PHYSIOLOGICAL AND QUALITY ASSESSMENT OF WHEAT (*Triticum aestivum* L.) CULTIVARS IN RESPONSE TO SOIL AND FOLIAR FERTILIZATION OF NITROGEN AND SULFUR

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ABSTRACT

Nitrogen and sulfur supplies have a strong influence on the physiological and phenological characteristics of crop as well as on the quality of wheat seed, which have the capacity to enhanced yield and production of wheat. In order to evaluate the physiological and quality assessment of wheat in response to soil and foliar fertilization of nitrogen and sulfur, a field trail was carried out having randomized complete block design with four replications and eight different treatments of nitrogen and sulfur combinations allotted to plots at different growth stages. Results indicated that highest emergence m^{-2} (136), maximum leaf area (37 cm^2) and maximum seedling dry weight (0.041 g) was recorded when the crop was fertilized with soil and foliar applied nitrogen and soil and foliar applied sulfur. Likewise maximum leaf area index (5.016 %) was observed with the fertilization of soil applied sulfur (treatment number-5), while maximum field emergence was recorded with fertilization of soil applied nitrogen (treatment number-3). Minimum number of unproductive tillers was obtained with the application of soil applied nitrogen (treatment number-3). Control practice resulted low emergence m^{-2} and maximum number of unproductive tillers. In all the recorded observations concerning the 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 vigorous seed of wheat through soil and foliar application of nitrogen and sulfur.

Keywords: wheat cultivars, leaf area index, leaf area, nitrogen, sulfur, seed vigor tests, un-productive tillers.

INTRODUCTION

Cereals are an important dietary source throughout the world, because they constitute the main protein and energy supply in the most countries (Bos *et al.,* 2005). Wheat is the major cereal crop which triggered green revolution in the Indian subcontinent, having a unique protein, which is consumed by humans and is grown around the world in diverse environment. Wheat is a staple food crop, cultivated in both irrigated and non-irrigated areas of the country.

Balanced nutrition is an essential component of nutrient management and plays a significant role in increasing crop production and its quality. For the major processes of plant development and yield formation the presence of nutrients like N, P, K, S and Mg etc in balance form are essential (Randhawa and Arora, 2000).

The soils of Pakistan are deficient in nitrogen and are supplemented with chemical fertilizers for enhancing crop productivity. Nitrogen fertilizers play a vital role in modern farm technology, however only 20 to 50 % of the soil applied nitrogen is recovered by annual crops (Bajwa, 1992). The left over nitrogen is lost from the soil system through denitrification, volatilization and leaching. Nitrogen rate, type of nitrogen and timing of its application are important factors to increase wheat yield and improve its flour quality (Garrido-Lestache *et al.,* 2005). Efficient remobilization of urea (N) to grain after foliar fertilization on wheat at optimum timings, i.e., at and after anthesis stage, has shown to increase grain protein content and improve bread-making quality (Tea *et al.,* 2007).

Both nitrogen and sulfur are important constituents of plant proteins and are required throughout the crop growth period from vegetative period to subsequent harvesting. Without adequate sulfur, crops can't reach their full potential in terms of yield, quality or protein content; nor can they make efficient use of applied nitrogen (Sahota, 2006). Reproductive growth of wheat appears to be more sensitive to sulfur deficiency than vegetative growth, with decreased grain size under sulfur limiting condition. In addition to the effects on yields, the sulfur status of wheat grain is an important parameter for the quality of wheat products (Zhao *et al.,* 1999a; Mcgrath, 2003). Sulfur deficiency in crop plants has been recognized as a limiting factor not only for crop growth and seed yield but also for poor quality of seed.

It has been observed that fertilizer efficiency particularly urea application through soil is not as effective as it is applied to plants through foliage along with soil application (Mosluh *et al.,* 1978). Such application methods ensure the availability of nutrients to crops for obtaining higher yield (Arif *et al.,* 2006). Foliar application of N and S at different growth stages of wheat enhanced the seed quality and ensures high yield.

