Original Research Article

Effect of Phosphorus and Sulphur on Chlorophyll, Nodulation, Soil Properties and Optimum Dose of Phosphorus and Sulphur for Clusterbean [Cyamopsis tetragonoloba (L.) Taub]

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A B S T R A C T

An experiment was conducted at Agronomy farm, S.K.N. College of Agriculture, Jobner, Jaipur during kharif, 2017. The experiment consisted 16 treatment combinations comprising of four levels each of phosphorus (0, 20, 40 and 60 kg P2O5 ha⁻¹) and sulphur (0, 20, 40 and 60 kg S ha⁻¹) was laid out in randomized block design and replicated thrice. Clusterbean variety RGC-1038 was used as a test crop. The results indicated that total chlorophyll content at 45 DAS, number of total and effective root nodules at flowering were observed significantly maximum upto 40 kg P2O5 ha⁻¹ level of phosphorus application over control and 20 kg P2O5 ha⁻¹. Whereas, the available N, P2O5 and S in soil after harvest of clusterbean were recorded significantly maximum with the application of 60 kg P2O5 ha⁻¹. Application of sulphur upto 40 kg ha⁻¹ resulted in significantly maximum increase in total chlorophyll content at 45 DAS, number of total and effective root nodules at flowering over control and 20 kg S ha⁻¹. Where as, significantly maximum available N, P2O5 and S in soil after harvest was recorded with the application of 60 kg S ha⁻¹. The optimum dose of phosphorus and sulphur as derived from response function curve were 49.31 and 50.64 kg ha⁻¹ respectively.

Keywords
Clusterbean, Phosphorus, Sulphur, Chlorophyll, Nodules

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Introduction

Clusterbean called as guar, is a draught tolerant annual legume crop. In India, clusterbean is grown for its green fodder and for the pods that are used as food and feed. Since it is a legume, it has soil enriching properties. The long deep tap root system enables the plant to grasp all the water available in the soil making it an ultimate drought resistant crop like other legumes and thus offer better scope for rainfed cropping and adds to the fertility of soil by fixing considerable amount of atmospheric nitrogen. Hence, Clusterbean is an excellent soil building crop with respect to available N.

Phosphorus content of soils is either low or medium. This is alarming because phosphorus is the backbone of balanced fertilizer use and it occupies a key place in intensive agriculture. The supply of phosphorus to legumes is more important than that of nitrogen because, latter is being fixed by
symbiosis with rhizobium bacteria. Indian soils are poor to medium in available phosphorus. Only about 30% of the applied phosphorus is available to crops and remaining part converted into insoluble phosphorus. The beneficial effects of phosphorus on nodulation, growth and yield of legume crops have been well established because apart from important role in root development, phosphorus is necessary for growth of rhizobium bacterial, responsible for nitrogen fixation in nodules. Phosphorus application to legumes not only benefits the current crop but also favourably affects the succeeding non-legume crop. Sulphur is an important essential plant nutrient whose deficiency was identified in soils of semi-arid regions, especially in Jodhpur, Udaipur and Jaipur districts of Rajasthan (Tandon, 1986). Importance of sulphur in Indian agriculture is being increasingly emphasized and has a great impact on legume production. Sulphur also promotes nodulation in legumes and favours solubilization of organic nitrogen and there is decrease in the quantity of insoluble nitrogen. Although not a constituent, sulphur helps in chlorophyll formation. Sulphur application increases drought and cold tolerance in plant due to the process of disulphide linkage. It also helps in control of diseases and pests. Gypsum has been found as an equally effective and cheapest source of sulphur for most of the crops, as in India huge deposits of gypsum are available, especially in Rajasthan.

