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Performance of soybean [*Glycine max* (L.) as influenced by different levels and method of nitrogen application under rainfed conditions.

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ABSTRACT
A field experiment was conducted during kharif season of 2014-15 in vertisols at research farm of R.A.K. College of Agriculture, Sehore (M.P.) to study the effect of different levels of nitrogen application on root nodulation, yield and nitrogen uptake by plant as influenced by method of application soybean. The experiment was laid out in Randomized Block Design with three replications having nine treatments. Observations were recorded for plant height, root length, number and dry weight of root nodules. All attributes were significantly influenced with the different treatments. Yield attributing characters i.e. number of pods per plant, grain yield per plant was significantly influenced with the different treatments, Highest number of pods and significantly higher grain yield per plant were produced by treatment 20 kg N/ha as basal + 20 kg N/ha as top dressed. Highest N-content (6.82%) and protein content in seed with application of 20 kg N/ha as basal + 20 kg N/ha as top dressing. application of 40 Kg N/ha as basal recorded highest N-content in straw it was significantly superior then all other treatments. Application of 20 kg N/ha as basal + 20 kg N/ha as top dressing recorded highest N-uptake and total N-uptake (seed + straw in soybean seed and application of 40 kg N/ha as basal recorded the highest N-uptake in soybean straw. The highest gain of available nitrogen was recorded by treatment foliar application of 2% urea.
Key words*: Nitrogen levels, Nodulation, Nitrogen uptake, Rainfed condition.*

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INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is the major Kharif crop of Madhya Pradesh or particularly in the central part of the country. Soybean crop at the beginning of pod formation requires more water and nutrients. At this stage, nitrogen demand of plants is more; therefore, to meet out the requirement, the nitrogen shall be supplied as top dressing or foliar application. As nodulation remain effective from 20 days to 60 days and there after senescence of nodules take place at the post flowering conditions. Hence, due to nodule degeneration, nitrogen requirement of crop at the later stages may be affected which may result in the productivity loss, Therefore, the application of nitrogen as top dressing or foliar application would be beneficial for obtaining optimum yield of soybean (Takahashi *et al.* 1992). Fertilizer-nitrogen application to soybean is based on two precepts of potential soil–nitrogen needs during soybean development. Periods in soybean development when soil- nitrogen is crucial are (i) during seedling development prior to nodule formation (Harper, 1974) and (ii) during periods of peak nitrogen demand such as pod fill (Diebert *et al*.1979). Starter- nitrogen application is directed at providing soybean with readily available soil nitrogen during seedling development and has been shown to increase soybean grain yields (Touchton and Rickerl, 1986). The period of high nitrogen requirement for soybean is during R_3 to R_6 and is characterized by peak nitrogen fixation. Harper (1974) reported both soil nitrogen and fixed nitrogen were needed for maximum soybean yield and that soybean plants at full bloom appear capable of responding to fertilizer nitrogen. Nitrogen additions during R_3 to R_6 have been shown to benefit soybean growth (Brevedan *et al*. 1987).

MATERIAL AND METHODS

The experiment was laid out in R.A.K. College of Agriculture, Sehore, R.V.S.K.V.V., Gwalior (Madhya Pradesh) under All India Coordinated Research Project on Soybean during *Kharif* 2014-15. The soil was medium black cotton soil (Vertisols) with (*p*H 7.78) and electric conductivity 0.32 dS/m, low in available N (220.4 kg/ha), and medium in available P (12.60 kg/ ha), available K (232 kg/ha) and available S (19.85 kg/ha).The experiment was laid out in Randomized Block Design with three replications having nine treatments. T_1 [control]; T_2 [20 kg N/ha as basal]; T_3 [30 kg N/ha as basal]; T_4 [40 kg N/ha as basal); T_5 [20 kg N/ha as top dressing at (R_5)]; T_6 [Foliar spray of 2% urea at (R_5)] ; T_7 [10 kg N/ha as basal + 10 kg N/ha as top dressing at (R_5)]; T_8 [20 kg N /ha basal application + 10 kg N/ha top dressing at (R_5)]; T_9 [20 kg N/ha basal application + 20 kg N/ha top dressing at (R_5)].The crop variety JS-95-60 was sown in the month of July (18 July 2014). In experimental plots, furrows were opened with the help of desi plough at the distance of 45 cm. Calculated quantity of fertilizers was applied plot wise as per treatments. Fertilizer doses were calculated on the basis of recommended dose of nutrients 20 kg N + 60 kg P_2O_5 + 20 kg K_2O + 20 kg S/ha through urea, single super phosphate and muriate of potash, respectively. In present experiment the nitrogen at different levels was applied in the form of urea in different methods like basal, top dressing and foliar application of nitrogen. Crop was raised following the recommended package of practices. Representative samples of soil, seed and straw were analyzed for nitrogen content in the plant and seed samples was determined by the micro kjeldhal method after digestion as suggested by Chapman and Prutt (1961). This seed nitrogen per cent was multiplied by the factor of 5.71 to get the percentage of protein in soybean seed. Observations were recorded on growth and yield attributes namely, dry weight per plant, crop growth rate, relative growth rate, pods per plant and 100 seed weight.
R_5 = Seed initiation stage

