

Original Research Article

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## Legume Friend Rhizobium and Booster Sulphur

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### ABSTRACT

An experiment was designed to assess the response of Sulphur to different Chickpea cultivars at Agriculture farm of Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot, Satna (M.P.). Three varieties (DCP 92-03, JG-16, JG-11) Seed Treatment in Chickpea with Rhizobium and three Sulphur levels (10, 20, 30 and 40 kg S /ha) along with all possible interactions were used. Experimental design following split plot design in three replications was employed data was collected on yield parameters. Rhizobium species form an endosymbiotic nitrogen-fixing association with roots of legumes and Parasponia. The bacteria colonize plant cells within root nodules, where they convert atmospheric nitrogen into ammonia using the enzyme nitrogenase and then provide organic nitrogenous compounds such as glutamine or ureides to the plant. Sulfur is an essential nutrient for plants because it is a constituent of the amino acids cysteine (Cys) and methionine (Met), metal cofactors, coenzymes, and secondary metabolites. The application of 40 kg S / ha-1 enhanced the plant height, number of branches, No. of nodule, nodule dry weight, no. of pods / plant, no. of seeds / plant, seed weight / plant, 100 seed weight (g) / plot, significantly over S1, S2, S3. This reflects the fact that sulphur only up to 30 kg / ha-1 (40 Kg S / ha-1) was sufficient to meet the requirement of the actively growing plants under the existing sulphur status of the soil.

#### Keywords

Rhizobium,  
Sulphur, Nodules

#### Article Info

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### Introduction

This Project work is about the bacterial genus and sulphur element. For the generic term that includes species in other genera, see *Rhizobia*. *Rhizobium* is a genus of Gram negative soil bacteria that fix nitrogen. Sulfur is an essential nutrient for plants because it is a constituent of the amino acids cysteine (Cys) and methionine (Met), metal cofactors,

coenzymes, and secondary metabolites (reviewed by Davidian and Kopriva, 2010). As occurs in other plants, sulfur deficiency in legumes decreases plant growth, photosynthesis, and yield. However, nodulated legumes have a high demand for sulfur and SNF is more sensitive to sulfur deficiency than is nitrate uptake (Zhao *et al.*, 1999; Varin *et al.*, 2010).

The plant, in turn, provides the bacteria with organic compounds made by photosynthesis. This mutually beneficial relationship is true of all of the rhizobia, of which the genus *Rhizobium* is a typical example. Martinus Beijerinck was the first to isolate and cultivate a microorganism from the nodules of legumes in 1888. He named it *Bacillus radicum*, which is now placed in Bergey's Manual of Determinative Bacteriology under the genus *Rhizobium*. *Rhizobium* forms a symbiotic relationship with certain plants such as legumes, fixing nitrogen from the air into ammonia, which acts as a natural fertilizer for the plants.

### **Experimental site and Location**

The experiment was conducted, during the Ravi season of 2015 – 16 at the Agriculture Farm of Mahatma Gandhi Chitrakoot, Gramodaya Vishwavidyalaya, Chitrakoot – Satna (Madhya Pradesh) located from 24°31' N latitude and 81° 15' E latitude. The experimental field is situated in the North Eastern part of Madhya Pradesh. All the facilities necessary for conducting the experiment, including labour and resources, which were necessary for normal cultivation were readily available in the department.

### **Experimental design and layout details**

To achieve the objectives the field experiment was conducted in split plot design with three replications having 12 treatments combination. The details of are given in Table 1.

### **Details of treatments**

#### **Main-plot treatment: Varieties-3**

1- DCP 92-03 (V<sub>1</sub>) 2- JG-16 (V<sub>2</sub>) 3- JG-11 (V<sub>3</sub>)

Variety - DCP 92-03, Source – IIPR, Year of Release/Notification – 1997, Area of adoption zone/ state - North West plane zone (Punjab, Haryana, Delhi, North Rajasthan, west U.P), Area yield (Q/ha)- 19-20, Days to Maturity - 145-150, Remarks - Lodging and wilt resistant yellowish Brown and excessive moisture conditions.

Variety - JG-16, Source – JNKV, Year of Release/Notification – 2001, Area of adoption zone/ state - M.P, Maharashtra, Gujarat, Area yield (Q/ha) - 18-20, Days to Maturity- 120-125, Remarks - JG 16: Maturity: 110-115 days, Seed size: 22-25 g, Seed yield: 18-20 q/ha, Semi spreading, profuse branching, dark green foliage, light brown, medium bold, attractive seed, rainfed.

