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SOIL SCIENCE

Fate of ^{15}N labeled nitrogen in maize grown with nutriseed pack using tracer technique

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Abstract

Deep placement of fertilizers will reduce the nutrient losses to the environment and increases the nutrient use efficiency. Nutriseed pack is a small tubular assembly consisting seed with bio-inoculants on top, manure pellet in middle and fertilizer pellet at bottom. A field study has been taken to determine nitrogen recovery and to evaluate the compatibility of phosphorus fertilizer sources with nitrogen sources which suited for Nutriseed pack by estimating the recovery of applied fertilizer N added as ^{15}N labeled urea with Nutriseed pack in maize and retention in soil using ^{15}N tracer technique in eastern block of Tamil Nadu Agricultural University, Coimbatore. This study has revealed that the actual nitrogen recovery from fertilizer and fertilizer nitrogen retention in the soil. The highest fertilizer nitrogen recovery (^{15}N) in leaves, stem and grain (47.95 %) was found with NP(DAP)K Nutriseed pack with phosphorus as Di ammonium phosphate placement recording the highest nitrogen use efficiency of 64.91%. Relatively low recovery of nitrogen (45.25%) was observed with NP(SSP)K Nutriseed pack with phosphorus as Single super phosphate placement recording 58.67% of nitrogen use efficiency. The labeled nitrogen retention in soil is recorded low in NP(DAP)K Nutriseed pack placement and comparatively high in NP(SSP)K Nutriseed pack placement.

Highlights

- Actual fertilizer nitrogen use efficiency of maize from Nutriseed pack and retention in soil have been analysed through ^{15}N tracer method in mass spectrometer.

Keywords: Deep placement, Nitrogen use efficiency, Nutriseed pack, Bio-inoculants, Nitrogen recovery, Labeled nitrogen

Nutriseed pack technique is an innovative approach of placing all nutrients just below the root in soluble and slow release form, which remain intact as a ready reserve, to supply nutrients in small and adequate amounts as and when the roots require the nutrients. This is a kind of deep placement of fertilizers. By design, Nutriseed Pack is a tubular packet having a fertilizer pellet at one side, a manure pellet in middle and seed with bio-inoculant mixture on other side. On placement in soil, seed in each pack germinate and tap the nutrients from the fertilizer and manure pellets throughout the crop period. Maize is one of the world's leading crops after wheat and rice. In India, it is cultivated over an area of about 9.4 million hectares with a production of about 24.35 million tonnes and productivity of 2.41 tonnes ha^{-1} of grain (Agricultural statistics at a glance-2014). The most important task for the future is to improve nutrient use efficiency or more precisely nitrogen use efficiency Grant, 2005. Nitrogen is the most important element required for plant growth and development. Variation in nitrogen availability can affect plant development and grain production in maize. Maize grain yield is linked to both higher nitrogen uptake and higher ability to utilize nitrogen accumulated in the plant in yield production (Luque *et al.* 2006). Worldwide, nitrogen use efficiency (NUE) for cereal production including wheat is approximately 33% (Raun and Johnson, 1999). Efficient use of N for maize production is important for increasing grain yield, maximizing economic return and minimizing NO_3 leaching (Gehl *et al.* 2005). A recent review of worldwide data on N use efficiency for cereal crops from researcher-managed experimental plots reported that single-year fertilizer N recovery efficiencies averaged 65% for corn, 57% for wheat, and 46% for rice (Ladha *et al.* 2005).

Nitrogen tracer (^{15}N) technique is an accurate tool to assess the N use efficiency and retention of added N in soil under different N management practices. Isotopic study by using ^{15}N can be used to determine N utilization from fertilizer by plant. Plant can obtain N from various sources, including soil, microbial fixation, and most importantly fertilizer. Fertilizer is applied as ^{15}N isotope to trace the amount of N derived from the fertilizer (NDFF). This information is very essential in developing fertilizer to meet the actual requirement of plant.

