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Contribution of soil and foliar fertilization of nitrogen and sulfur on physiological and quality assessment of wheat (*Triticum aestivum* L.)

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ABSTRACT

Nitrogen and sulfur supplies have a strong influence on the physical characteristics of crop as well as on the quality and quantity of wheat storage proteins, which play an important role in bread-making process. In order to evaluate the contribution of soil and foliar fertilization of nitrogen and sulfur on physiological and quality assessment of wheat, a field trail was carried out having randomized complete block design with four replications and eight different treatments of nitrogen and sulfur combinations were allotted to plots at different growth stages. Results indicated that highest protein content (12.82%), maximum moisture content (10.9%), maximum crop growth rate and maximum absolute growth rate were recorded when the wheat crop was fertilized with T_8 [Nitrogen @ 60 kg·ha^{-1} at sowing + 40 kg·ha^{-1} at tillering + 10 kg·ha^{-1} at anthesis (spray) + 10 kg·ha^{-1} after anthesis (spray)] + [Sulfur @ 15 kg·ha^{-1} at sowing + 10 kg·ha^{-1} at anthesis (spray) + 5 kg·ha^{-1} after anthesis (spray)], while control practice resulted low moisture content, low protein, minimum crop growth rate and low absolute growth rate. Among physiological components of wheat cultivars, leaf area index was enhanced when fertilization was done with T_5 (Sulfur @ 15 kg·ha^{-1} at sowing + 10 kg·ha^{-1} at anthesis + 5 kg·ha^{-1} after anthesis). In all the recorded observations, concerning experiment wheat cultivar Pirsabaq-2005 showed appreciable response as compared with other variety (Khyber-87). Thus it is possible to obtain maximum physiological traits as well as bread-making quality of wheat through soil and foliar application of nitrogen and sulfur.

Keywords: Absolute Growth Rate; Crop Growth Rate; Leaf Area Index; Moisture and Protein Contents

1. INTRODUCTION

Cereals are an important dietary protein source throughout the world, because they constitute the main protein and energy supply in most countries [1]. Wheat is one of the major cereal crops with a unique protein, which is consumed by the humans and is grown around the world in diverse environments. It has already been known that gluten proteins have a primary role in wheat flour quality. It is recognized that variation in protein contents and composition significantly affects wheat quality with subsequent influence on baking quality [2]. Nitrogen and sulfur contents of wheat influence the bread-making quality of wheat flour [3]. Studies have shown that foliar as well as soil application of nitrogen and sulfur on wheat at optimum timings (during and after anthesis) has increased grain protein contents and improved bread-making quality [4].

Nitrogen rate, type and timings of its application are important factors to increase wheat protein contents and

its yield [5]. In a study, Nitrogen spraying on leaves during 2 - 3 weeks after flowering has shown a significant increase in grain protein contents [4]. The metabolism of nitrogen and sulfur is closely interrelated and an optimal N/S ratio in grain has been shown to improve breadmaking quality. Differences in nitrogen and sulfur uptake and redistribution of nitrogen and sulfur influence protein content and composition as well as grain characteristics and dough properties. The current practice of applying large amounts of nitrogen fertilizers to cereal crops without considering sulfur requirements is becoming a concern for crop quality. With the increased use of sulfur-free fertilizers in modern cropping systems and the decrease of atmospheric sulfur emissions by industry, sulfur has become a major limiting factor for crop production. Research has also indicated that there is an increase of grain protein from 11% to 12% by spraying of 12 kg/ha of ammonium sulphate [4]. Although wheat has a relatively low sulfur requirement, still deficiencies have been observed in many countries of the world [6].

Nitrogen and sulfur fertilization boosts yield components, phenology and leaf traits. Crop biomass and crop growth rate are dependent on the ability of the canopy to intercept incoming photo synthetically active radiations, which is the function of leaf area index (LAI), leaf area duration and canopy architecture and then converts these radiation into new biomass. Nitrogen availability influences the efficiency of assimilated mobilization to sink during leaf senescence and thus affects leaf viability and activity [7]. Nitrogen addition increases the leaf architecture from 7% to 19% at the lower sulfur rate, but at the highest sulfur supply, these increments range from 20% to 35%, evidencing a clear interaction between both nutrients [4]. Sulfur effects are evident between anthesis and physiological maturity increasing CGR by 51% [4]. An increase in leaf photosynthesis is expected when sulfur supply is increased. LAI was reduced in crops grown under nitrogen deficiency. Increases in nitrogen and sulfur contents of soil affect all growth stages of the crops. For example an increase in nitrogen concentration at anthesis can result in an increase of LAI by as much as 62% and incoming photo synthetically active radiations by up to 20% [8].

