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LENTIL YIELD AND NODULATION IN RESPONSE TO FOLIAR S AND ZN COMBINED WITH NPK AND THEIR INTERACTION WITH FARMYARD MANURE

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ABSTRACT

Re-assessment of NPK recommendations as sole and mixed with other nutrients is necessary today because soil exhaustion continues with intensive cultivation. In line with, a study was carried out to assess the impact of mineral NPK with foliar S and Zn on lentil crop both in the presence and absence and farmyard manure (FYM) at the Agriculture Research Institute, Mingora, Swat during 2012. Using Randomized Complete Block Split Plot Design, FYM was allotted to main plots (-FYM and +FYM (10 t ha^{-1})) whilst NPK (kg ha^{-1}) and S and Zn (% solution) applied as; Control, NP (30:60), NPK (30:60:60), NPKS (NPK+3%S), NPKZn (NPK+3%Zn) and NPKSZn (NPK+S+Zn) were tested in sub plots. Combined NPK, S and Zn application (NPKSZn) improved the nodule number (NN) and nodule weight (NW) $plant^{-1}$ significantly (by 22 and 75%, respectively) over the control and resulted in 40, 49, 20 and 14% higher biological yield (BY), grain yield (GY), 1000-grain weight (GW) and harvest index (HI) over the control, respectively. The effect of FYM on lentil growth and nodulation, either as sole or in integration with inorganic nutrients, was non-significant. The maximum HI by NPKSZn indicated the both S and Zn have improved nutrients assimilation into the economic portion of the crop. However, the S effect on nodulation and nodule growth was greater than Zn. This study concluded that foliar application of 3% S and Zn solution is necessary along with NPK to improve the yield and nodulation in lentil.

Keywords: farmyard manure, foliar application, inorganic nutrients, lentil, nodulation, nodules weight

INTRODUCTION

Lentil (*Lens culinaris* M**.**) seed is referred as poor man's meat because of its rich nutritional value especially protein, carbohydrates, fiber, vitamin-A and B, potassium and iron and low sodium and fats (Anonymous, 2003). It is predominantly grown in South East Asia and consumed as thick soup made from whole or split grains fried and seasoned. The flour of lentil is also used to prepare

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soups, stew purees, and mixed with various cereals to bake bread and cakes and as a foodstuff for childrens (Williams and Singh, 1988). In the Western countries, lentil may be used in casseroles and as meat substitutes in vegetarian diets. Lentil, although known as a 'poor man's meat', is equally liked by all socio-economic groups (Bhatty, 1988).

Lentil is a high value cash crop of winter in Pakistan and is only second important after chickpea with cultivated area of 39000 ha, annual production of 21100 tons and average seed yield of 541 kg ha^{-1} (Anonymous, 2008). Comparing with growing demand of the rising population of the country, the current per hectare lentil yield is very low. On the other side, this low per hectare yield is unable to meet the economic needs of its growers. This along with attack of various diseases and infestation of weeds might be important reasons for decreasing lentil acreage in the country (Anonymous, 2008). The net impact of these circumstances is damper on national exchequer in the form of its imports from other countries especially India to meet our national requirements. Efforts are therefore required for scientific management of the crop mainly to improve its yield under the existing acreage and soil conditions.

Generally, plants require about a 10^{th} as much sulphur (S) as nitrogen (N), slightly less than they require phosphorus (P), but its deficiencies restrict plant growth as surely and severely as N deficiencies. It is generally called the fourth major nutrient after N, P and K. However, crop species and varieties vary in their S requirements. It works as antifungal when applied as foliar spray on crop. As a nutrient, S has most important role in the production of leguminous crops and its deficiency significantly affects the yield and quality of lentil since it is a building block for protein and enzymes and S-containing amino acids (Zhao *et al.,* 1999a,b). The S content normally varies from 0.2-0.5% in plant tissues. In mature wheat tissues, S level of 0.15% and an N/S ratio of 15 are considered as important. However, the requirement of S in leguminous crop is better with critical values of 0.16-0.20% S and N/S ratio of 17 in whole plants at the early flourishing stage. Decreasing S deposition and the use of more concentrated P fertilizers with less S content has led to reports of S deficiencies in lentil. Secondly, by nature, S is immobile in plants and its continuous supply to plant cells is needed from emergence till maturity. Its shortage is high in areas of oilseeds and pulses cultivation due to its higher removal by crops (Singh and Kumar, 2009). Without having ample S, crops cannot produce higher yield or protein content, nor can they proper utilize the applied N (Sahota, 2006).

