Influence of Various Levels and Application Methods of Sulphur and Zinc on Nodulation, Quality and Nutrient Content of Chickpea (Cicer arietinum L.)

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A B S T R A C T

A field experiment was conducted during rabi season 2007-08 and 2008-09 at Agricultural farm of Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot, Satna (M.P.) to study the root growth, nodulation and nutrient content of chickpea. Results revealed that root growth viz. root length, root dry weight/plant; nodulation viz. no. of nodules/plant, nodules dry weight/plant and seed yield were recorded significantly superior under basal application of sulphur 40 kg/ha and zinc 5 kg/ha during two years. Spray of zinc 0.5% and sulphur 2% produced significant greater grain yield over control during two respective years. The protein content of seed and protein yield was observed significantly more under basal application of sulphur 40 kg/ha and zinc 5 kg/ha during two years. However, foliar application of zinc 0.5% noted numerically higher protein content of seed while, protein yield was observed significantly higher under foliar spray of zinc 0.5% over S spray 2% and control. Sulphur and zinc content in grain were significantly improved with application of sulphur and recorded maximum under 40 kg S/ha however, basal application of zinc 5 kg/ha recorded significantly higher Zn content in grain. Basal application of 40 kg S/ha observed significantly superior sulphur and zinc content in soil however, S and Zn content in soil was significantly improved due to application of zinc 5 kg/ha. Foliar spray of S 2% observed significantly higher S in grain and soil, while Zn spray 0.5% recorded more Zn content in grain and soil.

Keywords
Chickpea, Root growth, Nodulation, Grain yield, Protein content, Protein yield, Nutrient content, Sulphur and zinc, Basal, Foliar application

Introduction

Chickpea (Cicer arietinum L.) is a premier rabi legume crop in India. It is grown in India of 10.56 million hectares areas and production of 11.23 million tonnes with productivity of 1063 kg/ha (Anonymous, 2018-19). Chickpea being a legume crop has got capacity of fixing nitrogen through Rhizobium bacteria which forms nodules on the roots for this crop. Rhizobium can fix N2 nitrogen actively only if plant is adequately supplied with all mineral elements essential for active growth. In this respect elements like P, Ca, S, Mo, Zn, Fe, Co and B play an important role (Shubba rao, 1997). The supplementation of secondary like sulphur and micronutrient like zinc along with Rhizobium in chickpea cultivars may increase
biological nitrogen fixation and there by its productivity.

Sulphur is considered to be important secondary nutrients, as it synthesizes sulphur containing amino acids. It also helps in synthesis of chlorophyll, nodules formation and growth of *Rhizobium* bacteria and to produce oxidation system in respiration. Due to crop identification and use of high analysis straight fertilizers, non S containing pesticides all these leads to deficiency of S in Indian soils. Out of 135 pulses growing districts, sulphur deficiency in 44 districts ranged between 40-60%, where other 43 districts showed 20-40 % deficiency (Tiwari and Mishra, 2000).

Zinc is an essential micronutrient and reduces toxicity of element like Fe altered metabolism of N, P, CHO and nucleic acid. It plays an important role in plant nutrition, which is involved in the biosynthesis of the plant hormones and Indole acetic acid (IAA) and it maintains the normal auxin concentration in tissues. It has in a vital role for the synthesis of protein and nucleic acid and helps in the utilization of N and P in plants. It also promotes nodulation and N-fixation in leguminous crops. It has been reported that large area under cultivation in India is becoming deficient in Zn. Despite of this fact not much work has been done regarding response of legumes to zinc in India. In pulse growing areas, the needs of zinc is increasing due to there continues depletion in the soil. Several workers reported the response of Zn from 2 to 20 kg/ha in the different parts of India to pulse crops like gram, green gram, soybean and Pigeon pea (Ali *et al.*, 2005).

An adequate supply of sulphur and zinc may lead to higher productivity of chickpea. Thus to study the basal and foliar application of sulphur and zinc on root growth, nodulation and sulphur and zinc content of chickpea.