The present study was therefore designed to investigate about contribution of soil and foliar fertilization of nitrogen and sulfur on physiological and quality assessment in two varieties of wheat.

2. MATERIALS AND METHODS

2.1. Site Description and Experimental Design

Experiment was conducted at New Developmental Farm of The University of Agriculture Peshawar, Pakistan during 2008 and 2009. A basal dose of Phosphorus (100 kg/ha) and potassium (60 kg/ha) was applied at sowing. Urea was applied as a source for nitrogen and ammonium sulphate as a source for sulfur. Half dose of both urea and ammonium sulphate was applied at the time of sowing and the remaining half dose at different growth stages. The experiment was laid out in randomized complete block design having 4 replications. Subplots size was 5 m × 3 m having 10 rows 5 m long & 30 cm apart. Two varieties Pirsabaq-2005 & Khyber-87 were used in the study.

2.2. Fertilizer Treatments

Details of the fertilizer treatments are as follows:

T_1 Control: without fertilization

T_2 Recommended dose of soil applied Nitrogen (60 kg·N/ha at sowing + 60 kg·N/ha at tillering)

T_3 Soil applied Nitrogen (60 kg·N/ha at sowing + 40 kg·N/ha at tillering + 10 kg·N/ha anthesis + 10 kg·N/ha after anthesis)

T_4 Soil + foliar applied Nitrogen [60 kg·N/ha at sowing + 40 kg·N/ha at tillering + 10 kg·N/ha at anthesis (foliar) + 10 kg·N/ha after anthesis (foliar)]

T_5 Soil applied Sulfur (15 kg·S/ha at sowing + 10 kg·S/ha at anthesis + 5 kg·S/ha after anthesis)

T_6 Soil + foliar applied Sulfur [(15 kg·S/ha at sowing + 10 kg·S/ha at anthesis (foliar) + 5 kg·S/ha after anthesis (foliar)]

T_7 Soil applied N + soil applied S (combination of soil applied N and soil applied S)

T_8 Soil and foliar applied N + soil and foliar applied S (combination of soil + foliar applied nitrogen and soil + foliar applied sulfur)

The moisture content was determined by taking 5.0 g of thoroughly homogenized sample in a previously dried and weighted china dish. The china dish containing sample was then allowed to dry in oven at 105˚C until a constant weight was obtained. Moisture content was calculated by the formula: (Final weight − Initial weight/total weight) × 100.

Protein in the sample was determined by Kjeldahl method. The samples were digested by heating with concentrated sulphuric acid in the presence of digestion mixture. The mixture was then made alkaline. Ammonium sulphate thus formed was collected in 2% boric acid solution and titrated against standard HCI. Total protein was calculated by multiplying the amount of nitrogen with appropriate factor (5.7) and the amount of protein was calculated.

LAI (tiller^{-1}) was calculated by multiplying leaf area tiller^{-1} over tillers m^{-2} and divided by 10,000.

For calculating CGR, 50 cm long row was harvested in each sub plot. Four samples each on one month interval was collected during the crop growing season from

tillering to physiological maturity. CGR was determined by using the formula:

$$CGR = \frac{(W_2 - W_1)}{(T_1 - T_2)} \times \frac{1}{GA}$$

AGR was derived from the CGR. AGR was determined by the formula.

$$AGR = \frac{(W_2 - W_1)}{(T_2 - T_1)}$$

2.3. Statistical Analysis

All data are presented as mean values of four replicates. Data were analyzed statistically for analysis of variance following the method of [9]. The significance of differences among means was compared by using Least Significance Difference Test [10].