The advantage of this technique is that the element is traceable. Isotopic ^{15}N is the most common stable isotope used in agriculture-related studies (IAEA, 2001). Asha (2003) made a pioneering approach of deep placing NPK fertilizers just below the germinating seedling with an aid of tubular holder called Nutriseed Holder, which contained sprouted seeds on top and fertilizers at bottom. This study with ^{15}N tracer demonstrated a 57.1% of fertilizer N recovery, which exceeded two folds of recovery noted for surface broadcast (26.1 %). Radhika *et al.* 2012 experimented ^{15}N tracer to examine the maize Nitrogen Use efficiency grown with Nutriseed pack which revealed that the highest (37%) NUE by placing Nutriseed pack in horizontal placement and reasoned out that fibrous root system of maize could easily avail the nutrients from the horizontal distribution of nutrients. These studies have proven that the placement of Nutriseed pack improved Nitrogen use efficiency. With these confirmations, a study has been taken to determine Nitrogen recovery by maize with different fertilizer sources in Nutriseed pack techniques to optimize the cost of fertilizers as well as to increase nutrient use efficiency.

Materials and methods

The ^{15}N tracer experiment was conducted in Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. The experimental soil was Periyanaickenpalayam soil series (*Vertic Ustropept*). Applications of labeled fertilizer N were made as per the treatment. ^{15}N applied plants were removed at harvest stage. Lower leaves, lower stem, upper leaves, upper stem, cob sheath and grains were separated, dried, weighed and ground for analysis. The post harvest soil samples were also collected. Plant parts and soil were subjected to ^{15}N assay.

Treatment details

^{15}N labeled urea (2.738 % atom ^{15}N excess) was used as a tracer for the preparation of fertilizer pellet of Nutriseed Pack as per the treatment.

Tr. No	Treatments
T_1	100% NPK (^{15}N Urea + DAP + MOP)
T_2	100% NPK (^{15}N Urea + SSP + MOP)

Details of ^{15}N tracer experiment

Crop and Variety	Maize, NK 6240
Duration (days)	105 – 110 days
N, P_2O_5,K_2O kg ha^{-1}	250 :75 :75
Date of Start	02 April 2013
Date of Completion	19 July 2013
Replications	12

Measurement of ^{15}N assay

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 into 2% boric acid solution containing double indicator (Bromocresol green and Methyl red) 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 acidified boric acid solution containing ^{15}N as ammonium sulphate was evaporated at 90°C on a sand bath to reduce the volume to about 3 ml. The contents were then evaporated at room temperature until it dried to flakes. The boric acid flakes were transferred to capped vials, 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 VG ISOGAS, installed in the Department of Soil Science and Agriculture Chemistry, TNAU, Coimbatore was used. Ratio analysis was performed as per the procedure outlined by Buresh *et al.* (1982) and Pruden *et al.* (1985).

Calculation of results

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

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

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,

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

Where,

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

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 or soil was calculated using the following equation.

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

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

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

$$\text{mg Ndff in plant part} = \frac{\text{\% Ndff}}{100} \times \text{(N uptake in plant part) (mg)}$$

$$\text{Total N uptake (mg plant}^{-1}) = \text{dry matter (mg plant}^{-1}) \times \text{N content (\%)}$$

Crop recovery of fertilizers N (N use efficiency) (%)

$$= \frac{\text{Sum of mgNdff of all plant parts N uptake (mg pot}^{-1})}{^{15}\text{N labeled urea N applied through Nutriseed Pack}} \times 100$$

Retention of fertilizer N in soil (%)

$$= \frac{\text{Total N in soil (mg) X \%Ndff}}{^{15}\text{N labeled urea N applied through Nutriseed Pack}} \times 100$$

Partitioning Efficiency (%)

$$= \frac{\text{mgNdff of plant part}}{\text{Total mgNdff of whole plant}} \times 100$$

Results and discussion

Tracer studies with labeled nitrogen conducted in field studies in hybrid maize crop brought out clear cut information on the extent of distribution of applied nitrogen in different plant parts of maize plant, nitrogen use efficiency and retention in soil (Table. 1).