**Materials and Methods**

The study was conducted at the Agriculture Farm Rajaula of Mahatma Gandhi Chitrakoot Gramodaya Vishwa Vidyalya, Chitrakoot, Satna (M.P.) during the *rabi* season of 2007-08 and 2008-09. The soil of experimental field was sandy loam with neutral in soil pH (7.4 and 7.5) and low in organic carbon (0.23 and 0.47%) and available N (103 and 198 kg N/ha), medium to high phosphorus (24.35 and 28.1 kg P/ha), medium in potassium (124 and 228 kg K/ha), sulphur with 47.16 and 52.68 kg S/ha and zinc 2.14 and 2.28 kg Zn/ha during two respective years. The experiment consisted three levels of sulphur (0, 20, 40 kg S/ha), two levels of zinc (0, 5 kg Z/ha) and three levels of foliar spray (water spray, sulphur 2.0 %, zinc 0.5 %). In all 18 treatments will be tested in RBD (factorial) with three replications. An uniform doses of NPK @ 20: 40: 20 kg N$_2$O$_5$: P$_2$O$_5$: K$_2$O/ha were applied as basal. Sulphur and zinc were applied as per treatment. However, foliar spray was done at initiation of flower and ten days after first spray. The chickpea variety Uday was sown on 11$^{th}$ Oct 2007 and 27$^{th}$ Sep 2008 in two respective years at a row spacing of 30 cm apart using seed rate 100 kg/ha. The plant to plant spacing was maintained 05 cm by thinning at 20 DAS. Crop was protected from weeds by using one hand weeding at 30 DAS. However, insect pest was controlled by spraying of Dimethoate @ 2 ml/litre water at pod formation stage. The crop was harvested on 25$^{th}$ March 2008 and 10$^{th}$ March 2009 in two respective years. The important growth parameters, yield attributes and yield were recorded at appropriate time as per standard procedure. Zinc was estimated by DTPA extractable zinc (Lindsay and Norvell 1978) with the help of Atomic Absorption
Spectrophotometer. Protein content in seed was estimated after the estimation of nitrogen percentage in seed by Kjeldhal method with the help of following formula:

$$\text{Protein content (\%) = Nitrogen content (\%) X 6.25.}$$

The experimental data was statistically analysed by Panse and Sukhatme (1985). The treatment differences were tested by using “F” test and critical differences at 5% probability.

**Results and Discussion**

**Root Growth**

Root length and root dry weight was obtained significantly higher under sulphur 40 kg/ha over control and 20 kg S/ha during 2007-08 and 2008-09. However, application of zinc 5 kg/ha recorded significantly superior root length and root dry weight than that of control during two years (Table 1). While, sulphur and zinc spray did not show any significant effect on these growth characters over water spray. The superior root growth might be due to basal application of sulphur and zinc which was associated with shoot parameters.

**Root Nodulation**

The formation of root nodules/ plant and dry weight of root nodules/ plant were observed statistically higher under sulphur 40 kg/ha than that of preceding doses at 45 and 60 days stages during two years (Table 1). However, application of zinc 5 kg/ha recorded significant superior root nodules/ plant and dry weight of root nodules/ plant at 45 and 60 days stages during 2007-08 and 2008-09. The formation of root nodules/ plant and dry weight of root nodules/ plant were found statistically at par in foliar spray of sulphur and zinc in chickpea. Such improvement in nodules formation might be possibly due to starter dose of sulphur and zinc application along with balance supply of nitrogen and phosphorus helps in establishment of the crop which ultimately leads to extensive development of root nodules and bacteria present in them for fulfilling the need of the crop for nitrogen (Sahu et al., 2002).

**Grain yield**

The grain yield of chickpea was significantly increased up to 40 kg S/ha (1923 and 2051 kg/ha) which showed 446 kg/ha and 595 kg/ha; and 122 kg/ha and 138 kg/ha higher over control and 20 kg S/ha, respectively during 2007-08 and 2008-09 (Table 2). However, the application of zinc 5 kg /ha recorded significant improvement in grain (1839 and 1968 kg/ha) of chickpea than that of no zinc during two respective years. Zinc application @ 5 kg Zn/ha produced 12.96 percent and 49.84 percent more seed yield compared with control during two respective years. The grain yield of chickpea was significantly increased with the application of zinc 0.5% (1806 and 1930 kg/ha) and sulphur 2% (1760 and 1803 kg/ha) and obtained 10.45 % and 14.45 % more over control during both the years. The improvement in seed yield by zinc application might be due to increase in synthesis of carbohydrate and protein and their translocation to the sink through efficient physiological activity in plants as evident from physiological parameters like shoot and root growth and yield contributing characters like number of primary and secondary branches, pods per plant, pod length, seeds per pod, seed weight per plant and 100 seeds weight. The results are in accordance with those reported by Puste and Jana (1995). Soil application of fertilizer leads to losses of nutrients in the form of leaching, volatilization and fixation affecting the nutrient use efficiency (Veerabhadrappa and Yeledhalli, 2005).
**Protein content and Protein yield**

