

Original Research Article

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## Influence of Various Levels of Zinc and Sulphur on Storage Proteins and Protein Quality of Lentil (*Lens culinaris*) Varieties

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### ABSTRACT

#### Keywords

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Influence of varying Sulphur and zinc levels were studied on storage proteins and protein quality of Lentil (*Lens culinaris*). Application of 30 kg S/ha enhanced the soluble proteins, tryptophan, methionine, albumin and globulin. Similarly zinc application @ 30 kg/ha had positive impact on the soluble proteins (albumin and globulins) and amino acids (lysine and tryptophan). The positive interaction between variety, zinc and Sulphur was observed on the tryptophan and albumin content. Lentil variety K – 75 had the maximum tryptophan and albumin content on application of 30 kg zinc and Sulphur fertilizers per hectare.

### Introduction

India is the largest producer, consumer, importer and processor of pulses in the world. Pulses are the basic ingredient in the diets of a majority of the Indian population, as they provide a perfect mix of vegetarian protein component of high biological value when supplemented with cereals (Ali and Gupta, 2012). Lentil (*Lens culinaris* Medik.) is one of the most nutritious cool season food legumes and ranks next only to chickpea. It is grown throughout the northern and central India for grains. Besides its utilisation as a dal, whole or dehulled grains are also used in various other preparations. It is one of the prominent

sources of vegetable protein in the Indo-Gangetic plain (IGP) region, essentially grown as a rainfed crop on the residual soil moisture of preceding crop (rice in-general) (Ali *et al.*, 2012 and Joshi 1998).

Increased popularity of high yielding varieties (HYV), surging crop intensity, use of Sulphur/zinc free fertilizers and limited dependence on organic manures have depleted soil reserves of these vital plant nutrients causing emergence of their deficiency in most of the soils all over India in general and UP in particular. These nutrients play a vital role in biosynthesis of proteins and amino acids. The application of Sulphur and zinc fertilizers has shown significant effects on yield, uptake of

nutrients and quality parameters in diverse crops. Research findings supporting the positive impact of zinc application have been reported earlier in wheat (Mishra, 2012), rice (Zeidan *et al.*, 2010; Wei *et al.*, 2012), maize (Potarzycki *et al.*, 2009), cluster-bean (Meena *et al.*, 2006), chickpea (Burman *et al.*, 2013).

Likewise, application of Sulphur leading to improvement in yield and nutritional quality of cereal (Salvagiotti and Miralles, 2008) has been extensively reported while studies elucidating its role on pulses are scarce. Lentil is one of the principle *rabi* season crop cultivated in Indian subcontinent. It serves as an important source of protein for large section of population who use pulse as a principal component in their daily diet. The protein quality in Lentil has a major drawback as it is deficient in Sulphur containing essential amino acids like methionine. In addition, it also has very low levels of cysteine.

During seed development in pulses, enormous quantities of proteins are accumulated over a short period of time. These accumulated proteins are of less diversity but constitute a major proportion of total seed protein in mature seeds. The globulin and albumins of pea, soybean, French bean, chickpea etc. have been extensively studied both qualitatively and quantitatively (Roy *et al.*, 2010; Wang *et al.*, 2008; Boye *et al.*, 2010). But literature on lentil regarding this aspect of protein quality is rare. Therefore, keeping this in focus the present study was carried out.

## **Materials and Methods**

### **Lentil Samples**

Four varieties of lentil (*Lens culinaris*) viz: L – 4076, K – 75, NDL – 1 and DPL – 15 were obtained from Student's Instructional Farm, NDU&T, Kumarganj, Faizabad. The

varieties were grown using standard agronomic practices in randomized block design having three replications. The N, P and K were applied at the rate of 20:50:40 kg/ha, respectively. Soil samples were analysed for Sulphur by turbidity method and zinc by DTPA extractable method to ascertain their availability before and after conducting the experiments.

### **Chemicals**

All chemicals used in the present investigation were of analytical grade and were purchased from Qualigen (India), SRL (India), or Sigma-Aldrich (USA).

### **Total Protein Content**

The total protein content of the lentil seeds was measured using a modified version of the Bradford assay (Bradford, 1976). The dye reagent (Bio-Rad protein assay kit II, Bio-Rad Laboratory, Hercules, CA) was diluted in 1:4 with distilled water after which 5 mL of the diluted dye was added to 50  $\mu$ L of the lentil seed samples, mixed thoroughly and incubated in the dark for 5 minutes. For the blank tube 50  $\mu$ L of distilled water was used instead of the lentil seed sample. All samples were analysed in triplicates and the absorbance was then measured at 595nm using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, NY).

A standard curve was prepared using bovine serum albumin (BSA) in different concentrations and the protein content was expressed as percent dry weight.

### **Quantitative determination of Protein Fractions**

Total protein of raw seed flour was extracted, based on the method outlined by Basha, Cherry, & Young (1976). Proteins were

purified by precipitation with 20% TCA and estimated according to Bradford assay (Bradford, 1976). The albumin and globulin fractions were separated, based on Murray (1979).

The protein fractions obtained were precipitated with TCA and re-dissolved in 0.2 N NaOH and protein content was determined according to Bradford assay (Bradford, 1976) and expressed as g/100g protein.

### **Determination of Amino acid composition**

Methionine content was determined according to the method of Horn *et al.*, (1946). Lysine was determined according to the method of Felker *et al.*, (1978) and the method proposed by Spies and Chamber (1949) was used to determine the tryptophan content. The results for amino acids were reported as and expressed as g/100g protein.

### **Statistical analysis**

Entire biochemical analysis was repeated two times. Two runs were performed from each extraction and all assays were carried out in triplicates (n = 12). Means, standard errors, and standard deviations were calculated from replicates using MS-Excel. The data were also analyzed for analysis of variance (ANOVA) using Statistical Analytical Software (SAS version 9.4; SAS Institute, Cary, NC).