Nitrogen and sulfur fertilization boosts yield components, phenology and leaf traits. Crop biomass and crop growth rate (CGR) are dependent on the ability of the canopy to intercept incoming photo- synthetically active radiations (IPAR), which is the function of leaf area index

(LAI), leaf area and canopy architecture and then convert 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 (Wagan 2003). Nitrogen addition increased the leaf architecture from 7 to 19 % at the lower sulfur rate, but at the highest sulfur supply, these increments ranged from 20 to 35%, evidencing a clear interaction between both nutrients (Fernado *et al*., 2008). Sulfur effects were evident between anthesis and physiological maturity increasing crop growth rate by 51% (Fernado *et al*., 2008). An increased in leaf photosynthesis is expected when sulfur supply is increased (Bushuk 1999). Research also reported that radiation use efficiency might increase when nitrogen and sulfur supply rise, but in lower magnitude with respect to LAI and LAD (Fernado *et al*., 2008). LAI was reduced in crops grown under nitrogen deficiency (Caviglia and Sardas 2001). 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 (IPAR) by up to 20 % (Salvagiotti and Miralles 2008).

The present study was therefore designed to investigate about Physiological and quality assessment of wheat cultivars in response to soil and foliar fertilization of nitrogen and sulfur.

MATERIALS AND METHODS

Site description and experimental design

Experiment was conducted at New Developmental Farm of Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan during 2008-09 and 2009-10. Soil of the experimental site is clay loam, low in nitrogen (0.03-0.04 %), low in organic matter (0.8-0.9 %), extractable phosphorus (6.57 mg kg^{-1}), exchangeable potassium (121 mg kg^{-1}) and alkaline in reaction with a pH of 8.0-8.2 (Amanullah *et al*., 2009). A basal dose of P (100 kg/ha) and K (60 kg/ha) was applied at sowing. Urea was applied as a source for nitrogen and ammonium sulphate was applied as a source for sulfur. In which half dose of urea and ammonium sulphate was applied at the time of sowing and the remaining half dose of both was applied at different growth stages. The experimental setup was randomized complete block (RCB) design having four replications. Subplots size was 5m x 3m having 10 rows 5m long and 30cm apart. Two varieties Pirsabaq-2005 and Khyber-87 were used.

Fertilizer treatments

Details of the fertilizer treatments are as follows:

Control: without fertilization (CK) (treatment number-1);
Recommended dose of N (60 kg N/ha at sowing + 60 kg N/ha at tillering) (treatment number-2);
Soil applied N (60 kg N/ha at sowing + 40 kg N/ha at tillering + 10 kg N/ha anthesis + 10 kg N/ha after anthesis) (treatment number-3);
Soil+ foliar applied N [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)] (treatment number-4);
Soil applied S (15 kg S/ha at sowing + 10 kg S/ha at anthesis + 5 kg S/ha after anthesis) (treatment number-5);
Soil+ foliar applied S [(15 kg S/ha at sowing + 10 kg S/ha at anthesis (foliar) + 5 kg S/ha after anthesis (foliar)] (treatment number-6);
Soil applied N + soil applied S (combination of soil applied N and soil applied S) (treatment number-7).
Soil and foliar applied N + soil and foliar applied S (combination of soil + foliar applied N and soil + foliar applied S) (treatment number-8).