3. RESULTS

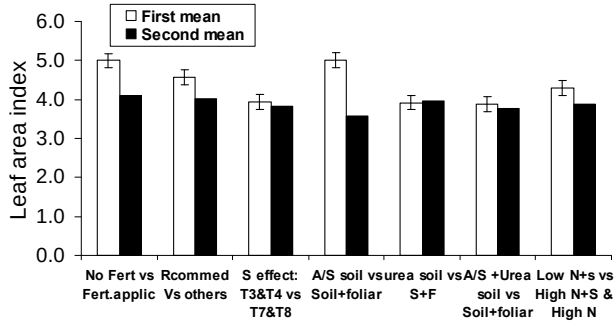
The presented data about LAI revealed that significant differences were found among different treatments of sulfur and nitrogen combinations **Table 1**. Data showed that highest LAI (5.01) was observed in T_5 (Sulfur @ 15 kg·ha^{-1} at sowing + 10 kg·ha^{-1} at anthesis + 5 kg·ha^{-1} after anthesis), while the lowest LAI (3.5) was found in T_6 (Sulfur @ 15 kg·ha^{-1} at sowing + 10 kg·ha^{-1} at anthesis + 5 kg·ha^{-1} after anthesis). Pirsabaq-2005 gave maximum LAI (5.1) as compared with the other variety. The interaction effects of variety and fertilizer was also recorded significant. Similarly the interaction of varieties and year was also found significant. From the means of planned comparison of two varieties it was observed that no fertilizer vs. fertilizers comparison showed significant result for LAI **Figure 1(a)**. Similarly the effects of sulfur alone treatment was also found significant.

It is evident from the data that crude protein contents are significantly affected by fertilizer treatments **Table 1**. The maximum crude protein contents (12.82%) were found in those plots which were treated with T_8 [Nitrogen @ 60 kg·ha^{-1} at sowing + 40 kg·ha^{-1} at tillering + 10 kg·ha^{-1} at anthesis (spray) and 10 kg·ha^{-1} after anthesis (spray)] + [Sulfur @ 15 kg·ha^{-1} at sowing + 10 kg·ha^{-1} at anthesis (spray) + 5 kg·ha^{-1} after anthesis (spray)], while control plots showed minimum crude protein contents (9.53%). Results showed that fertilizer treatments and both the varieties of wheat observed non significant affect on crude protein contents. Similarly no significant differences were found among the mean values of varieties on the concern parameter. However from the results of planned comparison for crude protein it is evident that the comparison of no fertilizer vs. fertilizer treatment gave significant association **Figure 1(b)**. Likewise significant differences were found among the mean values of recommended fertilizer vs. other fertilizer treatments comparison. The results of planned comparison of the

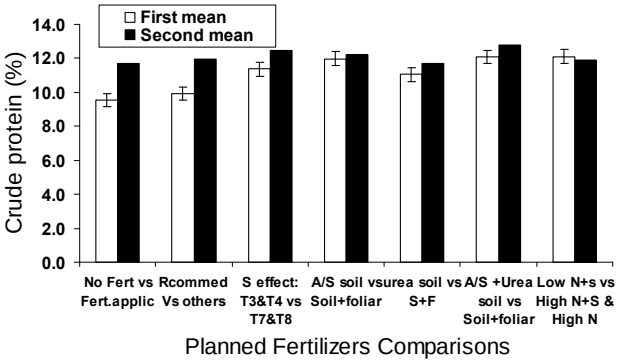
Table 1. Effects of various nitrogen and sulfur fertilizer treatments on physiological and quality assessment traits of two varieties of wheat.