Materials and Methods

The present study was conducted at Agronomy Farm and the plant and soil samples were analysed in Department of Soil Science and Agricultural Chemistry, S.K.N. College of Agriculture, Jobner (Rajasthan) during the kharif season, 2017. The average rainfall of this region is about 400 to 500 mm. The mean daily maximum and minimum temperatures during the growing crop season of clusterbean varied between 29.4 to 36.6 and 13.8 to 26.6 respectively. Similarly, mean daily relative humidity reached between 37 to 79%. The soil of experimental site (before kharif 2017) was loamy sand in texture with soil pH 8.2. Five plants were randomly selected from each plot of every replication. Total number of nodules per plant was recorded at flowering on the basis of another five randomly selected plants from each net plot and uprooted carefully, the soil mass embodying the roots of the plant was washed off by water and total nodules were counted. The mean value was recorded as total number of nodules per plant. Number of effective nodules was counted from same plants as taken for total number of nodules. Healthy pink coloured nodules were counted and mean value was recorded as effective number of nodules per plant.

The chlorophyll content in leaves at 45 DAS was determined as per the method advocated by Arnon (1949) by taking 50 mg fresh leaves; samples were homogenized in 80% acetone and aliquat was centrifuged for 10 minutes at 2000 rpm and the final volume was made to 10 ml. Absorbance of clear supernatant liquid was measured at 652 nm on spectronic-20.

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\text{Total chlorophyll A (652) x } 29 \times \text{Total volume (ml)} = \frac{\text{A (652) x Total volume (ml)}}{a \times 1000 \times \text{weight of sample}}
\]

Where, \(a\) is the path length = 1 cm.

Optimum dose of phosphorus and sulphur for yield of clusterbean under different phosphorus and sulphur levels will be worked out with the help of quadratic equation Croxton et al., (1973). To assess the fertility status of soil, the soil sample (0-15cm depth), from each plot at harvest of crop was taken. The samples were dried and passed through 2.0 mm plastic sieve to avoid metallic contamination for subsequent analysis and the samples were analysed as per standard methods.
Results and Discussion

Effect of phosphorus and sulphur on total chlorophyll content

A perusal of data presented in table-1 revealed that increasing levels of phosphorus upto 40 kg P$_2$O$_5$ ha$^{-1}$ significantly enhanced the total chlorophyll content at 45 DAS. Application of 40 kg P$_2$O$_5$ ha$^{-1}$ brought significant increase in total chlorophyll content at 45 DAS over control and 20 kg P$_2$O$_5$ ha$^{-1}$ by 13.86 and 6.48 percent, respectively. However, the application of 40 kg P$_2$O$_5$ ha$^{-1}$ and 60 kg P$_2$O$_5$ ha$^{-1}$ remained statistically at par with each other. Application of sulphur @ 40 kg ha$^{-1}$ significantly influenced the total chlorophyll content at 45 DAS over control and 20 kg S by 14.52 and 6.52 percent, respectively but it remained statistically at par with 60 kg S ha$^{-1}$.

Phosphorus is an essential constituent of all living organisms which play an important role in conservation and transfer of energy in metabolic reactions of living cells including biological energy transformations. The increase in photosynthetic activity in plant led to overall development in terms of growth. Thus, phosphorus fertilization enhanced the photosynthesis and other metabolic processes in the plant which ultimately enhanced the growth in terms of plant height, number of branches per plant and total chlorophyll content. These results are in close conformity with those of Ayub et al., (2012) and Raiger et al., (2017).

Sulphur being a constituent of succinyl Co-enzymes A which involved in chlorophyll formation and creates a balanced nutritional environment in the plant system in keeping the micronutrients physiologically active, has been very instrumental in increased chlorophyll synthesis in plant tissues. sulphur is one of the most important constituent of glutathione (glutamyl-cysteinyl-glycine) which though not a primary product of cellular metabolism, plays important role in detoxification of compounds, which are not favourable for growth. These findings are in close conformity with those of Kumar and Kumar (2013) and Raiger et al., (2017). The increase in plant height may be due to the beneficial effect of sulphur on the various metabolic activities and also play an important role in synthesis of sulphur containing amino acids, co-enzyme and increase in chlorophyll content and vitamins etc. in growing region and improving the photosynthetic activity ultimately enhancing cell division and there by resulting higher crop growth rate (Imsande, 1998).