Data on emergence m^{-2} were recorded randomly in each plot by counting number of plants emerged in per square meter area and were averaged. Unproductive tillers were counted in two central rows in each sub plot and were converted into unproductive tillers m^{-2}. LAI ($tiller^{-1}$) was calculated by multiplying leaf area $tiller^{-1}$ over tillers m^{-2} and divided by 10, 000. Leaf Area (cm^{-2}) was measured at anthesis stage with the help of leaf area meter (Licor, 2003) by picking all leaves from middle, bottom and top of the selected tillers from two central rows in each sub plot. The seedlings obtained after standard germination test were used to recorded data on seedling dry weight test. The plumules and radicals were detached from coleoptiles and were placed in paper bag, dried in oven at 60 ^{0}C for 24 hour and then recorded seedling dry weight. Field emergence trials were conducted at the new developmental farm, Khyber Pukhtunkhwa Agricultural University, Peshawar in March, 2010 and for second year in March, 2011. Four replications of 100 seeds of each variety were planted by hand each year in 4m long rows at a depth of 4-6 cm. The plots were furrow irrigated to maintain relatively uniform soil moisture conditions. Daily counts were made as soon as the seedlings begin to emerged and continued until emergence completed. Soil temperature and average maximum and minimum air temperature at soil surface and 1m above the soil were recorded daily.

Statistical analysis

All data are presented as mean values of four replicates. Data were analyzed statistically for analysis of variance following the method described by (Gomez 1984). MSTATC computer software was used to carry out statistical analysis (Russel and eisensmith 1983). The significance of differences among means was compared by using Least Significance Difference (LSD) test (Steel and Torrie 1997).

RESULTS AND DISCUSSIONS

Data regarding emergence m^{-2} of wheat varieties as affected by nitrogen and sulfur fertilizes application methods described that both nitrogen and sulfur fertilizer application had significantly affected the emergence m^{-2} (Table-1). High emergence m^{-2} (136) was found in treatment number 8 (soil+ foliar applied N and soil+ foliar applied S); whereas minimum emergence m^{-2} were recorded in control plots (Table-1). Maximum emergence from soil applied N and S may be due to higher availability and utilization of nitrogen during the process of germination and its subsequent emergence from the soil. Pirsabaq-2005 variety gave maximum emergence m^{-2} (130) as compared to khyber-87 (129). These results are in agreement with Jhonson, (2000), who stated that wheat is a crop that requires a relatively high amount of supplemental sulfur due to the incompatibility of conditions related to its emergence stage, when the rate of sulfur release from soil organic matter is slow. Interaction between fertilizer treatments and varieties was not significant but was significant between year and varieties. For determining the effect of sulfur alone, planned comparison was carried out for both varieties (Figure-1A). From the mean of this comparison it was derived that the effect of recommended dose vs. other treatments has significant effect on emergence m^{-2}. It may due to the fact that during vegetative and reproductive growth N and S are required for efficient growth to synthesize protein. The effect of soil applied urea vs. combination of soil and foliar applied urea was also observed with significant effect. Khan, *et al*., (2009) reported that nitrogen concentration within the plants increased by increasing nitrogen concentration either in soil or by foliar application of urea. While the interaction among year, variety and treatments as well as the interaction between year and treatments was found not significant.

Data recorded on unproductive tillers m^{-2} revealed that unproductive tillers m^{-2} of the two varieties were significantly affected by different fertilizers combination treatments. Minimum numbers of unproductive tillers (24) were recorded in plot treated with soil applied nitrogen (treatment number-3) (Table-1). It may due to the efficient utilization of nitrogen in the soil which restricted the production of unproductive tillers. These results are in conformity with those of Otteson, *et al*., (2007) who observed that split application of nitrogen at different growth stages increased number of productive tillers, while the maximum number of unproductive tillers (31 numbers) were found in control plots. Its main reason may that there was no application of nitrogen and sulfur. Similar results had shown by Clarson and Ramashwami, (1999) who investigated more number of tillers m^{-2} with the application of sulfur @ 45 kg/ ha compared to no sulfur treatment. The concern data also revealed significant effect of the two varieties. Khyber-87 showed more unproductive tillers (30 numbers) as compared with the other variety (Table-1). The possible reason might be the genetic potential of each variety for tiller production. It was also cleared from the results that the interaction between fertilizer treatment and varieties was found significant. From the means of the planned comparison of the two varieties elaborated that recommended dose vs. other fertilizers treatment showed significant effect (Figure-1B). It may be due to the fact that when ever there is either nitrogen or sulfur application; there must be reduction in the percentage of unproductive tillers. Similarly the comparison between no fertilizers vs. fertilizer application was also significant. The means of planned comparison also showed that soil applied sulfur vs. soil + foliar applied sulfur gave significant relationship on the concern parameter (Figure-1B). But these results are in contradiction with those of Jain, (1991) who explained that dry matter accumulation in different parts and total productive tillers from grand growth till maturity will be higher with the treatments receiving sulfur and nitrogen. It was also observed that soil applied urea vs. soil + foliar applied urea also presented significant association. The interaction of the two varieties with comparison of urea applied treatments as well as sulfur applied treatments was also found significant.

