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The Nature and Pattern of Nodulation and Dry Matter Production of *Crotolaria juncea* (Sunn hemp) and *Tephrosia purpurea*

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ABSTRACT. Sunn hemp and Tephrosia purpurea are two herbaceous legumes grown in paddy soils and market garden soils to improve the nitrogen fertility.

Studies on them showed the maximum nodulation (220 nodules/10 plants) occurred after six weeks in sunn hemp at 50% flowering stage while maximum nodulation (31/10 plants) occurred 12 – 13 weeks in Tephrosia purpurea. These plants were in the pod development stage. The mean dry weight of nodules per 10 plants was 0.6 g and 0.22 g for sunn hemp and Tephrosia respectively. In both cases the nodules started as small round whitish structures and at maturity they were coralloid. The nodules of Tephrosia were 8 – 12 mm at full growth and smaller in number, located mainly on the lateral roots while, in sunn hemp they were 9 – 10 mm and larger in number found mostly on the tap root near the surface of the soil.

In sunn hemp, nodules were shed by 10 weeks but in Tephrosia, nodules were shed by the 17th week. In both legumes inoculation of the seeds with freshly isolated respective rhizobia improved significantly their growth and the nodule weight. At maximum growth, nodulation was almost three times that of the uninoculated one.

Total nitrogen accumulation during maximum nodulation in sunn hemp was 34.33 and 23.27 Kg/ha in the seed inoculated and uninoculated respectively. The best time to plough in the plant tops into the soil is at the end of six weeks for Crotolaria and 12 weeks for Tephrosia. Combined nitrogen and specially urea significantly reduced nodulation in Sunn hemp. To obtain the maximum benefit, these crops must be grown in soil where the nitrogen has been depleted.

INTRODUCTION

The rapid rise in the cost of synthetic nitrogen fertilizers led to more emphasis being placed on the use of leguminous crops to supply nitrogen to the succeeding crops. The amount of residual nitrogen supplied to soil through the use of legumes is being received scant attention in developing countries. In Jaffna, sunn hemp and *Tephrosia purpurea* are two herbaceous legumes grown in paddy soils and market garden soils to improve the soil fertility. The former is systematically rotated with the main cash crops while, the latter is allowed to establish naturally. Inorder to assess the use of these two green manures, systematic studies have been carried out regarding the nature and pattern of nodulation under the local environmental conditions. Effect of inoculation and combined nitrogen application also studied to get the maximum benefit of these plants.

MATERIALS AND METHODS

Polythene bags (30 x 12 cm) were filled with the soil sample collected from garden soil (red loam) at Thirunelvely. Freshly harvested seeds of sunn hemp and surface sterilized seeds of *Tephrosia* were sown in bags. Seedlings were thinned to two per bag after germination. Ten samples from each plant were uprooted at weekly intervals and the pattern of nodulation, nodule weight and dry weight of shoot were recorded.

Seed inoculation

Seeds of *Crotolaria juncea* and *Tephrosia purpurea* were soaked in their respective rhizobial inoculum (10^7 cells / ml) for half an hour with frequent agitation inorder to produce evenly coated seeds. Seeds were then allowed to air dry on a clean paper protected from the direct exposure to sun, and sown in the soil in polythene bags. During maximum nodulation period, thirty plants were uprooted carefully and assessed for the nodule formation and dry weight production. Seeds soaked in sterile water for half an hour were used as control for the nodule assessment.

In a field trial, 150 g of sunn hemp seeds inoculated with 75 ml (10^7 cells/ml) of its respective rhizobia were broadcasted in plots of 3m x 3m for seed treatment. Uninoculated seeds of the same amount were

maintained as control. Nitrogen content of the plant, nodule and soil were estimated during the 4th, 6th and 8th weeks after the germination of seeds.

Combined nitrogen

Effect of combined nitrogen in the forms of nitrate (KNO_3), ammonium [$(NH_4)_2\ SO_4$] and urea was tested in the seedlings of sunn hemp grown on seedling agar medium in test tubes. Inorganic nitrogen was incorporated with seedling agar medium as 50, 100 and 150ppm. The medium was prepared in slants. Healthy seedlings of *Crotolaria juncea* grown on water agar plates were transferred to the slants three days after germination. Inoculation was done after one week of the establishment of the seedlings on the slants. Two ml the liquid inoculum (10^8 cells/ml) was added to each slant and the required amount of distilled water was added throughout the experimental period to maintain the seedlings on slants. Six replicates were maintained and the nodulating ability was assessed periodically for five weeks after the inoculation.