Variety - JG-11, Source – JNKV, Year of Release/Notification -1997, Area of adoption zone/, state - M.P, Area yield (Q/ha) -13-15 Days to Maturity -120-125, Remarks- Seed pinkish, JG 11 (ICCV 93954) is a desi chickpea varieties developed through collaborative breeding efforts of ICRISAT with Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh, India, and released in 1999.

This is the most preferred variety by farmers in India because of its early maturity (95–100 d) and higher yield (up to 2.5 t ha<sup>-1</sup> in rain-fed and up to 3.5 t /ha<sup>-1</sup> under irrigated conditions) than the other varieties with an attractive large seed (22 g per 100 seed).JG 11 brought the chickpea revolution in Andhra Pradesh by covering 70% area with potential yield of 36.0 q/ha by replacing age old wide adopted variety Annagiri (Table 2).

#### **Sub-plot treatment: Sulphur levels – 4**

1. 10 kg S/ha (S<sub>1</sub>). 2. 20 kg S/ha (S<sub>2</sub>).  
3. 30kg S/ha (S<sub>3</sub>). 4. 40 kg S/ha (S<sub>4</sub>).

## **Treatment combination**

### **Nodulation**

Three plants at random were uprooted from each pot causing minimum damage to the roots at 75 DAS of the crop. The roots were thoroughly washed with a jet of water, and then nodules were removed from roots with the help of forceps. The effective nodules were counted and data recorded for three plants uprooted from each plot. These nodules were dried at  $70 \pm 1^\circ\text{C}$  for 2-3 days. Then oven dry weight of nodules was recorded.

## **Results and Discussion**

### **Nodulation observation**

Observations on nodulation were taken on 75 DAS stage and results of these parameters are presented in Table 4.

### **Nodules number**

The data presented in table 4 indicated that the different chickpea cultivars and sulphur levels increased the nodule number and their dry weight/plant significantly at 75 DAS stage

### **Effect of varieties**

The nodule number noted in the range of 6.01 -11.16. par plant under different varieties of chickpea. Whereas, variety DCP 92-03 ( $V_1$ ) also recorded significantly higher (11.16 / Plant) nodule number over JG-16 ( $V_2$ ) and JG-11 ( $V_3$ ).

### **Effect of sulphur levels**

It is seen from the data in Table 4, that the increasing level of sulphur increased the nodule number per plant significantly. Maximum nodule number (08.39 / plant) was

observed in 40 kg S/ha ( $S_4$ ) levels which was significantly superior over 10 kg S / ha ( $S_1$ ) and 20 kg S/ha ( $S_2$ ) levels but statistically at par with 30 kg S/ha ( $S_3$ ) level.

### **Interaction effects**

The interaction effect due to varieties and sulphur levels on nodule number plant was found to the non -significant at 75 DAS stages of growth.

### **Nodule dry wright**

Data on nodule dry weight have been set out in Table 4.

### **Effect of varieties**

The nodule dry weight observed in the range of 6.8 mg–13.8 mg per plant under different varieties of chickpea. Variety DCP 92-03 ( $V_1$ ) recorded maximum nodule dry weight (13.8 mg / plant) which was significantly superior over remaining two varieties under tested. Whereas, JG-16 ( $V_2$ ) also recorded higher nodule dry wright over JG-11 ( $V_3$ ) of significance.

### **Effect of sulphur levels**

It is seen from the data (Table 4) that the increasing level of sulphur increased the nodule dry weight per plant significantly. Maximum nodule dry weight (9.96 g /plant) was noted with 40 kg S/ha ( $S_4$ ) level which was significantly superior over 10 kg S / ha ( $S_1$ ) and 20 kg S/ha ( $S_2$ ) but statistically at par with 30 kg S/ha ( $S_3$ ) level.

### **Interaction effects**

The interaction effect due to varieties and sulphur levels on nodule dry weight / plant was found to the non-significant at 75 DAS stages of growth.

### Economics

Recommendation and adoption of any practices by cultivators depends upon its economics. Therefore, it becomes essential to work out economics of the treatments tested for judging the best treatments under study, for getting higher net profit per hectare.