The presented data about leaf area index (LAI) revealed that significant differences in LAI were found among different treatments of sulfur and nitrogen combinations (Table-1). Data showed that highest LAI (5.01) was observed in soil + foliar applied sulfur. This may be due to that growth characters i.e., plant height and flag leaf area gradually increased by increasing sulfur fertilization. These results are in line with those of (Seadh *et al*., 2007) who observed that sulfur fertilization enhanced LAI, while the lowest LAI (3.5) was found in treatment number-6 (foliar applied sulfur). 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 (Figure- 1C). The reason may be that the increase in growth due to nitrogen fertilization may be attributed to the role of nitrogen in increasing division and elongation of cells as well as activation of metabolic and photosynthesis process. Likewise the comparison between recommended fertilizers vs. other fertilizers also presented significant result (Figure-1C). It may be due to the fact that increase in N as well as S contents of the soil affects all growth stages of the wheat crop. Such results are supported by (Salvagiotti and Miralles 2008) who stated that increase in N: S concentration at anthesis stage can results increased in LAI by as much as 62 %. Similarly the effects of sulfur alone treatment was also found significant. It might be due to the fact that 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.

Perusals of data regarding leaf area indicate that different fertilizers treatments combinations have significant relationship with leaf area (Table-1). Maximum leaf area (36.88 cm^2) was obtained from plots treated with soil and foliar applied N + soil and foliar applied S, whereas minimum leaf area (25.45 cm^2) was obtained from control plots. This might be due to limited nutrient availability in control treated plots, which leads to fewer tillers production and smaller leaf area. Van der werf, (1996) reported that leaf area is determined by nitrogen and sulfur accumulation and it's partitioning within the leaves. No significant differences were found among two varieties on leaf area. Results from the planned mean comparison of fertilizer treatment on two varieties observed that the comparison of no fertilizer vs. fertilizer application showed significant effect on leaf area (Figure-1D). The probable reason may be due to the fact that N and S fertilization boosts the phenology and leaf traits by influencing the efficiency of assimilated mobilization to sink during leaf area development. Ellen, (1987) reported an increase in leaf length and width due to S and N application. Similarly the comparison of recommended vs. other fertilizers application also reported significant effect. This might be due to the efficient utilization of N in plants, which is the component of chlorophyll content and resulted in maximum leaf area. Thomas and Gausman, (1977) suggested that leaf area varied with different N application rates. It was also evident from the means planned comparison that the effect of sulfur alone treatment was found significant. The higher response of leaf area to sulfur might be the inadequacy of sulfur in the soil. These results are in agreement with those of Fernado and Danial, (2008) who reported 20 % increased in leaf area due to sulfur addition. While all the interactions of varieties with fertilizer treatments showed non significant results.

