50% flowering stage; 50% MS = 50% maturity stage

Achenes per head were positively affected with boron application of 4.0 kg ha^{-1} under all nitrogen nutrition levels. Soil applied boron with 2.0 kg ha^{-1} also offered better response in terms of maximum achenes per head but its effect was less than 4.0 kg ha^{-1} (Table 5). With 100% RN nutrition, maximum number of achenes per head was produced with 2.0 and 4.0 kg ha^{-1} boron application rates as compared to the control during the year of 2012. Similarly, with 75% RN, boron rate of 4.0 kg ha^{-1} provided highest number of achenes, but plants treated with 2.0 kg B ha^{-1} revealed maximum number of achenes per head under 50% RN nutrition. The increase in boron application rate up to the limit of 6.0 kg ha^{-1} resulted in reducing number of achenes per head among all rates for nitrogen levels (Table 5). Crop plants produced heavier achenes when they were treated with boron rate of 4.0 kg ha^{-1} after 100% RN nutrition over un-treated control in both years; while during 2011, boron rate of 2.0 kg ha^{-1} behaved statistically similar under same level of nitrogen nutrition in relation to heavier achenes (Table 5). With 75% RN nutrition, maximum 1000-achene weight was also recorded with boron level of 4.0 kg ha^{-1} parallel to the control treatment in both years. Further enhancement in boron rate beyond 4.0 kg ha^{-1} resulted in significant decrease in 1000-achene weight with all nitrogen levels. Under nitrogen nutrition of 50% RN, 4.0 kg ha^{-1} boron application ensured improved achene weight during 2011, while in next year, 2.0 kg ha^{-1} boron application stayed dominant for heavier achenes (Table 5). Combined effect of divergent nitrogen and boron nutrition levels was measured significant in improving the achene yield of sunflower (Table 5). In 2011, boron rate of 4.0 kg ha^{-1} performed best in improving the achene yield when plants were grown with 100% RN nutrition. While in 2012, the maximum achene yield was exposed with 2.0 kg ha^{-1} boron application rate over control, but it was not statistically differed with 4.0 kg ha^{-1} boron rate. Similarly, with 75% RN nutrition, maximum improvement in achene yield was ensured with boron application of 4.0 kg ha^{-1} over control during both years (Table 5). Under 50% RN, boron rate of 4.0 kg ha^{-1} responded well in producing highest achene yield in 2011; while in the next year, 2.0 kg ha^{-1} boron rate ensured highest achene yield in producing maximum achene yield (Table 5).

Table 3. Phenology of sunflower as affected by soil applied nitrogen and boron fertilization

Treatments	50% IES		50% FS		50% MS	
	2011	2012	2011	2012	2011	2012
Nitrogen levels (kg ha^{-1})						
50% RN	35.74 c	34.08 c	58.08 c	57.00 c	100.83 c	98.66 c
75% RN	39.25 b	37.16 b	61.50 b	60.16 b	103.84 b	101.83 b
100% RN	42.16 a	40.83 a	66.58 a	64.50 a	108.08 a	105.92 a
CD ($p \leq 0.05$)	0.98	0.97	1.29	1.31	1.13	1.01
Boron rates (kg ha^{-1})						
0	38.00 c	35.44 c	60.11 c	58.77 c	102.22 c	100.66 c
2.0	39.22 b	37.89 b	62.44 b	60.76 b	105.11 a	102.33 b
4.0	40.55 a	39.33 a	64.00 a	62.44 a	105.89 a	103.67 a
6.0	38.44 bc	36.78 b	61.66 b	60.22 bc	103.78 b	101.89 b
CD ($p \leq 0.05$)	1.14	1.12	1.49	1.52	1.30	1.16
Interaction	ns	ns	ns	ns	ns	ns

Main effect means for any trait not sharing common letter(s) in their respective column differ significantly ($p \leq 0.05$). CD = Critical difference; ns = non-significant; 50% IES = 50% inflorescence emergence stage, 50% FS = 50% flowering stage, 50% MS =50% maturity stage.

