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^{15}N studies on fertilizer nitrogen derivation, quantity and translocation efficiency in groundnut (*Arachis hypogaea* L.)

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ABSTRACT

A field experiment was carried out at Regional Research Station, Tamil Nadu Agricultural University, Vridhachalam to quantify the amount of nitrogen fixed by different parts of groundnut and investigate their response to organic and inorganic fertilizer by ^{15}N techniques. Distribution of fertilizer N from source to sink assessed by ^{15}N tracer clearly indicated that kernel was the major sink attracting N in groundnut. The fertilizer N absorbed by the different plant parts were high when N was split applied at early growth stages and moved to kernel at harvest. Based on the results, it could be stated that split application of N is effective to fulfill the crop requirement of N for maturity of kernel and oil content.

Key words: Ammonium sulphate, groundnut, ^{15}N tracer, urea

To sustain soil fertility in agricultural systems, nutrients exported through agricultural products or lost to the environment need to be replaced. The *Rhizobium* – legume symbiosis provides potentially an alternative to N fertilizers to balance N losses through its ability to fix atmospheric nitrogen. Hence there is a need to use precise methods to measure fertilizer nitrogen derivation, quantity and translocation efficiency in groundnut under field conditions. There are several concepts that are key to conserving nutrients in a soil-plant system. It is important to make nutrient applications concurrent to crop uptake and utilization patterns. Therefore, the time (stage of crop growth), method, and rates of application are all very important in achieving optimum crop uptake and utilization of the applied nutrients. ^{15}N as a tracer can be used to understand the plant demand of N during different phenological stages, as well as its distribution within the plant (Wallace *et al.*, 2007). Olson and Kurtz (1982) described plant use and efficiency of fertilizer N as a function of 1) time of application 2) rate of the N applied and 3) precipitation and climate-related variables. Bloom *et al.* (1988) observed that the apparent recovery of N by winter wheat (measured as the difference between uptake from fertilized and unfertilized crops) varied from 40 to 88 %. The recovery of applied ^{15}N by the crop differed substantially depending on the rate and method of N application (Recous *et al.*, 1988). There are numerous reports of the use of ^{15}N isotope for the estimation of biological N_2 fixation (BNF) (Hauck and Bystrom, 1970). In field studies, BNF was estimated by applying ^{15}N-enriched salts to the soil and measuring the ^{15}N uptake. The objective of this study was to quantify the amount of nitrogen fixed by different parts of groundnut and investigate their response to organic and inorganic fertilizer.

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MATERIALS AND METHODS

The study was conducted at Regional Research Station, Vridhachalam. This is located at 11° 3'N latitude and 79° 26'E longitude at an altitude of 42.67 m above mean sea level. The experimental area falls under north eastern agro climatic sub zone of Tamil Nadu. The soil of the experimental field was sandy loam in texture; the pH was almost neutral 6.8±7.0 and the organic matter content very low. Three combinations of nutrient management were evaluated for this study along with poultry manure (5 t ha^{-1}) and farm yard manure (12.5 t ha^{-1}). Along with this, recommended dose of (17:34:54 kg NPK ha^{-1}) were applied as per the schedule. Nitrogen was applied as ^{15}N tagged urea and ammonium sulphate (10 % atom ^{15}N excess) at the recommended dose and applied uniformly in microplots, made of PVC pipe bits. Microplots were located in the center of the plot and each microplot of 1 x 1 m was established. Applications of labeled fertilizer N were made as per the treatment schedule. Samples were collected at 30, 60 days after sowing (DAS) and at harvest stage. After harvest leaf, stem, flower, root, kernel and shell were separated and kept for analysis. The post harvest soil samples were also collected and subjected to ^{15}N assay. The soil samples were taken from each microplots at a plough depth of 0-20 cm in two layers *viz.*, 0-10 cm (surface) and 10-20 cm (sub surface) at 30, 60 DAS and at harvest.