The presented data regarding seedling dry weight revealed that significant differences were found among the mean values of different fertilizer treatment combinations (Table-1). This might be due both nitrogen and sulfur application enhanced the protein content and resulted in heavier seedlings. Lowe and Ries, (1973) reported positive correlation of macro and micro nutrients and seedling dry weight. They found that seedlings developed from high protein grain showed increased water absorption and oxygen consumption during germination and produced heavier seedlings. Maximum seedlings weight (0.041g) was recorded from the sample which was treated with treatment number-8 (soil and foliar applied urea + soil and foliar applied sulfur), whereas minimum seedlings dry weight of 0.034g was recorded from treatment number-3 (soil applied nitrogen). The interaction between different fertilizer treatments and varieties of wheat were also significant for seedling dry weight. Maximum seedling dry weight (0.045g) was produced by treatment number-7 (soil applied nitrogen + soil applied sulfur). Planned means comparison of the two varieties showed that the comparison between no fertilizers vs. fertilizer combination gave significant results. It was also found that the effect of sulfur alone showed significant effect (Figure-1E). This might be due to that sulfur fertilization improved the potential of seed to produce more protein contents as well as increased it efficiency to absorbed water during germination which leads to healthier seedlings. Idres and Aslam, (1975) reported that among various agro-management practices which are used to raised successful crop, nutrients supplementation seems to more promising practice as it stimulates germination and subsequent seedling growth both under normal and saline soil conditions. An increase in root and shoot dry weight by micronutrients treated seeds has been reported by Shaban and Eid, (1984). The planned means comparison results that ammonium sulphate soil applied vs. soil + foliar applied combination produced significant results. Similarly the comparison among the means of urea soil applied vs. soil + foliar applied combinations also reported significant results (Figure-1E). It might be due to that low nitrogen always gave seedlings with smaller dry weight. Metivie, (1976) concluded that the superior growth of high nitrogen seedlings is associated with greater enlargement of the first leaf lamina and high protein content as well as high rate of photosynthesis which leads to more rapid rate of dry matter accumulation in seedlings.

Data regarding field emergence test revealed significant effect to different fertilizer treatment combinations. Maximum field emergence of 83.12% and 83.25% were obtained from the seed lots treated with treatment number-6 (soil and foliar applied sulfur) and 3 (soil applied nitrogen). The reason may be due to the fact that fertilization of crop with different nutrients increased the potential of seed to germinate and emerged efficiently in field. McDonald, (2000) reported that seed treatment with different nutrients as well as priming techniques has been developed and used extensively to improved seed germination and field emergence of seedlings. It is evident from the Table-1 that the interaction between fertilizer treatment and varieties of wheat were significant. Maximum field emergence (84.5 %) was obtained from pirsabaq-2005 treated with treatment- 3 (soil applied nitrogen). It may be due to proper timings and rate of fertilization of crop with different nutrients as well as genotypic make up of seed lots which greatly associated with vigor of seed lots. Moreno-Martinez, *et al*., (2000) stated that field emergence in maize cultivars for field emergence and germination. Analysis of variance showed non significant differences among the mean values of varieties with FE test. The interaction of varieties with no fertilizer vs. fertilizer application comparison showed significant results and also the interaction of varieties with sulfur alone treatment were found significant. Planned means comparison of two wheat varieties elaborated that comparison between no fertilizers vs. fertilizer combination presented significant results (Figure-1F). Ali, *et al*., (2001) reported that sulfur and nitrogen has an essential role in the synthesis of protein and a wide variety of metabolites that are crucial for seedling emergence in the field. Similarly the comparison between urea and ammonium sulphate soil applied vs. soil and foliar application of both fertilizers gave significant association with the concern data (Figure-1F).

Table-1. Response of various N and S fertilizer treatment combinations on physiological and seed quality of two wheat cultivars.

