EFFECTS OF SEASONAL VARIATION AND NITROGEN TREATMENTS ON NODULATION AND NITROGEN FIXATION BEHAVIOUR IN *PONGAMIA PINNATA*

S. P. Chaukiyal, S. K. Sheel* & T. C. Pokhriyal

Plant Physiology, Botany Division, Forest Research Institute, Dehra Dun - 248 006, India

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CHAUKIYAL, S. P., SHEEL, S. K. & POKHRIYAL, T. C. 2000. Effects of seasonal variation and nitrogen treatments on nodulation and nitrogen fixation behaviour in *Pongamia pinnata*. Nodulation behaviour and nitrogenase activity were studied in relation to nitrogen treatment (i.e. 0, 40, 80, 100 kg N ha^{-1}) and seasonal variations in *Pongamia pinnata* seedlings. A marked increase in the nodule biomass per plant and nitrogenase activity was observed in the rainy season followed by winter and summer. Two peaks, one small in early summer and another large during the rainy season, were observed in nitrogenase (g^{-1} fresh wt. h^{-1} and pl^{-1} h^{-1}) activity. However,no definite trend was observed due to the different nitrogen treatments on the nodule biomass and nitrogenase activity, but 40 kgN ha^{-1} was observed to be more effective than the others.

Key words: Nodule biomass - nitrogenase activity - seasonal variation - nitrogen treatments - *Pongamia pinnata*

CHAUKIYAL, S.P., SHEEL, S. K. & POKHRIYAL, T. C. 2000. Kesan perubahan musim dan rawatan nitrogen terhadap tingkah laku pembintilan dan pengikatan nitrogen dalam *Pongamia pinnata*. Kajian mengenai kaitan tingkah laku pembintilan dan aktiviti nitrogenase dengan rawatan nitrogen (iaitu 0, 40, 80, 100 kg N ha^{-1}) dan perubahan musim dalam biji benih *Pongamia pinnata.* Pertambahan yang ditandakan dalam nodul biojisim setiap pokok dan aktiviti nitrogenase dicerap pada musim hujan diikuti musim sejuk dan musim panas. Dua perbezaan yang nyata, satu kecil pada awal musim panas dan satu lagi besar pada musim hujan dicerap dalam aktiviti nitrogenase (g^{-1} berat segar h^{-1} dan setiap pokok h^{-1}). Bagaimanapun, tiada trend khusus yang dicerap akibat daripada perbezaan rawatan nitrogen yang berbeza terhadap biojisim nodul dan aktiviti nitrogenase, tetapi 40 kg N ha^{-1} didapati lebih aktif daripada yang lain.

Introduction

Low fertility is an important problem in establishing vegetation on degraded lands. Since nitrogen is generally deficient in these lands, afforestation can be accomplished using nitrogen fixing species, which can utilise atmospheric and soil nitrogen more efficiently to meet their requirements for proper growth and

*Statistics Section, F.R.I., Dehra Dun, India.

development. Intensive harvesting of wood will always increase nitrogen and organic matter losses from forest sites. If the fertility of such soil is to be maintained, it must be replenished either by fresh supply of fertilisers, which are costly and scarce, especially for developing countries like India, or alternatively, by planting such tree species which enrich the soil nitrogen status regularly. Furthermore, fertiliser nitrogen is lost rapidly due to leaching, runoff and volatilisation, causing water and atmospheric pollution (Bohlool 1990).

It is well established that excessive nitrogen application reduces root hair infection (Munns 1968, Dazzo & Brill 1978), nodule number and weight (Dart & Mercer 1965, Summerfield *et al.* 1977) and total amount and form of nitrogenous compounds (Dart & Wildon, 1970). On the other hand, Sehgal *et al.* (1992 a, b) reported an increase in the various growth parameters and nitrogenase activity and rhizosphere microflora due to lower doses of nitrogen treatments in *Enterolobium timbouva.*

The amount of N taken up by field crops varies during the growing season (Goh & Haynes 1986, Mengel & Kirkby 1987). Initially the emerging crop has a small demand for N but the requirement increases rapidly as the plant grows. However, Mangual-Crespo *et al.* (1987) and Ssali and Keya (1982) reported that the addition of a small amount of N stimulates nodulation and increases yield. In many situations soil N cannot match plant requirements and during this phase, fertilisers may be required to maximise crop growth rates. Therefore, in the present paper an attempt was made to study the effects of seasonal variations and nitrogen treatments on the nodulation behaviour and nitrogenase activity in *Pongamia pinnata* Pierre, a very common species being used for agro-social forestry plantation programmes.