Treatment details

T_1 – Recommended dose of fertilizers (17:34:54 kg NPK ha^{-1}) at basal application

T_2 – Poultry manure @ 5 t ha^{-1} + split application of 50 % N & K as basal + 25 % N and K at 20 DAS + 25 % N and K at 45 DAS (30:60:95 kg NPK ha^{-1}) + combined nutrient spray at 25 and 35 DAS

T_3 –Farm yard manure @ 12.5 t ha^{-1} + split application of 50 % N & K as basal + 25 % N and K at 20 DAS + 25 % N and K at 45 DAS (30:60:95 kg NPK ha^{-1}) + combined nutrient spray at 25 and 35 DAS

Recommended dose of P fertilizers were applied as basal in T_1 and T_2

Measurement of ^{15}N assay

Sample collection

Plant samples were collected at 30, 60 DAS and at harvest stage and separated in to leaves, stem, floral parts, root, kernel and shell. Post harvest soil samples were also collected. Plants and soil samples were subjected to ^{15}N assay.

Sample preparation

The plant and soil samples were digested by adopting regular kjeldahl digestion procedure to convert N in to ammonia form (Bremner, 1965). The digested samples were distilled in to 2 percent boric acid solution containing double indicator and titrated with 0.1N H_2SO_4 to obtain in the form of ammonium sulphate, a suitable material for ^{15}N analysis (Buresh *et al.*, 1982). The soil and plant samples were subjected to available N estimation (Subbiah and Asija, 1956). The acidified boric acid solution containing ^{15}N as ammonium sulphate was evaporated at 60°C on a sand bath to reduce the volume to about 3.0 ml. The contents were transferred to a glass vials and again evaporated carefully to dryness in a hot air oven at 60°C. Later, the vial were capped, stored and used for ^{15}N assay.

$^{15}N/^{14}N$ ratio analysis

The ammonium sulphate samples were allowed to react with sodium hypobromite solution to evolve N_2 in the inlet system of mass spectrometer. Inside the mass spectrometer, N_2 was ionized for $^{15}N/^{14}N$ ratio measurement. The mass spectrometer model micromass 622 VS ISOGAS, was used. Ratio analyses were performed as per the procedures outlined by Buresh *et al.* (1982) and Pruden *et al.* (1985).

Calculation of results

Isotopic abundance was expressed in term of ^{15}N atom percent and calculated using the following relationship.

$$^{15}N \text{ atom } \% = \frac{R}{R+2} \times 100 \text{ where,}$$

R=Measured ratio between the ion currents corresponding to the mass 28 ($^{14}N^{14}N$ and mass 29 ($^{14}N^{15}N$)

Further,

$$R = \frac{m}{(M+1)100}$$

m = Current of minor ion beam ($^{14}N^{15}$)

M = Current of major ion beam ($^{14}N\ ^{14}N$)

The labeled ^{15}N recovered by crop as well as retained in soil were calculated as detailed in the technical document IAEA (1983).

The fraction of N derived from ^{15}N fertilizer (fNdff) in the plant and soil was calculated using the following equation.

$$fNdff = \frac{\%\ ^{15}N \text{ atom excess in plant or soil}}{\%\ ^{15}N \text{ atom excess in fertilizer}}$$

$$\text{Per cent Ndff} = fNdff \times 100$$

Accounting for ^{15}N recovery in plant and ^{15}N retained in soil were made with the following formulae:

$$\text{kg Ndff pot}^{-1} = \frac{\%\ Ndff \times (N \text{ uptake / plant) (kg)}}{1000}$$

$$\text{Total \% Ndff (Whole plant)} = \frac{\text{kg Ndff (leaves)} + \text{(kg Ndff (stem)+ (kg Ndff (kernal)+(kg Ndff (shell)+ (kg Ndff (root)}}{\text{Total N uptake (kg pot}^{-1})} \times 100$$

Total mg Ndff = Total % Ndff X Total N uptake

$$\text{Total N uptake (kg pot}^{-1}) = \text{Dry matter (mg pot}^{-1}) \times N \text{ content (\%)}$$

$$\text{Partitioning efficiency (\%)} = \frac{\text{kg Ndff of plant part}}{\text{Total kg Ndff of whole plant}} \times 100$$

RESULTS AND DISCUSSION

Recovery (%) and quantity of N (kg Ndff) derived from ^{15}N labelled fertilizer in soil

Among the treatments, poultry manure @ 5 t ha^{-1} + split application of 50 % N & K as basal + 25 % N and K at 20 DAS + 25 % N and K at 45 DAS + combined nutrient spray at 25 and 35 DAS registered the highest recovery per cent of (22.38, 13.45 and 16.82 % respectively at 30, 60 DAS and at harvest respectively) in 0-10 cm layer (Table 1). Basal application of recommended dose of fertilizers (30:60:95 kg NPK ha^{-1}) resulted in low N of recovery %. Similar to recovery % of ^{15}N fertilizers, quantity of N (kg Ndff) was also observed (Table 2).

