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RESEARCH ARTICLE

EFFECT OF DIFFERENT FERTILIZERS IN YIELD AND NODULATION OF COWPEA (*VIGNA UNGUICULATA*) UNDER MULCHED AND UN-MULCHED FIELD CONDITION IN CHITWAN DISTRICT, NEPAL

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ABSTRACT

To study the effects on nodulation and yield of Cowpea *(Vigna Unguiculata),* a research had been conducted in the sandy loam soil of the horticulture farm of Agriculture and Forestry University in 2018. The experiment was laid out in factorial 2×5 Randomized complete block design with ten treatments and 3 replications which consists; 1st factor - mulched and un-mulched condition, 2nd factor - Rhizobium inoculation (R) at 1gm/ 16.66 gm seed, Phosphorus (P) at recommended dose of 120kg/ha ,use of R plus P(RP),RP plus Molybdenum (RPMo) at 2 gm Molybdenum per kg seed and control with no such application. The analysis of the findings showed that mulched and RPMo treatment were found significantly superior than other treatments in case of main effects of yield attributes like plant height, canopy diameter and stem number. The highest fresh weight of pods per plant i.e. 312.61 gm and 312.53 gm and no of nodules per plant i.e. 121.63 and 129.33 were found in mulched and RPMo treatment, significant at $p<0.001$ and $p<0.05$ respectively. The rise in number of nodules had higher significant positive correlation ($p<0.01$) with yield and yield attributes. There were no any significant interaction effects between the two factors.

KEYWORDS

Vigna Unguiculata, fresh weight, correlation, treatments.

1. INTRODUCTION

Legumes are the important food crops in relation to nutrition. Grain legumes occupy 10.22% of the cultivated area which is equal to 0.316 million ha with 0.27 mt production and productivity of 0.85 t/ha [1]. Per capita consumption of grain legumes in Nepal is very low which is around 10 kg/annum or 27g/capita/day [2] and is three times less than minimum requirement prescribed by World health Organization [3]. Cowpea (Vignaunguiculata (L.) Walp.) is an important legume vegetable crop of Nepal.It is warm season crop grown in many parts of the country with humid as well as sub-tropical climate. It has numerous uses like vegetable, pulse, green manuring and fodder crop [4].

Adequate quantity of nutrients is very critical for proper growth and yield potential of cowpea. Nitrogen, which is a major constituent of protein and chlorophyll, must be adequately supplied to the crop in time. In its roots, there are numerous nodules containing Rhizobium bacteria which form symbiotic association with the plants. Rhizobium creates symbiotic mutualism with the legumes. The bacteria differentiate morphologically into bacteroids inside the nodules and using enzyme nitrogenase, they fix atmospheric nitrogen into ammonium. Ammonium is then converted into amino acids like glutamine and asparagine which is exported to the plant. In exchange, the plant supplies the bacteria with carbohydrates in the form of organic acids. Though pulses are naturally capable of fixing atmospheric nitrogen, they need a small basal dose of nitrogenous fertilizer for quick and better start.

Nitrogenous fertilizer is very critical to the crop as it is the major component of amino acid as well as protein. Biological nitrogen fixation (BNF) technology can be used in the form of Rhizobium inoculants in grain legumes as an alternative of nitrogenous fertilizer. Leguminous crops meet up their N requirement through BNF depending on proper growth, development and also leghemoglobin content of the root nodules [5]. Next to nitrogen, phosphorus is regarded as the important plant nutrient, since it is needed by the leguminous crop for rapid and healthy root development, which becomes helpful in greater nodulation by Rhizobium bacteria. Phosphorus is vital for the production of protein, phospholipids and phytin in the legumes [6]. Similarly, the application of Mo also plays the key role in the yield of cowpea because it is directly involved in nitrogen fixing enzymes nitrogenase and N reduction enzyme, nitrate reductase especially for legumes forming root nodules. Sharma et al. (1988) observed that molybdenum was responsible for the formation of nodule tissue and increase in nitrogen fixation and without adequate quantities of molybdenum, nitrogen fixation could not occur, and microbial activity was depressed [7]. Similarly, mulching had positive effects on soil moisture conservation and improved cowpea yield [8].

