Influence of Foliar Application of Water Soluble Fertilizers on Nodule Count and Rhizosphere Microbial Population in Green gram (Vigna radiata L.)

M. Bhavya¹, C. J. Sridhara¹, M. S. Nandish²*, N. S. Mavarkar¹, Y. Suchitha² and B. S. Sumithra¹

¹Department of Agronomy, College of Agriculture, UAHS, Shivamogga, Karnataka, India
²Department of Agriculture Microbiology, College of Agriculture, UAHS, Shivamogga, Karnataka, India

*Corresponding author

A B S T R A C T

A field experiment was conducted during late Kharif 2018 on sandy loam soils at College of Agriculture, University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, to study the effect of foliar application of water soluble fertilizers on nodule count and microbial population in green gram (Vigna radiata L.). The experiment was laid out in a Randomized Complete Block Design comprising of 3 replications and 13 treatment combinations consisting of three water soluble fertilizers along with package of practice (viz., 2 % DAP, 1 % 19:19:19, 2 % 19:19:19, 1 % monopotassium phosphate, 2 % monopotassium phosphate, 1% 19:19:19 +1 % monopotassium phosphate). All water soluble fertilizers were given as foliar spray at two crop growth stages (30 and 45 DAS). Results showed that foliar application of monopotassium phosphate and 19:19:19 each @ 1 per cent at 30 and 45 DAS along with package of practice recorded significantly higher number of nodules (36.92) at 45 DAS and the same treatment recorded significantly higher bacterial population (49.24 cfu g⁻¹ of soil and 46.12 cfu g⁻¹ of soil at 10⁻⁵ and 10⁻⁶ dilution, respectively), fungi population (17.13 cfu g⁻¹ of soil and 14.97 cfu g⁻¹ of soil at 10⁻³ and 10⁻⁴ dilution, respectively) and actinomycetes population (10.97 cfu g⁻¹ of soil and 8.93 cfu g⁻¹ of soil at 10⁻² and 10⁻³ dilution, respectively) as compared to other treatments.

Keywords
Green gram, Water soluble fertilizers, cfu (colony forming units) and nodule count

Introduction

The green gram (Vigna radiata L.) is one of the thirteen food legume grown in India and third most important pulse crop after chickpea and pigeon pea. In India, it occupies 4.32 lakh hectare area with the production of 21.65 lakh tonnes with the productivity of 5.46 q ha⁻¹(Anon, 2017). The important green gram growing states are Rajasthan, Madhya Pradesh, Uttar Pradesh, Odisha, Maharashtra, Karnataka and Bihar. It is an excellent source of high quality protein with easy digestibility, consumed as whole grains, dal, sprouted and in variety of ways. Amongst the pulses, green gram ranks second in the nutritive value. It contains about 24 to 25 per cent protein, this being about two third of protein content of...
soybean, twice that of wheat and thrice that of rice. The protein is comparatively rich in lysine, which is deficient in cereal grains. Hence, a diet combining green gram and cereal grains forms a balanced amino acid diet.

Green gram, like other pulses are considered as subsidiary crops. It is often grown on marginal lands and is usually intercropped with other pulses or planted on bunds. As a crop of secondary importance in many of these systems, it receives little or no purchased high cost inputs and the crop is mainly grown in rainfed condition with poor management practices and also due to various physiological, biochemical as well as inherent factors associated with the crop. Apart from the genetic makeup, the physiological factor viz., slow rate of dry matter accumulation during pre-flowering phase, low partitioning efficiency of assimilates to grains, poor pod setting due to the flower abscission, onset of leaf senescence during the period of pod development and lack of nutrients during critical stage of crop growth, coupled with a number of disease and pest (Mahala et al., 2001) constitute the major constraints for the poor yield. Hence, the productivity of green gram in our country is far below the potential and provides substantial scope for improvement with the adoption of improved agronomic practices. One among them is the foliar application of organic and inorganic sources of nutrients for exploiting genetic potential of the crop is considered to be an efficient and economic method of supplementing the part of nutrient requirements at critical stages.