Deficiency and response to Zinc (Zn) in lentil have been reported from various parts of the world, Pakistani soil is no exception because of its addition to the soil in negligible quantities. Its availability in soil in minute amount is critical for crops numerous growth and reproductive functions. It is required for many purposes like cell expansion, carbohydrate and protein development, and for root growth hormones; therefore it must be applied to the crop by the 4-5 leaf growth stage. The same as, it is necessary for pollen function in lentil because low supply of Zinc may affect the germination of pollen grains, their size and viability, size of the anthers, growth of pollen tubes, the pollen producing capacity, the setting of seeds and their viability. Its (Zn) requirement for reproduction can be met through its supplementation at the time of initiation of the reproductive phase (Pandey, *et al*., 2006). Zinc adjusts and regulates the function of carbonic anhydrase, an enzyme that regulates plant growth. Zinc is also a part of several other enzymes such as superoxide dismutase and catalase, which avoids oxidative strain in plant cells. However, various other roles of Zn in plants are; like, making of auxin, an essential growth hormone; also regulates starch arrangement and appropriate root growth; formation of chlorophyll and carbohydrates; and enable plants to endure lower air temperatures.

Since, the current inorganic fertilizer recommendations for lentil were made by more than two decades ago when soil was not so much intensively cultivated and exhausted with respect to nutrients. This study, therefore, hypothesizes that because of deficiency of other macro and trace elements, the current inorganic NPK recommendations for lentil cannot explore the full potential of the lentil crop. Exploration of other nutrients as yield controlling factors are, therefore, essentially required. This study was planned to determine the effect of S and Zn foliar application along with inorganic NPK either in the presence or absence of farmyard manure on lentil growth and yield. The objective was to select best combination of the applied nutrients and amendments for improved lentil yield from a given piece of land.

MATERIALS AND METHODS

The trial was conducted during winter 2012 at the Agriculture Research Institute (34^o 78' 54” N and 72^o 34' 71” E), Mingora, Swat, Pakistan, using an RCB Design with split plot arrangement. The details of experimental/study are given in Table 1. Treatments consisted of farmyard manure (FYM) in main plots as present at the rate of 10 t ha^{-1} (+FYM) and absent (-FYM) whilst NPK (kg ha^{-1}) and S and Zn (% solution) applied as; Control, NP (30:60), NPK(30:60:60), NPKS (NPK+3%S), NPKZn (NPK+3%Zn) and NPKSZn (NPK+S+Zn) were tested in sub plots. All treatments were replicated three times in plots with 12m*4m size.

Table 1. Characteristics of the experimental site

Property	Unit	Value
Textural class	-	Silt loam
Bulk density	Mg m^{-3}	1.36
Available water holding capacity	%	19.5
pH	-	6.8
E C	dS m^{-1}	0.45
Lime	(%)	1.3
Organic matter	(%)	1.16
Organic carbon	(%)	0.67
Total N	(%)	0.05
C/N ratio	-	22.42
Mineral N	(mg kg^{-1})	13.52
Extractable P	(mg kg^{-1})	4.45
Extractable K	(mg kg^{-1})	56
Extractable Zn	(mg kg^{-1})	3.2
SO_4	(mg kg^{-1})	8.8

The field was ploughed twice and prepared for cultivation. The FYM was obtained from a local dairy farm (Characteristics are given in Table 2) and applied to designated plots 15 days before crop sowing whilst inorganic NPK was applied at the time of sowing. The 3% S and Zn prepared from K_2SO_4 and Zn $(NO_3)_2$, respectively, were applied as foliar spray on 16 March 2012. Sources for inorganic NPK were Urea, SSP and K_2SO_4. Lentil (candidate variety) was sown on 1st December 2011 and harvested on 7th June 2012. The following parameters were recorded:

Table 2. Characteristics of farmyard manure used in the experiment

Property	Unit	Vlaue
Moisture	g kg^{-1}	150
Total Organic C	g kg^{-1}	458
Total N	g kg^{-1}	12
C:N ratio	-	38.2
Total volatile solids	g kg^{-1}	178
Total ash	g kg^{-1}	214
Total P	g kg^{-1}	7.2
Total K	g kg^{-1}	32.8
Total SO_4	g kg^{-1}	6.5
Total Zn	g kg^{-1}	2.4

For mineral N, 10g soil sample was extracted with 50 ml 1N KCL solution (Mulvaney, 1996) and the extract was distilled through Kjeldhal procedure. The pH and electrical conductivity (EC) of the sample were measured in 1:5 (w/v) suspension using digital pH (InoLab, WTW Series, Germany) and EC (Jenway, UK) meters as described by Mclean (1982) and Rhoades (1996), respectively. Total organic C was measured with Walkely and Black procedure (Nelson and Sommers, 1996). Soil sample (10g) was extracted with 1N ammonium bicarbonate diethylene triamine penta acetic acid (AB-DTPA) (1.97g DTPA+ 79.06 g NH_4HCO_3 dissolved in 1L) solution for mineral P, K and Zn, estimation as described in Soltanpour and Schwab (1977). For SO_4-S determination the procedure of Williams and Steinbergs (1959) was adopted. A 5 g sample was shaken with 25 mL of 0.15% $CaCl_2$ $2H_2O$ for 30 minutes and filtered by Whatman 42 No filter paper. A 10 mL from the extract was added with 1 mL mixed acid reagent (HCl 6 *M*; 496.8 mL+503.2 mL DI water) and 5 mL of 70% sorbitol solution and finally added with 1 g of $BaCl_2.2H_2O$ cystal and shaked for 1 minute. A blank was also prepared in the same way without aliquot. A 100 mg L^{-1} standard stock solution (0.543 g K_2SO_4 in 1L distilled water) was used to prepare 5, 10, 15, 20, 30, 40 and 50 mg L^{-1} standard solutions. Absorbance was read at 470 nm on spectrophotometer and the concentration of SO_4-S in the sample was determined from the calibration curve. Kjeldhal method was adopted for N (total) in FYM samples (Bremner and Mulvaney, 1982). Nitric acid (HNO_3)-Perchloric acid ($HClO_4$) digestion method of A.O.A.C (1995) was used for digestion of FYM samples for the determination of total P, K, SO_4 and Zn. For P, spectrophotometer at 880 nm, for total K, flame photometer and for micro-nutrients determination, the atomic absorption spectrophotometer (Perkin Elmer Model 2380, USA) were used. Moisture content (ω) is percentage of moisture in the sample is based on the amount of mass lost from the FYM sample at 110 °C after 24 hours. For total volatile solid (TVS) in the FYM, a 1g FYM sample was burnt at 950 C^o for 11 minutes in an electric furnace in the absence of oxygen. Total volatile solid were determined as (McLaughlin *et al.*, 2009):

$$\text{TVS } (\%) = \frac{M_{950}}{M_s} \text{ X } 100$$

Where Ms is the weight of sample, M_{950} is the weight of total volatile solids. For total cash determination, oven dried FYM sample (1 g) was combusted gradually in presence of O_2 upto 575 C^o for 3 hours in an electric furnace till ash formation and weighed (M_{575}). Ash was calculated as (Sluiter *et al*., 2005):

$$\text{Ash } (\%) = \frac{M_{575}}{M_s} \text{ X } 100$$

Total C content was determined as (McLaughlin *et al.*, 2009):

Total C (%) = 100 - (TVS% + Ash% + ω)

C/N ratio in the compost sample was then determined by dividing the total C content (g kg^{-1}) in the compost by the total N content expressed in the same units.