		LAI			CP (%)			MC (%)		
		Varieties			Varieties			Varieties		
Fertilizer Treatments		**PS-2005**	**Khyber-87**	**Mean**	**PS-2005**	**Khyber-87**	**Mean**	**PS-2005**	**Khyber-87**	**Mean**
Control (CK)	**(T_1)**	6.89a	3.10fg	4.999a	9.303	9.767	9.535d	8.0	8.0	8.0d
Recommended dose	**(T_2)**	5.17b	3.97cde	4.577a	9.799	10.059	9.929d	8.0	8.0	8.0d
Soil applied N	**(T_3)**	4.50bc	3.33efg	3.917b	11.727	10.374	11.05c	8.0	8.0	8.0d
Soil and foliar applied N	**(T_4)**	3.82cdef	4.10cde	3.969b	12.211	11.181	11.696bc	9.0	8.75	8.9c
Soil and foliar applied N	**(T_4)**	7.10a	2.92g	5.016a	12.322	11.658	11.99ab	10.0	10.0	10.0b
Soil and foliar applied S	**(T_6)**	5.19b	1.94h	3.567b	12.289	12.201	12.24bc	10.0	10.0	10.0b
Combination of T_3 & T_5	**(T_7)**	4.21cd	3.54defg	3.883b	12.335	11.825	12.08ab	10.0	10.0	10.0b
Combination of T_4 & T_6	**(T_8)**	3.86cdef	3.65cdefg	3.763b	12.249	13.390	12.82a	11.0	10.75	10.9a
Mean		5.10	10.9a	-	11.49	11.30	-	9.25	**9.18**	-
2008-09		5.2a	3.06c	4.13	11.49	11.57	11.56			
2009-10		4.99a	3.58b	4.29	11.49	11.03	11.26	9.25	9.18	9.21
LSD										
F			0.604			0.9058			0.126	
V × F			0.855			-			-	
Y × V			0.4273			-			-	
Interactions			**P-Value**			**P-Value**			**P-Value**	
Y × F			0.3125			0.1000			0.1000	
Y × V × F			0.1333			0.4764			0.1000	

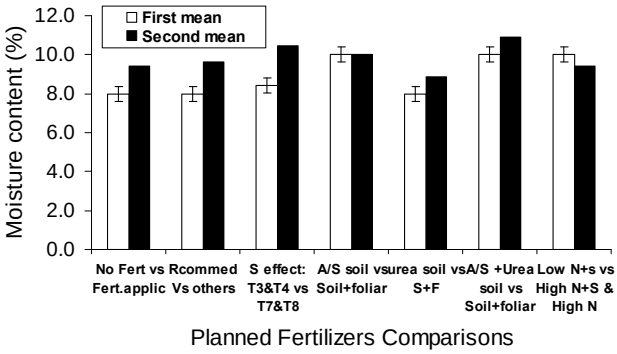
*Mean of the same category followed by different letters is significantly different ($P \geq 0.05$) using LSD test; LAI = Leaf Area Index, CP = Crude Protein, MC = Moisture Content, Y = Year, F = Fertilizer treatments; V = Variety, PS-2005 = Pirsabaq-2005, LSD = Least Significant Difference.



(a)



(b)



(c)

Figure 1. Planned comparison of the leaf area index (a), crude protein (b) and moisture content (c) of wheat as affected by the rate and method of sulfur and nitrogen application.

fertilizer treatments on two varieties also proposed significant affect of sulfur alone treatment on crude protein contents.

Data concerning moisture contents is depicted in **Table 1** which elaborated that different fertilizer treatment combinations reported highly significant effect on moisture content of the wheat flour. Maximum moisture contents (10.9%) were observed in the flour sample which was treated with T_8 [Nitrogen @ 60 kg·ha^{-1} at sowing + 40 kg·ha^{-1} at tillering + 10 kg·ha^{-1} at anthesis (spray) + 10 kg·ha^{-1} after anthesis (spray)] + [Sulfur @ 15 kg·ha^{-1} at sowing + 10 kg·ha^{-1} at anthesis (spray) + 5 kg·ha^{-1} after anthesis (spray)], while minimum moisture contents (8%) was recorded in control. Regarding varieties maximum moisture contents were observed in Pirsabaq-2005 compared with other variety. While the interaction between fertilizer treatments and varieties showed no significant relationship. Data from the planned comparison of fertilizer treatments on two varieties suggested that no fertilizer vs. fertilizer application comparison gave highly significant effect on moisture contents **Figure 1(c)**. Likewise recommended dose vs. other treatments also proposed highly significant effect. Similarly from the results of the planned comparison it is recorded that the effect of sulfur alone treatment also observed highly significant results.