RESULTS AND DISCUSSION

Nodulation was observed after one week and three weeks of the germination of seeds of sunn hemp and *Tephrosia* respectively. Nodules were first formed on the main axis of the root system of sunn hemp and when the plants became older, they were observed in the lateral roots, while, in *Tephrosia*, almost all nodules were located in the lateral roots during all growth stages. In both cases the nodules started as small round whitish structures and at maturity, they were corolloid. The colour of the nodules changed to pink during the 4th and 6th week of the growth period of *Crotolaria juncea* and *Tephrosia purpurea* respectively, indicating the initiation of active nitrogen fixing period. The active period was continued further to 2 – 3 weeks in sunn hemp and 6 – 7 weeks in *Tephrosia*. Maximum nodulation was (220/10 plants) observed in sunn hemp after 6 weeks at 50% flowering stage while, in *Tephrosia* it was occurred after 12 – 13 weeks at the pod development stage (31 nodules/10 plants). The size and weight of nodules during maximum nodulation period also vary in both plants. The mean dry weight of nodules per 10 plants was 0.6 g and 0.22 g and were 9 – 10 mm and 8 – 12 mm size at full growth for sunn hemp and *Tephrosia* respectively. Nodules in the fully mature stage turned to brown due to

the conversion of haemoglobin to meth – haemoglobin. After passing this stage, nodules were detached from the root system of the plants. This was occurred by 10 weeks in *Crotolaria* and 17th week in *Tephrosia*. Table 1 shows the pattern of nodulation of the above two plants. Since sunn hemp has a short life cycle, the active period for fixation is also limited to a very short period compared to *Tephrosia purpurea*. However, size, weight and number of nodules in sunn hemp were high compared to *Tephrosia* during the maximum nodulation period.

Table 1. Number of nodules produced on the root system of *Crotolaria juncea* & *Tephrosia purpurea* during different growth periods.

Age of plant (Weeks)	Number of nodules / 10 plants	
	Sunn hemp	*Tephrosia* sp.
1	–	–
2	35	–
3	72	–
4	97	7
5	129	5
6	220	6
7	224	17
8	186	27
9	163	25
10	130	29
11	–	26
12	–	31
13	–	23
14	–	28
15	–	–
16	–	–
17	–	23

Changes in dry weight of plants and nodules of the two plants are shown in Figure 1. Positive correlation was seen between the dry matter production and nodules development in both plants. Linear correlation

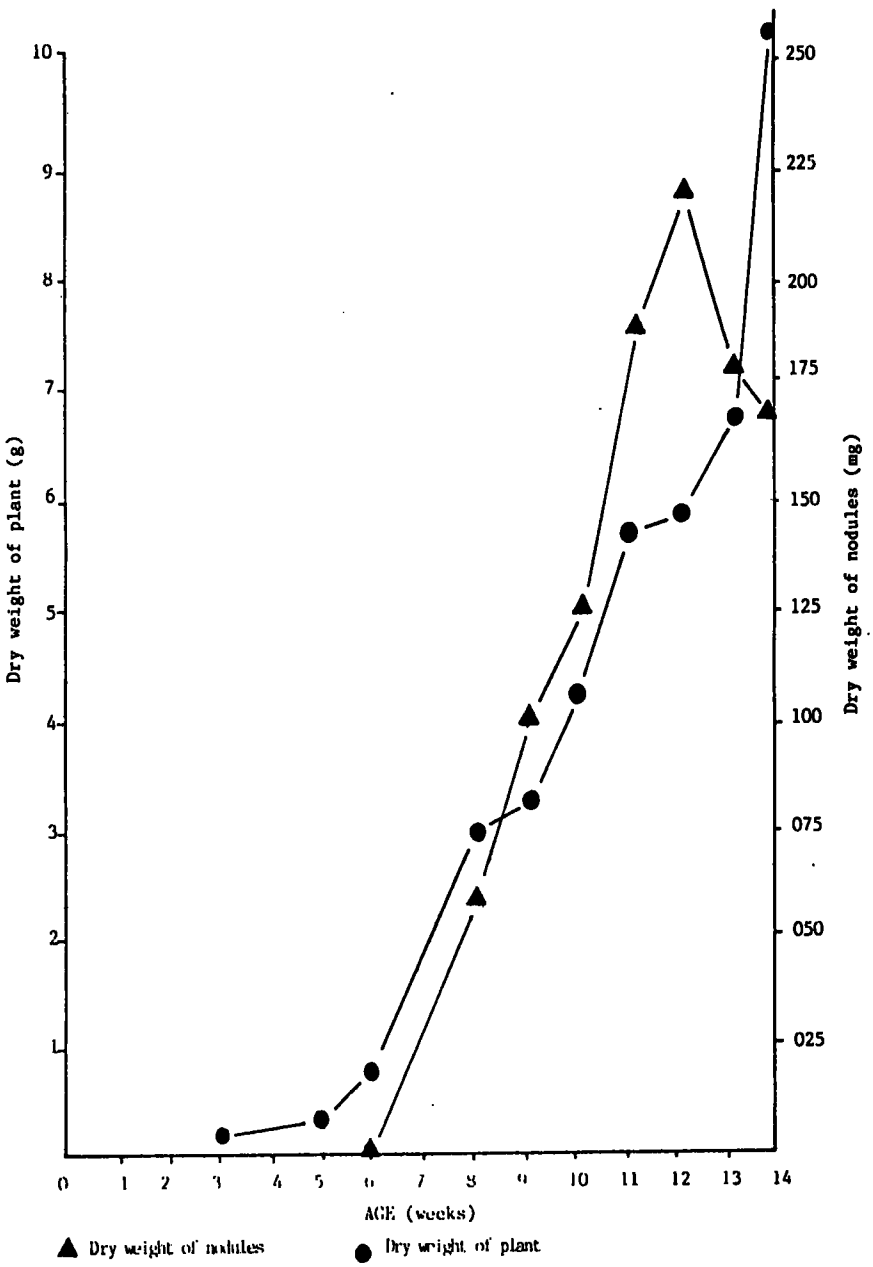

Fig.1. Changes in dry weight of plant and nodules with age of the plant.