Fertilizer treatments	Emergence m^{-2}			Unproductive tillers			Leaf area index			Leaf area (cm^2)			Seedling dry weight test			Field emergence test		
	Varieties			Varieties			Varieties			Varieties			Varieties			Varieties		
	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean
Control treatment (T1)	119	120	120d	32cd	30e	31a	6.89a	3.1gh	5.0a	26.6	24.4	25.5e	0.042a	0.035b	0.039a	79f	83abc	81a
Recommended dose (T2)	121	119	120d	28ef	29de	29bc	5.17b	4cde	4ab	27.0	29.0	27.9d	0.040a	0.042a	0.041a	83.7abc	80def	81.8a
Soil applied N (T3)	130	129	130c	23h	24gh	24d	4.50c	3.3fgh	4bc	33.0	33.0	32.9c	0.030b	0.038b	0.038b	84.5a	82bcde	83a
Soil and foliar applied N (T4)	134	131	133b	26g	34b	30ab	3.82def	4cde	4bc	33.0	34.6	34.0bc	0.037b	0.032b	0.032b	82bcde	78f	80a
Soil applied S (T5)	133	132	133b	33bc	29e	31a	7.10a	3.0h	5a	35.1	35.0	35.1ab	0.037b	0.032b	0.032b	84ab	80def	82a
Soil and foliar applied S (T6)	133	133	133b	27f	28ef	28c	5.19b	2.0i	3.5c	35.3	34.6	35.0ab	0.040a	0.040a	0.040a	82.7bcde	83abc	83a
Combination of T3 and T5 (T7)	135	134	134ab	23h	37a	30ab	4.21cd	3efg	4bc	34.3	34.0	34.1bc	0.045a	0.032b	0.032b	80def	80ef	80a
Combination of T4 and T6(T8)	137	136	136a	24h	32d	28c	3.9def	3.6defg	3.7bc	36.8	36.9	36.8a	0.040a	0.042a	0.042a	81cde	83abc	82a
Mean	130	129	-	27	30	-	5.1	3.3	-	32.7	32.7	-	0.039	0.037	-	82	81	-
2008-09	131a	129b	130a	27	31	29	5.2a	3.06c	4.13	32.9	32.6	32.7	0.039	0.037	0.038	82	81	81.7
2009-10	129b	129b	129b	27	30	29	5.0a	3.58b	4.29	32.5	32.7	32.6	0.039	0.037	0.038	82	81	81.7
LSD																		
Fertilizer treatments	2.493				2.084			0.8311			1.936			0.03141			3.245	
V x F	-				1.473			0.5877			-			0.02221			2.298	
Y x V	1.245				-			0.4273			-			-			-	
Interactions	P-value				P-value			P-value			P-value			P-value			P-value	
Y x F	0.9825				0.3119			0.3125			0.0241			1.0000			1.0000	
Y x V x F	0.8853				0.8961			0.1333			0.8604			1.0000			1.0000	

Mean of the same category followed by different letters are significantly different ($P \geq 0.05$) using LSD test.
V1 = Pir Sabaq-2005
V2 = Khyber-87
Y = Year
F = Fertilizer Treatments
V= Variety
LSD = Least Significant Difference

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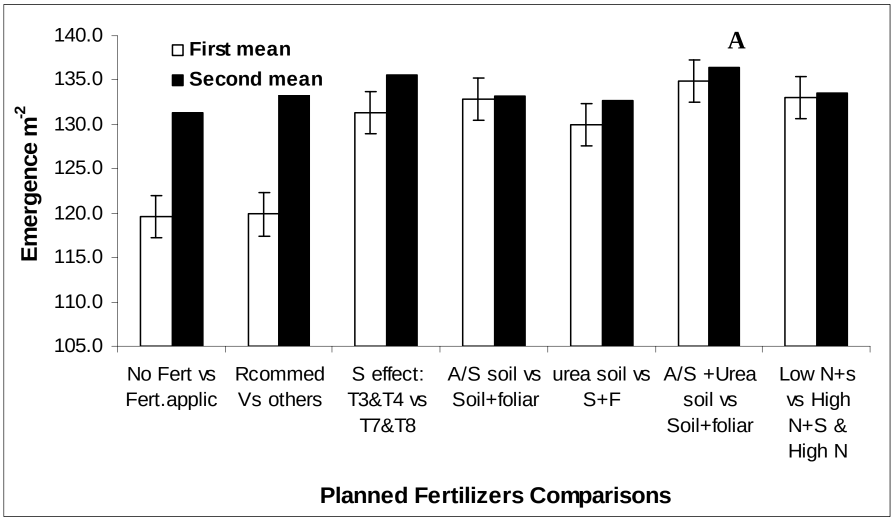

