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EFFECT OF DIFFERENT LEVELS OF NITROGEN AND PHOSPHORUS ON ATMOSPHERIC NITROGEN FIXATION AND COMPUTATION BY VARIOUS METHODS IN FOUR SPECIES OF *Acacia*

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Abstract

Greenhouse study was carried out on sandy clay loam soil (Typic Haplustalf) of Coimbatore. Tamil Nadu to determine the amount of nitrogen fixed from the atmosphere by different species of *Acacia* by various methods by the application of nitrogenous and phosphatic fertilizers in combination with N_2 fixing organisms. The accuracy of various methods of N_2 fixation were evaluated and the results predicted that 'A' value technique was comparatively best suitable method for tree crops closely followed by dilution method. Among the four *Acacia* species. *A. nilotica* derived maximum amount of nitrogen from the atmosphere followed by *A. planifrons* due to the application of ^{15}N urea (75%)+ SSP (100%)+ Rhizobium + VAM.

KEY WORDS: Atmospheric nitrogen fixation, Different methods, *Acacia* species

The tree legumes do fix nitrogen through symbiotic association with nitrogen fixing organisms, usually nodule forming bacteria of the genus *Rhizobium*. At present, more than 640 trees species are known to fix nitrogen (Halliday and Nakao, 1982). Besides, they play a very important role in soil conservation, improvement of soil fertility through nitrogen fixation, rapid nutrient cycling, addition of organic matter and stabilization of soil by their deep root system, thus make the soil more fertile by conserving soil moisture too.

Vesicular - arbuscular mycorrhiza also improves growth, nodulation and nitrogen fixation in *Rhizobium* - legume association by supplying adequate phosphorus to roots.

An accurate method of measuring N_2 fixation is essential for evaluation of usefulness of different N_2 fixing plants. Work on these aspects are scanty and research focus on nodulation and nitrogen fixation with combined application of microbial inoculants and inorganic fertilisers and quantification of nitrogen fixation using ^{15}N labelling techniques in forest trees would open up new vistas for formulating strategies for fertilization to forest trees.

MATERIALS AND METHODS

Bulk soil samples were collected from physically degraded wastelands of Coimbatore district of Tamil Nadu which belongs to sandy clay loam, mixed (non-acid), isohyperthermic, Typic Haplustalf (Sommayanur series).

The treatment combinations consisting of N and P management practices using ^{15}N urea alone and single super phosphate (SSP) alone or in combination with bio - inoculants *viz*., *Rhizobium* and VAM. The N and P treatments included different levels of recommended fertiliser dose (9:18:9 kg N, P_2O_5 and K_2O / 1,00,000 seedlings). The experiment was laid out in completely randomised design (CRD) replicated three times separately for four *Acacia* species. Flat sized polybags (150-200 gauge thickness) of size 5" X 7" were filled with the pot mixture (Soil : FYM = 3:1 ratio) leaving one centimeter at the top of the polybag. The seeds of the selected *Acacia* species were scarified in Conc. H_2SO_4 for 10 minutes, thoroughly rinsed with sterile water and dried under shade. The treated seeds were sown in the polybags and the seedlings were raised for six months.

The labelled urea (45.79%N) containing 5.00028 per cent atom excess was used as ^{15}N

source and SSP was used as phosphorus source. *Rhizobium leguminosarum* in peat medium and *Glomus fasciulatum* (VAM) in vermiculite medium were used as bio-softwares imposed by seedling dipping for half an hour and soil application (mixing with pot mixture) respectively.

The fraction and amount of labelled N absorbed by the plant after six months of seedling growth are calculated using the following equations:

(i) Fraction of N derived from fertiliser (fNdff)

$$= \frac{\%^{15}\text{ N atom excess in plant}}{\%^{15}\text{ N atom excess in fertiliser}}$$

(ii) Percent N derived from fertiliser (% Ndff)

$$= \text{fNdff} \times 100$$

(iii) Amount of labelled N recovered by plants (mg Ndff)

$$= \frac{\%\text{Ndff} \times \text{N uptake / plant (mg)}}{10^{2}}$$

(iv) N use efficiency (NUE %)

$$= \frac{\text{Total N uptake} \times 100}{\text{Fertilizer N applied}}$$

Using the above parameters, the amount of N_2 derived from atmosphere was computed by different methods as follows.