Dry matter

High dry matter of upper leaf (18.05 g plant^{-1}), lower leaf (33.36 g plant^{-1}), upper stem (20.83 g plant^{-1}), lower stem (38.88 g plant^{-1}), cob sheath (13.44 g plant^{-1}) and grain (170.52 g plant^{-1}) were found with NP(DAP)K Nutriseed pack placement. Relatively low dry matter of upper leaf (16.41g plant^{-1}), lower leaf (31.81 g plant^{-1}), upper stem (17.44 g plant^{-1}), lower stem (36.09 g plant^{-1}), cob sheath (11.41 g plant^{-1}) and grain (153.86 g plant^{-1}) were found with NP(SSP)K Nutriseed pack placement. The increased dry matter production with NP(DAP)K Nutriseed pack placement might be due to the cumulative effect of both Urea as well as DAP.

Concentration of labeled nitrogen in plant parts (%Ndff)

The Concentration of labeled nitrogen in plant parts of maize varied with sources of fertilizers used with Nutriseed Pack placement. Relatively low %Ndff was found in upper leaf (33.86 %), lower leaf (29.17 %), upper stem (34.62 %), lower stem (38.88 %), cob sheath (40.95 %) and grain (58.42 %) were found with NP(DAP)K Nutriseed pack placement. High

Table 1: Recovery of added fertilizer ^{15}N in maize plant parts and in post harvest soil

Treatment	Plant part	Dry weight g	N content	N uptake g/plant	%Ndff	mg N added	mgNdff	Recovery in Plant (%)	PE (%)	NUE
^{15}N Recovery in Crop										
NP(DAP)K	Upper leaf	18.05	1.16	0.21	33.86	1986	71.1	3.58	5.52	64.91 (67.15)*
	Lower leaf	33.36	1.18	0.39	29.17	1986	113.8	5.73	8.82	
	Upper stem	20.83	0.66	0.14	34.62	1986	48.5	2.44	3.76	
	Lower stem	38.88	0.54	0.21	39.55	1986	83.1	4.18	6.44	
	Sheath	13.44	0.35	0.05	40.95	1986	20.5	1.03	1.59	
	Grain	170.52	1.05	1.63	58.42	1986	952.2	47.95	73.87	
NP(SSP)K	Upper leaf	16.41	1.14	0.19	36.25	2250	68.9	3.06	5.22	58.67
	Lower leaf	31.81	1.09	0.35	35.40	2250	123.9	5.51	9.39	
	Upper stem	17.44	0.37	0.06	38.44	2250	23.1	1.03	1.75	
	Lower stem	36.09	0.49	0.18	43.00	2250	77.4	3.44	5.86	
	Sheath	11.41	0.15	0.02	43.68	2250	8.7	0.39	0.66	
	Grain	153.86	0.96	1.63	62.46	2250	1018.1	45.25	77.14	
Soil Retention of fertilizer ^{15}N (%)										
	Soil	Weight g	N %	N (g)	%Ndff	Fert N	mgNdff	Retention		
NP(DAP)K	Root zone	500	0.096	0.332	33.9	1986	112.5	5.67		
NP(SSP)K	Root zone	500	0.081	0.327	42.6	2250	139.3	6.19		

** includes N contributed by urea and DAP*

PE – Partitioning Efficiency (%)

%Ndff was observed in upper leaf (36.25 %), lower leaf (35.40 %), upper stem (38.44 %), lower stem (43.00 %), cob sheath (43.68 %) and grain (62.46 %) with NP(SSP)K Nutriseed pack placement. With the use of ^{15}N tracer the exact pathway of movement of N from source to sink has been identified in plant in the present study. Grain N, being the ultimate sink of N in plant, gained much of the applied N from the major source of lower leaves and lower stem. Uptake of N in lower leaf and stem was high, and fertilizer N contributed for the same. This has been evidenced from %Ndff of stem which was moderate.