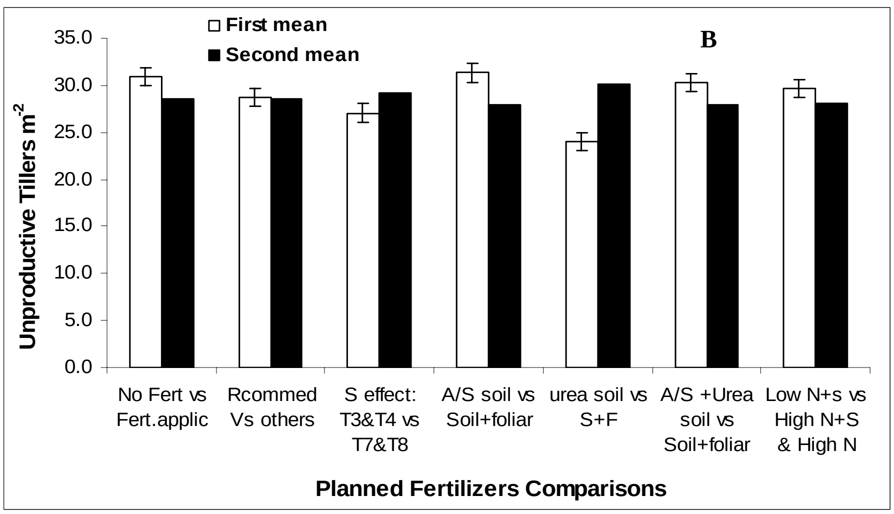

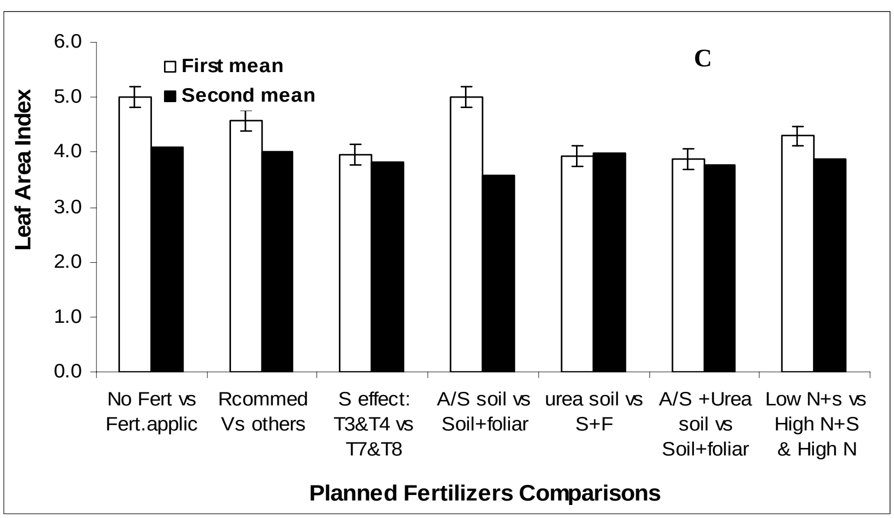

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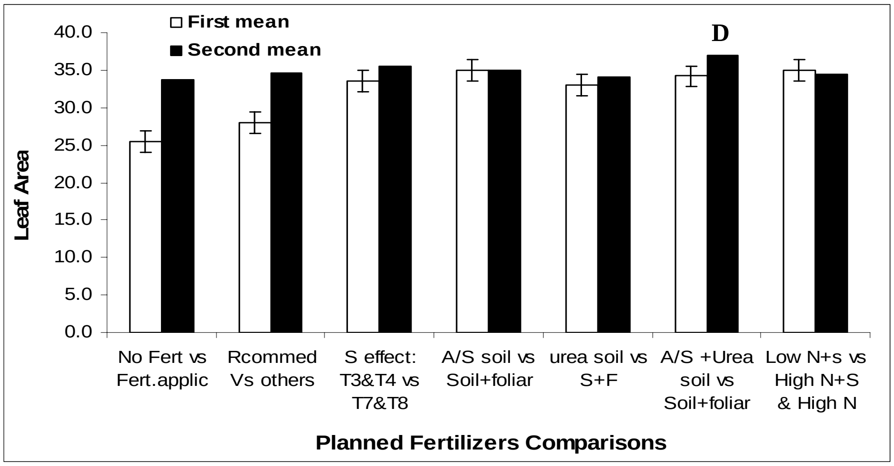