Various literatures had been taken into consideration to examine the effect of rhizobium, Phosphorus and Molybdenum in the yield and yield parameters of cowpea crop [9-12]. They had reported that use of Rhizobium or Phosphorus or Molybdenum increases the yield and yield parameters in cowpea. In our country, there is limited research and study in this field. Along with this study, this research had objective to find the effect of different fertilizers like Rhizobium, Phosphorus and Molybdenum in the yield and nodulation of cowpea crop under both field condition i.e. under mulched and un-mulched condition.

2. MATERIALS AND METHODS

2.1 Experimental site

The experiment on effect of Rhizobium inoculation, phosphorus and molybdenum in yield, yield attributes and nodulation of cowpea was carried out from May 9 to July 9, 2018 at olericulture farm of Agriculture and Forestry University situated at Rampur, Chitwan, Nepal.

2.1.2 Climatic condition at experimental site

The data regarding maximum, minimum temperature and rainfall during the experiment period of about 3 months from May to July 2018 was recorded from weather station of National Maize Research Centre located at about just less than half kilometer far from the experimental site. The total rainfall of 380 mm was recorded during the cropping. The maximum temperature during the cropping period ranges from 27-37°C whereas the minimum temperature ranges from 19-28°C.

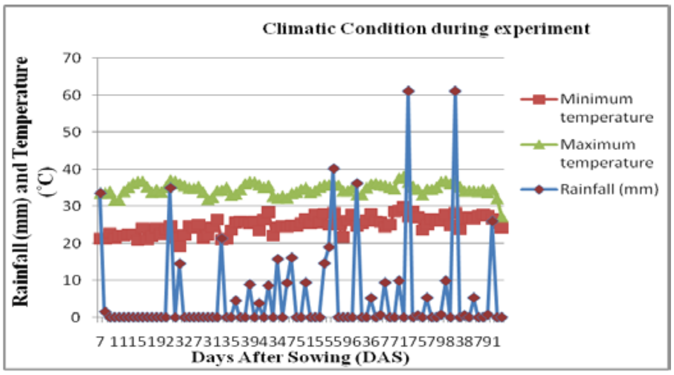

Figure 1 : Climatic condition of experimental site during the experimental period

2.1.3 Physical and chemical soil properties of experimental site

Soil from the experimental site was collected before conducting experiment from all replications and mixed well to make a perfect sample size for testing its physical and chemical soil properties (Table 1). It was tested on the soil testing lab of Regional Soil Testing Laboratory, Pokhara.

Table 1: Physical and chemical soil properties of the experimental site.

S.N	Properties	Average content	Category
	Physical properties		
1	Sand (%)	70.1	
2	Silt (%)	14.5	
3	Clay	7.2	
4	Textural class		Sandy Loam
	Chemical properties		
1.	Organic matter (%)	5.81	High
2.	Nitrogen (%)	0.2	Slightly higher
3.	Soil pH	5.8	Slightly acidic
4.	Average P_20_5 (kg/hac)	100	Medium
5.	Average K_20_5 (kg/hac)	246.5	

2.2 Experimental Design and Layout

The field experiment was carried out on factorial RCBD with two factors, three replications and ten treatments in total land area of 282.75m²(32.5m*8.7m) at olericulture farm of Agriculture and Forestry University. Each replication consists of ten treatments plot each of area 5.13m²(2.7m*1.9m) that were placed through randomization. The distance of 1m and 0.5m was maintained between replications and plots respectively. The description on factors and treatments used were given below:

Table 2: Assignment of different factors used in the experiment.

S.N.	1st Factor	Abbreviation
1	Mulching	M
2	No mulching	M0
S.N	**2nd Factor**	**Abbreviation**
1	Control	C
2	Rhizobium inoculation	MR
3	Rhizobium inoculation and phosphorus	RP
4	Phosphorus	P
5	Rhizobium inoculation, phosphorus and molybdenum	RPMo

Table 3: Different treatments and their abbreviations used in the experiment.