Effect of phosphorus and sulphur on nodulation

Total nodules

Data given in table-1 revealed that the number of total nodules per plant at flowering was influenced significantly with increasing levels of phosphorus up to 40 kg P$_2$O$_5$ ha$^{-1}$, which was found at par with 60 kg P$_2$O$_5$ ha$^{-1}$. Application of phosphorus @ 40 kg ha$^{-1}$ significantly increased the number of total nodules per plant by 22.30 and 13.31 percent over control and 20 kg P$_2$O$_5$ ha$^{-1}$, respectively. Application of sulphur @ 40 kg ha$^{-1}$ significantly increased the number of total nodules per plant by 22.30 and 13.31 percent over control and 20 kg P$_2$O$_5$ ha$^{-1}$, respectively.

Effective nodules

Data further showed in table-1 that application of 40 kg P$_2$O$_5$ ha$^{-1}$ recorded significant increase in number of effective nodules per plant by 19.21 and 7.83 percent over control and 20 kg P$_2$O$_5$ ha$^{-1}$, respectively. Application of 40 kg S ha$^{-1}$ recorded significant increase in number of effective nodules.
nODULES PER PLANT BY 24.53 AND 12.30 PERCENT OVER CONTROL AND 20 KG S HA⁻¹, RESPECTIVELY WHICH WAS FOUND STATISTICALLY AT PAR WITH 60 KG S HA⁻¹.

**Table 1** Effect of phosphorus and sulphur on total chlorophyll content at 45 DAS, number of total and effective root nodules per plant at flowering of clusterbean

| Treatments | Total Chlorophyll content (mg/g) | Total nodules | Effective root nodules |
|------------|----------------------------------|---------------|------------------------|
| Phosphorus (P₂O₅) |                                  |               |                        |
| P₀        | 1.803                           | 18.02         | 13.74                  |
| P₂₀       | 1.928                           | 19.45         | 15.19                  |
| P₄₀       | 2.053                           | 22.04         | 16.38                  |
| P₆₀       | 2.132                           | 23.30         | 17.27                  |
| SEm⁺      | 0.043                           | 0.46          | 0.34                   |
| CD (P=0.05%) | 0.124                          | 1.32          | 0.98                   |
| Sulphur   |                                  |               |                        |
| S₀        | 1.797                           | 18.61         | 14.31                  |
| S₂₀       | 1.932                           | 20.27         | 14.87                  |
| S₄₀       | 2.058                           | 21.63         | 16.70                  |
| S₆₀       | 2.129                           | 22.30         | 17.60                  |
| SEm⁺      | 0.043                           | 0.46          | 0.34                   |
| CD (P=0.05%) | 0.124                          | 1.32          | 0.98                   |

**Table 2** Effect of phosphorus and sulphur on organic carbon and available N, P₂O₅, K₂O and S in soil after harvest

| Treatments | Organic carbon (%) | Available N (kg/ha) | Available P₂O₅ (kg/ha) | Available K₂O (kg/ha) | Available S (mg/kg) |
|------------|--------------------|---------------------|------------------------|-----------------------|---------------------|
| Phosphorus (P₂O₅) |                    |                     |                        |                       |                     |
| P₀        | 0.199              | 114.18              | 15.26                  | 136.12                | 8.29                |
| P₂₀       | 0.200              | 121.14              | 16.78                  | 138.58                | 9.41                |
| P₄₀       | 0.202              | 124.96              | 18.10                  | 141.05                | 10.28               |
| P₆₀       | 0.205              | 128.76              | 20.20                  | 141.35                | 10.92               |
| SEm⁺      | 0.002              | 1.27                | 0.33                   | 2.09                  | 0.13                |
| CD (P=0.05%) | NS                | 3.67                | 0.96                   | NS                    | 0.38                |
| Sulphur   |                    |                     |                        |                       |                     |
| S₀        | 0.197              | 114.86              | 15.03                  | 135.21                | 7.39                |
| S₂₀       | 0.201              | 120.91              | 17.01                  | 138.33                | 9.70                |
| S₄₀       | 0.202              | 124.64              | 18.44                  | 141.72                | 10.41               |
| S₆₀       | 0.206              | 128.63              | 19.85                  | 141.84                | 11.40               |
| SEm⁺      | 0.002              | 1.27                | 0.33                   | 2.09                  | 0.13                |
| CD (P=0.05%) | NS                | 3.67                | 0.96                   | NS                    | 0.38                |