Soil analysis

Estimation of available nitrogen

Indigestion tube of automatic Kjeldhal instrument 1 g of soil was taken. To this 20 ml of distilled water was added and subsequently install the digestion tube in the instrument and then select the program no. 01 for estimation of available nitrogen and run the machine, Automatically 25 ml of boric acid was came in the beaker placed inside the instrument followed by 25 ml of 0.32% $KMnO_4$ and 25 ml of 40% NaOH. The distillation processes took 9 minutes for completion and automatically stopped after 9 minutes. The sample was removed and titrated against 0.02 N H_2SO_4 until original pinkish shade appeared. Blank reading (without soil) was also taken for the final calculation.

Plant Analysis

Determination of total nitrogen in straw and grain

Nitrogen in plant samples was determined by the kjeldhal method.

Extraction

0.5 gram straw and 0.5 gm of oven dried grain samples were taken separately, add 3 g of digest mixture (copper sulphate and potassium sulphate in ratio 1:5) and 10 ml of concentrated H_2SO_4 added into digestion tube of digestion assembly of Pelican make. The contents were digested initially at 150 ^{0}C for 2 hours and thereafter at high temperature (300-400 ^{0}C) till the content of digestion tube becomes clear solution of light green colour.

Distillation

10 ml of the digested material was taken and transferred in vacuum jacket of micro kjeldahl distillation tube. In conical flask, 10 ml of 4% boric acid solution containing bromocresol green and methyl red indicators was taken to which the condenser outlet from where ammonia comes. After adding the aliquot, the funnel of the apparatus was washed with 2-3 ml of deionized water and 10 ml of 40% NaOH solution was added. 100 ml aliquot was distilled to the flask containing 10 ml of boric acid. After distillation the distillate was titrated against 0.02 N H_2SO_4. Blank was also run.

Nitrogen content

Nitrogen content in the plant and seed samples was determined by the micro kjeldhal method after digestion as suggested by Chapman and Prutt (1961).

$$N\,\% = \frac{(\text{ml of acid used for sample} - \text{ml of acid used for blank}) \times 14 \times N \times 10^4 \quad x100}{\text{Weight of sample}}$$

Where, N = Normality of H_2SO_4

Calculation of Nitrogen uptake in seed and straw

Nitrogen uptake of soybean seed was computed from the following formula
Nitrogen uptake (kg/ha) = {Nitrogen content (%) × seed yield (kg/ha)}/100.

RESULTS AND DISCUSSION

Results obtained in present investigation entitled "effect of different levels of nitrogen application on root nodulation, yield and nitrogen uptake by plant as influenced by method of application soybean" have been examined critically and discussed here. In soybean, growth characters like plant height, root length, dry weight and number of nodules are very important parameters, which directly influence the grain yield. Plant height is an important character of the vegetative phase and indirectly influences the yield components.

Plant height as a measure of crop growth was recorded at successive stages of crop growth *i.e.* 30, 45, 60, DAS and at maturity. The analyzed data is presented in table 1.

A linear increase in plant height was observed with the advancement in age in all the nitrogen treated plots. The rate of growth in height was faster in the beginning up to 60 DAS, thereafter, it was slowed down. The increase in the height was at the fastest rate between 30 to 60^{th} day stage. The height of the plant approached at maximum value on maturity.