Materials and methods

Matured pods of *P. pinnata* were collected from healthy trees distributed around the Forest Research Institute Campus at Dehra Dun, India. Seeds were separated from the pods and sown in germination boxes. Healthy and uniform seedlings were transplanted into earthern (30 cm diameter) pots filled with 5 kg mixture of soil:sand:farmyard manure (2:1:1 ratio) and one plant was maintained in each pot. The plants were kept in the open under natural conditions. Seedlings were divided into four groups containing 50 plants each. Four nitrogen treatments (i.e. 0, 40, 80 and 100 kg N ha^{-1}) were applied in two equal split doses in the months of July and September respectively. Irrigation and other cultural practices were carried out uniformly in all treatments as and when required. At monthly intervals (from November 1995 to October 1996), three plants in each treatment were harvested and nodulated roots were excavated with the help of regulated water pressure, so that damage could be minimised.

Nitrogenase activity (E.C.1.7.99.2) was estimated monthly by the acetylene-ethylene reduction assay as described earlier by Hardy *et al.* (1968). For seasonal variation studies, observations from November to February, March to June and July to October were pooled and considered as winter, summer and rainy seasons respectively. The data recorded under each parameter were analysed by two-way ANOVA to see the significance of differences between nitrogen levels, months and seasonal variations.

Results

A progressive increase in the plant height was observed from May to October in all nitrogen treatments. Maximum and minimum shoot heights were recorded in October and April respectively (Figure 1a). Overall, maximum plant height was recorded in N-40 as compared to N-80 and N-100 treatments. Significantly higher values were observed during the rainy season than during winter and summer. No definite pattern in plant height was observed due to different nitrogen treatments (Table 1a).

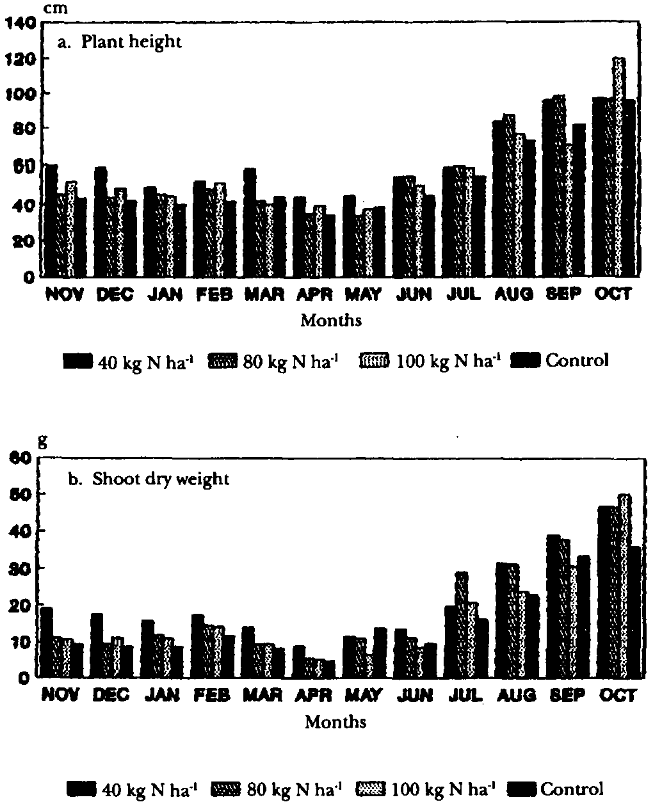

Figure 1 (a & b). Effect of different N treatments on plant height and shoot dry weight per plant in *Pongamia pinnata*

Table 1. Changes in plant height, nodule number and dry weight per plant as affected by different nitrogen treatments and seasons in *Pongamia pinnata* seedlings