S.N.	Treatments	Abbreviation
1	Mulching with control (T1)	MC
2	Mulching with Rhizobium inoculation (T2)	MR
3	Mulching with Rhizobium inoculation and phosphorus (T3)	MRP
4	Mulching with phosphorus (T4)	MP
5	Mulching with Rhizobium inoculation, phosphorus and molybdenum (T5)	MRPMo
6	No mulching with control (T6)	M0C
7	No mulching with Rhizobium inoculation (T7)	M0R
8	No mulching with Rhizobium inoculation and phosphorus (T8)	M0RP
9	No mulching with phosphorus (T9)	M0P
10	No mulching with Rhizobium inoculation, phosphorus and molybdenum (T10)	M0RPMo

2.3 Seed treatment with Rhizobium and molybdenum

For the experiment, around 200gm seeds of Karma stick less variety of cowpea was taken. The seeds were soaked in water for 4 hrs. The mixture of water and molasses was boiled and let down for cool. After that about 120 gm seeds were soaked into that mixture for 1 minute to make the sticky surface of seed for better contact with inoculants. The soaked seeds were taken out and made well mixed with the soil mass culture Rhizobium inoculants (@1gm/16.66gm of seed), brought from Nepal Agriculture Research Council (NARC) situated in Khumaltar, Lalitpur, Nepal. For the molybdenum treatment, about 40 gm of Rhizobium inoculated seeds were taken and treated with molybdenum powder (@2gm/kg of seed) separately. And treated seeds were let under shade condition for dry for 1-2 hrs.

2.4 Fertilization, spacing and sowing of seed

The plots were well ploughed, fertilized, leveled and finally seeds were sown by maintaining proper spacing. During fertilization, farmyard manure (@20kg/plot) was supplied into all plots. The chemical fertilizer was used @ 20:120:40 kg NPK/ha, where the nitrogen rate was decreased by four parts from the recommended dose (@ 80:120:40 kg NPK/ha). In case of phosphorus treatments, recommended dose of phosphorus (@20:120:40kgNPK/ha) was used and for non-phosphorus treatment the recommended dose was reduced into half (@20:60:40 kg NPK/ha). Urea, single super phosphate and miurate of potash were used as a source of nitrogen, phosphorus and potassium respectively. All calculated dose of fertilizer was applied as basal application. The seed was shown into plots after fertilization by maintaining row to row spacing of 60cm and plant to plant spacing of 40cm. Twenty-five number of plant population were maintained in each plot.

2.5 Data collection and analysis

The five plants were tagged randomly from each plot for collection of data. The data regarding to plant height, leaves number, stem diameter and canopy length were taken at 15, 30 and 60 days after sowing whereas two non-tagged plant were uprooted from each plot at flowering stage for counting nodules number.

The collected data were entered in Microsoft excel and analyzed using R Stat software. The means were compared by using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

3. RESULTS AND DISCUSSIONS

3.1 Canopy length

Canopy length is one of the important parameters that influenced the plant yield. There were comparable differences between treatments and control in case of canopy length (Table 4). The length of canopy was found significantly higher in mulching (40.88, 65.20 and 71.78 cm) as compared to no mulching (32.30, 56.39 and 64.58 cm) at 15, 30 and 60 days after sowing (DAS) respectively. The insignificant result on canopy length was found in between the second factor treatments but the highest canopy length was found in RPMo treatment (37.40, 61.68 and 71.54 cm) at 15, 30 and 60 days after sowing respectively as compared to other treatment. In case of interaction between first and second factor, there was no any significant differences among the different treatment combinations but highest canopy length was found in mulching with RPMo (42.26, 67.00 and 74.71 cm) at 15, 30 and 60 DAS respectively as compared to other treatment combinations.