Table 4. Plant height, stem diameter and head diameter of sunflower as affected by soil applied nitrogen and boron fertilization

Boron rates (kg ha^{-1})	Plant height (cm)				Stem diameter (cm)				Head diameter (cm)			
	50% RN	75% RN	100% RN	Means	50% RN	75% RN	100% RN	Means	50% RN	75% RN	100% RN	Means
						2011						
0	167.83^{ns}	190.78	195.70	184.77 c	1.43^{ns}	1.76	2.26	1.82 c	10.69^{ns}	15.00	19.08	14.92 c
2.0	182.76	197.01	205.02	194.93 a	1.58	1.94	2.32	1.95 b	11.64	16.57	21.03	16.41 a
4.0	184.58	198.63	206.71	196.64 a	1.67	2.07	2.41	2.05 a	12.05	16.83	22.07	16.98 a
6.0	183.74	184.71	201.08	189.84 b	1.50	1.83	2.28	1.87 c	10.99	15.51	20.25	15.58 b
Means	179.73 c	192.78 b	202.13 a		1.55 c	1.90 b	2.32 a		11.34 c	15.97 b	20.61 a	
CD ($p \leq 0.05$)	Nitrogen levels = 4.35; Boron rates = 5.02				Nitrogen levels = 0.04; Boron rates = 0.06				Nitrogen levels = 0.56; Boron rates = 0.64			
						2012						
0	165.59^{ns}	177.96	190.83	178.13 c	1.26^{ns}	1.59	2.11	1.65 d	9.44 b	13.63 c	17.72 c	13.59
2.0	174.63	186.28	194.27	185.06 ab	1.43	1.79	2.20	1.81 b	10.39 a	14.68 b	19.06 b	14.71
4.0	176.60	190.62	199.76	188.99 a	1.53	1.91	2.25	1.89 a	10.76 a	15.39 a	20.94 a	15.69
6.0	173.71	182.57	192.80	183.03 b	1.35	1.72	2.13	1.73 c	9.65 b	14.35 b	18.21 c	14.07
Means	172.63 c	184.35 b	194.42 a		1.39 c	1.75 b	2.17 a		10.06	14.51	18.98	
CD ($p \leq 0.05$)	Nitrogen levels = 3.79; Boron rates = 4.37				Nitrogen levels = 0.05; Boron rates = 0.06				Nitrogen levels × Boron rates = 0.70			

Interaction and main effect means for any trait not sharing common letter(s) in their respective column differ significantly ($p \leq 0.05$). CD = Critical difference; ns = non-significant.

Table 5. Achenes/head, 1000-achene weight and achene yield of sunflower as affected by soil applied nitrogen and boron fertilization

Boron rates (kg ha^{-1})	Achenes/head			1000-achene weight (g)			Achene yield (kg ha^{-1})		
	50% RN	75% RN	100% RN	50% RN	75% RN	100% RN	50% RN	75% RN	100% RN
					2011				
0	724 c	934 d	1131 c	30.21 c	38.52 d	50.41 c	1719 c	2301 c	2905 c
2.0	795 b	992 b	1186 b	32.82 b	43.93 b	53.97 a	1917 b	2459 b	3165 b
4.0	833 a	1028 a	1210 a	34.79 a	45.84 a	55.29 a	2023 a	2664 a	3285 a
6.0	739 c	968 c	1170 b	32.41 b	41.24 c	52.44 b	1763 c	2415 b	3107 b
CD ($p \leq 0.05$)	Nitrogen levels × Boron rates = 20.62			Nitrogen levels × Boron rates = 1.44			Nitrogen levels × Boron rates = 59.77		
					2012				
0	680 b	871 c	1082 c	27.09 c	36.35 c	46.03 d	1580 c	2167 c	2826 c
2.0	753 a	936 b	1143 a	31.66 a	39.70 b	51.00 b	1819 a	2328 b	3131 a
4.0	772 a	994 a	1162 a	31.91 a	42.64 a	52.85 a	1879 a	2591 a	3186 a
6.0	699 b	917 b	1116 b	29.28 b	38.69 b	49.02 c	1675 b	2269 b	2914 b
CD ($p \leq 0.05$)	Nitrogen levels × Boron rates = 21.03			Nitrogen levels × Boron rates = 1.03			Nitrogen levels × Boron rates = 60.06		

Interaction and main effect means for any trait not sharing common letter(s) in their respective column differ significantly ($p \leq 0.05$). CD = Critical difference; ns = non-significant.