The statistical analysis of data showed that the plant height at all stages was significantly affected by the treatments. At 60 DAS, treatment 40 kg N/ha as basal (T_4) was recorded maximum plant height, which was significantly at par with T_3 treatments and higher than rest of the treatments. Treatment 20 kg N/ha as top dressing (T_5) was recorded the shorter plant height than all other treatments except control treatment (T_1)

It is revealed that the effect of treatments on root length per plant was significant at all the stages (table 2). At 30 and 45 DAS, application of 40kg N/ha as basal was recorded highest root length (15.34 cm and 19.98 cm), which was significantly superior to all the remaining treatments.

Similarly at 60 DAS, treatment 40kg N/ha as basal was recorded highest root length (26.22 cm), which was significantly at par with T_3, T_9 and higher than all other treatments. The lowest root length (19.44cm) was recorded in control treatment.

At maturity, treatment 40Kg N/ha as basal application was recorded maximum root length (26.16 cm) which was significantly at par with T_3 treatment and higher than all other treatment and The lowest root length (20 cm) was recorded in control treatment.

Data indicated in table -3 that at30 DAS, application of 40kg N/ha as basal was recorded the maximum number of nodules (15.55), which were significantly higher than rest of the treatments. At 45 DAS, maximum number of nodules were recorded in treatment T_4 (40kg N/ha as basal), which were significantly at par with T_3 and T_9 treatments and significantly superior to all other treatments. Similarly, at 60 DAS maximum number of nodules were recorded in treatment T_4 (40kg N/n ha as basal), which were significantly at par with T_3, T_7 and T_9 treatments and significantly superior to all other treatments. The minimum number of root nodules was recorded in control treatment (T_1).

Similarly maximum nodules dry weight per plant recorded at 60 DAS. Maximum number of root nodules were recorded by the application of higher dose of nitrogen i.e. 40 kg N/ha as basal application (Table 4). At 60 DAS, nodules dry weight decrease due to N_2-fixation activity started to decrease after the R_5 stage, when the plants absorbed N most actively.

The yield of soybean per unit is influenced to a most important component of yield is governed by initiation and differentiation of buds into floral or vegetative growth and the number of pods are results of floral buds in each plant. Plant nutrient play an important role in bud initiation and differentiation, deficiency of nitrogen leads to reduction in bud differentiation and excessive nitrogen leads to more vegetative growth, while balance nutrition may also give rise to more flowers and finally more yield.

The result indicates that in table 5 effect of methods of nitrogen application was found significantly maximum grain yield per hectare was produced by 20 kg N/ha as basal+20kg N/ha as top dressing, which was higher than control treatment. It might be due to the growth characters influenced the plant to have good production of dry matter in early stages and that eventually raised and partitioned to the reproductive units.

As nodulation remain effective from 20 days to 60 days and there after senescence of nodules take place at the post flowering conditions. Hence, due to nodule degeneration, nitrogen requirement of crop at the later stages may be affected which may result in the productivity loss, Therefore, the application of nitrogen as top dressing or foliar application would be beneficial for obtaining optimum yield of soybean. The increase in grain yield of soybean due to nitrogen application may be because of the fact that nitrogen plays an important role in the synthesis of chlorophyll and amino- acids which are the indispensable ingredients of the process of autotrophization(Takahashi *et al.*1992). (Takahashi *et al.*1992).

Total uptake of nitrogen (kg/ha)

Application of 20 kg N/ha as basal + 20 kg N/ha as top dressing recorded highest N-uptake (143.23 kg/ha) and total N-uptake (seed + straw in soybean seed and application of 40 kg N/ha as basal (T_4)

recorded the highest N-uptake (40.72 kg/ha) in soybean straw. The highest gain of available nitrogen (174.27 kg/ha) was recorded by treatment foliar application of 2% urea (Table 4).

Nitrogen content

Nitrogen content (%) in seed

Data on N-content in seeds are presented in table 6. The data in table 16 showed significant effect of treatments on N-content in seed. Application of 20 kg N/ha as basal + 20 kg N/ha as top dressing recorded significantly highest N-content (6.82%), then all the treatments except T_8 treatment. The lowest N content in seed was recorded in control (5.12%)

Nitrogen content (%) in straw

The data of N-content (%) in straw influenced by the treatments and presented in table 6. Application 0f 40 Kg N/ha as basal recorded highest N-content (1.54%) in straw and was significantly superior then all the treatments except over T_3 treatment.

