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Effect of *Rhizobium* inoculum and N-fertilizer on bio-mass production of cowpea (*Vigna unguiculata*) forage at different stages of maturity

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Abstract

An experiment was conducted for a period of 60 (sixty) days during March to May 2003 to study the effect of *Rhizobium* inoculum and N-fertilizer on bio-mass production in 3 (three) stages (40d, 50d and 60d) of maturity of cowpea plants following a randomized block design (RBD). There were six treatments as: T_0 (uninoculated control), T_1 (inoculated), T_2 (25 kg N/ha), T_3 (inoculated + 25 kg N/ha), T_4 (35 kg N/ha) and T_5 (45 kg N/ha) having three replication in each treatment. With the advancement of plant age, yield of cowpea forage on green as well as dry basis increased linearly. The maximum green biomass (265 q/ha) production was attained in treatment T_5 at 60 days of plant age against 200 q/h in untreated control (T_0). Similar trend was observed in total dry matter and organic matter production ($P<0.05$) at harvest age of 60 days. Crude protein in different treatments raged from 5.39 to 4.74, 4.24 to 6.64 and 4.04 to 6.35 q/ha in 40, 50 and 60 days of plant age respectively. Maximum yield of crude protein attained at 50% flowering stage (50d) of maturity in all treatments. *Rhizobium* inoculum and/or nitrogen fertilizer application had significant ($P<0.01$) effect on green matter, DM, OM, and CP yield of cowpea forage. *Rhizobium* inoculum with or without N-fertilizer or nitrogen fertilizer alone significantly ($P<0.01$) and progressively increased plant height over the control but decreased leaf and stem ratio at all stages of maturity. Inoculum + N fertilizer and N-fertilizer alone with the application of 35 to 45 kg N/ha from urea showed a tendency to enhance rate of branching compared to control ($P<0.05$).

Keywords: Cowpea, *Rhizobium* inoculum, N-fertilizer, bio-mass production, stages of maturity

Introduction

In a typical small-holder farm in Bangladesh approximately 90% of the cattle feed supply comes from poor quality roughage, mostly of rice straw and small quantity of green grass with little concentrates (Tareque and Saadullah, 1988). Rice straw as such is deficient in readily fermentable carbohydrate, protein, minerals and vitamins. As a result, growth rates and milk production of the animal consuming rice straw alone are generally low and often only about 10% of the genetic potentiality of the animal (Leng, 1995). On the straw based diet, supplementation of green legume forage is often recommended for optimization of rumen environment (Preston and Leng, 1987) or even to meet the maintenance requirement of animal (Ranjhan and Singh, 1993). Green forages are easily digestible, palatable, being slightly laxative in action, and above all, provide fresh nutrients in a most natural form resulting in efficient utilization of the entire feed. Among the forages, legumes are important in supplying the most demanding and quality nutrients like protein, minerals and vitamins to the animals. Farmers of some areas of Bangladesh grow cowpea (*vigna unguiculata*) as a leguminous summer vegetable and after collecting fruits; the plants are used as animal feed. On the other hand, in Bathan areas of Pabna district (milk pocket area) the cattle owners grow cowpea as fodder crop for feeding dairy animals in winter season. Of the legume forage crops cowpea is important due to its rapid growth and high productivity.

Inoculation of leguminous seed with *Rhizobium* bacteria is a common method employed to reduce the required amount of costly N-fertilizer. Cowpea has good capacity to fix atmospheric nitrogen through root nodule bacteria called *Bradyrhizobium. Bradyrizobium* bacteria in symbiotic association with the leguminous plants are able to fix dinitrogen ($N \equiv N$) from the atmosphere. Air of the atmosphere contains about 78% nitrogen in the element form ($N \equiv N$). This large amount of nitrogen can not be used by plants unless it is converted to available form (NH_4^+, $N0_3^-$). Fortunately, the *Bradyrhizobium japonicum* bacteria can fix atmospheric nitrogen (about 300 kg/ha/yr) in symbiosis with legume plants which contributes significantly to the N-status of the plant ranging from 25 to 75 per cent of its requirement (Keyser and Fudi, 1992).