During the last few years, there is a steady trend to reduce the use of mineral fertilizers, especially soil applied nitrogen, phosphorus and potassium and supplement the mineral nutrition through non-conventional methods (Haytova, 2013). These facts create preconditions to increase the importance of foliar fertilization as an alternative to meet plant nutrient demand during the growing season. The interest on foliar fertilization has risen as a result of many advantages such as quick and efficient utilization of nutrients, elimination of losses through leaching, fixation and regulating the uptake of nutrients by plant (Manonmani and Srimathi, 2009). Foliar feeding is often the most effective and economical way to improve plant nutrient deficiency (Dixit and Elamathi, 2007). Among the methods of fertilizer application, foliar nutrition is recognized as an important method of fertilization, since foliar nutrients usually penetrate the leaf cuticle or stomata and enter the cells facilitating easy and rapid utilization of nutrients. Hence, it is the fastest way to boost up crop growth (Latha and Nadanassababady, 2003). Under rainfed condition when the availability of moisture becomes scarce the application of fertilizers as foliar spray resulted in efficient absorption and usage. Though foliar spray is not a substitute to soil application but it certainly act as a supplement to soil application. In almost all the pulses, the extent of flower drop determine the yield and yield attributing characters. Retention of flowers by the plant gives higher yield more than expected yield. According to several studies conducted in different crops by different scientists over the world revealed that, retention of flowers is possible through foliar application of growth regulators as well as macro nutrients during flower initiation and pod development stages along with soil application of micro nutrients (Chaurasia et al., 2005).

It is also known that active nodulation of pulse crop stops after 45 to 50 days after sowing and at that time, the positive effect of supplying pulse with supplementary nitrogen was found to have beneficial effects on enhancing growth and increasing seed yield.
by quick supply of nitrogen. Based on the past work done by different researchers and in view of greater need for using foliar application of water soluble fertilizers in pulse production. The present investigation was conducted with an aim to study the influence of foliar application of water soluble fertilizers on nodule count and microbial population in green gram in late Kharif.

Materials and Methods

A field experiment was conducted during late Kharif-2018 at College of Agriculture, UAHS, Shivamogga. The experimental site was situated at 13° 58’ to 14° 1’ North latitude and 75° 34’ to 75° 42’ East longitude with an altitude of 650 m above the mean sea level comes under Southern Transition Zone of Karnataka. The experiment was laid out in a Randomized Complete Block Design (RCBD) comprising 3 replication and 13 treatments viz., T1: Farmers practice (50 kg DAP acre−1 as basal application), T2: Package of practice (RDF of 13:25:25 kg N, P2O5 and K2O ha−1 + 7.5 t FYM ha−1 + 10 kg ZnSO4 ha−1), T3: T2 + DAP spray @ 2 % at 30 and 45 DAS, T4: T2 + 19:19:19 @ 1 % at 30 DAS, T5: T2 + 19:19:19 @ 1 % at 30 and 45 DAS, T6: T2 + 19:19:19 @ 2 % at 30 DAS, T7: T2 + 19:19:19 @ 2 % at 30 and 45 DAS, T8: T2 + monopotassium phosphate @ 1 % at 30 DAS, T9: T2 + monopotassium phosphate @ 1 % at 30 and 45 DAS, T10: T2 + monopotassium phosphate @ 2 % at 30 and 45 DAS, T11: T2 + monopotassium phosphate @ 2 % at 30 DAS and 19:19:19 each @ 1 % at 30 and 45 DAS, T12: T2 + monopotassium phosphate and 19:19:19 each @ 1 % at 30 and 45 DAS. Seeds were dibbled at 5 cm depth with a spacing of 30 cm x 10 cm. Irrespective of treatments, basal dose of fertilizer 13:25:25N: P2O5: K2O kg ha−1 in the form of urea, superphosphate and muriate of potash were supplied to all plots. Foliar application of required quantity of NPK (19:19:19) and monopotassium phosphate (MPP) and DAP were given in two sprays at 30 and 45 DAS (Plate 1). The analysis of variance was carried out using the Randomized Complete Block Design (Gomez and Gomez, 1984).