CONCLUSION

Investigation showed that root nodulation, grain yield and nitrogen uptake influenced by different levels and methods of nitrogen application in soybean nitrogen influenced the better root development of the plant and it might profuse nodulation on account of increase in the rhizobial activity in the rhizosphere under the influence of method of nitrogen application, which in turn resulted in the formation of active and more number of nodules /plant. Nitrogen influenced the grain yield through source-sink relationship resulting in higher production of photosynthates and their increased translocation to reproductive parts. Nitrogen content (%) in seed and straw and total uptake of nitrogen also influence by different treatment due to different methods and levels of nitrogen application. Treatment (T_9) 20 kg N/ha as basal +20 kg N/ha as top dressing gave highest yield (2112 kg/ha).

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Table- 1 Plant height (cm) influenced by different levels and methods of nitrogen application

Treatments	Plant height (cm)			
	30 DAS	45 DAS	60 DAS	Maturity
T_1 0 kg N/ha as basal	18.44	24.16	32.00	33.16
T_2 20 kg N/ha as basal	21.60	28.30	36.16	36.50
T_3 30 kg N/ha as basal	22.16	35.33	39.50	40.16
T_4 40 kg N/ha as basal	23.10	36.03	40.86	42.20
T_5 20 kg N/ha as top dressing	19.33	26.33	33.46	34.80
T_6 Foliar application of 2% Urea	18.90	25.33	34.83	37.50
T_7 10 kg N/ha as basal+ 10 kg N/ha as top dressing	20.66	26.5	35.46	37.13
T_8 20 kg N/ha as basal+10 kg N/ha as top dressing	21.76	28.33	36.16	38.16
T_9 20 kg N/ha as basal + 20 kg N/ha as top dressing	22.00	28.83	37.00	39.33
S.Em ±	0.40	0.98	0.90	0.83
C.D at 5%	1.19	2.93	2.71	2.47

Table- 2 Root length per plant (cm) influenced by different levels methods of nitrogen application

Treatments	Root length per plant (cm)			
	30 DAS	45 DAS	60 DAS	At maturity
T_1 0 kg N/ha as basal	11.27	16.60	19.44	20.00
T_2 20 kg N/ha as basal	12.32	19.39	20.85	23.11
T_3 30 kg N/ha as basal	14.35	19.87	25.88	25.11
T_4 40 kg N/ha as basal	15.34	19.98	26.22	26.16
T_5 20 kg N/ha as top dressing	11.85	16.63	23.27	23.00
T_6 Foliar application of 2% Urea	11.94	17.64	24.16	23.66
T_7 10 kg N/ha as basal+ 10 kg N/ha as top dressing	12.02	19.19	21.44	22.33
T_8 20 kg N/ha as basal+10 kg N/ha as top dressing	12.49	19.56	23.27	23.00
T_9 20 kg N/ha as basal + 20 kg N/ha as top dressing	13.63	19.71	24.77	24.00
S.Em ±	0.35	0.50	0.55	0.46
C.D at 5%	1.05	1.48	1.66	1.38

Table -3 Root nodules/plant (no) influenced by different levels methods of nitrogen application

Treatments	Root nodules/plant (no)		
	30 DAS	45 DAS	60 DAS
T_1 0 kg N/ha as basal	12.44	24.88	25.77
T_2 20 kg N/ha as basal	13.55	27.22	29.00
T_3 30 kg N/ha as basal	14.77	29.10	35.55
T_4 40 kg N/ha as basal	15.55	30.88	36.11
T_5 20 kg N/ha as top dressing	12.55	25.00	30.77
T_6 Foliar application of 2% Urea	12.77	25.44	31.32
T_7 10 kg N/ha as basal+ 10 kg N/ha as top dressing	13.21	25.88	33.77
T_8 20 kg N/ha as basal+10 kg N/ha as top dressing	14.44	28.44	31.22
T_9 20 kg N/ha as basal + 20 kg N/ha as top dressing	14.55	28.77	34.00
S.Em ±	0.37	0.76	0.86
C.D at 5%	1.09	2.26	2.56

Table -4 Dry weight of root nodules/plant (mg) influenced by different methods of nitrogen application