Plants grown with lowest rate of 50% RN nutrition showed highest percentage of achene oil contents in both years. The minimum oil contents were recorded when plants were supplemented with 100% RN. Oil contents of sunflower seeds were declined as the nitrogen nutrition was increased (Table 6). Similarly, boron application also reduced the achene oil contents, as the higher values for percent oil contents were exposed for those plants that stayed untreated with boron (Table 6). Least oil contents were produced with boron application of 4.0 kg ha^{-1} in 2011, while in next year, minimum oil contents were produced with 2.0 kg ha^{-1} boron application rate followed by 4.0 kg ha^{-1}. The interaction study of nitrogen and boron for stearic acid, palmitic acid, oleic acid and linoleic acid could not achieve the level of significance in both years. The maximum stearic acid percentage was obtained with 50% RN nutrition, while the higher rate of 100% RN helped more to reduce the stearic acid in sunflower oil during both years (Table 6). Boron supplemented seeds with 2.0 and 4.0 kg ha^{-1} indicated least stearic acid in 2011, which was followed by highest rate for boron nutrition of 6.0 kg ha^{-1} over control. The treated seeds with all boron rates exposed minimum stearic acid percentage over control during the study year of 2012 (Table 6). Palmitic acid was increased with higher nitrogen nutrition rates as its maximum concentration was exhibited with 100% RN nutrition (Table 6). The lowest rate for 50% RN nutrition showed poor response in relation to improve the palmitic acid percentage in both years. Among diverse boron application rates, 4.0 kg ha^{-1} proved to be best for increasing palmitic acid concentration which was followed by 2.0 kg ha^{-1} of boron rate. Increment in boron rate up to 4.0 kg ha^{-1} enhanced the palmitic acid concentration, but further boron addition reduced its percentage during both years of experimentation (Table 6). Oleic acid concentration was reduced following the order of 50% RN > 75% RN > 100% RN nutrition levels during both years. Plants grown without boron showed maximum concentration for oleic acid in achene oil, while minimum was recorded with 4.0 kg ha^{-1} boron fertilization during 2011. The trend was different for 2012, as lowest concentration of oleic acid was recorded with both 2.0 and 4.0 kg ha^{-1} boron rates in contrast to the control (Table 6). The higher linoleic acid concentration was observed when plants were fertilized with 100% RN, and it declined progressively with reducing rates for nitrogen level in both years. In 2011, 4.0 kg ha^{-1} boron rate presented maximum linoleic acid contents in achene oil, followed by 2.0 and 6.0 kg ha^{-1} as compared to no boron application. The boron rates of 2.0 and 4.0 kg ha^{-1} depicted highest concentration for linoleic acid during 2012 (Table 6).

During 2011, the maximum achene protein contents were exhibited for seeds that amended with 4.0 kg ha^{-1} boron application rate, then supplemented with 100% RN nutrition, but was followed by the seeds grown with 2.0 kg ha^{-1} boron under same level of nitrogen nutrition in next year (Figure 2). Seeds harvested from treated plants of 2.0 kg ha^{-1} boron rate followed by 4.0 kg ha^{-1} with 75% RN, ensured maximum percentage for achene protein contents parallel to the seeds harvested from control plots in 2011, while only 2.0 kg ha^{-1} boron level remained best for 2012. In 2011, soil applied boron rate of 4.0 kg ha^{-1} with 50% RN nutrition resulted in superior development of seed protein contents, while in 2012, the maximum achene protein contents were recorded with 2.0 kg ha^{-1} boron rate (Figure 2). The maximum benefit cost ration (BCR) was attained when plants were grown under 100% RN nutrition with boron application of 2.0 kg ha^{-1}. The boron rate of 4.0 kg ha^{-1} regarded superior in giving higher BCR values with 75% RN nutrition. The lower BCR was recorded in amended plants of 50% RN with all boron application rates during both years of study (Table 7).