Number of nodules per plant

The number of nodules per plant at 30 and 45 DAS was recorded by carefully uprooting five randomly selected plants in each plot and the average of five plants was expressed as a number of nodules per plant. The selected plants were saturated with water on the previous evening of observation. Next morning, selected five plants were carefully lifted along with the adhering soil mass. Enough care was taken to keep the root system intact so that none of the nodules were lost. All the nodules were collected and counted after carefully washing-off the soil. The collected nodules where scrutinized to note their effectiveness. Nodules were crushed to see the presence of pink colour which indicates the presence of leghaemoglobin.

Enumeration of rhizosphere microorganism in soil

The microbial population in the soil after harvest was determined by standard dilution plate count method. Soil samples from different treatments were collected separately from each replication and then they were pooled. Ten grams of pooled soil (treatment wise) weighed and mixed in 90 ml sterilized water blank to give 10−1 dilutions. Subsequent dilutions up to 10−6 were made by transferring serially 1 ml of each dilution to 9 ml sterilized water blanks. The population of bacteria, fungi and actinomycetes were estimated by serial dilution plate count technique and by taking 1ml from selected dilution of 10−2, 10−3,
10^{-4}, 10^{-5}, 10^{-6} were transferred aseptically to petridishes and the desired melted agar media were added to their respective dilutions. Plating on appropriate media viz., Nutrient agar, Martin’s Rose Bengal agar and Kuster’s agar media, respectively. The inoculated plates were kept for incubation at 30 °C ± 10 °C for a week time and emerged colonies were counted Cochran (1950) and expressed in colony forming units per gram of soil (cfu×g\(^{-1}\)of soil).

**Results and Discussion**

**Effect of foliar application of water soluble fertilizers on nodule count in green gram**

The data on number of nodules plant\(^{-1}\) at 30 DAS did not indicate significant variation for foliar application of water soluble fertilizers (Table 1). Number of nodules plant\(^{-1}\) in the study varied from 12.98 to 13.76. However, numerically highest number of nodules plant\(^{-1}\) (13.76) was recorded with T\(_2\)+monopotassium phosphate and 19:19:19 each @ 1 per cent at 30 and 45 DAS and the lowest number of nodules plant\(^{-1}\) (12.98) was observed in the treatment applied with only 50 kg DAP acre\(^{-1}\) as basal dose (Farmers practice).

Number of nodules plant\(^{-1}\) showed significant variation at 45 DAS due to treatments (Table 1). Significantly higher number of nodules plant\(^{-1}\) (36.92) was noticed in the treatment received with T\(_2\)+monopotassium phosphate and 19:19:19 each @ 1 per cent at 30 and 45 DAS, which was on par with T\(_2\)+monopotassium phosphate and 19:19:19 each @ 1 per cent at 30 DAS (36.45) compared to package of practice (26.58). However, significantly lowest number of nodules plant\(^{-1}\) (24.24) was recorded with the farmers practice. It might be due to the fact that pulses often experience nitrogen deficiency at later stage, because nitrogen fixation usually declines at reproductive stage and this is preceded by a decrease in fixation rate per unit weight of root nodules which probably results from bacteriod decay in the oldest nodules or in other words, gradual degeneration of root nodules. The results were in agreement with the finding of Nithukumari et al., (2018), Yadav and Choudhary (2012) and Gupta et al., (2011). This might be also due to the application of additional nitrogen and phosphorus which influence better root development of the plant and it might leads to profuse nodulation on account of increase in the rhizobial activity in the rhizosphere under the influence of foliar nutrition of 1 per cent monopotassium phosphate and 19:19:19 at 30 and 45 DAS (Fig.1) which in turn resulted in the formation of active and more number of nodules. These results are in accordance with the findings of Kachlam (2017), Hiwale (2015).