There is not enough consistent scientific information on the yield response of cowpea fodder production to the application of nitrogen fertilizer alone, inoculation of *Rhizobium* bacteria, *as* well as the effect of stage of maturity on bio-mass production. Therefore, the present investigation was carried out to study the effect of *Rhizobium* inoculum and N-fertilizer on biomass yield and plant characteristics of cowpea (*Vigna unguiculata*) at different stages of maturity.

Materials and methods

Experimental site

Cowpea (*Vigna unguiculata*) fodder production and its related activities were carried out in the Animal Nutrition Field Laboratory, Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh during the period from March to May 2003. The soil of the experimental plot was of silt loam texture, neutral in reaction (P^H 7.0) and contained 0.12% nitrogen (N) 0.07% phosphorus (P) and 1.76% organic matter (OM). The land was flat, moderately drained and was above flood levels.

Preparation of inoculum materials

The seeds of cowpea was collected from a local market. Immature and unhealthy seeds were isolated manually and only mature uniform seeds were used in the experiment. Inoculum was collected from Bio-fertilizer Production Laboratory of Bangladesh Institute of Nuclear Agriculture, Mymensingh which was prepared following the method of Vincent (1974). Yeast extract mannitol liquid medium was prepared in 500 ml conical flask containing 5 g mannitol, 0.5 g K_2HPO_4, 0.2 g $MgSO_4$ with $7H_2O$, 0.1 g NaCl, 1.0 g yeast extract and 1 litre distilled water. The liquid medium was sterilized at 15 lb pressure for 15 minutes. A loopful of the respective individual pure cultures was inoculated in 300 ml of the yeast extract mannitol media in 500 ml conical flasks. The inoculum of the flasks were then allowed to grow for 5 – 7 days on a rotary shaker at room temperature. The mixed culture was prepared by mixing equal quantity of fully-grown viable cells ($>10^8$/ml) of broth in an aseptic condition. From the readily prepared broth, 25 ml were taken out by sterile syringe and injected into the sterilized peat bag. The inoculated materials of the packets were then incubated at 28^0C for two weeks and were made ready for cowpea seed inoculation.

Design of the experiment

The experiment was arranged in a randomized block design (RBD) having three replications in each treatment for each stage of maturity. There were six treatments as: T_0 (uninoculated and unfertilised control), T_1 (inoculated), T_2 (25 kg N/ha), T_3 (inoculated + 25 kg N/ha), T_4 (35 kg N/ha) and T5 (45 kg N/ha). The plot size for each replicate was 3m×2m. The treatments were randomly distributed in each block at each stage of growth.

Preparation of land

The land was ploughed and cross-ploughed six times with bullock drawn country plough followed by laddering, harrowing and planking to obtain the desirable filth. The corners of the land were spaded and visible larger clods were broken into small pieces. All the weeds and stubbles of the previous crop were removed. A basal dose of P (40 kg P_2O_5), K (30 kg K_2O) and cow dung (1,000 Kg) per ha were applied at the time of land preparation. The fertilizer was mixed properly and land was levelled and divided into three blocks and each block was again sub divided into eighteen small units. Fifty percent of the required nitrogen fertilizer in the form of urea [(NH_2) CO] was applied immediately before sowing of seeds and remaining 50%was applied at the plant age of 35 days.

Seed rate and method of sowing

After land preparation and fertilizer application cowpea seeds were coated with the *Rhizobium* inoculums (10^8 bacteria /g) at the rate of 100 g/kg seed. For proper binding of inoculum with the seeds, small amount of molasses were added and kept for 30 minutes in open space for drying and then sown in the plots maintaining a row spacing of 20 cm and plant spacing of 5 cm behind a plough having continuous sowing in a row. Sixty g of seeds were sown in each plot (3m x 2 m) with the seed rate of 100 kg/ha. After sowing, seeds were covered with well pulverized soil followed by a light pressure of hand. Seeds were germinated from 4th day after sowing and full germination was observed by 8th day of sowing. No irrigation or insecticide was used. The excess rainwater was drained out.