### **Results and Discussion**

The content of protein, tryptophan, lysine, methionine, globulin and albumin increased significantly with increasing levels of Sulphur upto 30kg/ha (Table 1). The increasing trend in the protein fractions may be attributed to the fact that Sulphur stimulates the biosynthesis of proteins and Sulphur containing amino acids which is reflected during the assay of the samples. Similar trend

was observed for tryptophan and lysine also. Sulphur being an essential structural component of amino acids, co-enzymes involved in protein synthesis may have led to the increasing trend in protein and amino acids visible in the present investigation. Similar findings have been reported by Mishra *et al.*, (2012), Raikwar *et al.*, (2012) in wheat and barley respectively. Jamal *et al.*, (2005) reported comparable results in soybean and Chiaiese *et al.*, (2004) in chickpea.

Albumin content also increased significantly with increasing levels of Sulphur up to 30Kg/Ha. This may be due to high amino acid content particularly Sulphur containing amino acid methionine which is an important constituent of albumin protein fraction. On the contrary globulin fraction did not show any increase. The results are in accordance to the findings of Sharma and Sharma (2014); Sharma *et al.*, (2013).

There was significant increase in all the parameters except protein, albumin and globulin content (table 1). Application of zinc significantly increased tryptophan content. Tryptophan is an essential amino acid containing aromatic ring and zinc in directly involved in the synthesis of this amino acid. Tryptophan is a precursor of auxin hormone and is therefore important in normal growth of the plants also.

The role of zinc as cofactors of several enzymes is well established. Similar findings were observed by Mishra (2012) in wheat, Wang &Daun (2004) in *Pisum sativum* and Togay *et al.*, (2004) in *Phaseolus vulgaris* L. A significant increase in lysine content was also observed by increasing zinc doses at 30Kg/ha, which in turn results in increased globulin content. Findings of Togay *et al.*, (2004) in *Phaseolus vulgaris* L. support the results obtained in this investigation.

**Table.1** Biochemical composition of lentil as influenced by varieties, sulphur and zinc levels

Treatment	Protein (% DW)	Tryptophan (g/100g protein)	Lysine (g/100g protein)	Methionine (g/100g protein)	Albumin (g/100g protein)	Globulin (g/100g protein)
<b>Variety</b>						
DPL – 15	24.79	0.63	1.97	0.48	8.36	59.20
NDL -1	24.94	0.96	1.98	0.48	9.00	60.04
K – 75	26.16	0.99	1.97	0.54	9.34	60.06
L – 4076	24.48	0.60	1.93	0.48	8.12	59.45
CD at 5 %	0.51	0.02	0.04	0.01	0.17	1.28
<b>Sulphur levels (in Kg/ha)</b>						
Control	24.71	0.77	1.87	0.48	8.57	57.95
10	25.05	0.80	1.97	0.49	8.65	59.70
20	25.16	0.81	2.01	0.50	8.77	60.26
30	25.45	0.82	2.02	0.51	8.83	60.84
CD at 5 %	0.51	0.01	0.04	0.01	0.16	1.27
<b>Zinc levels (Kg/ha)</b>						
Control	25.02	0.75	1.89	0.49	8.65	57.89
10	25.04	0.81	1.96	0.49	8.71	60.30
20	25.23	0.82	1.21	0.50	8.76	60.76
30	25.08	0.81	1.99	0.48	8.70	59.80
CD at 5 %	0.51	0.02	0.03	0.01	0.17	1.28

**Table.2** Interaction of variety and sulphur on tryptophan (g/100g protein) in lentil varieties

Zinc levels (Kg/ha)	DPL – 15	NDL -1	K – 75	L – 4076
Control	0.63	0.65	1.05	0.63
10	0.53	0.65	1.01	1.01
20	0.63	0.65	1.08	0.89
30	0.63	0.62	1.11	0.97
CD at 5 %	0.03			

**Table.3** Interaction of variety and sulphur on albumin (g/100g protein) in lentil varieties

Sulphur levels (Kg/ha)	DPL – 15	NDL -1	K – 75	L – 4076
Control	8.09	8.78	9.33	8.10
10	8.29	9.01	9.32	7.96
20	8.09	9.44	9.45	8.10
30	8.96	8.78	9.26	8.33
CD at 5 %	0.03			

The variety K – 75 proved significantly superior to rest of the three varieties (DPL – 15, NDL – 1, L – 4076) with respect to all the

protein quality parameters and storage proteins (Table – 1). The varietal difference among the three samples studied may be

attributed to difference in genetic constitution. The results are in concordance to the findings reported by Mishra (2012), Wang and Daun (2004).

The interactive effect of zinc and varieties was significant and it was maximum in the variety K – 75 followed by NDL – 1 with 30, 40 and 20 Kg Zn/ha (table 2). Similar observations were also reported by Dwivedi *et al.*, (2002).

The combined response of different levels of Sulphur and varieties were also noticed in albumin content (Table 3). The variety K – 75 performed better with 20 Kg S/ha. Our results are in accordance with the findings of Wang and Daun (2004).

The findings of the investigation indicate that lentil is a reasonable source of protein with the variety K – 75 having the highest protein content. It is also concluded that application of sulphur and zinc fertilizers at 30Kg/ha was the best dose that led to maximum protein content, albumin and globulin fractions. The application of zinc and sulphur fertilizer also led to increase in essential amino acids lysine, tryptophan and methionine reasonably well in all the varieties investigated. Overall it may be concluded that zinc and sulphur application @ 30Kg/ha can be used to improve both quantity and quality of varieties of lentil.

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