Treatments	Dry weight of root nodules/plant (mg)		
	30 DAS	45 DAS	60 DAS
T_1 0 kg N/ha as basal	58.66	147.33	123.87
T_2 20 kg N/ha as basal	81.00	170.55	143.66
T_3 30 kg N/ha as basal	91.10	185.66	155.66
T_4 40 kg N/ha as basal	94.66	193.21	155.77
T_5 20 kg N/ha as top dressing	59.32	147.99	138.66
T_6 Foliar application of 2% Urea	61.99	154.21	125.55
T_7 10 kg N/ha as basal+ 10 kg N/ha as top dressing	71.77	158.11	149.53
T_8 20 kg N/ha as basal+10 kg N/ha as top dressing	81.88	175.33	164.10
T_9 20 kg N/ha as basal + 20 kg N/ha as top dressing	83.22	182.88	170.99
S.Em ±	2.82	3.56	5.48
C.D at 5%	8.44	10.67	16.40

Table -5 Crop Yield parameter as influenced by different levels and methods of nitrogen application

Treatments	Grain yield (kg/ha)	Straw yield (kg/ha)	Biological yield (Kg/ha)
T_1 0 kg N/ha as basal	1706	2154	3860
T_2 20 kg N/ha as basal	1953	2238	4191
T_3 30 kg N/ha as basal	1989	2146	4135
T_4 40 kg N/ha as basal	2029	2649	4678
T_5 20 kg N/ha as top dressing	1984	2489	4473
T_6 Foliar application of 2% Urea	2004	2580	4584
T_7 10 kg N/ha as basal+ 10 kg N/ha as top dressing	2018	1969	3987
T_8 20 kg N/ha as basal+10 kg N/ha as top dressing	2071	2526	4597
T_9 20 kg N/ha as basal + 20 kg N/ha as top dressing	2112	2559	4671
S.Em ±	44.00	55.00	67.75
C.D at 5%	130.00	166.00	203.10

Table- 6: Nitrogen status in soil after harvest as influenced by different levels and methods of nitrogen application

S. No	Treatments	Initial N (kg/ha	N-added through fertilizer(kg/ha	Total N (a+b)=c	Crop removal N-balance (kg/ha)	Theoretical N-balance kg/ha	Actual N after harvest kg/ha	Loss/gain of available N(kg/ha
		A	B	C	D	e = (c-d)	F	g = (f-e)
1.	0 kg N/ha	220.40	0	220.40	109.03	111.37	226.40	115.03
2.	20 kg N/ha as basal	220.40	20	240.40	149.02	91.38	232.80	141.42
3.	30 kg N/ha as basal	220.40	30	250.40	137.06	113.34	228.40	115.06
4.	40 kg N/ha as basal	220.40	40	260.40	161.20	99.2	236.40	137.20
5	20 kg N/ha as topdressing	220.40	20	240.40	150.02	90.98	242.60	151.62
6.	Foliar application of 2% urea	220.40	12	232.40	160.17	72.23	246.50	174.27
7.	10 kg N/ha as basal+ 10 kg as top dressing	220.40	20	240.40	148.73	91.67	231.80	140.13
8.	20 kg N/ha as basal+10 kg N/ha as top dressing	220.40	30	250.40	164.23	86.17	248.20	162.03
9.	20 kg N/ha as basal + 20 kg N/ha as top dressing	220.40	40	260.40	174.9	85.5	248.60	163.10

Table -7 Effects of methods of nitrogen application on nitrogen content (%) in seed, straw and total uptake

Treatments	N content (%) in seed	N content (%) in straw	N uptake in seed	N uptake in straw	Total uptake
T_1 0 kg N/ha as basal	5.12	1.01	87.28	21.75	109.03
T_2 20 kg N/ha as basal	6.18	1.27	120.69	28.33	149.02
T_3 30 kg N/ha as basal	5.38	1.41	106.92	30.14	137.06
T_4 40 kg N/ha as basal	5.94	1.54	120.48	40.72	161.20
T_5 20 kg N/ha as top dressing	6.16	1.12	122.23	27.79	150.02
T_6 Foliar application of 2% urea	6.42	1.22	128.62	31.55	160.17
T_7 10 kg N/ha as basal+ 10 kg as top dressing	6.22	1.18	125.51	23.22	148.73
T_8 20 kg N/ha as basal+10 kg N/ha as top dressing	6.58	1.09	136.73	27.50	164.23
T_9 20 kg N/ha as basal + 20 kg N/ha as top dressing	6.82	1.24	143.23	31.67	174.9
SEm±	0.13	0.05	3.05	1.38	3.31
CD at 5%	0.37	0.16	9.13	4.13	9.90

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