NS = Non significant
Table 3 Seed yield (Y) as a function of phosphorus fertilization \((Y = b_0 + b_1 X + b_2 X^2)\)

| S.No. | Study parameters                         | Phosphorus |
|-------|------------------------------------------|------------|
| 1     | Partial regression coefficients          |            |
| b_0   | 829.40                                   |            |
| b_1   | 10.97                                    |            |
| b_2   | -0.095                                   |            |
| 2     | Coefficients of multiple correlation (R) | 0.999      |
| 3     | Optimum level of phosphorus (kg/ha)      | 49.315     |
| 4     | Yield at optimum level (kg/ha)           | 1139.35    |
| 5     | Response at optimum level (kg/ha)        | 309.95     |

* Significant at 5% level of significance

Table 4 Seed yield (Y) as a function of sulphur fertilization \((Y = b_0 + b_1 X + b_2 X^2)\)

| S.No. | Study parameters                         | Sulphur    |
|-------|------------------------------------------|------------|
| 1     | Partial regression coefficients          |            |
| b_0   | 775.65                                   |            |
| b_1   | 15.3075                                  |            |
| b_2   | -0.14938                                 |            |
| 2     | Coefficients of multiple correlation (R) | 0.9955     |
| 3     | Optimum level of sulphur (kg/ha)         | 50.64077   |
| 4     | Yield at optimum level (kg/ha)           | 1167.76    |
| 5     | Response at optimum level (kg/ha)        | 392.11     |

* Significant at 5% level of significance

Fig. 1 Response of clusterbean to phosphorus fertilization

\[
Y = 829.40 + 10.97X - 0.095X^2
\]

Optimum Level= 49.325
The increase in number of nodules per plant might be attributed to the optimum supply of available phosphorus to plants resulting in better development of root nodules because rhizobium in an aerobic bacteria and leghaemoglobin provide oxygen to bacteriods. Leghaemoglobin content of nodules probably increased due to application of phosphorus and it consistently made the nodules effective. Phosphorus also being the constituent of nucleic acid and different forms of protein, might have stimulated cell division resulting in increased growth of plants. Similar, results was reported by Yadav (2011) and Raiger et al., (2017) in clusterbean.

**Effect of phosphorus and sulphur on soil properties**

The organic carbon and available potassium content of soil after harvest of crop was not affected significantly due to the application of different levels of phosphorus and sulphur (Table-2).

**Available nitrogen**

Data given in table-2 showed that significantly maximum available nitrogen (128.76 kg ha$^{-1}$) was recorded under the application of 60 kg P$_{2}$O$_5$ ha$^{-1}$ while minimum (114.18 kg ha$^{-1}$) was recorded under P$_0$. The available nitrogen content in soil under P$_{60}$ was significantly increased by 12.76, 6.29 and 3.04 percent over P$_0$, P$_{20}$ and P$_{40}$ respectively. The available nitrogen content (128.63 kg ha$^{-1}$) in soil after harvest under S$_{60}$ was significantly increased by 11.98, 6.38 and 3.20 percent over S$_0$, S$_{20}$ and S$_{40}$ respectively.