(i) Based on N derived from soil

$$\%\text{ Ndfs} = \frac{\%\ ^{15}\text{N atom excess (plant)}}{\%\ ^{15}\text{N atom excess (soil)}} \times 100$$

$$\%\text{ Ndfa} = 100 - \%\text{ Ndfs}$$

(ii) Based on N derived from fertilisers (dilution method)

$$\%\text{ Ndfa} = \frac{\%^{15}\text{N atom excess in fixing plant}}{\%\ ^{15}\text{N atom excess in non - fixing plant}} \times 100$$

(iii) 'A' value technique

$$\text{ANF or AF} = \frac{\%\text{ Ndfs}}{\%\text{ Ndff}} \times \text{Amount of fertiliser N applied}$$

Amount of N_2 fixed = (ANF -AF) RF

Where RF = NUE

Cassia siamea was used as non - fixing reference plant. The amount of N_2 fixed after arriving % Ndfa was computed as.

mg Ndfa = % Ndfa x N uptake of fixing plant (mg)

RESULTS AND DISCUSSION

Effect of nitrogen on N_2 fixation

The four species of *Acacia* exhibited a similar trend with reference to nitrogen fixation as judged by four methods. The triple combination of

Table 1. Effect of various levels of inorganic and biofertilizers on nitrogen derived from fertilisers (% Ndff) and N use efficiency

Treatments	*A. nilotica*		*A. leucophloea*		*A. holoceresia*		*A. planifrons*	
	% Ndff	NUE (%)	% Ndff	NUE (%)	% Ndff	NUE (%)	% Ndff	NUE (%)
Control	2.85	12.5	2.30	6.18	1.85	4.40	2.65	8.27
15 N Urea (50%)'R	2.49	36.2	1.93	24.5	1.48	17.6	2.28	30.4
15 N Urea (75%)'R	2.10	43.9	1.56	29.8	1.11	20.1	1.91	37.2
15 N Urea (100%)'R	2.59	29.6	2.03	19.2	1.58	13.4	2.38	23.7
15 N Urea (50%)'R+V	2.21	40.2	1.66	29.5	1.22	20.4	2.01	36.9
15 N Urea (75%)'R+V	1.90	40.3	1.36	26.6	0.91	16.9	1.71	34.4
15 N Urea (100%)'R+V	2.50	35.0	1.95	24.3	1.50	16.8	2.30	28.9
SSP (50%)+V	2.19	44.3	1.65	30.6	1.20	30.9	2.00	37.8
SSP (75%)+V	2.23	44.4	1.69	32.0	1.22	20.9	2.02	37.9
SSP (100%)+V	2.52	30.8	1.96	21.0	1.54	14.3	2.32	25.2
CD (0.05)	0.05	1.31	0.08	1.16	0.07	2.25	0.08	2.27

optimum mineral N (75%) with two bio-inoculants ranked top in the nitrogen fixing potential registering the values of 176, 146, 127 and 150mg/seedling by *Acacia nilotica, Acacia leucophloea, Acacia holoceresia* and by *Acacia planifrons* (Table. 1). Again the incorporation of higher N dose depressed this potential.

The dual inoculation with *Rhizobium* and VAM helped in the higher accumulation of ureides, allantoin and allantoic acid in cell sap of these nodulated leguminous tree saplings. The essentiality and importance of N nutrition conjoint with bio-fertilizers and its bearing nodulation and nitrogen fixation is well reflected by the close correlation (*A. nilotica*, r=0.82**; *A. leucophloea*, r=0.66**;

A. holoceresia, r=0.64**; *A. planifrons*,r=0.63**)

Effect of phosphorus on N_2 fixation

Application of phosphorus significantly increased the amount of nitrogen derived from atmosphere and the increase was particularly at a low level of phosphorus (SSP (50%) with VAM). At higher levels, it was interesting that there was considerable decrease in % Ndfa and the amount of N_2 fixed seedling $^{-1}$. The % Ndfa was improved by P addition, only at lower dose especially 20kg P_2O_5 ha^{-1} (Sanginga *et al.* 1995).

Although the P effect on plant growth was a major factor in increasing N_2 fixation, it was well manifested only at a lower level for the greater symbiotic ecosystem. The role of phosphorus at lower level in maximizing the percent nitrogen derived from the atmosphere is further confirmed by the positive correlation between the respective P uptake and nodule number (*A. nilotica* r = 0.78** and r = 0.65**; *A. holoceresia* : r = 0.74** and r = 0.79**; *A. planifrons*: r = 0.78** and r = 0.76**).