Quantity of labeled nitrogen in plant parts (mgNdff)

The quantity of labeled nitrogen in plant parts of maize varied with sources of fertilizers used with Nutriseed Pack placement. Relatively low mgNdff in upper leaf (71.1 mg), lower leaf (113.8 g), upper stem (48.5 mg), lower stem (83.1 mg), cob sheath (20.5 mg) and grain (952.2 mg) were found with NP(DAP)K Nutriseed pack placement. High mgNdff was observed in upper leaf (68.9 mg), lower leaf (123.9 mg), upper stem (23.1 mg), lower stem (77.4 mg), cob sheath (8.7 mg) and grain (1018.1 mg) with NP(SSP)K Nutriseed pack placement. The function of fertilizer N in contributing the development of different plant parts and resulting in grain yield has been distinctly brought out by mgNdff values. There were very high mgNdff values in grain and it was substantially low in other parts. On the other hand, considerably low mgNdff in upper leaf and upper stem was found since these parts were still functional and did not involve in the partitioning of N to grain. These results clearly reflect the continuous contribution of fertilizer N throughout the crop period when fertilizer N was placed at depth by means of Nutriseed pack, and its release was regulated.

Recovery of labeled nitrogen in plant parts and N use efficiency

Recovery of labeled N in plant parts of maize varied with sources of fertilizers used with Nutriseed Pack placement. Very high recovery of ^{15}N in in upper leaf (3.58 %), lower leaf (113.8 %), upper stem

(48.5 %), lower stem (4.18 %), cob sheath (1.03 %) and grain (47.95 %) were found with NP(DAP)K Nutriseed pack placement recording the highest nitrogen use efficiency of 64.91% due to the contribution of urea alone and 67.15% due to the contribution of urea and diammonium phosphate. Relatively low recovery of nitrogen was observed in upper leaf (3.06 %), lower leaf (5.51 %), upper stem (1.03 %), lower stem (3.44 %), cob sheath (0.39 %) and grain (45.25 %) with NP(SSP)K Nutriseed pack placement recording overall nitrogen use efficiency of 58.67% due to the contribution of nitrogen from the only source of urea. The true efficiency of added N fertilizer measured as ^{15}N recovery % (NUE) was high with NP(DAP)K recording 64.91% assessed by the contribution of urea. Placement of Nutriseed Pack with NP(DAP) K registered the highest overall N use efficiency (67.15%) assessed by the contribution of urea and diammonium phosphate. Placement of Nutriseed Pack with NP(SSP)K registered relatively high ^{15}N recovery (58.67%). Close placement of nutrients in the pellets of Nutriseed Pack near the surface where roots proliferate caused very high mgNdff that has been reflected in ^{15}N recovery under Nutriseed pack placement

Partitioning Efficiency of applied N in plant parts (%)

Ability of maize plant to accumulate the added fertilizer N in plant parts of maize was measured with ^{15}N tracer as partitioning efficiency. Partitioning efficiency varied with sources of fertilizers used with Nutriseed Pack placement. Relatively low partitioning of ^{15}N was found in upper leaf (5.52 %), lower leaf (8.82 %), upper stem (3.76 %), lower stem (6.44 %), cob sheath (1.59 %) and grain (73.87 %) with NP(DAP)K Nutriseed pack placement. Partitioning efficiency was relatively high with NP(SSP)K Nutriseed pack placement as found in upper leaf (5.22 %), lower leaf (9.39 %), upper stem (1.75 %), lower stem (5.86 %), cob sheath (0.66 %) and grain (77.14 %).

Retention of Labeled nitrogen in soil

With respect to retention of applied labeled nitrogen, 5.67% N was retained in placement of Nutriseed Pack with NP(DAP)K followed by NP(SSP)K in which 6.19% N was retained. Due to application of fertilizers by deep placed Nutriseed pack and subsequent utilization by crop total N did not build up much. However considerable portion of added N was recovered. The highest amount of soil retention was found in placement of Nutriseed pack with NP(DAP)K registering 5.67% followed by NP(SSP)K registering 6.19%.

Conclusion

Recovery of labeled N in plant parts of maize varied with sources of fertilizers used with Nutriseed Pack placement. Very high recovery of ^{15}N in leaves, stem and grain (47.95 %) was found with NP(DAP) K Nutriseed pack placement recording the highest nitrogen use efficiency of 64.91%. Relatively low recovery of nitrogen was observed with NP(SSP) K Nutriseed pack placement recording 58.67%. This study has revealing that the placement of NP(DAP)K Nutriseed pack has registered the highest nitrogen use efficiency compared to NP(SSP) K Nutriseed pack.

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