Study of plant characteristics

Cowpea forage was harvested at three stages of maturity such as pre-flowering (40 d), 50% flower (50 d) and full flowering (60 d) stages. Plot wise green fodder production under six treatments was recorded immediately after harvesting by talking weight of forage samples in a pan balance. In every harvest twenty-five plants were randomly selected from each plot and full length of each plant was measured using a metre scale. Plant height was measured in centimetre from the base to the tip of the plant and branching of plant was also counted. Leaves were separated from stems and weight was recorded separately.

Statistical analysis

The data were analyzed using MSTAT statistical program to compute analysis of variance for a randomized block design and differences among the treatment means were determined by the least significant difference test (Steel and Torrie, 1980).

Results and Discussion

Green biomass yield

In pre-flowering stage green matter yield among the treatments ranged from 150 to 189 q/h (Table 1) and the differences were highly significant ($P<0.01$). With increasing plant age, the yield of green fodder biomass also increased. The green forage yield for treatments T_0 to T_5 ranged from 181 to 250 q/ha at 50% flowering condition (Table 2) and at full flower stage (60 d) it ranged between 200 to 265 q/ha (Table 3) and the differences were highly significant ($P<0.01$).

In pre-flowering stage, green forage yield was maximum (189.00 q/ha) with the treatment (T_5) having the nitrogen doses of 45 kg/ha, while the lowest yield (150 q/ha) was observed in T_0 (control). Similarly, the highest values (250.00 and 265.00 q/ha) of green matter yield was found in treatment T_5 and lowest values (181.00 and 200.00 q/ha) in treatment T_0 (control) at 50% flowering and full flower stages respectively. Results obtained by application of only *Rhizobium* inoculant (T_1) did not differ significantly from that of control (T_0), but inoculum + N-fertilizer application (T_3) showed positive effect on green matter yield at different stages of maturity of cowpea forage. The lack of response in treatment T_1 may be due to higher level of N (0.12%) in soils and possibly that root nodule bacteria did not promote atmospheric nitrogen fixation and utilization for improved growth, development and yield of forage. Sultana (1999) found the highest green forage yield of cowpea fodder at 98 day of maturity (332 q/ha) compare to 277 q/ha at 78 days in the present experiment. Total production of cowpea forage increased with increasing level of nitrogen fertilizer at pre-flowering, 50% flowering and full flower stages. The application of increased level of N fertilizer presumably increased the availability of soil nitrogen which might have enhanced the meristimatic growth and resulted in higher forage yield.

Table 1. Bio-mass yield and plant characteristics of cowpea forage at pre-flowering age (40d)

Parameters	Treatments						SED	Level of significance
	T_0	T_1	T_2	T_3	T_4	T_5		
Green forage yield (q/ha)	150.00^a	156.67^a	162.50^b	176.00^c	187.00^d	189.00^d	1.31	**
Dry matter yield (q/ha)	22.67^a	24.02^b	25.42^c	29.07^d	29.96^{de}	30.92^e	0.42	**
Organic matter yield (q/ha)	20.67^a	21.93^{ab}	23.22^b	26.57^c	27.47^{dc}	28.28^d	0.41	**
CP yields (q/ha)	3.39^a	3.60^a	3.82^{ab}	4.38^{bc}	4.51^{bc}	4.74^c	0.24	*
Plant height (cm)	17.51^a	21.27^b	22.74^c	24.62^d	25.43^d	27.41^e	0.42	**
Leaf-stem ratio	2.05^a	2.05^a	1.77^b	1.69^b	1.61^b	1.68^b	0.08	**
No. of branching per plant	3.00	3.00	3.00	3.20	3.40	3.70	0.27	NS

Treatments: T_0=control; T_1=inoculated; T_2=25 kg N/ha; T_3=inoculated+25 kg N/ha; T_4=35kg N/ha; T_5=45 kg N/ha.