Table 6. Fatty acid profile of sunflower oil as affected by soil applied nitrogen and boron fertilization

Treatments	Achene oil contents (%)		Saturated fatty acids: Stearic acid (%)		Saturated fatty acids: Palmitic acid (%)		Un-saturated fatty acids: Oleic acid (%)		Un-saturated fatty acids: Linoleic acid (%)	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Nitrogen levels (kg ha^{-1})										
50% RN	43.95 a	42.85 a	4.18 a	3.87 a	5.33 c	5.01 c	12.97 a	11.56 a	61.44 c	56.71 c
75% RN	39.32 b	37.49 b	3.74 b	3.46 b	6.62 b	6.34 b	12.49 b	10.96 b	64.73 b	60.33 b
100% RN	34.89 c	33.58 c	3.05 c	2.78 c	7.71 a	7.45 a	11.97 c	10.45 c	67.98 a	63.08 a
CD ($p \leq 0.05$)	1.05	1.21	0.06	0.05	0.13	0.10	0.08	0.11	0.56	0.54
Boron rates (kg ha^{-1})										
0	41.13 a	39.39 a	3.76 a	3.48 a	6.35 c	6.10 c	12.65 a	11.27 a	63.88 c	58.96 c
2.0	39.27 b	36.67 b	3.61 c	3.34 bc	6.58 b	6.28 b	12.45 b	10.86 c	64.89 b	60.56 a
4.0	37.94 c	37.21 b	3.57 c	3.28 c	6.75 a	6.45 a	12.27 c	10.74 c	65.56 a	60.93 a
6.0	39.21 b	38.62 a	3.68 b	3.40 b	6.52 b	6.23 b	12.54 b	11.09 b	64.54 b	59.71 b
CD ($p \leq 0.05$)	1.20	1.39	0.07	0.07	0.15	0.12	0.10	0.13	0.65	0.62
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Main effect means for any trait not sharing common letter(s) in their respective column differ significantly ($p \leq 0.05$). CD = Critical difference; ns = non-significant.

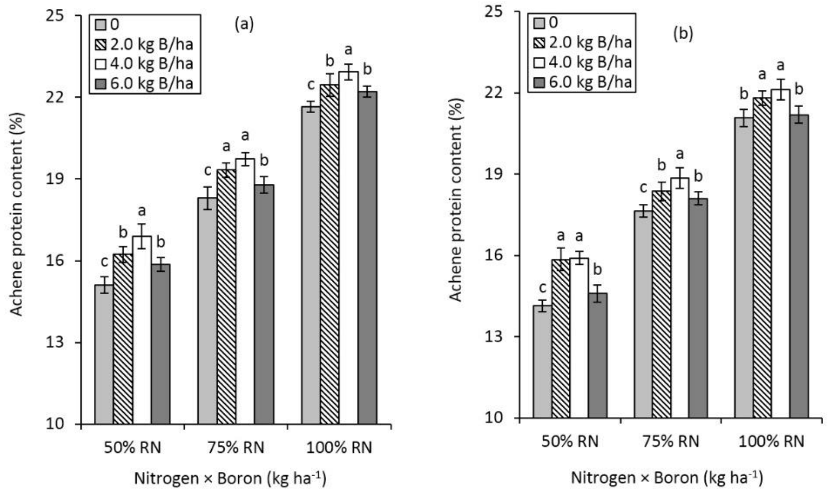

Figure 2. Achene protein content (%) of sunflower as affected by soil applied nitrogen and boron fertilization during 2011 (a) and 2012 (b)

Table 7. Economic analysis of sunflower as affected by soil applied nitrogen and boron fertilization