Characters	Season	Bar diagram				Level of significance
(a) Height (cm)	Winter	N-40 54.75	N-100 48.96	N-80 45.79	N-0 38.67	**
	Summer	N-40 50.08	N-100 41.43	N-80 41.38	N-0 39.42	ns
	Rainy	N-80 85.67	N-40 84.08	N-100 81.50	N-0 76.42	ns
	Pooled treat	N-40 62.97	N-80 57.61	N-100 57.43	N-0 51.50	**
	Pooled season	Rainy 81.92	Winter 47.04	Summer 43.18		***
(b) Nodule dry weight (g)	Winter	N-40 1.78	N-80 1.58	N-100 1.53	N-0 1.43	ns
	Summer	N-0 1.38	N-40 1.32	N-80 1.15	N-100 1.14	ns
	Rainy	N-0 2.50	N-80 2.46	N-40 2.45	N-100 2.08	ns
	Pooled treat	N-40 1.79	N-0 1.70	N-80 1.64	N-100 1.54	ns
	Pooled season	Rainy > 2.37	Winter > 1.57	Summer 1.24		***
(c) Nodule number	Winter	N-40 12.0	N-80 11.0	N-100 10.0	N-0 9.0	ns
	Summer	N-40 7.0	N-0 7.0	N-80 7.0	N-100 6.0	ns
	Rainy	N-40 22.0	N-0 18.0	N-80 17.0	N-100 17.0	ns
	Pooled treat	N-40 13.79	N-80 11.56	N-0 11.54	N-100 11.12	***
	Pooled season	Rainy > 19.0	Winter > 11.0	Summer 7.0		*

* significant at 5% level.
** significant at 1% level.
*** significant at .1% level.
ns = not significant.

Table 2. Effect of seasonal changes on nitrogenase activity and shoot dry weight as affected by different nitrogen treatments in *P. pinnata* seedlings

Characters	Season	Bar diagram				Level of significance
(a) C_2H_2 reduced g^{-1} fr. wt. h^{-1}	Winter	N-100 6.07	N-80 4.59	N-0 4.50	N-40 4.20	ns
	Summer	N-80 5.63	N-0 5.58	N-40 4.24	N-100 3.11	ns
	Rainy	N-40 151.44	N-0 148.22	N-80 141.16	N-100 86.43	ns
	Pooled treat	N-0 15.49	N-80 15.39	N-40 13.92	N-100 11.78	ns
	Pooled season	Rainy 128.644	Summer 4.7884	Winter 4.5129		***
(b) C_2H_2 reduced pl^{-1} h^{-1}	Winter	N-100 16.553	N-40 13.217	N-80 13.171	N-100 9.928	ns
	Summer	N-0 6.644	N-40 5.932	N-80 3.399	N-100 2.854	*
	Rainy	N-0 941.76	N-40 911.95	N-80 652.97	N-100 359.38	ns
	Pooled treat	N-40 1.6181	N-0 1.5978	N-80 1.4886	N-100 1.410	ns
	Pooled season	Rainy > 2.8261	Winter > 1.1141	Summer 0.6457		***
(c) Shoot dry weight (g)	Winter	N-40 3.33	N-100 2.910	N-80 2.854	N-0 2.656	**
	Summer	N-40 2.900	N-80 2.531	N-0 2.501	N-100 2.335	*
	Rainy	N-80 4.655	N-40 4.345	N-100 4.092	N-0 4.043	ns
	Pooled treat	N-40 17.64	N-80 14.87	N-100 12.84	N-0 12.52	**
	Pooled season	Rainy 28.41	Winter 11.89	Summer 8.71		***

* significant at 5% level.
** significant at 1% level.
*** significant at .1% level.
ns = not significant.

The minimum shoot dry weights were observed in April (N-0, 4.83; N-40, 8.97; N-80, 5.47; N-100, 5.13) and the maximum in October (N-0,35.85; N-40, 46.75; N-80, 46.71; N-100, 50.14 g). Shoot biomass remained almost constant during winter followed by a gradual increase up to the rainy season (Figure 1b). The season-wise analysis indicated that the variations between N-40 and other treatments during winter and summer seasons were significant. The rainy season was observed to be the best time for shoot biomass development compared to summer and winter (Table 2c).