**Available phosphorus**

The highest available phosphorus (20.20 kg ha$^{-1}$) was recorded under P$_{60}$, which was increased by 32.37, 20.38 and 11.60 percent over P$_0$, P$_{20}$ and P$_{40}$ respectively. The significantly maximum available phosphorus (19.85 kg ha$^{-1}$) content in soil after harvest was recorded under S$_{60}$. Where as, minimum (15.03 kg ha$^{-1}$) was under S$_0$. The increase in available phosphorus due to application of level S$_{60}$ was 32.06, 16.69 and 7.64 percent over S$_0$, S$_{20}$ and S$_{40}$ respectively.

**Available sulphur**

It is evident from the data given in table-2 that available sulphur content of soil after harvest of crop increased significantly with increasing levels of phosphorus. The maximum sulphur content (10.92 mg kg$^{-1}$) was recorded with the level P$_{60}$, which was increased by 31.72, 16.04 and 6.22 percent, over P$_0$, P$_{20}$ and P$_{40}$ respectively. The application of the level S$_{60}$ enhanced the available sulphur by 54.26, 17.52 and 9.51 percent as compared to S$_0$, S$_{20}$
and S$_{40}$ respectively. Clusterbean is legume crop and phosphorus application increase activity of soil microorganisms, more efficiency of nitrogen fixation (Yadav et al., 2014). Phosphorus also increased root nodulation which might have promoted microbial activity and thereby higher mineralization. Further, the release of organic acids and hormones due to phosphorus bacterial activity might have helped in the availability of nutrients. Similar findings were also reported by Yadav (2011), Bhatt et al., (2013) and Raiger et al., (2017). Intensive cultivation without sulphur fertilization resulted in a decline of 30-60% of sulphur content of soil when no sulphur was applied. Application of sulphur has been reported to help in lowering the soil pH, which is the principle reason for greater availability and mobility of nutrients. Similar results were also reported by Raiger et al., (2017).

**Optimum dose of phosphorus**

To describe the relationship between yield of clusterbean (Y) and applied phosphorus, Since the main effect of P on yield of clusterbean was found significant (Table-3 and Fig-1), it was considered appropriate to establish a relationship describing the yield of clusterbean as a function of main effect of P fertilization. The relationship of the type $Y = b_0 + b_1P + b_2P^2$ describing yield as a function of P derived from the observed data was curvilinear and presented in table-3 and fig-1. The estimated optimum level of phosphorus recording the predicted yield of 1139.35 kg ha$^{-1}$ has been worked out to be 49.31 kg ha$^{-1}$.

**Optimum dose of sulphur**

To describe the relationship between yield of clusterbean (Y) and applied sulphur, Since the main effect of S on yield of clusterbean was found significant (Table-4 and Fig-2), it was considered appropriate to establish a relationship describing the yield of clusterbean as a function of main effect of S fertilization. The relationship of the type $Y = b_0 + b_1S + b_2S^2$ describing yield as a function of S derived from the observed data was curvilinear and presented in table-4 and Fig-2. The estimated optimum level of sulphur recording the predicted yield of 1167.76 kg ha$^{-1}$ have been worked out to be 50.64 kg ha$^{-1}$.

Based on the result of one-year experimentation, it may be concluded that total chlorophyll content at 45 DAS, total and effective root nodules per plant at flowering increased significantly with the application of 40 kg P$_2$O$_5$ ha$^{-1}$ and 40 kg S ha$^{-1}$ over lower levels. Available nitrogen, phosphorus and sulphur status in soil after harvest of clusterbean crop were recorded as significantly maximum with the application of phosphorus @ 60 kg ha$^{-1}$ and sulphur @ 60 kg ha$^{-1}$ over rest of the treatments. Where as, optimum dose of phosphorus and sulphur for clusterbean crop were computed as 49.31 kg P$_2$O$_5$ ha$^{-1}$ and 50.64 kg S ha$^{-1}$ respectively.

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