3.2 Plant height

Plant height is also one of the yields attributing parameter that influenced the plant yield. There were comparable differences between treatments and control in case of plant height which is presented in Table 4. The plant height was found significantly higher in mulching (25.34, 56.48 and 80.27 cm) as compared to no mulching (21.67, 50.47 and 69.48 cm) at 15, 30 and

60 DAS respectively. The insignificant result on plant height was found in between the second factor treatments at 15 and 30 DAS. At 60 DAS, plant height was found significantly higher in the RPMo (78.79cm) which was at par with RP (78.72cm) and R treatment (75.88cm) respectively. Plant height was found statistically lowest in control. In case of interaction between first and second factor, there was no any significant differences among the different treatment combinations but highest plant height was found in MP (26.00cm) at 15 DAS, MRPMo(58.6cm) at 30DAS and in MRP (85.91 cm) at 60 DAS as compared to other treatment combinations.

Table 4: Effects of different fertilizers on canopy length and plant height at different days after sowing.

	CANOPY (cm)			PLANT HEIGHT (cm)		
	15 DAS	30 DAS	60 DAS	15 DAS	30 DAS	60 DAS
Factor 1st						
M	40.88	65.20	71.78	25.34	56.48	80.27
M0	32.30	56.39	64.58	21.67	50.47	69.48
LOS	***	**	*	***	*	*
LSD	2.21	5.1	5.88	1.7	4.91	4.45
Factor 2nd						
C	36.21	57.91	61.32	22.58	50.48	67.55b
R	36.31	62.07	69.12	23.33	53.46	75.88a
RP	36.31	61.37	69.42	23.16	53.63	78.72a
P	36.71	60.95	69.49	24.23	53.71	73.44ab
RPMo	37.40	61.68	71.54	24.22	56.10	78.79a
LOS	NS	NS	NS	NS	NS	*
LSD	3.49	8.06	9.3	2.69	7.77	7.04
Interaction (1ST*2ND)						
MC	40.05	62.85	68.74	24.60	54.60	70.83
MR	40.20	65.73	72.04	25.56	55.66	80.08
MRP	40.93	65.81	70.41	24.80	56.06	85.91
MP	40.96	64.60	73.01	26.00	57.50	80.28
MRPMo	42.26	67.00	74.71	25.73	58.60	84.25
M0C	32.37	52.96	53.91	20.56	46.36	64.26
M0R	32.43	58.42	66.20	21.10	51.26	71.69
M0RP	31.70	56.93	68.43	21.53	51.20	71.52
M0P	32.46	57.30	65.96	22.46	49.93	66.61
M0RPMo	32.53	56.36	68.37	22.71	53.60	73.33
LOS	NS	NS	NS	NS	NS	NS
LSD	4.94	11.4	13.2	3.8	11	9.96
SEM(±)	0.9744	1.303	1.5045	0.5004	1.2419	1.6232
CV (%)	7.87	10.9	11.3	9.43	12	7.75

(*Means with the same letter do not differ significantly at p= 0.05 by DMRT. CV = Coefficient of variation. LSD= least significant difference, SEM= Standard error of mean.LOS= level of significance.DAS= Days after sowing.*)

3.3 Stem diameter

There were comparable differences between treatments and control in case of stem diameter (Table 5). The stem diameter was found significantly higher in mulching (0.83 and 1.31cm) as compared to no mulching (0.73 and 1.14cm) at 30 and 60 DAS respectively. The insignificant result on stem diameter was found in between the second factor treatments at 30 DAS and 60 DAS but the highest stem diameter (0.79cm) was found RPMo and P treatment at 30 DAS whereas highest stem diameter (1.29cm) was found in RPMotreatment at 60 DAS. In case of interaction between first and second factor, there was no any significant differences among the different treatment combinations but highest stem diameter (0.84cm) was found in MP and MRPMo treatment at 30DAS whereas the highest stem diameter (1.35cm) was found in MRPMo treatment at 60DAS.