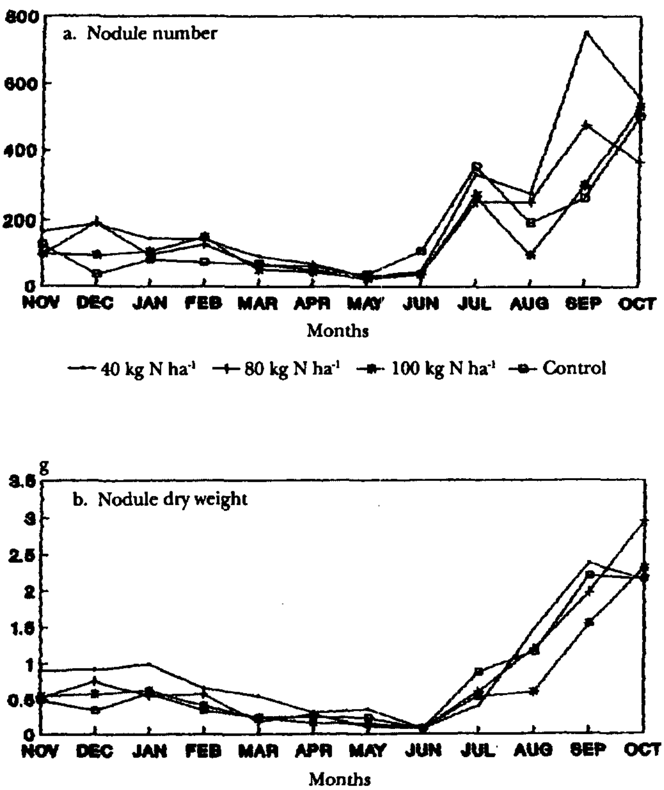

Figure 2 (a & b). Effect of different N treatments on nodule number and nodule biomass per plant in *P. pinnata*

An increase in nodule number per plant was observed from late summer to the late rainy season (June to October) followed by a sharp decrease during winter (Figure 2a). Minimum numbers of nodules per plant were recorded during summer. Maximum values, i.e. 503 in N-0 and 533 in N-100 (in October), 751 in N-40 and 477 in N-80 (in September); and minimum, i.e. 36 in N-0, 28 in N-40, 31 in N-80 and 24 in N-100 (all in May); were observed.

Nitrogen treatments and seasonal variation effects on the nodule formation were observed to be non-significant. However, on the basis of annual averages, significantly higher values were observed in N-40 as compared to N-0, N-80 and N-100. The numbers of nodules per plant were observed to be significantly higher during the rainy season followed by winter and summer (Table 1c).

An increase in the dry weight of nodule biomass was recorded from July to October followed by a gradual decrease in the subsequent months in all treatments. Maximum dry weights were recorded in N-80 (2.95 g), N-100 (2.30 g), N-0 (2.15 g) in the month of October and N-40 (2.38 g) in September. However, minimum nodule weights were recorded in June in N-0 (0.08 g), N-40(0.07 g), N-80(0.07 g) and N-100(0.07 g)(Figure 2b).

New nodule initiation started from June onwards and continuously increased up to the end of the rainy season. On the basis of annual average values, the best performance was observed in N-40 treatment. No significant differences were noticed in nodule dry weight in different seasons. However, maximum values were recorded during the rainy season followed by a decrease in the winter and summer seasons (Table 1b).

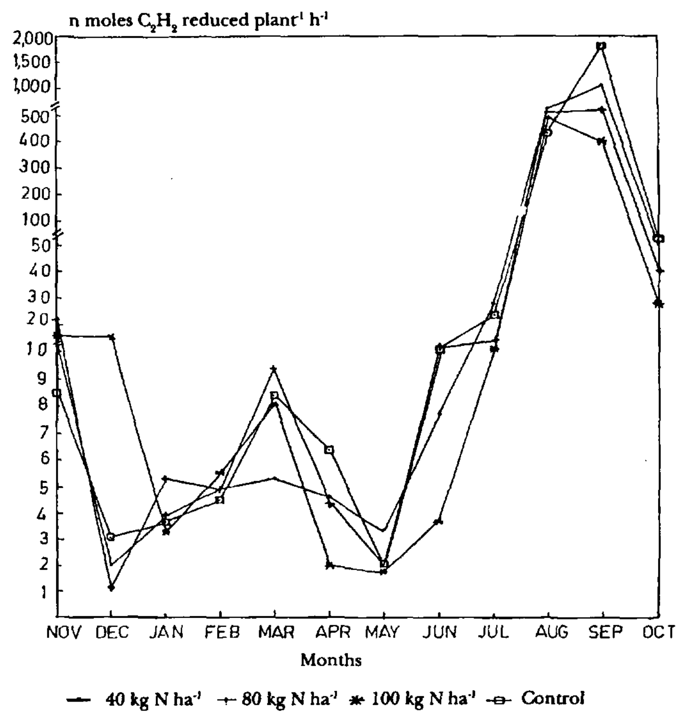

Figure 3. Changes in nitrogenase (n moles C_2H_2 reduced g^{-1} fresh wt. h^{-1}) activitiy as influenced by nitrogen treatments and seasonal variations in *P. pinnata*

In nitrogenase (g^{-1} fresh wt. h^{-1} and $plant^{-1}$ h^{-1}) activity, two peaks (one small and broad in the early summer and another large and sharp in the rainy season) were observed. Minimum nitrogenase activities as influenced by N treatments were observed in May (i.e. N-100, 1.69 n moles g^{-1} fresh wt. h^{-1} and 0.77 n moles $plant^{-1}$ h^{-1}) and maximum in September (N-0, 1883.2 n moles g^{-1} fresh wt. h^{-1} and 16814.0 n moles $plant^{-1}$ h^{-1}) (Figures 3 & 4).