Three plants were tagged randomly at third week of germination for recording data on plant height, number of nodules and nodule weight. Plant height was determined at flowering from the base to the top of the plant. The plants were carefully uprooted and the attached soil was washed. Nodules were separated with a forceps and counted. The nodules were oven dried at 70^oC for 48 hours and dry weight of the nodules per plant was recorded. At maturity of the pods, three central rows were harvested from each sub plot. Followed by, the bundles were sun-dried and weighed by spring balance for calculating biological yield. The data were converted into kg ha^{-1}. Grain yield was recorded after threshing of plants from each treatment and then converted into kg ha^{-1}. 1000-grain weight were collected from each plot and weighed separately. Harvest index were calculated and expressed in percentage for each plot using the following formula:

$$\text{Harvest index } (\%) = \frac{\text{Grain yield}}{\text{Biological yield}} X \text{ } 100$$

Statistical analysis

The collected data were analyzed statistically as proposed by Gomez and Gomez (1984) and treatment means (if) significantly differed were compared using Least Significant Difference (LSD) test at 5% level of significance.

RESULTS AND DISCUSSION

Results (Table 3) indicated that soil and foliar plant nutrition (NPK, S and Zn) did not affect the number of plants ft^{-2}, but it significantly (P<0.05) increased the nodules number (NN) and nodules weight (NW) $plant^{-1}$. The combined NPK and S (NPKS) and NPK and Zn (NPKZn) treatments enhancement in NN was 20 and 13% and in NW was 50 and 42% over the control, respectively (Table 3). But the combined application of NPK with foliar S and Zn (NPKSZn) recorded 22%

higher NN and 75% higher NW compared to the control. These results showed importance of the required nutrition for successful nodulation and nodules growth. These results further showed that S effect on nodulation and its growth was higher by 7 and 8% than Zn, respectively. Nutrients application not only increase crop nutrition but improve the soil properties both of which are required factors for nodulation and nodule growth. Results (Table 3) further indicated that +FYM resulted in 17% more plant, 1% higher NN and 5% more NW compared to –FYM, the variation was, however, non-significant. It has been demonstrated by Ahmad *et al.* (2014) and Ahmad and Khan (2014) that FYM addition improves both physical and chemical properties of the soil resulting in improved stand establishment. Since FYM is a source of nutrients, its release upon decomposition is uncontrolled. Higher release may render lentil plants to survive only on these for its growth and needs not to expand additional energy on nodules formation, lower release however, may cause improper plant nutrition and lower nodulation. Nutrients application in required amounts improves the survival of plants because of improved vigor or stress resistance. Mehrotra *et al.* (1973) reported best lentil wilt pathogen control with 80 ppm of Mn and Zn salt foliar sprays. Singh *et al.* (2013) reported efficient foliar application of S and Zn for powdery mildew disease management in lentil. Inorganic nutrients application (soil and foliar), improve soil fertility and result in higher C sequestration in soils because of higher biological mass production both above and below ground. Higher soil C content is associated with favorable soil environment resulting in improved stand establishment. Since foliar application of Zn and S was carried out at the mid growing season, their effect on number of plants ft^{-2} was non-evident. Otieno *et al*. (2009) reported no effect of manure addition on number of nodules under long rain seasons and significantly higher number of nodules under short rain season. Results further showed no interaction between the nutrient application and FYM to affect number of plants per unit area (ft^{-2}), NN and NW ($plant^{-1}$) of the lentil crop (Table 3)

Table 3. Effect of NPK along with foliar S and Zn and FYM on number of plants (ft^{-2}), nodulation and nodules weight ($plant^{-1}$)

Treatments	**No. of plants (ft^{-2})**	**No. of nodules ($plant^{-1}$)**	**Nodule weight (g $plant^{-1}$)**
Control	13.55	15.33b	0.12d
NP	14.11	15.77b	0.14cd
NPK	15.33	16.11b	0.16bc
NPKZn	15. 52	17.33ab	0.17bc
NPKS	15.77	18.44ab	0.18ab
NPKZnS	15.33	18.77a	0.21a
LSD (P<0.05)	ns	2.34	0.322
+FYM	16.33	17.31	0.20
-FYM	14	17.08	0.19
T-test$_{(p<0.05)}$	0.064	0.943	0.723
Nutrients * FYM	ns	ns	ns

Table 4. Probabilities and LSD values for yield parameters affected by nutrients (NPK combined with foliar S and Zn), FYM and their interaction.