**Effect of foliar application of water soluble fertilizers on microbial population on green gram**

In order to know the influence of foliar application of water soluble fertilizers on the microbial population of the experimental soil, both initial and final population was assessed by serial dilution pour plate method. The perusal of Table 2 reveals the initial bacterial population of 19.23 cfu g\(^{-1}\) of soil and 19.01 cfu g\(^{-1}\) of soil at 10\(^{-5}\) and 10\(^{-6}\) dilution, respectively and fungi population of 10.50 cfu g\(^{-1}\) of soil and 10.13 cfu g\(^{-1}\) of soil at 10\(^{-3}\) and 10\(^{-4}\) dilution, respectively whereas the actinomycetes population of 4.61 cfu g\(^{-1}\) of soil and 3.31 cfu g\(^{-1}\) of soil at 10\(^{-2}\) and 10\(^{-3}\) dilution, respectively was observed. The study revealed that microbial population varied significantly due to foliar application of water soluble fertilizers as compared to initial microbial population of the experimental soil (Table 3).
Table 1: Number of nodules plant$^{-1}$ as influenced by foliar application of water soluble fertilizers at different growth stages of green gram

| Treatments | 30 DAS | 45 DAS |
|------------|--------|--------|
| T1 Farmers practice | 12.98 | 24.24 |
| T2 POP | 13.60 | 26.58 |
| T3 T2 + DAP spray @ 2 % at 30 and 45 DAS | 13.82 | 29.48 |
| T4 T2 + 19:19:19 @ 1 % at 30 DAS | 13.74 | 30.15 |
| T5 T2 + 19:19:19 @ 1 % at 30 and 45 DAS | 13.78 | 30.47 |
| T6 T2 + 19:19:19 @ 2 % at 30 DAS | 13.64 | 30.95 |
| T7 T2 + 19:19:19 @ 2 % at 30 and 45 DAS | 13.72 | 31.46 |
| T8 T2 + MPP @ 1 % at 30 DAS | 13.84 | 33.71 |
| T9 T2 + MPP @ 1 % at 30 and 45 DAS | 13.68 | 34.01 |
| T10 T2 + MPP @ 2 % at 30 DAS | 13.79 | 34.78 |
| T11 T2 + MPP @ 2 % at 30 and 45 DAS | 13.81 | 35.31 |
| T12 T2 + MPP and 19:19:19 each @ 1% at 30 DAS | 13.72 | 36.45 |
| T13 T2 + MPP and 19:19:19 each @ 1% at 30 and 45 DAS | 13.76 | 36.92 |
| S.Em.± | 0.23 | 0.49 |
| CD (P=0.05) | NS | 1.42 |

Note: Farmers practice - 50 kg DAP acre$^{-1}$ as basal application, POP (Package of practice) - 13:25:25 kg N, P$_2$O$_5$, K$_2$O ha$^{-1}$ + 7.5 t FYM ha$^{-1}$ + 10 kg ZnSO$_4$ ha$^{-1}$, DAP - Di ammonium phosphate, MPP- Mono potassium phosphate, DAS - Days after sowing

Table 2: Initial microbial populations of the experimental soil

| Sl. No. | Sample | Bacterial population | Fungi population | Actinomycetes population |
|---------|--------|----------------------|------------------|-------------------------|
|         |        | $cfu \times 10^5$ g soil$^{-1}$ | $cfu \times 10^6$ g soil$^{-1}$ | $cfu \times 10^5$ g soil$^{-1}$ | $cfu \times 10^4$ g soil$^{-1}$ | $cfu \times 10^2$ g soil$^{-1}$ | $cfu \times 10^3$ g soil$^{-1}$ |
| 1.      | Experimental soil | 19.23 | 19.01 | 10.50 | 10.13 | 4.61 | 3.31 |
Table.3 Influence of foliar application of water soluble fertilizers on microbial population of green gram after harvest