The concern data proposed that maximum crop growth rate (23.3 g/m^2/day) was observed from the application of T_8 [Nitrogen @ 60 kg·ha^{-1} at sowing + 40 kg·ha^{-1} at tillering + 10 kg·ha^{-1} at anthesis (spray) + 10 kg·ha^{-1} after anthesis (spray)] + [Sulfur @ 15 kg·ha^{-1} at sowing + 10 kg·ha^{-1} at anthesis (spray) + 5 kg·ha^{-1} after anthesis (spray)], while control treatment was observed with minimum growth rate (3 g/m^2/day) **Figure 2**. It is also evident from the data that significant differences found among the means of interaction of crop growth rate and fertilizer treatments. The trend gradually increased with fertilizer application and reached to the plateau at third sampling period and then started decreasing frequently in later sampling periods **Figure 3**. The trend lines in the **Figure 3** explained that maximum dry matter was accumulated by the fertilization of soil and foliar applied nitrogen and soil and foliar applied sulfur. Data regarding absolute growth rate proposed that maximum AGR (2.1 g/day) was observed from the application of T_8 [Nitrogen @ 60 kg·ha^{-1} at sowing + 40 kg·ha^{-1} at tillering + 10 kg·ha^{-1} at anthesis (spray) + 10 kg·ha^{-1} after anthesis (spray)] + [Sulfur @ 15 kg·ha^{-1} at sowing + 10 kg·ha^{-1} at anthesis (spray) + 5 kg·ha^{-1} after anthesis (spray)], while control

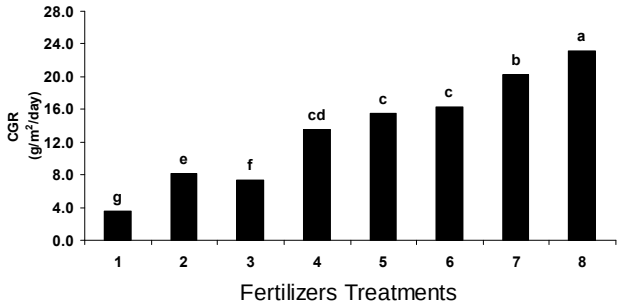


Figure 2. Seasonal mean of crop growth rate (CGR) of sulfur and nitrogen application to two different wheat cultivars (Pirsabaq-2005 and Khyber-87) during 2008-09 and 2009-10. **1** = control or ck; **2** = Recommended dose of nitrogen; **3** = soil applied nitrogen; **4** = soil and foliar applied nitrogen; **5** = soil applied sulfur; **6** = soil and foliar applied sulfur; **7**= soil applied nitrogen + soil applied sulfur; **8** = soil and foliar applied nitrogen + soil and foliar applied sulfur.

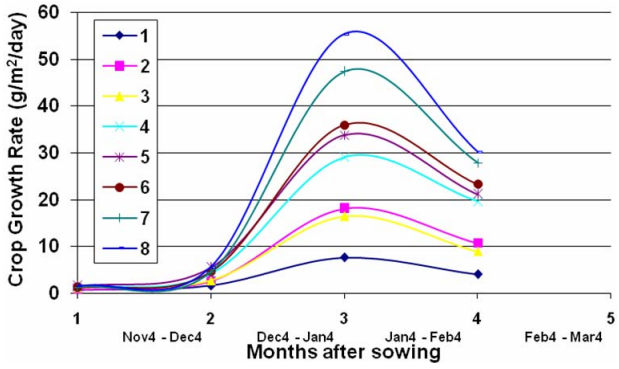


Figure 3. Seasonal trends of mean crop growth rate (CGR) of sulfur and nitrogen application to two wheat varieties (Pirs baq-2005 and Khyber-87) during 2008-09 and 2009-10. **1** = control or ck; **2** = Recommended dose of nitrogen; **3** = soil applied nitrogen; **4** = soil and foliar applied nitrogen; **5** = soil applied sulfur; **6** = soil and foliar applied sulfur; **7** = soil applied nitrogen + soil applied sulfur; **8** = soil and foliar applied nitrogen + soil and foliar applied sulfur.