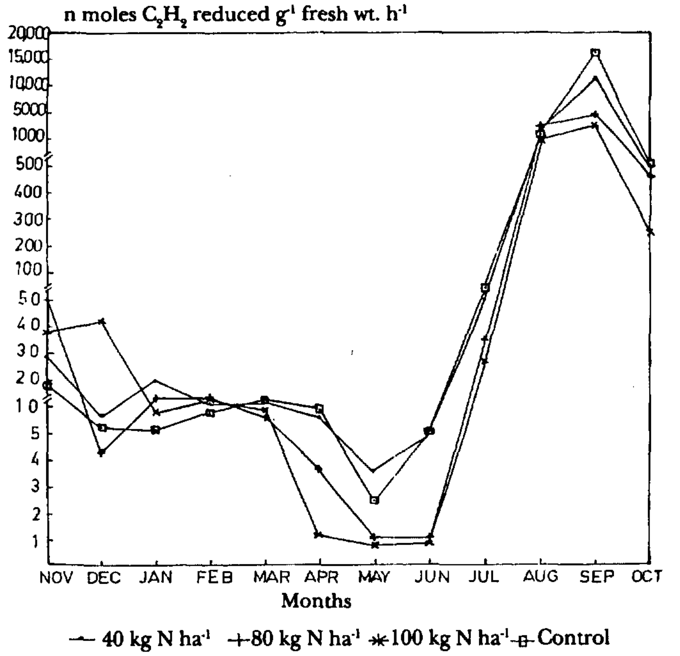

Figure 4. Changes in total nitrogenase (n moles C_2H_2 reduced pl^{-1} h^{-1}) activity as influenced by nitrogen treatments and seasonal variations in *P. pinnata*

No significant differences due to nitrogen fertiliser application on the nodular nitrogenase activity were observed. However, nitrogen treatments do not differ significantly among themselves except in summer. Maximum nitrogenase activity was observed during the rainy season followed by winter and summer (Tables 2a & b).

Discussion

The inhibitory effect of combined nitrogen on rhizobial infection, nodule development and nitrogen fixation by *Rhizobium*-legume symbiosis has been well documented by Sprent (1976), Munns (1977), Bisseling *et al.* (1978), Dazzo *et al.* (1981), and Gibson and Jordan (1983). In *P. pinnata* maximum increase in the nodule biomass and nitrogenase activity was observed at N-40 treatment and thereafter it decreased with increase in the nitrogen doses. Almost similar observations were earlier reported by Sehgal *et al.* (1992 a,b) in *Enterolobium timbouva* and by Johnson and Bongarten (1991) in *Robinia pseudoacacia*.

Initiation of fresh nodules in *P. pinnata* took place during summer when plants had to sustain adverse environmental conditions and ultimately resulted in a marked suppression in nodule formation and nitrogenase activity during this period. Pate (1961) and Bergersen *et al.* (1965) also reported the initiation of new nodules with the start of the new growing season. Slogar *et al.* (1975) observed a close correlation between the acetylene reduction rate of field grown soybean with air temperature and other environmental variables. The nitrogenase activity in the newly formed nodules remained initially low due to the prevailing atmospheric conditions as already reported by Kao and Boersma (1971), Sprent (1972), and Engine and Sprent (1973). Similarly, Hardy *et al.* (1968) and Waughman (1977) also observed variation in acetylene reduction with the species and temperature conditions. After the completion of the stress period and with the forthcoming rainy season, the atmospheric conditions would be more favourable to overall growth and other metabolic activities. These results support the findings reported by Pokhriyal *et al.* (1990, 1991) in *Acacia catechu* and *Dalbergia sissoo.*