Recovery (%) and quantity of N (kg Ndff) derived from ^{15}N labelled fertilizer in the crop

The effect of treatments was remarkable on derivation of applied labeled ^{15}N in leaves, stem, flower, kernel, shell and root estimated at 30, 60 DAS and harvest stage. Application of N at higher levels enhanced greater fertilizer N accumulation in plant parts. Poultry manure @ 5 t ha^{-1} + split application of 50 % N & K as basal + 25 % N and K at 20 DAS + 25 % N and K at 45 DAS + combined nutrient spray at 25 and 35 DAS was very much effective and accumulated higher quantity of fertilizer N (kg Ndff) in all plant parts at all the crop growth stages (Table 3). Similar to quantity of N (kg Ndff), recovery % of

^{15}N fertilizers was highest with application of poultry manure @ 5 t ha^{-1} + split application of 50 % N & K as basal + 25 % N and K at 20 DAS + 25 % N and K at 45 DAS + combined nutrient spray at 25 and 35 DAS at all the stages (Table 4).

Partitioning efficiency of fertilizer N within groundnut plant

The contribution of fertilizer N absorbed by plant for the development and maturity was assessed by ^{15}N partitioning was in different plant parts like leaves, stem, flower, root, kernel and shell. Poultry manure @ 5 t ha^{-1} + N three splits- split application of 50 % N & K as basal + 25 % N and K at 20 DAS + 25 % N and K at 45 DAS + combined nutrient spray at 25 and 35 DAS register highest partitioning efficiency in leaf (44.40 %), kernel (25.0 %) and shell (9.43 %) at 60 DAS and in kernel (30.15%) and shell (20.52 %) at harvest (Table 5).

Nitrogen tracer (^{15}N) technique is an accurate tool to assess the absorption and partitioning of added N under different N management practices. Assaying ^{15}N tracer added as labelled urea and ammonium sulphate in leaves, stem, flower, root, kernel and shell of groundnut brought out the superiority of split N management on the derivation of fertilizer N from soil to plant parts, in terms of partitioning efficiency, crop recovery and quantity of fertilizer N in soil.

The total N content of the soil decreased gradually with increasing soil depth. In the microplots where ^{15}N labelled fertilizer was surface applied very few quantity of the applied ^{15}N moved downwards. The majority of the ^{15}N was still found in the top soil even at harvest creating a decrease of ^{15}N content with increasing soil depth. The ^{15}N signature of the mineral N in the natural abundance plots was not significantly affected by soil depth but was much greater than that of total soil N. Silvertooth *et al.* (2001) reported that the fertilizer N recovery levels declined dramatically below 60 cm depth of soil.

As parameters indicative of labeled N derivation, the fertilizer N composition (%Ndff) in plant and fertilizer N uptake (kgNdff) showed distinctly the influential role of split N management on fertilizer N accumulation particularly in kernel and shell. Splitting of N as basal and two top dressing might have supplied fertilizer N for longer period spreading over the growth phase of groundnut and hence the observed substantial accumulation in leaves. This led translocation to kernel and shell. In addition to accumulation in stem, root and flower. These results were similar to the studies conducted in Arizona (Navarro *et al.,* 1997) and Norton and Silvertooth *et al.* (2001) which also demonstrated greater levels of N use efficiency with split application.

Split application of N fertilizers in groundnut resulted in high N content in leaf, stem, kernel and shell. About 40 to 50 % of applied ^{15}N was absorbed only in leaf and stem and out of which 30-35 % was translocated to kernel. Only 20-25 per cent of ^{15}N was absorbed through shell and rest was translocated to vegetative parts. A similar result was observed by Wuest and Cassman (1992).