coefficient was found to be 0.708 for sunn hemp and 0.5469 for *Tephrosia* indicating that a high correlation was shown in sunn hemp compared to the other. Maximum dry matter was produced during the maximum nodulation period as the nitrogen is actively fixed during this period and translocated to shoot resulting high dry matter content.

Dry weight of plant tops and nodules of seed inoculation significantly differed from those of the control ones in both plants in pot experiment. Pahalwan and Tripathi (1984) reported that the nodulation and accumulation of nitrogen increased due to seed inoculation. At maximum nodulation period, seed inoculation improved nodule formation by two fold in sunn hemp and three fold in *Tephrosia*. When the seeds were inoculated with their respective rhizobia, the nodules were formed in early stages of growth and their number and weight also increased than that of the uninoculated control. Table 2 shows the effect of seed inoculation on these plants.

Increase in nitrogen content of plants, nodule and soil by seed inoculation led to more nitrogen accumulation in soil when sunn hemp was grown under field conditions (Table 3). Similar results were also obtained by Pal & Saxena (1976). Seed inoculation resulted in increase seedling vigour and enhanced green manure production.

Table 2. Effect of seed inoculation on growth and nodulation of *Crotolaria juncea* & *Tephrosia purpurea* during the initial active period of nitrogen fixation.

	Sunn hemp		Tephrosia sp.	
	Seed inoculation	Control	Seed inoculation	Control
Mean dry weight of plant (g)	1.61 ±0.12	0.88 ±0.11	0.72	0.70
Mean fresh weight of nodules (g)/plant	0.22 ±0.02	0.12 ±0.01	0.15	0.061
Mean height of Shoot (cm)	43.37 ±4.33	29.96 ±1.03	22.48	19.81

Nitrogen accumulation rate during the 6th week of the growth period of sunn hemp was found to be 34.33 kg/ha & 23.27 kg/ha in seed treated and control respectively. Thus an increase of 11 kg/ha can be achieved by inoculating the seed with its respective active rhizobial strain.

Table 3. Percentage nitrogen contribution to soil and the amount if nitrogen contribution by shoots and nodules of *Crotolaria juncea* at different growth periods when inoculated by Rhizobium sp.

Age of the plant (weeks)		Amount of nitrogen contribution g / 100 plants	
	shoot	Seed inoculation	Control
	shoot	1.2421	0.9324
2	nodules	0.0181	0.0030
	soil (%)	0.166	0.162
	shoot	10.9917	5.5134
4	nodules	0.0947	0.0400
	soils (%)	0.105	0.091
	shoot	30.9077	23.5900
6	nodules	0.0591	0.0287
	soils (%)	0.168	0.142
	shoot	111.0882	95.2091
8	nodules	0.0018	0.0005
	soil (%)	0.098	0.999

When combined nitrogen was incorporated in seedling agar medium, 50ppm of nitrate and ammonium nitrogen & 100ppm of nitrate nitrogen did not affect nodule formation compared to that of the control, while, 150ppm of all forms of nitrogen and all concentrations of urea affected nodulation. Urea severely affected nodule formation in *Crotolaria* seedlings. Only a single nodule was observed in the root system of the seedling incorporated with 50ppm of urea. Investigation made by

Chaillou *et al.* (1986) agreed with our observation. They reported that ammonium nitrogen nutrition is known to depress plant growth compared with nitrate nitrogen nutrition. This was due to more energy requirement needed for the synthesis and accumulation of aminoacids for the plants grown in NH_4^+ fed medium.

Table 4 shows that the nodule formation was severely inhibited under the influence of combined nitrogen in the form of urea under the field conditions. Eventhough the initial dry matter production was not affected in urea treatment, later stages showed a marked decrease in the dry matter production. The data showed that during the early stages of plant development, dependence solely on nitrogen fixation is an expensive process compared with the nitrogen supplied plants. At mature stages, symbiotic activity contributed more nitrogen and thereby they showed more vigorous growth than the plants which had supplied with nitrogen where nodulation was severely affected.

Table 4. Effect of combined nitrogen (urea) on the growth and nodulation of *Crotolaria juncea* at different growth periods under field conditions.

	Urea	Control
Fresh weight of nodules (g) / plant		
4th week	0.008 a	0.023 b
6th week	0.018 a	0.170 b
8th week	0.014 a	0.054 b
Dry weight of plant (g)		
4th week	0.93 a	0.96 a
6th week	1.42 a	2.45 b
8th week	2.02 a	5.25 b

n = 20. Values denoted by different letters in each row are significantly different.

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