| Treatment details                        | Microbial populations of the experimental soil after harvest | Bacterial population | Fungi population | Actinomycetes population |
|------------------------------------------|-------------------------------------------------------------|----------------------|------------------|--------------------------|
|                                          |                                                             | $\text{cfu} \times 10^{-5}$ | $\text{cfu} \times 10^{-6}$ | $\text{cfu} \times 10^{-3}$ | $\text{cfu} \times 10^{-4}$ | $\text{cfu} \times 10^{-2}$ | $\text{cfu} \times 10^{-3}$ |
| T₁ Farmers practice                      |                                                             | 23.23                | 20.33             | 11.31                    | 8.41                      | 5.23                    | 2.33                    |
| T₂ POP                                   |                                                             | 25.41                | 22.41             | 11.46                    | 9.15                      | 4.89                    | 2.89                    |
| T₃ RDF + DAP spray@ 1.5 % at 30 and 45 DAS |                                                             | 29.34                | 26.24             | 12.78                    | 9.87                      | 6.36                    | 3.45                    |
| T₄ RDF + 19:19:19 @ 1 % at 30 DAS         |                                                             | 34.45                | 31.33             | 12.80                    | 10.15                     | 7.67                    | 4.69                    |
| T₅ RDF + 19:19:19 @ 1 % at 30 and 45 DAS  |                                                             | 34.65                | 31.67             | 13.08                    | 10.63                     | 7.42                    | 4.97                    |
| T₆ RDF + 19:19:19 @ 2 % at 30 DAS         |                                                             | 35.63                | 32.54             | 13.13                    | 10.97                     | 7.94                    | 5.78                    |
| T₇ RDF + 19:19:19 @ 2 % at 30 and 45 DAS  |                                                             | 37.79                | 34.67             | 13.68                    | 11.54                     | 8.29                    | 6.15                    |
| T₈ RDF + MPP @ 1 % at 30 DAS              |                                                             | 38.77                | 35.67             | 14.41                    | 11.87                     | 9.51                    | 6.64                    |
| T₉ RDF + MPP @ 1 % at 30 an               |                                                             | 41.00                | 37.83             | 15.05                    | 12.65                     | 9.37                    | 6.97                    |
| T₁₀ RDF + MPP @ 2 % at 30 DAS             |                                                             | 41.78                | 38.66             | 15.53                    | 13.08                     | 9.98                    | 7.53                    |
| T₁₁ RDF + MPP @ 2 % at 30 a               |                                                             | 43.31                | 40.33             | 16.15                    | 13.97                     | 10.12                   | 7.94                    |
| T₁₂ RDF + MPP and 19:19:19 ea             |                                                             | 48.34                | 45.00             | 16.89                    | 14.78                     | 10.78                   | 8.67                    |
| T₁₃ RDF + MPP and 19:19:19 each @ 1% at 30 and 45 DAS | | 49.24                | 46.12             | 17.13                    | 14.97                     | 10.97                   | 8.93                    |
| S. Em.±                                  |                                                             | 0.61                 | 0.60              | 0.49                     | 0.26                      | 0.14                    | 0.22                    |
| CD (P= 0.05 )                            |                                                             | 1.79                 | 1.75              | 1.43                     | 0.77                      | 0.42                    | 0.66                    |

Note: Farmers practice - 50 kg DAP acre$^{-1}$ as basal application, POP (Package of practice) - 13:25:25 kg N, P₂O₅, K₂O ha$^{-1}$ + 7.5 t FYM ha$^{-1}$ + 10 kg ZnSO₄ ha$^{-1}$, DAP - Di ammonium phosphate, MPP- Mono potassium phosphate, DAS - Days after sowing.
**Fig. 1** Influence of foliar application of water soluble fertilizers on number of nodules plant\(^{-1}\) at different growth stages of green gram.