The distribution of fertilizer N from source to sink assessed by ^{15}N tracer clearly indicated that kernel was the major sink attracting N in groundnut. The fertilizer N absorbed by the different plant parts was also high when N was applied in splits at early stages and moved to kernel at harvest. Based on the results, it could be stated that split application of N is an effective tool to fulfill the requirement of N for maturity of the kernel.

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Table 1: Recovery (%) of N derived from 15N labeled fertilizer in soil by groundnut

Treatment	30 DAS		60 DAS		Harvest	
	0 to 10 cm	10 to 20 cm	0 to 10 cm	10 to 20 cm	0 to 10 cm	10 to 20 cm
T_1	13.87	5.60	10.56	4.21	12.43	3.12
T_2	22.38	4.47	13.45	10.45	16.82	5.43
T_3	18.45	5.41	10.34	8.76	13.78	9.47

Table 2: Quantity of N derived from 15N labeled fertilizer in soil by groundnut (kg Ndff)

Treatment	30 DAS		60 DAS		Harvest	
	0 to 10 cm	10 to 20 cm	0 to 10 cm	10 to 20 cm	0 to 10 cm	10 to 20 cm
T_1	4.16	1.02	3.45	2.12	2.62	1.76
T_2	6.71	1.44	4.05	2.88	3.57	2.84
T_3	7.87	1.62	3.34	2.78	3.09	2.43

Table 3: Recovery (%) N derived from ^{15}N labeled fertilizer by groundnut at 30, 60 DAS and at harvest stage

Treatment	T_1			T_2			T_3		
	30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest
Leaf	20.00	14.23	3.01	25.52	15.04	4.10	20.56	14.12	3.26
Stem	2.63	8.32	2.05	3.80	12.22	5.99	4.09	10.27	3.68
Root	0.12	1.45	2.87	0.89	3.23	2.32	0.76	2.07	1.90
Flower	1.45	1.12	0.56	4.61	3.34	2.11	3.10	2.87	1.78
Kernel	0.00	2.76	3.57	0.00	8.43	5.94	0.00	7.21	5.67
Shell	0.00	2.12	2.32	0.00	5.83	3.67	0.00	4.86	2.37
Total	**24.20**	**30.00**	**14.38**	**34.82**	**48.09**	**24.13**	**28.51**	**41.40**	**18.66**

Table 4: Quantity of N derived from ^{15}N labeled fertilizer by groundnut at 30, 60 DAS at harvest stage (kg Ndff)

Treatment	T_1			T_2			T_3		
	30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest
Leaf	2.53	2.34	1.28	3.66	3.11	1.34	2.99	2.89	1.01
Stem	1.08	2.50	0.61	2.14	4.67	1.80	2.13	3.61	1.40
Root	0.03	1.45	0.43	0.06	2.73	0.70	0.26	1.78	0.45
Flower	0.78	0.52	0.21	2.38	1.65	0.78	1.92	0.87	0.43
Kernel	0.00	0.69	0.90	0.00	4.64	2.82	0.00	3.04	1.92
Shell	0.00	0.33	0.70	0.00	1.75	1.92	0.00	1.16	1.21
Total	**4.43**	**7.83**	**4.14**	**8.24**	**18.54**	**9.35**	**7.30**	**13.35**	**6.43**

Table 5: Partitioning efficiency of ^{15}N fertilizers by groundnut at 30, 60 DAS and at harvest

Treatment	T_1			T_2			T_3		
	30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest
Leaf	57.11	29.89	30.97	44.40	16.77	14.33	40.96	21.64	15.76
Stem	24.49	31.93	14.87	25.98	25.18	19.22	29.14	27.03	21.79
Root	0.79	18.52	10.40	0.74	14.72	7.45	3.60	13.33	7.01
Flower	17.61	6.64	5.08	28.89	8.90	8.34	26.30	6.52	6.69
Kernel	0.00	8.81	21.76	0.00	25.00	30.15	0.00	22.80	29.91
Shell	0.00	4.21	16.92	0.00	9.43	20.52	0.00	8.68	18.84