Parameter	Nutrients			FYM	(Nut. * FYM)	
	F value	Prob.	LSD(P<0.05)	T-Test Prob.	F value	Prob.
BY (t ha^{-1})	12.52	0.000	1.7	0.109	0.958	0.4662
GY (kg ha^{-1})	23.36	0.000	107.6	0.906	1.383	0.2726
GW (g)	30.91	0.000	1.58	0.091	1.2	0.3448
HI (%)	3.805	0.014	1.24	0.108	1.061	0.4106

Prob.: probability, Nut: nutrients, BY: Biological yield, GY: Grain yield, GW: 1000 grain weight, HI: Harvest index

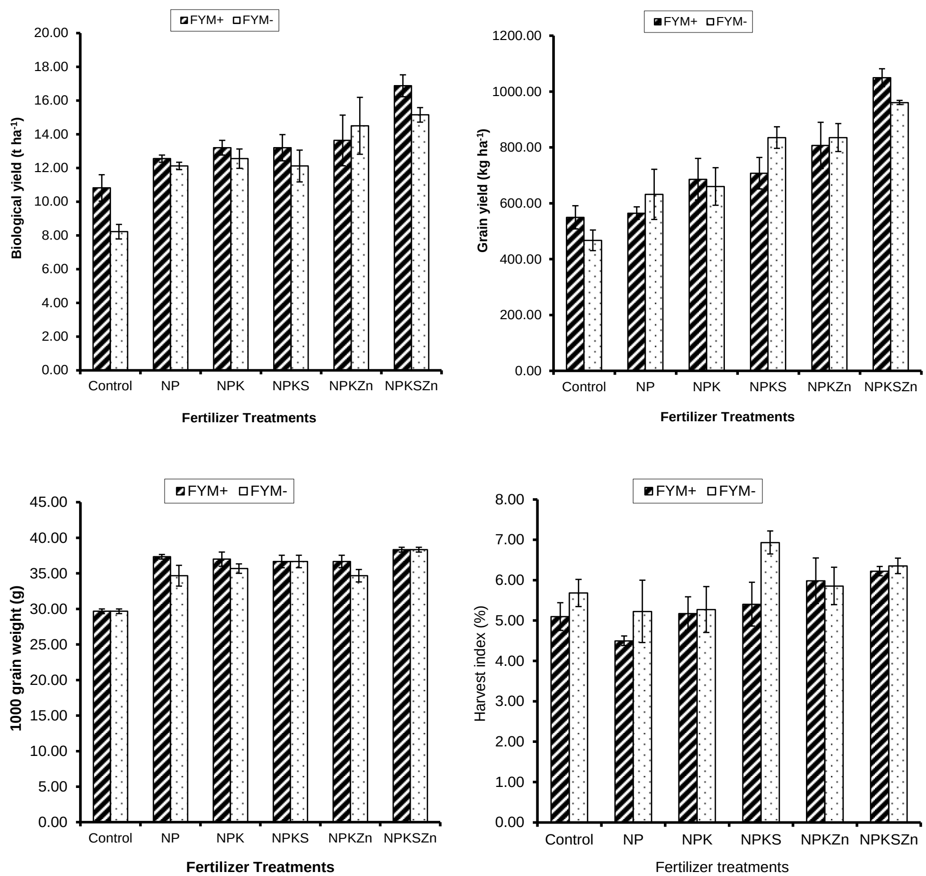

Figure 1. Effect of NPK and foliar S and Zn in the presence and absence of FYM on lentil yield's parameters