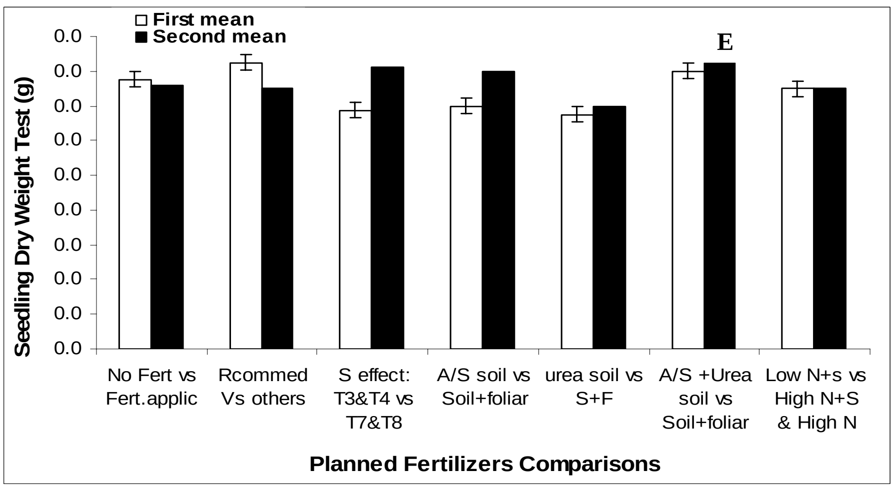

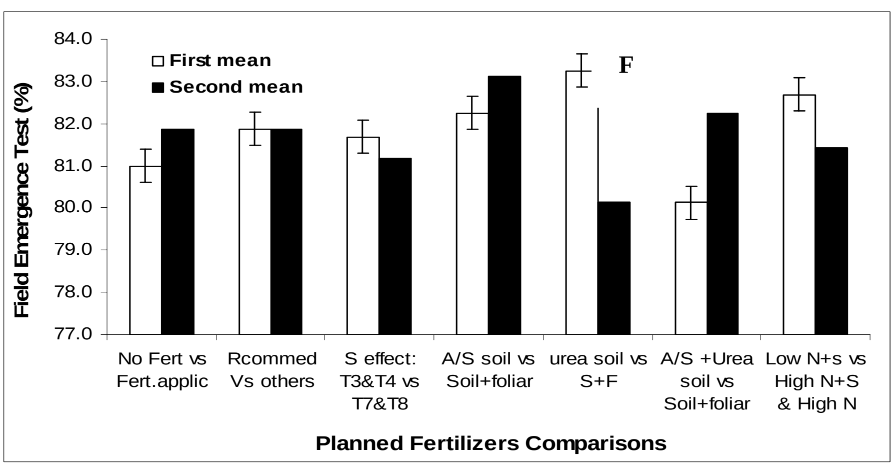

Figure-1. Planned comparison of the emergence m^{-2}(A), un productive tillers (B), LAI (C) leaf area (D), seedling dry weight test (E), field emergence test (F) of wheat as affected by the rate and method of sulfur and nitrogen application.

CONCLUSIONS

This work proposed that foliar and soil application of nitrogen and sulfur at various growth stages of wheat improved the ability of grain for best vigor, viability and strong source-sink relationship to minimize risk of leachates losses. 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 leaf area at anthesis and after anthesis stages of growth. Both the varieties showed appreciable response towards N and S application over growth factors.

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