### Effect of sulphur levels

Under different levels of sulphur 40 kg S/ha<sup>-1</sup> (S<sub>4</sub>) gave highest gross and net income of Rs.83289 / ha<sup>-1</sup> and Rs.57574 / ha<sup>-1</sup> which is followed by 30 kg S / ha<sup>-1</sup> (S<sub>3</sub>) with Gross returns (Rs.66416/ha<sup>-1</sup> and Net returns Rs.41401) and minimum (Gross returns Rs.47871 and Net returns Rs.24255/ha<sup>-1</sup>) under 10 kg S / ha<sup>-1</sup> (S<sub>1</sub>).

Highest net income of Rs.57574 / ha<sup>-1</sup> and B:C ratio (3.14) were recorded with 40 kg S / ha<sup>-1</sup> (S<sub>4</sub>) respectively (Table 5).

### Effect of sulphur

It is evident from the results reported in foregoing pages, that the application of 40 kg S/ha<sup>-1</sup> enhanced the plant height, number of branches, No. of nodule, nodule dry weight, no. of pods/plant, no. of seeds/plant, seed weight/plant, 100 seed weight (g)/plot, significantly over S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>. This reflects the fact that sulphur only up to 30 kg/ha<sup>-1</sup> (40 Kg S/ha<sup>-1</sup>) was sufficient to meet the requirement of the actively growing plants under the existing sulphur status of the soil.

**Table.1** Experimental design and layout details

1	Design	Split plot
2	Treatments	12 (3 varieties and 4 Sulphur level)
3	Replications	03
4	Number of plots	36
5	Gross plot size	5.0 m x 3.0 m
6	Net plot size	4.0 m x 2.10 m
7	Replication border	1.0 m
8	Distance between plots	0.5 m
9	Distance between plots	30 cm
10	100% RDF	20 kg N + 40 kg P <sub>2</sub> O <sub>5</sub> + 20 kg K <sub>2</sub> O/ha
11	Seed rate	80 kg /ha

**Table.2** Treatment combinations and symbols used

S. No.	Treatments				
1	T <sub>1</sub>	S <sub>1</sub> V <sub>1</sub>	7	T <sub>7</sub>	S <sub>3</sub> V <sub>1</sub>
2	T <sub>2</sub>	S <sub>2</sub> V <sub>2</sub>	8	T <sub>8</sub>	S <sub>4</sub> V <sub>2</sub>
3	T <sub>3</sub>	S <sub>3</sub> V <sub>3</sub>	9	T <sub>9</sub>	S <sub>1</sub> V <sub>3</sub>
4	T <sub>4</sub>	S <sub>4</sub> V <sub>1</sub>	10	T <sub>10</sub>	S <sub>2</sub> V <sub>1</sub>
5	T <sub>5</sub>	S <sub>1</sub> V <sub>2</sub>	11	T <sub>11</sub>	S <sub>3</sub> V <sub>2</sub>
6	T <sub>6</sub>	S <sub>2</sub> V <sub>2</sub>	12	T <sub>12</sub>	S <sub>4</sub> V <sub>3</sub>

**Table.3** Details of pre and post sowing operations

S. No	Operation	Dates
1	Harrowing & Ploughing	30/10/2015
2	Ploughing –II & Leveling (Planking)	06/11/2015
3	Layout formation and soil sample	06/11/2015
4	Fertilizer application (basal) and Sowing	07/11/2015
5	Date of irrigation	17/11/2015
6	Resowing	21/11/2015
7	Thinning	05/12/2015
8	First hand weeding	08/12/2015
9	Date of Second irrigation	19/12/2015
10	First observed taken	21/12/2015
11	Second observed taken	06/01/2016
12	Third observation taken	22/01/2016
13	Nodulation study	05/02/2016
14	Date of Third irrigation	10/02/2016
15	Second hand weeding	20/02/2016
16	Harvesting	20/03/2016