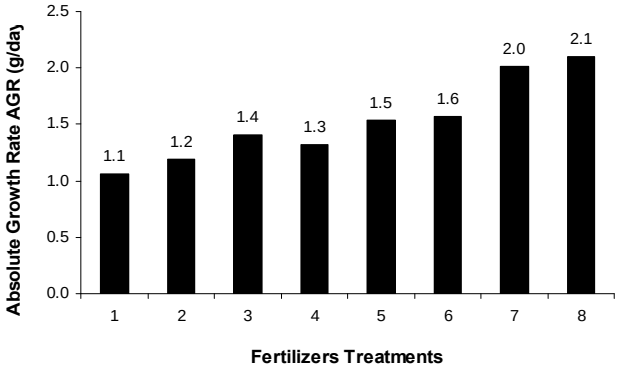


Figure 4. Seasonal mean of absolute growth rate (AGR) of sulfur and nitrogen application to two different wheat cultivars (Pirsabaq-2005 and Khyber-87) during 2008-09 and 2009-10. **1** = control or ck; **2** = Recommended dose of nitrogen; **3** = soil applied nitrogen; **4** = soil and foliar applied nitrogen; **5** = soil applied sulfur; **6** = soil and foliar applied sulfur; **7** = soil applied nitrogen + soil applied sulfur; **8** = soil and foliar applied nitrogen + soil and foliar applied sulfur.

treatment was observed with minimum growth rate (1.1 g/day) **Figure 4**. The data also declared that significant difference found among the means of interaction of absolute growth rate and fertilizer treatments. The trend gradually increased with fertilizer application and reached to the peak point at third sampling period and then started decreasing gradually in later sampling intervals **Figure 5**. The trend lines in the **Figure 5** explained that maximum dry matter was accumulated by the fertilization of T_8 [Nitrogen @ 60 kg·ha^{-1} at sowing + 40 kg·ha^{-1} at tillering + 10 kg·ha^{-1} at anthesis (spray) + 10 kg·ha^{-1} after anthesis (spray)] + [Sulfur @ 15 kg·ha^{-1} at sowing + 10 kg·ha^{-1} at anthesis (spray) + 5 kg·ha^{-1} after anthesis (spray)].

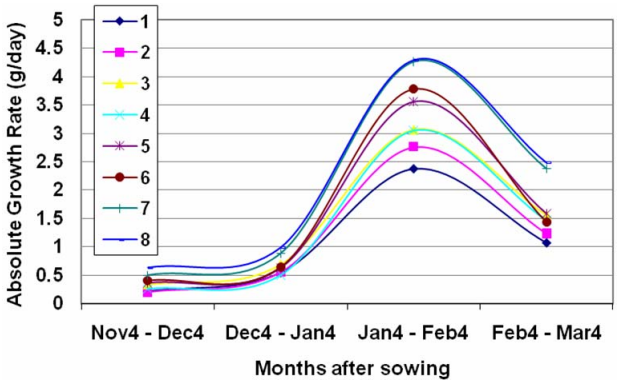


Figure 5. Seasonal trends of mean absolute growth rate (AGR) of sulfur and nitrogen application to two wheat varieties (Pirsabaq-2005 and Khyber-87) during 2008-09 and 2009-10. **1** = control or ck; **2** = Recommended dose of nitrogen; **3** = soil applied nitrogen; **4** = soil and foliar applied nitrogen; **5** = soil applied sulfur; **6** = soil and foliar applied sulfur; **7** = soil applied nitrogen + soil applied sulfur; **8** = soil and foliar applied nitrogen + soil and foliar applied sulfur.

4. DISCUSSION

Maximum LAI was counted through growth characters *i.e*. plant height and flag leaf area gradually increased by increasing sulfur fertilization. These results are in line with those of [8], who observed that sulfur fertilization enhanced LAI, The reason may be that the increase in growth due to nitrogen fertilization attributed to the role of nitrogen in increasing division and elongation of cells as well as activation of metabolic and photosynthesis process. Increase in nitrogen as well as sulfur contents of the soil affects all growth stages of the wheat crop. Such results are supported by [8], who stated that increase in N:S concentration at anthesis stage can resulted increased in LAI by as much as 62%. Similarly the effects of sulfur alone treatment was also found significant. It might be due to fertilizer application to wheat specifically sulfur during various stages of development greatly increased leaf area by delaying leaf senescence and maintained the process of photosynthesis which greatly enhanced LAI.