SED = Standard error of difference; NS = Non significant, *P<0.05; **P<0.01

^{abcde}Mean values with different superscripts in each row differ significantly (P<0.05)

Dry matter and organic matter yield

Yield of dry matter ranged between 23 and 31; 29 and 43; 33 and 46 q/ha at pre flowering (40 d), 50% flowering (50d) and full flower (60d) stages respectively (Table 1). Application of higher doses of nitrogen fertilizer had positive effect on dry matter yield of the cowpea forage. Stage of maturity showed a positive effect on DM content of cowpea forage. In the present study the DM content increased from 30.92 (40 d) to 46.32 q/ha as the forages were harvested at 60 days of maturity. Similarly Khan *et.al.,* (1992) observed increased DM yield of cowpea fodder with advancement of harvesting days of the plants. There was significant (P<0.01) difference among the treatment groups at all maturity stages. Although *Rhizobium* inoculum and/or N-fertilizer showed positive effect on DM yield but higher doses of N-application did not show significant effect on DM yield of cowpea forage.

It is revealed from Tables 1, 2 and 3 that organic matter yield was the highest for treatment T_5 at all stages of maturity where 45 kg N/ha was applied. Yield of organic matter at 40, 50 and 60 days of plant age ranged from 21 to 28; 26 to 39 and 30 to 42 q/ha respectively and the differences were statistically significant (P<0.01). The results on the yield of DM and OM are consistent with the existing reports of Khan et. al.,(1992) and Sultana (1999).

Crude protein (CP) yield

Yield of CP of different treatments are presented in Table 1 to 3. The results showed that there was no significant difference among untreated (T_0), *Rhizobium* inoculum, (T_1) and 25 kg N/ha (T_2) treatment groups at pre-flowering (40 d) but application of *Rhizobium* inoculum alone or with N-fertilizer or N fertilizer alone showed significantly ($P<0.01$) higher CP yield in comparison to untreated cowpea forage at 50 and 60 days of age. It can be seen from the Tables that maximum yield of crude protein was attained at 50% flowering stage in all treatments. Patel *et al.,* (1977) reported that the green biomass, dry matter and crude protein yield of cowpea variety raged from 196 to 494, 29.1 to 58.4 and 5.28 to 10.29 q/ha which are in agreement with the present findings.

Plant characteristics

Plant height: Tables 1, 2 & 3 show that plant height obtained from the application of *Rhizobium* inoculums along with 25 kg N/ha (T_3) or 35 kg N/ha (T_4) was almost similar at three stages of maturity. The treatment 45 kg N/ha (T_5) produced higher plant height (42.30 cm) at 60 d than those of 40 and 50 d of plant growth and the differences in every stage of plant growth were highly significant ($P<0.01$). Plant height recorded at 50 d of growth (Table 2) was almost similar among the treatments as that of plant height recorded at 60 d (Table 3) of growth of fodder except treatment T_5.

Table 2. Bio-mass yield and plant characteristics of cowpea forage at 50% flowering age (50 d)

Parameters	Treatments						SED	Level of significance
	T_0	T_1	T_2	T_3	T_4	T_5		
Green forage yield (q/ha)	181.00^a	186.06^{ab}	190.25^b	226.00^c	243.00^d	250.00^d	2.51	**
Dry matter yield (q/ha)	28.82^a	30.83^b	31.21^b	37.69^c	40.12^d	42.58^e	0.38	**
Organic matter yield (q/ha)	26.29^a	28.14^{ab}	28.53^b	34.41^c	36.77^d	38.96^e	0.57	**
CP yields (q/ha)	4.24^a	4.56^b	4.72^b	5.79^c	6.27^d	6.64^e	0.10	**
Plant height (cm)	21.29^a	23.53^b	26.46^c	31.72^d	32.77^d	34.80^e	0.54	**
Leaf-stem ratio	1.73	1.65	1.59	1.62	1.67	1.66	0.10	NS
No. of branching per plant	3.11	3.20	3.50	3.80	4.00	4.50	0.31	NS

Treatments: T_0=control; T_1=inoculated; T_2=25 kg N/ha; T_3=inoculated+25 kg N/ha; T_4=35kg N/ha T_5=45 kg N/ha.
SED = Standard error of difference; NS = Non significant, **$P<0.01$
^{abcde}Mean values with different superscripts in each row differ significantly ($P<0.01$)

Khan *et al.* (1992) reported the similar results of increased plant height and production of cowpea fodder due to application of nitrogen fertilizer with inoculums. In the present experiment the progressive increases in plant height with the increase in nitrogen fertilizer doses might be due to higher intake of nitrogen by the plants from soil and more tissue protein synthesis resulting higher plant growth. This result is in consistent with the results of Gami *et al.* (1986) who reported that the plant height was increased due to application of N fertilizer.