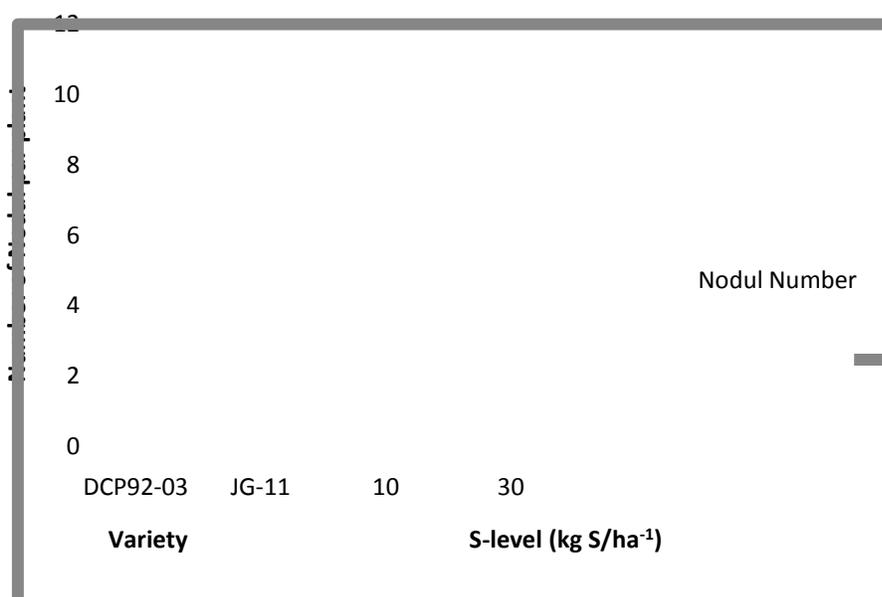
**Table.4** Effect of varieties and sulphur levels on nodulations of chickpea. (75 DAS)

Treatment	Number of nodulation at 75 DAS	
(A) Cultivars	Nodule number	Nodule dry weight (mg)
V <sub>1</sub> : DCP 92-03	11.16	13.8
V <sub>2</sub> : JG-16	6.37	7.0
V <sub>3</sub> : JG-11	6.01	6.8
S.E m. <sub>±</sub>	0.3438	.2163
C.D.(P=0.05)	1.0216	.6426
(B) Sulphur levels (kg S/ha <sup>-1</sup> )		
S <sub>1</sub> :10	6.58	8.38
S <sub>2</sub> :20	7.66	8.95
S <sub>3</sub> :30	8.21	9.65
S <sub>4</sub> :40	8.93	9.96
S.E. m. <sub>±</sub>	0.22	0.21
C.D. (P=0.05)	0.68	0.61
Interaction	(N.S)	(N.S)