Treatments		Gross Income (Rs. ha^{-1})	Variable Cost (Rs. ha^{-1})	Fixed cost (Rs. ha^{-1})	Total Cost (Rs. ha^{-1})	Net Income (Rs. ha^{-1})	BCR[a]
				2011			
50% RN	0	78906	5142	70961	76103	2803	1.04
	2.0	87867	9186	70961	80147	7720	1.10
	4.0	92622	12480	70961	83441	9181	1.11
	6.0	80949	15774	70961	86735	-5786	0.93
75% RN	0	105537	7151	70961	78112	27425	1.35
	2.0	112734	11195	70961	82156	30578	1.37
	4.0	121902	14489	70961	85450	36452	1.43
	6.0	110665	17783	70961	88744	21921	1.25
100% RN	0	133195	9159	70961	80120	53075	1.66
	2.0	144938	13203	70961	84164	60774	1.72
	4.0	150306	16497	70961	87458	62848	1.72
	6.0	142336	19791	70961	90752	51584	1.57
				2012			
50% RN	0	69123	4945	69300	74245	-5122	0.93
	2.0	79356	8585	69300	77885	1471	1.02
	4.0	81966	11527	69300	80827	1139	1.01
	6.0	73208	14468	69300	83768	-10560	0.87
75% RN	0	94636	6893	69300	76193	18443	1.24
	2.0	101596	10533	69300	79833	21763	1.27
	4.0	112815	13475	69300	82775	30040	1.36
	6.0	99011	16416	69300	85716	13295	1.16
100% RN	0	123280	8841	69300	78141	45139	1.58
	2.0	136368	12481	69300	81781	54587	1.67
	4.0	138753	15423	69300	84723	54030	1.64
	6.0	127073	18364	69300	87664	39409	1.45

[a]BCR = Benefit cost ratio.

DISCUSSION

Prolonged duration of different phenophases of sunflower was perceived with nitrogen and boron nutrition in present study. Extended 50% inflorescence emergence, 50% flowering and 50% maturity stages might be due to stimulating effect of nitrogen and boron nutrition on plant growth and productivity plus photosynthetic efficiency through increased thylakoid and stomatal protein contents in leaves (Shekhawat and Shivay, 2012). Increased time to sunflower growth stages was also responsive to boron due to its involvement in cell wall synthesis and structure, protein, nucleic acid and carbohydrate metabolism, sugar transport and membrane functions and integrity. A facilitative response of nitrogen to extend the plant growth stages was due to its involvement in structural support of cell membrane as well as in non-structural components of enzymes, nucleic acids, amino acids and chlorophyll pigments (Goldbach et al., 2001; Seilsepour and Rashidi, 2011).

In current study, nitrogen application in combination with boron considerably improved the growth and yield performance of sunflower. Significant taller plants were acquired with nitrogen application because of quick and vigorous growth regarding shoot and root development (Ozer et al., 2004). In fact, nitrogen stabilizes the carbon assimilation and mineral acquisition through source sink relationship, nutrients and water supply. The nitrogen supplied plants grows quickly and retain maximum shoot length by means of higher canopy area development, CO_2 exchange rate and photosynthetic capacity (Tonev, 2006). The positive effect of boron on enhanced plant height was considered due to higher photosynthetic efficiency in plants by increased metabolic activities. Its initial supply also diminishes the lignification, due to which plant cells endures to grow exclusively at tips with elongation of hypocotyl and epicotyls (Shekhawat and Shivay, 2012). Improvement in stem diameter has also been anticipated by nitrogen and boron because of its role in chlorophyll and nucleic acid synthesis, cells differentiation and elongation, turgidity and lignin biosynthesis (Hellal et al., 2009). Increase in head size with nitrogen and boron was also described in present results. Sadiq et al. (2000) also exposed the increase in capitulum diameter with nitrogen and boron application by means of efficient assimilates partitioning towards reproductive parts. Further, its application also regulates the photosynthetic efficiency and the process of respiration by maintaining protein and carbohydrate metabolism. Supply of boron actually increased the development of capitulum size through inhibiting the unnecessary conversion of sugars into starch. Its deficiency leads towards callus formation in sieve tubes which restricted the sugar borate complex, eventually depressing sink capacity and dropping head size (Shekhawat and Shivay, 2008).