The inorganic NPK fertilizer and its combination with foliar application of S and Zn significantly affected biological yield (BY, *P*<0.01), grain yield (GY, *P*<0.05), 1000-grains weight (GW, *P*<0.05) and harvest index (HI, *P*<0.05) of the lentil crop (Table 4). The highest biological yield (16.0 t ha^{-1}), grain yield (1005 kg ha^{-1}), 1000-grain weight (38.3 g) and harvest index (6.2%) were obtained with NPK and foliar S and Zn combined application (NPKSZn). The application of NPK combined with foliar Zn (NPKZn) resulted the second highest BY (14.1 t ha^{-1}) and GGY (821 kg ha^{-1}) after NPKSZn whilst the application of NPK combined with foliar S (NPKS) resulted the second highest GW (36.7g) and HI (6.17%) after NPKSZn. The control showed the lowest BY (9.5 t ha^{-1}), GY (509 kg ha^{-1}), GW (29.7 g) and HI (4.9%) amongst treatments. The NPKSZn edge in BY, GY, GW and HI over rest of treatments ranged from 12-40%, 18-49%, 4-20% and 1-22%, respectively (Figure 1).

Reported the effectiveness of higher doses of S (300 kg ha^{-1}) along with Zn fertilization on grain and straw yield of lentil. Azad *et al. (*1993) stated that intermediate rate of soil application of Zn (12.5 kg $ZnSO_4$ ha^{-1}) was effective for higher grain yield whilst higher rate of soil application of Zn (25 kg $ZnSO_4$ ha^{-1}) resulted in higher biological yield of the lentil crop. But our results showed that foliar application of Zn was effective for both biological and grain yield increases compared to foliar application of S. Pandey *et al.* (2006) stressed the availability of Zn as a critical requirement for pollen function in lentil, the deficiency of which greatly reduce the seed setting and affect their viability. Based on his findings he asserted the specific role of Zn in lentils reproduction that can be met through Zn supplementation at the time of initiation of the reproductive phase for obtaining higher seed yield. Zeidan *et al.* (2010) reported improved grain yield, straw yield, 1000-grain weight and number of grain $spike^{-1}$ after Zn application with other micronutrients. However, results regarding 1000-grain weight and harvest index showed that foliar S application was more effective for grain filling and growth and thus for increase in crop economic portion. Sulphur, being integral requirement for nodule formation, might have increased nodule activity and N fixation at flowering and reproductive stage and have increased accumulation of assimilates in the economic portion of the crop. Singh *et al.* (2013) asserted the efficient S and Zn management as crucial for boosting lentil production through the control of powdery mildew disease in lentil. Similarly, Heidarian *et al.* (2011) also reported the good impact of Fe and Zn foliar application on grain yield, harvest index, first pod height, number of pods per plant, number of seeds per pod and 1000-grain weight, therefore highly recommended Zn+Fe combination treatment for better yields.

Farmyard manure application increased the GW by 3%, however, decreased the GY by 0.6% and HI by 11% (Figure 1) over the -FYM. However, all these variations were non-significant. Increase in BY could be attributed to increased number of plant ft^{-2} in +FYM plots perhaps due to improved soil environment and moisture availability (Ahmad and Khan, 2014) that could prove helpful to withstand the stress. The additional nutrients released from FYM decomposition should have provided a chance of luxurious vegetative growth to the crop. Since, lentil, being leguminous crop, it improves the fertility of poor soils through N fixation (Schmidtke *et al.*, 2004; Quddus *et al.*, 2014), its own performance on an N fertile soils remain poor with respect to N fixation and seed production. Otieno *et al.* (2009) reported significantly fewer and lighter nodules with N application. Chemining'wa *et al.* (2004); Gentili *et al.* (2006) and Pons *et al.* (2007) have reported inhibitory effect of the added N to nodulation and N Fixation. Therefore, in such cases when available N in soil get exhausted mostly during vegetative growth, and N falls short of the requirements for seed setting due to very nominal N fixation, seeds develop poorly and are small sized reducing total yield and 1000-grain weight (as 0.6% and 11% in our study, respectively). Results further indicated that the interaction between FYM and fertilizer treatment on BY, GY, GW and HI was non-significant.

CONCLUSION

Lower harvest index with NPK fertilizer application suggests its use mostly during vegetative growth. However, its combination with foliar S and Zn increased the harvest index of the crop significantly. This study indicates that S effects on nodulation; whilst for higher harvest index, Zn application was necessary. Based on these results, the study, recommends 3% S and Zn solution for foliar application along with NPK fertilizer for raising lentil production.

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