Nitrogen derived from fertiliser (Ndff) and nitrogen use efficiency (NUE)

The percent nitrogen derived from fertilizers ranged from 0.91 to 2.85 irrespective of species (Table 1). Among different species, the value extended from 1.90 to 2.85, 1.36, to 2.30, 0.91 to 1.85 and from 1.71 to 2.65 percent for *A. nilotica, A. leucophloea, A. holoceresia* and for *A. planifrons* respectively. The % Ndff was low in the treatment ^{15}N urea(75%) + Rhizobium + VAM which recorded the maximum amount of N_2 fixed from the atmosphere. The reason attributed to the fact that the legumes derive most of the nitrogen from the atmosphere through nodulation and N_2 fixation rather than from soil or fertiliser as reported by Sanginga *et al.* (1986). Similar trend of results were observed in nitrogen use efficiency.

Table 2. Effect of various levels of inorganic and biofertilizers on nitrogen fixation (mg/seedling) by different methods

Treatments	*A. nilotica*			*A. leucophloea*			*A. holoceresia*			*A. planifrons*		
	% Ndfs	Dilution	'A' Value	% Ndfs	Dilution	'A' Value	% Ndfs	Dilution	'A' Value	% Ndfs	Dilution	'A' Value
Control	26.8	26.8	27.8	14.8	15.0	15.2	11.6	10.9	11.3	18.0	18.5	18.8
15 N Urea (50%)'R	106	106	109	85.1	89.7	88.6	71.7	77.0	74.9	41.2	94.8	96.0
15 N Urea (75%)'R	162	162	165	138	144	143	117	124	122	144	148	150
15 N Urea (100%)'R	78.3	79.3	81.4	59.4	62.8	82.0	47.4	50.5	49.7	64.4	68.0	67.1
15 N Urea (50%)'R+V	144	146	150	120	122	125	103	106	108	127	135	132
15 N Urea (75%)'R+V	167	177	176	141	150	146	122	124	127	148	149	153
15 N Urea (100%)'R+V	99.5	106	103	81.8	82.9	85.1	66.7	68.7	69.7	83.6	84.8	87.0
SSP (50%)+V	153	155	158	130	137	135	111	115	116	136	145	141
SSP (75%)+V	149	150	154	125	127	130	108	111	111	131	138	136
SSP (100%)+V	85.8	86.9	88	67.6	68.5	70.4	52.9	53.7	55.4	70.5	72.4	73.4
CD (0.05)	3.21	7.01	3.11	2.52	5.21	2.91	3.61	4.07	2.75	3.25	4.26	2.82

Nitrogen Fixation by Various Methods

The highest amount of nitrogen was fixed from the atmosphere by *A. nilotica* followed by other tree species registering 167, 177 and 176 mg/seedling by % Ndfs, isotope dilution and 'A' value technique respectively (Table.2). *Acacia nilotica* performed better in shoot and root growth, nodulation and in turn in N_2 fixation compared to other three species.

Among the three methods, isotope dilution technique and 'A' value method recorded higher estimate of N_2 fixation followed by the method based on% Ndfs. In most cases, all the three produced statistically similar estimates of N_2 fixation. According to Danso (1991), the isotope dilution and 'A' value methods appear to be the most accurate. The 'A' value technique has more underlying assumptions and is more complicated conceptually and mathematically. The method based on % Ndfs gave little bit lower estimates of N_2 fixation due to the immobilization of initial enrichment in soil (Kadiata *et al.*, 1995).

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BIOLOGICAL NITROGEN FIXATION IN *Casuarina equisetifolia* USING NATURAL ^{15}N ISOTOPIC METHOD

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ABSTRACT

Casuarina equisetifolia seedlings, uninoculated or inoculated with *Frankia* strain Cc2 and *Glomus fasciculatum* were grown for 3 months in pots, harvested and N_2 fixation was measured using ^{15}N isotopic method. Maximum Ndfa percent was 83.3 when inoculated with *Frankia* and Vesicular Arbuscular Mycorrhiza at 10kg fertilizer N level. The modest amount of nitrogen fixed was attributed to the low soil fertility and the result of this experiment confirm that *Frankia* strain Ce 2 can be confidently recommended to inoculate *Casuarinas* along with *Glomus fasciculatum* in the field.

KEY WORDS: *Casuarina equisetifolia*, Frankia, N_2 Fixation, ^{15}N, VA-mycorrhiza

Casuarina species are frequently reported to fix large amounts of N, upto 100 kg N ha^{-1} year^{-1}, although exact measures are difficult to be obtained owing to (methotodological limitations (Shearer and Kohl, 1986; Sougoufara *et al.*, 1990) *Casuarina equisetifolia* has potential for use in biomass production, land reclamation, and for rotational agriculture to improve the N status in soil. The outstanding ability of *Casuarina equisetifolia* to thrive in poor N deficient soils is due to their association with *Frankia*, the symbiotic N_2 fixing actinomycete forming nodules on their roots.