3.4 Leaves number

There were comparable differences between treatments and control in case of leaves number which is presented in Table 5. The leaves number was found significantly higher in mulching (30.88, 83.60 and 95.07) as compared to no mulching (23.69, 70.72 and 82.40) at 15, 30 and 60 DAS respectively. The insignificant result on leaves number was found in between the second factor treatments at 15, 30 and 60 DAS but the highest leaves number was found in RPMo (28.50 and 97.00) at 15 and 60 DAS respectively whereas highest leaves number was found in P treatment (80.5) at 30 DAS. In case of interaction between first and second actor, there was no any significant differences among the different treatment combinations but highest leaves number was found in MRP (31.86), MP (86.8) and MRPMo (100.75) at 15, 30 and 60 DAS respectively.

Table 5: Effects of different fertilizers on stem diameter and no of leaves in different days after sowing.

Treatments	STEM DIAMETER (CM)		NO. OF LEAVES		
	15 DAS	30 DAS	15 DAS	30 DAS	60 DAS
Factor 1st					
M	0.83	1.31	30.88	83.60	95.07
M0	0.73	1.14	23.69	70.72	82.40
LOS	**	**	**	***	*
LSD	0.056	0.096	3.87	6.75	12.1
Factor 2nd					
C	0.75	1.15	25.76	73.1	79.36
R	0.78	1.26	26.86	78.7	87.33
RP	0.78	1.19	28.36	75.5	91.95
P	0.79	1.23	26.93	80.5	88.02
RPMo	0.79	1.29	28.50	78.0	97.00
LOS	NS	NS	NS	NS	NS
LSD	0.089	0.152	6.12	10.7	19.1
Interaction (1ST*2ND)					
MC	0.82	1.28	29.73	79.2	83.33
MR	0.80	1.28	30.73	79.2	94.00
MRP	0.83	1.32	31.86	81.0	99.66
MP	0.84	1.31	31.33	86.8	97.61
MRPMo	0.84	1.35	30.73	85.6	100.75
M0C	0.67	1.03	21.80	67.0	75.40
M0R	0.75	1.24	23.00	72.0	80.66
M0RP	0.73	1.06	24.86	70.0	84.25
M0P	0.74	1.16	22.53	74.2	78.43
M0RPMo	0.74	1.24	26.26	70.4	93.25
LOS	NS	NS	NS	NS	NS
LSD	0.127	0.215	8.65	15.1	27
SEM(±)	0.0156	0.027	1.117	1.8159	2.8516
CV (%)	9.53	10.2	18.5	11.4	17.8

(*Means with the same letter do not differ significantly at p= 0.05 by DMRT. CV = Coefficient of variation. LSD= least significant difference, SEM= Standard error of mean.LOS= level of significance.DAS= Days after sowing*)

3.5 Pod Yield (Fresh weight of pods per plant)

There were significant differences between treatments and control in case of pod yield (Table 6). Mulching treatment was found significantly higher ($P<0.001$) than un-mulching treatment with 312.61 gm average pod yield per plant. Similarly, in case of second factor, RPMo with 312.53 gm of pod yield per plant was found significantly highest ($P<0.05$) than other treatments which was at par with RP (296.47 gm) and R (276.78 gm). All the treatments were found significantly higher than the control. There was no any significant interaction between the two factors but the highest pod yield per plant was found in MRPMo and lowest in MOC. There was high positive correlation between pod yield and no of nodules with the correlation coefficient r value of 0.54 ($P<0.01$) which is presented in Table 7 and figure 2.

Table 6: Effects of different fertilizers on cowpea pod yield per plant and no of nodules per plant.

Treatments	Yield/Plant(gm) (1st+2nd+3rd)	Number of Nodules Per Plant (flowering stage)
Factor 1st		
M	312.61^{a}	121.63^{a}
M0	250.99^{b}	82.83^{b}
LOS	***	***
LSD	25.2	14.5
Factor 2nd		
C	253.62^{c}	77.92^{c}
R	276.78^{abc}	95.00^{bc}
RP	296.47^{ab}	115.25^{ab}
P	269.62^{bc}	93.67^{bc}
RPMo	312.53^{a}	129.33^{a}
LOS	*	**
LSD	39.9	22.9
Interaction (1ST*2ND)		
MC	278.90	93.50
MR	311.10	115.00
MRP	328.47	134.50