In the present study in *P. pinnata,* N treatment effect and seasonal behaviour interactions were non-significant, but significantly higher nodule numbers per plant were observed in the rainy season followed by winter and summer. Palmgren *et al.* (1985) also reported no significant effect on nodule biomass due to low N fertiliser doses, whereas higher N doses either inhibited nodules or killed alder clones. A marked increase in nitrogenase activity was observed during the rainy season followed by winter and summer. However, no definite trend in nitrogenase activity was observed due to the different N treatments and seasonal behaviour in this study. Maximum nitrogenase activity was observed in the N-40 treatment followed by a decrease with increasing N doses in the summer and rainy seasons (Figure 5). Nitrogen supply at initial stages may result in rapid initial growth so that after the depletion of nitrogen, the plant is capable of nitrogen fixation at a higher rate than the normal (Mahon & Child 1979). Reduction in nodule growth and nitrogenase activity due to high doses of nitrogen application in *P. pinnata* may be due to inhibition in the root hair infection as already explained earlier by Virtanen *et al.* (1955), Munns (1968), Gibson and Jordon (1983), Dazzo and Brill, (1978), Minchin *et al.* (1981), Varade and Pokhriyal (1985), Sawhney *et al.* (1985, 1991) and Sehgal *et al.* (1992a,b) in various nitrogen fixing tree species. The hypothesis of Johnson and Bongarten (1991) also supports the view that total acetylene reduction and nodule biomass in response to low concentrations of NO_3^- result from increases in plant growth due to N fertilisation; decreases in nodule biomass observed with higher N additions are due to diminished biomass allocation to nodules. The concentrations of soil N that yield maximum total acetylene reduction and nodule biomass will depend on species, plant age and environmental conditions. However, the reason for the reverse trend during winter season may probably be due to low temperature, when most physiological activities remain low and the competition for photosynthates and recycling of metabolites could only be available to the nodules with higher N treatments and less nitrogen fixation activity in this case. The other reason may be due to senescence of nodules during this stage when nitrogen fixing bacteroid growth and multiplication are hampered and host cells degenerate (Kneen *et al.* 1990). Chaukiyal (1994) observed that the initial yellow-pink colour of the nodules turned dark brownish with age. That the change in colour from pink to greenish-brown is a sure sign of nodule senescence was demonstrated by Pfeiffer *et al.* (1983). Our observations support the view of Sawhney *et al.* (1991) that low nitrogenase activity during winter in pigeon pea may be due to the dark textured nodules accumulating five times more nitrite than the pinkish nodules, and that in the presence of nitrite their rate of C_2H_2 reduction was also depressed to a far greater extent. However, more experimental work is needed in this direction.

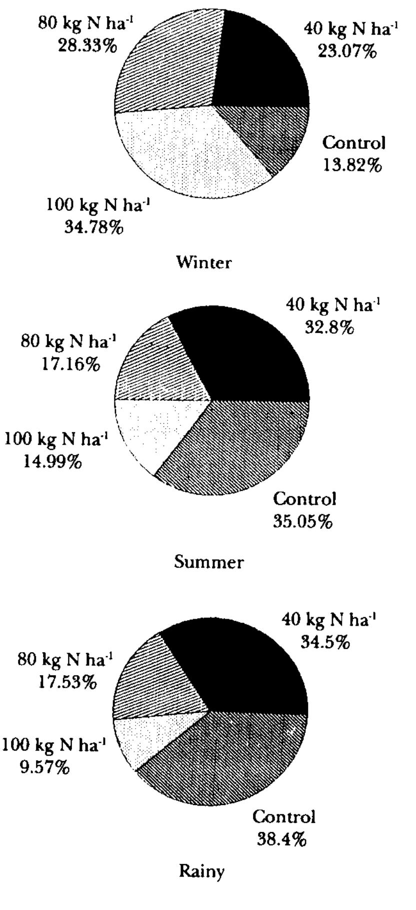

Figure 5. Effect of seasonal variations and nitrogen treatments on nitrogenase activity in *P. pinnata*

The responsiveness of crops to N fertiliser is extremely variable and depends on species, soil and environmental conditions (Williams & Haynes 1995). Fertiliser recommendation would be easier if soil N availability throughout the growing season can be predicted. To maximise the utility of nitrogenous fertilisers in leguminous tree crops, nitrogen should be ideally applied in split doses depending upon the requirements of the plants as confirmed in the case of *P. pinnata.* The major aim of tree growers in applying N fertilisers is to obtain increased plant yields with economic returns from the additional expense of applying fertiliser. The application of excessive amounts of fertiliser N is undesirable as this leads to an economic loss of N and environment problems.

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