The protein content of seed (21.53 and 23.49%) and protein yield (415 and 482 kg/ha) was observed significantly more under basal application of sulphur 40 kg/ha than that of control and 20 kg S/ha during both the years (Table 2). This could be ascribed that sulphur is a constituent of three essential amino acids (Cysteine, Cystine and Methionine) which promotes the more accumulation of protein content in grain of chickpea. Sulphur application improved yield and quality of cowpea (Thuan, and Rana, 2010) and mungbean (Ram and Katiyar, 2018).

Zinc application 5 kg/ha recorded significant superior protein content of seed (20.82 and 22.80%) and of protein yield (385 and 450 kg/ha), than that of no zinc application. This might be due to zinc application enhance protein and carbohydrates synthesis and their transportation to the site of seed formation. Similar results were reported by Adsul et al., (2020). Micronutrients application also increased the protein harvest maximum values were recorded 6 kg Zn/ha followed by 1 kg Mo/ha and 0.5 kg B/ha as compared to no micronutrients application in French bean (Singh et al., 2006). More N uptake by plant resulted in more protein yield in seed. The results of similar kind were also reported by Deo et al., (2002).

Foliar application of zinc 0.5% noted significantly higher protein content of seed than that of no zinc application (20.99) during 2007-08 and statistically at par in 2007-08. Protein yield was observed significantly higher under foliar spray of zinc 0.5 percent over S spray 2% and control. Such increase could be ascribed due to more proportionate increase N content in grain. This possible due to more increase in nitrogenase activity in the root nodules might have increased the N fixation and there by N content in grain. The results are in agreement with the findings of Abd EL-Kader and Mona (2013), Alam et al., (1999) and Chandra (1995). In this concern Ved et al. (2002) stated that foliar applied zinc enhances photosynthesis at early growth of mungbean plants, improves their nitrogen fixation, grain protein and yields.

**Nutrient content**

**Sulphur and zinc content in grain**

The sulphur contents in grain was significantly increased with application of sulphur and it was maximum at 40 kg S/ha (0.22 and 0.24%) during 2007-08 and 2008-09 (Table 3). This might be due to more availability of available S to plant resulted more uptake and S content in grain. Sulphur is a constituent of three essential amino acids (Cysteine, Cystine and Methionine) and also a constituent of ferrodoxin-containing Nitrogenase, which takes part in the biological nitrogen fixation resulting highest number of nodules/ plant and nitrogen and sulphur content in the soil. Similar finding was also reported by Khan and Prakash (2014). This trend was perhaps to establish favourable N:S ratio in the vegetative tissue of the plant (Kachhave et al., 1997).

Application of zinc 5 kg/ha recorded numerically higher S content in seed. Foliar spray of S 2% significantly increased S content in seed over control and Zn 0.5%. Zinc content in grain was significantly increased and obtained superior under 40 kg S/ha during two years. However, the maximum contents of Zn in grain (36.94 and 36.08 mg/kg) was recorded with application of 5 kg Zn/ha during 2006-07 and 2008-09, respectively. Foliar spray of Zn 0.5% conspicuously increased Zn content over control and S spray 2%. Similar results have been reported by Bansal (1992) in wheat crop.
**Table 1** Effect of sulphur and zinc as basal and foliar application on root growth and nodulation of chickpea