| Treatments                | Number of nodules plant\(^{-1}\) |
|---------------------------|----------------------------------|
| T1 Farmers practice       | 12.98                            |
| T2 Package of practice    | 13.6                             |
| T3 T2 + DAP spray @ 2 % at 30 and 45 DAS | 13.82                        |
| T4 T2 + 19:19:19 @ 1 % at 30 DAS | 13.74                        |
| T5 T2 + 19:19:19 @ 1 % at 30 and 45 DAS | 13.78                        |
| T6 T2 + 19:19:19 @ 2 % at 30 DAS | 13.64                        |
| T7 T2 + 19:19:19 @ 2 % at 30 and 45 DAS | 13.72                        |
| T8 T2 + MPP @ 1 % at 30 DAS | 30.15                            |
| T9 T2 + MPP @ 1 % at 30 DAS | 30.47                            |
| T10 T2 + MPP @ 2 % at 30 DAS | 30.95                            |
| T11 T2 + MPP @ 2 % at 30 and 45 DAS | 31.46                        |
| T12 T2 + MPP and 19:19:19 each @ 1% at 30 DAS | 34.01                        |
| T13 T2 + MPP and 19:19:19 each @ 1% at 30 and 45 | 34.78                        |
**Fig. 2** Influence of foliar application of water soluble fertilizers on microbial population in green gram at harvest

![Graph showing microbial population](image)

| Treatment | Description |
|-----------|-------------|
| T<sub>1</sub> | Farmers practice |
| T<sub>2</sub> | Package of practice |
| T<sub>3</sub> | T<sub>2</sub> + DAP spray @ 2% at 30 and 45 DAS |
| T<sub>4</sub> | T<sub>2</sub> + 19:19:19 @ 1% at 30 DAS |
| T<sub>5</sub> | T<sub>2</sub> + 19:19:19 @ 1% at 30 and 45 DAS |
| T<sub>6</sub> | T<sub>2</sub> + 19:19:19 @ 2% at 30 DAS |
| T<sub>7</sub> | T<sub>2</sub> + 19:19:19 @ 2% at 30 and 45 DAS |
| T<sub>8</sub> | T<sub>2</sub> + MPP @ 1% at 30 DAS |
| T<sub>9</sub> | T<sub>2</sub> + MPP @ 1% at 30 and 45 DAS |
| T<sub>10</sub> | T<sub>2</sub> + MPP @ 2% at 30 DAS |
| T<sub>11</sub> | T<sub>2</sub> + MPP @ 2% at 30 and 45 DAS |
| T<sub>12</sub> | T<sub>2</sub> + MPP and 19:19:19 each @ 1% at 30 DAS |
| T<sub>13</sub> | T<sub>2</sub> + MPP and 19:19:19 each @ 1% at 30 and 45 DAS |

2390
Combined foliar application of mono potassium phosphate and 19:19:19 each at 1 per cent at 30 and 45 DAS recorded significantly higher bacterial population (49.24 cfug$^{-1}$ of soil and 46.12 cfu$^{-1}$ of soil at $10^{-5}$ and $10^{-6}$ dilution, respectively), fungi population (17.13 cfu$^{-1}$ of soil and 14.97 cfu$^{-1}$ of soil at $10^{-3}$ and $10^{-4}$ dilution, respectively) and actinomycetes population (10.97 cfu$^{-1}$ of soil and 8.93 cfu$^{-1}$ of soil at $10^{-2}$ and $10^{-3}$ dilution, respectively) (Fig. 2) as compared to other treatments. This increase in microbial population might be due to production of root exudates due to the luxuriant growth of crop as reflected by higher dry matter production resulted in higher microbial population (Hamayun and Chaudhary, 2014).

From the results it can be concluded that foliar application of monopotassium phosphate and 19:19:19 each at 1 per cent at 30 and 45 DAS along with package of practice in late Kharif found ideal for achieving higher nodule count and microbial population in green gram.

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