Nitrogen and sulfur applied by foliar spray at anthesis stage is transported and accumulated in grains, provided support for protein synthesis. Similar results were reported by [6], who suggested that a synergistic effect between nitrogen and sulfur fertilizers is observed, suggesting an interaction between the metabolism of both the elements. Likewise significant differences were found among the mean values of recommended fertilizer vs. other fertilizer treatments comparison. The possible reason may be due to fact that nitrogen applied as urea displayed maximum crude protein in wheat flour, also urea retained in the soil for longer time resulted that plants have more chance for uptake and hence improved the

crude protein contents in the flour. Matching results were reported by [7] who proposed that nitrogen application in the form of urea enhanced the crude protein contents in wheat flour. The results of planned comparison of the fertilizer treatment on two varieties also proposed significant affect of sulfur alone treatment on crude protein contents. Reason for the concern observation may be due that sulfur application either as foliar spray or through soil application modified dough mixing properties and enhanced protein storage in grain which resulted in better bread making characteristics. Nitrogen and sulfur application enhanced the potential of the crop for good moisture absorption which contributed in quality assessment characters of flour. These observations are in agreement with those of [4], who reported that wheat grown under different nitrogen and sulfur levels produced grain with varying protein and moisture contents. The probable reason may be that when moisture contents are available and sulfur supply is also sufficient then there is enhancement in grain protein content which further improved the flour quality. These results are similar with those of [11] who explained very high grain protein contents can be attained through sufficient moisture and nitrogen contents availability. Sulfur has the ability to improve the moisture contents of wheat flour which ultimately promoted the rheological properties of flour. These results are in line with those of [12] who proposed that sulfur positively affects the development, stability, softening and quality of dough as well as bread volume.

The calculated mean values of fertilizer treatments fitted against clustered columns showed that significant differences were recorded among the mean values of all the fertilizer treatment for crop growth rate. It is evident from the data that there is a consistent increased in dry matter accumulation of the crop from sowing to physiological maturity with different fertilizer treatments application. The reason may be that crop growth rate was increased by both nitrogen and sulfur application both as foliar and soil application and the interaction between both the nutrients were evident after anthesis. Addition of nitrogen and sulfur enhanced CGR from emergence to anthesis and then declined at later sampling intervals. It is matching to what was reported in other studies [13], who explained that sulfur effects were evident between anthesis and physiological maturity increasing CGR by 51%. Therefore the positive interactions between nitrogen and sulfur in higher nitrogen use efficiency when the crop had no sulfur deficiency. The probable reason may be that there was a significant interaction between both the nutrients for CGR, the response to nitrogen fertilization was different depending on sulfur fertilization. The higher the nitrogen rate the greater the effect of sulfur addition for growth rate of crop. These results fit well with those of [11], who reported that CGR increased before anthesis with the addition of nitrogen with some impact of sulfur.

The mean values of fertilizer treatments drawn in column graph presented that significant differences were recorded among the mean values of all the fertilizer treatments for absolute growth rate. It is clear from the data that there is a sequential increased in dry matter accumulation of the crop from sowing to physiological maturity with different fertilizer treatments application. Sulfur and nitrogen fertilization increased AGR from emergence to anthesis and then decreased at later sampling periods. These results are similar to what was observed in other research [14], who proposed that the AGR was affected by the type and method of fertilizer application during all plant growing periods. At the first growth period from transplanting to flowering, application of nitrogen increased AGR. The reason may be that there was a significant association between nitrogen and sulfur for AGR. These reports are in line with those of [15], who observed that AGR increased with efficient utilization of radiation use efficiency as well as various nitrogen levels at different growth stages of wheat crop, while control treatment was observed with minimum AGR. From the mean of planned comparison of the two varieties recorded significant association of no fertilizer vs. fertilizer treatment. Likewise sulfur alone effect for AGR also presented significant results.

5. CONCLUSION

This work proposed that foliar and soil application of nitrogen and sulfur at various growth stages of wheat improved the rheological properties of dough, extensibility of flour and ultimately bread making quality of wheat. The fertilization of nitrogen and sulfur at anthesis stage enhanced the gluten content as well as improved the moisture absorption ability of grain. It was also concluded that foliar application of nitrogen at tillering stage influenced the leaf architecture by maximizing the LAI. Simultaneous application of nitrogen and sulfur also hastened the dry matter accumulation which resulted in maximum CGR at anthesis and after anthesis stages of growth.

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