MP	293.17	105.00
MRPMo	351.40	160.17
M0C	228.33	62.33
M0R	242.45	75.00
M0RP	264.48	96.00
M0P	246.06	82.33
M0RPMo	273.65	98.50
LOS	NS	NS
LSD	56.5	32.4
SEM(±)	8.890	5.8703
CV (%)	11.7	18.5

(*Means with the same letter do not differ significantly at p= 0.05 by DMRT. CV = Coefficient of variation. LSD= least significant difference, SEM= Standard error of mean. LOS= level of significance.DAS= Days after sowing*)

3.6 Number of Nodules

Number of nodules was found significantly higher in case of mulching than un-mulched field which is depicted in Table 6. In case of 2nd factor, it was found significantly highest (P<0.01) in RPMo with 129.33 nodule numbers which was at par with RP treatment (115.25 nodule number). Similarly, RP treatment was found significantly similar with R and P treatments in case of nodule number. whereas control was found significantly lowest. There was no any significant interaction between two factors but the nodule number was found highest in MRPMo and lowest in MOC. There was a high significant positive correlation (P<0.01) between the no of nodules and the yield parameters like stem diameter, plant height, canopy and no of leaves and also yield (Table 7). The increase in yield was therefore due to increase in no of nodules since increase in nodule corresponded linearly to the increase in yield and yield parameters.

Table 7: Correlation between different parameters recorded during the experiment.

SN	PARAMETER (i)	PARAMETER (ii)	COEFFICIENT OF CORRELATION (r value)
1	Root nodule	Yield	0.5437988**
2	Root nodule	Canopy	0.5860812**
3	Root nodule	Leaves number	0.534542**
4	Root nodule	Plant height	0.6963344**
5	Root nodule	Stem Diameter	0.4492855**
	n=30		r value at $P_{0.05}$= 0.306
			r value at $P_{0.01}$ = 0.423

** indicates r value > critical r value at P=0.01.

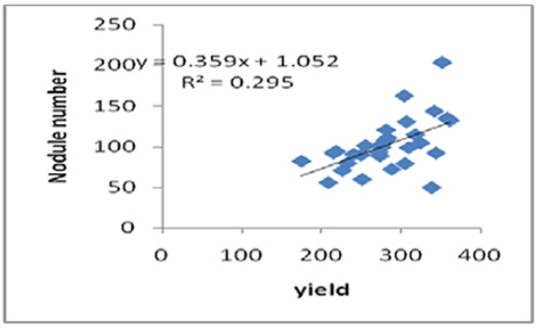

Figure 2: Relationship between nodule number and yield.

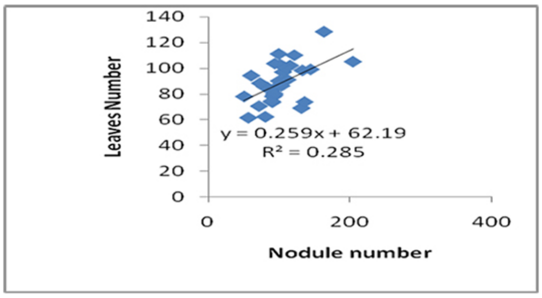

Figure 3: Relationship between nodule number and leaves number.