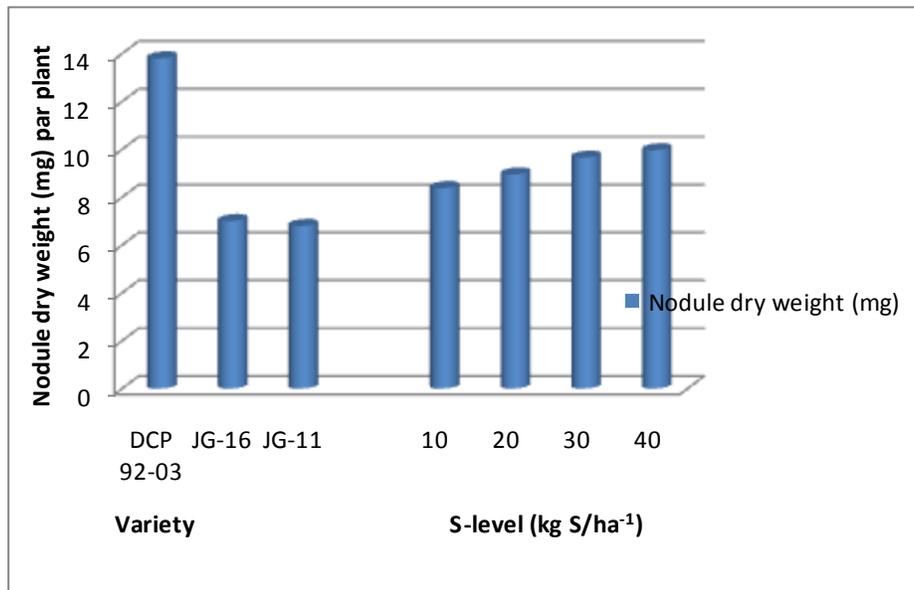
**Table.5 Economics**

Treatment	Cost of Cultivation (Rs./ha <sup>-1</sup> )	Gross returns (Rs./ha <sup>-1</sup> )	Net returns (Rs./ha <sup>-1</sup> )	B:C Ratio
V <sub>1</sub> DCP 92-03	27465	104989	77524	3.805
V <sub>2</sub> JG-16	23265	42247	18982	1.8025
V <sub>3</sub> JG- 11	23265	46147	22882	1.98
S <sub>1</sub> 10 kg S / ha <sup>-1</sup>	23616	47871	24255	1.97
S <sub>2</sub> 20 kg S / ha <sup>-1</sup>	24315	60268	35953	2.41
S <sub>3</sub> 30 kg S / ha <sup>-1</sup>	25015	66416	41401	2.8
S <sub>4</sub> 40 kg S / ha <sup>-1</sup>	25715	83289	57574	3.14
V <sub>1</sub> S <sub>1</sub>	26416	75464	49048	2.86
V <sub>1</sub> S <sub>2</sub>	27115	98306	71191	3.63
V <sub>1</sub> S <sub>3</sub>	27815	106711	78896	3.84
V <sub>1</sub> S <sub>4</sub>	28515	139474	110959	4.89
V <sub>2</sub> S <sub>1</sub>	22216	29940	7724	1.35
V <sub>2</sub> S <sub>2</sub>	22915	38498	15583	1.68
V <sub>2</sub> S <sub>3</sub>	23615	41375	17760	1.75
V <sub>2</sub> S <sub>4</sub>	24315	59176	34861	2.43
V <sub>3</sub> S <sub>1</sub>	22216	38208	15992	1.72
V <sub>3</sub> S <sub>2</sub>	22915	44001	21086	1.92
V <sub>3</sub> S <sub>3</sub>	23615	51163	27548	2.17
V <sub>3</sub> S <sub>4</sub>	24315	51218	26903	2.11

**Fig.1 Nodules number**



**Fig.2** Nodule dry weight (g)



An improvement in plant growth parameters by increasing doses of sulphur might be because of its role in the formation of amino acids viz, cysteine, methionine and synthesis of protein, vitamin and chlorophyll. The findings corroborates with the findings of Shri Krishna *et al.*, (2004), Muhammad Islam *et al.*, (2013), Togay *et al.*, (2008), Sher Singh (2004), Shivakumar (2001), Dwivedi *et al.*, (1982), Chaudhary and Goswami (2005) and Sharma and Kushwah (2011) in chickpea crop in different parts of India.

Maximum nodule number (08.39 / plant) was observed in 40 kg S / ha (S<sub>4</sub>) levels which was significantly superior over 10 kg S / ha<sup>-1</sup> (S<sub>1</sub>) and 20 kg S / ha<sup>-1</sup> (S<sub>2</sub>) levels but statistically at par with 30 kg S / ha<sup>-1</sup> (S<sub>3</sub>) level.

Maximum nodule dry weight (9.66 mg /plant) was noted with 40 kg S/ha (S<sub>4</sub>) level which was significantly superior over 10 kg S / ha<sup>-1</sup> (S<sub>1</sub>) and 20 kg S / ha<sup>-1</sup> (S<sub>2</sub>) but statistically at par with 30 kg S / ha<sup>-1</sup> (S<sub>3</sub>) level.

The improvement in nodules per plant and consequently their dry weight might be due to

adequate supply of sulphur to plants which helps in stimulation the Rhizobium bacteria for nodule formation. These results confirmed the findings of Kasturikrishna and Sher singh (2004), Shivkumar (2001), Muhammad Islam (2013), Togay (2008). Deo and Khaldelwal (2009) and Sharma and Kushwah (2011) The probable reason may be due to adequate supply of sulphur might have played an important physiological role by enhancing cell multiplication, elongation, expansion and chlorophyll biosynthesis, which is turn increased the assimilate production to be used for root development also.

These results confirmed the findings of Kasturkrishna and Ahlawat (2000), and Sharma and Kushwah (2011) it is revealed from the results reported in foregoing pages that the sulphur addition 40 kg/ha<sup>-1</sup> significantly increased seed yield of chickpea. This increase in seed yield might have resulted from the efficient and greater partitioning of metabolites and adequate translocation of photosynthesis towards the developing reproductive structure (Fig. 1 and 2).

Although increase the straw yield significantly but the difference between straw yields under various sulphur levels was statistically at par. the straw yield might because of higher growth parameters as well as greater nodulation and root development. The probable reason may be due to adequate supply of all the nutrients, which resulted in greater accumulation of carbohydrates, amino acids and their translocation to the productive organs, which, in turn improved all the growth and yield attributing characters. The findings corroborates with the findings of Mondal *et al.*, (2005), Chaudhary and Goswami (2005), Kumar *et al.*, (2009). Tripathi *et al.*, (2011) and Sharma and Kushwaha (2011).

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