Leaf-stem ratio and number of branching per plant

At pre-flowering stage, treatment effects on leaf-stem ratio of cowpea fodder differed significantly (P<0.01), however at 50% flower and full flowering stage of maturity leaf-stem ratio within the treatments did not differ significantly (P>0.05). From the results the Table 1 it was observed that the highest value of leaf-stem ratio was 2.05 in case of both control (T_0) and *Rhizobium* inoculated treatment (T_1). But leaf-stem ratio in treatment T_3, T_4 and T_5 were similar and did not differ significantly (P>0.05). It is revealed from Table 1, 2 and 3 that number of branches per plant ranged between 3.0 to 3.7; 3.1 to 4.5; 3.1 to 4.8 at pre-flowering, 50% flowering and full flower stages, respectively. Using inoculums and nitrogen fertilizer from urea did not show any significant effect on branching of plant at 40 and 50 days of plant growth. However, there was highly significant (P<0.01) differences at 60 days of plant age regarding branching, although no significant differences were observed among treatment T_0, T_1 and T_2 and also in treatments T_3, T_4 and T_5. From the present findings, it is evident that the *Rhizobium* inoculum and level of N-fertilizer increased plant height (P<0.01) and number of branch per plant but decreased leaf stem ratio over uninoculated control (T_0). Sikinyi and Kimani (1988) in their experiment with pigeonpea observed similar trend of production. Riewe and Lipkke, 1969 stated that with the stage of maturity, the building up of structural material occurs at faster rate and decrease in leaf stem ratio and accelerated rate of accumulating cell wall material in plants.

Table 3. Bio-mass yield and plant characteristics of cowpea forage at full flowering age (60 d)

Parameters	Treatments						SED	Level of significance
	T_0	T_1	T_2	T_3	T_4	T_5		
Green forage yield (q/ha)	200.00^a	205.00^b	211.00^c	246.00^d	261.00^e	265.00^e	1.29	**
Dry matter yield (q/ha)	32.55^a	33.59^a	35.97^b	42.81^c	45.46^d	46.32^d	0.41	**
Organic matter yield (q/ha)	29.68^a	30.62^a	32.77^b	38.87^c	41.35^d	42.19^d	0.11	**
CP yields (q/ha)	4.04^a	4.23^b	4.79^c	5.72^d	6.15^e	6.35^f	0.06	**
Plant height (cm)	22.25^a	24.21^a	27.50^b	33.57^c	35.37^c	42.30^d	8.78	**
Leaf-stem ratio	1.72	1.63	1.59	1.62	1.66	1.60	0.06	NS
No. of branching per plant	3.11^a	3.20^a	3.60^{ab}	4.30^{bc}	4.32^{bc}	4.82^c	0.23	**

Treatments: T_0=control; T_1=inoculated; T_2=25 kg N/ha; T_3=inoculated+25 kg N/ha; T_4=35kg N/ha; T_5=45 kg N/ha. SED = Standard error of difference; NS = Non significant, **P<0.01

[abcdef]Mean values with different superscripts in each row differ significantly (P<0.01)

Considering biomass and nutrient yield it may be suggested to cultivate cowpea (*Vigna unguiculata*) forage using 45 kg N/ha from urea fertilizer. Fifty percent of it should be given at the time of land preparation along with 40kg P_2O_5,30 Kg K_2O and 1,000 kg cow dung per ha and rest 50% (22.5 Kg N) at 35 d of plant age and harvested at the plant age of 60 days. If *Rhizobium* inoculum is available, it may be suggested to treat the cowpea seed with inoculum and apply fertilizer grade urea at a rate of 25kgN/ha.

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