Increased number of achenes/capitulum with nitrogen and boron application was due to its role in pollen tube formation by cross linking and muco-de-esterification of cell wall pectins (Renukadevi et al., 2002). Under well nitrogen availability, boron has good impact on better development of pollens, pollen germination, anther viability, anthesis, cell division, enhanced enzymatic activity and more availability of sugars (Garge et al., 1979), end resulted in production of more seeds. Transport of photo-assimilates towards achenes was triggered rapidly with nitrogen and boron nutrition management. Heavier achenes attributable to nitrogen and boron fertilization was due to its key function in amino acids, cellulose, chlorophyll, nucleoproteins, kinins, auxins and also provides a rigid cell wall to the plants, led towards higher growth and photosynthates production (Awais et al., 2013). Application of nitrogen and boron sustains the proper functioning of enzymes and integrity of plasma membrane that drops starch contents in leaves and promotes photosynthates translocation towards seeds, finally enhancing 1000-achene weight (Mazher et al., 2006). Significant effects on achene yield were existed in our study when sunflower crop treated with nitrogen and boron nutrition. Improved achene yield with nitrogen and boron nutrition was due to its imperative role in pollen germination, flowering, proper seed setting, fruiting processes, sugar metabolism and balance between net photosynthetic rate and respiration (Khan et al., 2006). Its removal alters the cell wall structure integrity, caused hardening of plasma membrane led to reduce its activity for better transportation of photosynthates, eventually reduced seed yield. Application of nitrogen encourages sugar production which leads towards more export of sugars from leaves to the storage organs as in roots and seeds. This accumulation of sugars improves the yield of sunflower (Noppakoonwong et al., 1997; Yu et al., 2002).

Current results exhibited decline in achene oil contents with nitrogen and boron fertilization. In fact, balanced nitrogen and boron nutrition caused synthesis of protein in seeds (Bredenhann, 1999), while this reduction was due to opposite correlation between oil contents and protein synthesis via dilution effect. A negative correlation might be ascribed towards transportation of sugars effecting achene oil synthesis. After boron fertilization and seed setting, protein is synthesized then oil production starts. Hence, exogenous supply of boron specifically after pollination or in grain filling period may diminish achene oil contents (Salisbury and Ross, 1994; Ceyhan et al., 2008). Application of boron also resulted in more uptake of nitrogen by seeds which successively incorporated in protein synthesis. Adequate application of nitrogen develops the protein precursors, due to which maximum photosynthates utilized for protein synthesis, while lesser amount is accessible for fats synthesis (Ahmad, 2007). Exogenous applied nitrogen and boron had stimulatory influence on quantity of nucleoproteins and also involved in biological cell activities like isolated placenta nuclei (transcription) and wheat germ extract (translation), which greatly improved mRNA synthesis and consequently enhanced protein contents (Dzondo-Gadet et al., 2002). Significant decline in stearic and oleic acid while increase in palmitic and linoleic acid concentrations with nitrogen and boron fertilization were recorded in present study. Palmitic acid showed superior response when plants were grown in nitrogen amended plots but inverse behavior was noted for stearic acid (Steer and Seiler, 1990). Through involvement of enzymatic activity of $\Delta 12$ desaturase for conversion of oleic acid to linoleic acid ultimately enhanced linoleic acid concentration (Ali et al., 2009). Application of boron distinctly enhanced the unsaturated fatty acids (oleic and linoleic) contents and lessened the contents of saturated fatty acids (stearic and palmitic). Use of boron also hinders the acetate incorporation into lipids (Rani et al., 2006), which improves the ratio of unsaturated fatty acids to saturated fatty acids.

CONCLUSIONS

Integrated use of boron with nitrogen had positive impact on sunflower growth and productivity in calcareous soils. Sunflower phenology, achene yield, its components and oil quality traits (stearic, palmitic, oleic and linoleic acids) were substantially improved with boron nutrition under adequate nitrogen supply. The boron rate of 2.0 kg ha^{-1} with 100% RN nutrition economically performed best for improving maximum growth and yield of sunflower. The boron rate of 4.0 kg ha^{-1} was considered best under reduced nitrogen nutrition of 75% RN and 50% RN levels.

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