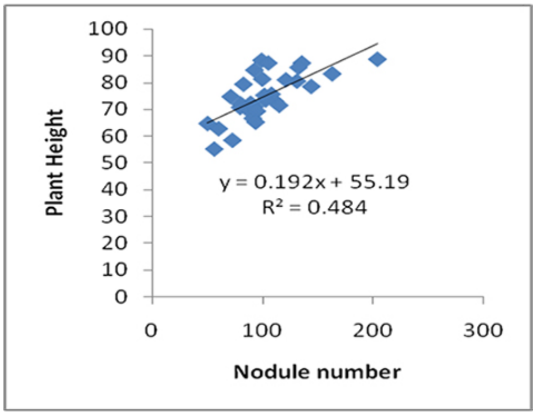

Figure 4: Relationship between nodule number and plant height

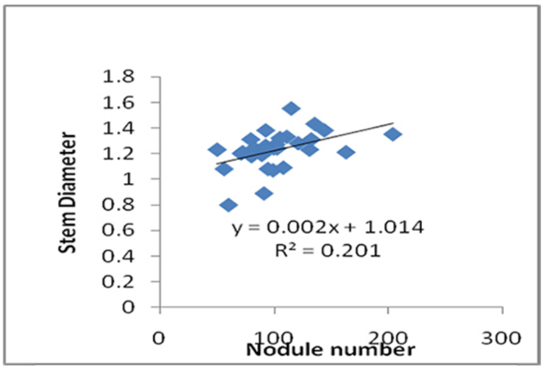

Figure 5: Relationship between nodule number and stem diameter

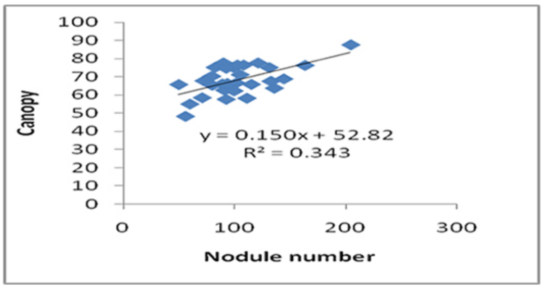

Figure 6: Relationship between nodule number and canopy

In case of 1st factor, the findings revealed that there was significant increase in yield and yield attributes of cowpea due to mulching. Similar findings were reported by Kamara, D.S. (1981) who reported that mulching had positive effects on soil moisture conservation and improved cowpea yield [13]. In case of 2nd factor, the findings showed that treatments with inoculation of rhizobium, Phosphorus and Molybdenum application had got the highest no of nodules. Rahman et al. (2008) also reported the similar result in his experiment in Mungbean which showed that seed inoculation with Rhizobium significantly increased nodule number as compared to that of the non-inoculated plants of mungbean [14]. Similarly, Zaman et al. (1996) observed that there was higher nodule number with the application of Mo @1.0 kg/ha [15]. Chawdhury et al. (1998) reported that with the phosphorus application at the rate of 60kg P_2O_5/ha had significantly increases the nodulation of legumes. Similarly, along with the rise in no of nodules, there was also increase in yield parameters like stem diameter, plant height, canopy and no of leaves. Similar results was also reported by Singh et al. (2007) which showed that rhizobium inoculation with phosphorus application enhanced the growth parameters of cowpea. Othman W.M. W. (1994) also reported that rhizobium inoculation increased the number of nodules and nitrogen fixation causing increase in growth and growth parameters.

Similarly, there was significant increase in pod yield of cowpea with the applications of rhizobium, Phosphorus and Molybdenum than control. Similar results were reported by Jain et al. (1993) and Singh et al. (2007) that rhizobium inoculation, nitrogen and phosphorus application enhanced grain and straw yield of cowpea which was due to increasing growth and yield attributing characters. Pandey et al. (2015) also reported that there was increase in yield and yield attributes under combined inoculation of seeds (PSB and rhizobium) with phosphatic fertilizer in cowpea [16]. Muhammad et al. (2004) and Malik et at. (2002 & 2003) reported that the number of pods per plant of legume increased with rhizobuim inoculant [17, 18]. Landge et al. (2002) reported that rhizobium inoculant in association with P and Mo led to increase the number of seeds per pod of legumes [19].

4. CONCLUSION AND RECOMMENDATION

The experimental findings revealed that the combined use of rhizobium at 1gm/16.66 gm seed, phosphorus at recommended dose of 120kg/ha and Molybdenum at 2 gm per kg seed increases the yield parameters like plant height, stem diameter, number of leaves, canopy length in cowpea. There was also significant increase in pod yield and number of nodules in these combined treatments. Similarly, mulching was also found to be an efficient technology in increasing yield and yield attributes of cowpea. So, this paper recommends using mulching and combined use of rhizobium, phosphorus and molybdenum in efficient proportion for increasing yield and fulfilling the demands of legumes in developing countries.

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