| Treatment | Root length/plant (cm) | Root dry weight/plant (g) | No. of Nodules/plant | Nodules dry weight/plant (mg) |
|-----------|------------------------|---------------------------|----------------------|-------------------------------|
|           | 60 DAS 2007-08 | 60 DAS 2008-09 | 45 DAS 2007-08 | 60 DAS 2008-09 | 45 DAS 2007-08 | 60 DAS 2008-09 |
| S Level (kg S /ha) | | | | | | |
| 0         | 11.91      | 12.07 | 0.57 | 1.29 | 8  | 10 | 8  | 11 | 19.15 | 19.37 | 22.81 | 24.46 |
| 20        | 14.10      | 13.89 | 0.67 | 2.49 | 13 | 15 | 14 | 16 | 28.91 | 31.20 | 39.33 | 39.83 |
| 40        | 16.06      | 15.93 | 0.83 | 2.91 | 14 | 18 | 15 | 19 | 39.31 | 35.72 | 43.28 | 43.03 |
| SE m±     | 0.18       | 0.20  | 0.02 | 0.06 | 0.32 | 0.33 | 0.17 | 0.33 | 0.45 | 0.67 | 0.68 | 0.66 |
| CD (P=0.5)| 0.51       | 0.58  | 0.05 | 0.17 | 0.93 | 0.95 | 0.50 | 0.94 | 1.28 | 1.94 | 1.95 | 1.91 |
| Zn level (kg Zn /ha) | | | | | | |
| 0         | 13.35      | 13.24 | 0.63 | 2.12 | 10 | 11 | 11 | 13 | 26.52 | 23.68 | 32.22 | 30.38 |
| 5         | 14.69      | 14.69 | 0.75 | 2.34 | 13 | 17 | 13 | 18 | 31.73 | 33.85 | 38.06 | 41.17 |
| SE m±     | 0.15       | 0.16  | 0.01 | 0.05 | 0.26 | 0.27 | 0.14 | 0.27 | 0.36 | 0.55 | 0.56 | 0.54 |
| CD (P=0.5)| 0.42       | 0.47  | 0.04 | 0.14 | 0.76 | 0.77 | 0.40 | 0.77 | 1.05 | 1.58 | 1.60 | 1.56 |
| Foliar Spray | | | | | | |
| Water spray | 14.07   | 13.94 | 0.68 | 2.24 | 11 | 14 | 12 | 15 | 27.91 | 28.49 | 32.97 | 35.05 |
| S -spray 2% | 14.01   | 13.81 | 0.70 | 2.23 | 12 | 14 | 12 | 16 | 29.26 | 28.79 | 35.29 | 35.60 |
| Zn spray 0.5% | 13.99  | 14.13 | 0.70 | 2.23 | 12 | 14 | 12 | 16 | 30.20 | 29.01 | 37.16 | 36.68 |
| SE m±     | 0.18       | 0.20  | 0.02 | 0.06 | 0.32 | 0.33 | 0.17 | 0.33 | 0.45 | 0.67 | 0.68 | 0.66 |
| CD (P=0.5)| NS         | NS    | NS   | NS   | NS  | NS  | NS  | NS  | 1.28 | NS   | NS   | NS   |
### Table 2: Effect of sulphur and zinc as basal and foliar application on grain yield and quality parameters of chickpea

| Treatment     | Seed yield (kg/ha) | Protein content (%) | Protein yield (kg/ha) |
|---------------|--------------------|---------------------|-----------------------|
|               | 2007-08 | 2008-09 | 2007-08 | 2008-09 | 2007-08 | 2008-09 |
| **S Level(kg S/ha)** |         |         |         |         |         |         |
| 0             | 1477     | 1456    | 18.20   | 21.05   | 271     | 308     |
| 20            | 1801     | 1913    | 19.58   | 22.76   | 353     | 435     |
| 40            | 1923     | 2051    | 21.53   | 23.49   | 415     | 482     |
| SE m±         | 26.44    | 23.30   | 0.39    | 0.19    | 8.86    | 6.79    |
| CD (P=0.5)    | 75.99    | 66.98   | 1.13    | 0.56    | 25.46   | 19.54   |
| **Zn level (kg Zn/ha)** |         |         |         |         |         |         |
| 0             | 1628     | 1646    | 18.71   | 22.06   | 308     | 367     |
| 5             | 1839     | 1968    | 20.82   | 22.80   | 385     | 450     |
| 40            | 1923     | 2051    | 21.53   | 23.49   | 415     | 482     |
| SE m±         | 26.44    | 23.30   | 0.39    | 0.19    | 8.86    | 6.79    |
| CD (P=0.5)    | 75.99    | 66.98   | 1.13    | 0.56    | 25.46   | 19.54   |
| **Foliar Spray** |         |         |         |         |         |         |
| Water spray   | 1635     | 1687    | 19.23   | 22.28   | 319     | 380     |
| S spray 2%    | 1760     | 1803    | 19.08   | 22.32   | 339     | 406     |
| Zn spray 0.5% | 1806     | 1930    | 20.99   | 22.69   | 381     | 440     |
| SE m±         | 26.44    | 23.30   | 0.39    | 0.19    | 8.86    | 6.79    |
| CD (P=0.5)    | 75.99    | 66.98   | 1.13    | 0.56    | 25.46   | 19.54   |

### Table 3: Effect of sulphur and zinc as basal and foliar application on contents of sulphur and zinc in grain and soil

| Treatment     | S content in grain (%) | Zn content in grain (mg/kg) | S content in soil (mg/kg) | Zn content in soil (mg/kg) |
|---------------|------------------------|-----------------------------|---------------------------|-----------------------------|
|               | 2007-08 | 2008-09 | 2007-08 | 2008-09 | 2007-08 | 2008-09 | 2007-08 | 2008-09 |
| **S Level (kg S/ha)** |         |         |         |         |         |         |         |         |
| 0             | 0.18     | 0.18    | 32.57   | 32.71   | 6.54    | 6.60    | 0.56    | 0.60    |
| 20            | 0.21     | 0.23    | 33.60   | 33.14   | 11.23   | 11.74   | 0.60    | 0.62    |
| 40            | 0.22     | 0.24    | 36.17   | 35.90   | 20.61   | 21.89   | 0.68    | 0.69    |
| SE m±         | 0.01     | 0.004   | 0.79    | 0.61    | 0.24    | 0.18    | 0.01    | 0.01    |
| CD (P=0.5)    | 0.02     | 0.01    | 2.26    | 1.77    | 0.68    | 0.53    | 0.03    | 0.03    |
| **Zn level (kg Zn/ha)** |         |         |         |         |         |         |         |         |
| 0             | 0.20     | 0.21    | 31.29   | 31.75   | 12.62   | 12.98   | 0.45    | 0.49    |
| 5             | 0.21     | 0.22    | 36.94   | 36.08   | 12.96   | 13.83   | 0.78    | 0.78    |
| SE m±         | 0.005    | 0.003   | 0.64    | 0.50    | 0.19    | 0.15    | 0.01    | 0.01    |
| CD (P=0.5)    | NS       | 0.01    | 1.85    | 1.44    | 0.56    | 0.43    | 0.02    | 0.03    |
| **Foliar Spray** |         |         |         |         |         |         |         |         |
| Water spray   | 0.19     | 0.21    | 32.80   | 32.19   | 12.70   | 13.34   | 0.55    | 0.57    |
| S - spray 2%  | 0.23     | 0.23    | 34.01   | 33.27   | 13.72   | 14.24   | 0.55    | 0.60    |
| Zn - spray 0.5% | 0.19   | 0.21    | 35.54   | 36.29   | 11.96   | 12.64   | 0.75    | 0.75    |
| SE m±         | 0.01     | 0.004   | 0.79    | 0.61    | 0.24    | 0.18    | 0.01    | 0.01    |
| CD (P=0.5)    | 0.02     | 0.01    | NS      | 1.77    | 0.68    | 0.53    | 0.03    | 0.03    |
Increased availability of Zn in the soil on the addition of water soluble EDTA Zn resulted in increase in its contents in the grains. Increased availability of Zn in the soil on the addition of water soluble ZnCl₂ resulted in increase in its contents in the grains. Similar findings have also been reported by Tripathi et al., (1997) and Dev et al., (1992).

**Sulphur and zinc content in soil**

Sulphur (20.61 and 21.89 mg/kg) and zinc contents (0.68 and 0.69 mg/kg) in soil after harvest of crop were found significantly higher in 40 kg S /ha over control and 20 kg S/ha during both the years (Table 3). However, addition of 5 kg Zn/ha recorded significantly superior sulphur (12.96 and 13.83 mg/kg) and Zn contents (0.78 and 0.78 mg/kg) in soil during 2007-08 and 2008-09, respectively. Foliar spray of S 2% increased S content in soil however, zinc spray 0.5 enhanced Zn content in soil. The increase in S and Zn content in soil might be due drifted particles of sulphur or zinc spray on the soil. In case of greater bioavailability of the grain zinc derived from foliar applications than from soil, agronomic biofortification would be a very attractive and useful strategy in solving zinc deficiency related health problems globally and effectively (Yosefi1 et al., 2011; Abd El-Baky et al., 2010; Cakmak, 2008).

Thus, it can be concluded that sulphur 40 kg/ha and zinc 5 kg/ha application were found equally most effective in respect of root growth, nodulation, yield, quality and S and Zn content in grain of chickpea and for soil status of S and Zn. Foliar applications of S 2% and Zn 0.5% are more suitable than the soil application due to the rapid overcoming on deficiency, easy to use, reduce the toxicity caused by accumulation and prevent of elements stabilization in the soil.

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