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Using ^{15}N Isotope Technique for Study the Balance of Nitrogen Fertilizer in Young Rubber Tree -*Hevea Brasilliencis*

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Abstract

The pot experiment was conducted in late rainy season in the South of Vietnam to determine influence of 2gN/pot to %Na.e, Ndff and movement of N fertilizer in soil by using ^{15}N isotope technique. There were big differences of ^{15}Na.e, Ndff and Fertilizer N utilization in leaf, stem and root. ^{15}Na.e of stem and leaf at 0.19% were higher than the one in root at 0.16%. Similarly, amount of Ndff also was highest in leaf at 0.331 g/pot. Total amount of N derived from fertilizer was 0.432g/pot and it means fertilizer N utilization was 21.09% while loses occupied very high at 44.75%. At the end of the study 90 days, N fertilizer had residue in soil at 25% and approximately 9.16% of N fertilizer was unaccounted.

Keywords: ^{15}Na.e; Ndff; N fertilizer; rubber RRIV 214; ^{15}N isotope.

1. Introduction

Rubber tree -*Hevea brasilliencis* has been planted widely in the South of Vietnam. It also considers as an important plant for economic earning. Urea is the major N fertilizer used in rubber plantation both for immature and mature plant. With long period of time for premature stage – about seven years, this period often gets increased due to wrong N fertilizer use. Application of higher doses of NPK during early years was reported to reduce the immature period of rubber [4].

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The same opinion of Adiwiganda *et al*., 1995, and Sihotang, 1993 showed that the immature period of rubber tree can be shortened by optimum fertilizer application [2]. Suitable N fertilizer recommendation is generally based mainly on the requirement of macro nutrient N combines with P, K, and Mg rate [1], while the micro nutrient is considered small and can be satisfied by the soil. Study in the balance of nitrogen fertilizer in young rubber tree -*Hevea brasilliencis* especially in Haplic acrisol supplies and improves knowledge of rubber nutrition that had been ignored in the past. Consequently, minimizing loses and increasing N use efficiency for rubber tree in Vietnam.

Therefore, by using N-15 dilution techniques with urea N-15, the objectives of this study were 1).To determine movement downward of N fertilizer in haplic acrisol soil by time; (2) To determine dynamic change of fertilizer- N in leaf, root and stem of young rubber tree to compare nitrogen derived from fertilizer and (3) To determine the balance of nitrogen fertilizer in young rubber tree RRIV 214.

2. Materials and methods

Pot experiment was conducted in experimental farm of Ho Chi Minh City University of Agriculture and Forestry, Thu Duc District, Ho Chi Minh City, Vietnam 2014-2015. The rubber tree used was RRIV 214 with one whorled leave. Twenty kilograms of the haplic acrisol soil samples were filled into polythene pots (40cm diameter and 50cm height). Each plastic pot contained 1 plant. The properties of the soil and figure of meteorological experiment farm were listed in table 1 and table 2.

Table 1: The properties of the soil

pH	Organic matter (%)	Total N (%)	Total P (%)	Total K (%)	Sand (%)	Slit (%)	Clay (%)
5.19	2.68	0.09	0.07	0.05	33	58.6	2.4

(Source: CNT, Biology Department)

Table 2: Figure of meteorological experiment farm

Month	Temperature (^{0}C)			Total rainfall (mm)	Humidity (%)	The total number of hours of sunshine (hour)
	Average	Max	Min			
Oct 2014	28,3	35,0	23,8	342,1	80	97,2
Nov 2014	28,1	35,0	24,0	306,5	80	109,6
Dec 2014	28,8	35,4	23,6	267,4	76	223,7
Jan 2015	27,9	35,3	21,2	82,2	72	291,4

(Source: Hydrometeorological Observatory of Vietnam Southern region, 2015)

2.1 Pot experimental design

Urea-^{15}N-$CO(NH_2)_2$ - was used as a tracer. Fertilizer at 4.3g urea-^{15}N/pot (equivalence 2gN/pot) - normal nitrogen fertilizer rate was applied one time at the beginning. The experiments were arranged in randomized block design with 5 replications.

^{15}N abundance of all urea-^{15}N labeled fertilizers was 10.16%. The unfertilized scion was used as the control in this study. All treatments received adequate nutrients including 11.0 g SSP/pot (P fertilizer: superphosphate, available P_2O_5 16%); and 1.9 g KCl/pot (K fertilizer: KCl, K_2O content 63%) to ensure that N was the only limiting nutrient on rubber tree growth.

2.2 Sampling and analysis

The study duration was 90 days. Rubber plants were collected in separately leaves, stem and root at the end of the study. Soil samples in depth 0-20cm and 20cm-40cm were collected at 15 day after fertilizer application (DAF), 30 DAF, 60 DAF and 90 DAF then dried and grinded to investigate dynamic change of fertilizer in oranges of the plant and in soil depth.

Chemical treatment of ^{15}N-labelled materials for total nitrogen and ^{15}N content was conducted in Biotechnology Department, Center for Nuclear Techniques in Ho Chi Minh City, Vietnam. Total nitrogen was measured by Kjeldahl-Rittenberg oxidation method. ^{15}N-labelled was identified by emission spectrometer NOI 7PC.

Nitrogen in the plant derived from fertilizer (Ndff) on % was calculated as

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

2.3 Statistical analysis:

Statistical procedures were carried out with the software package MSTATC. Analysis of variance (ANOVA) was performed by using Duncan comparison of the means of each treatment.

3. Result and discussion

3.1 N delivered from fertilizer in plant

Results in table 3 shows that there were significant differences in dry matter, %N total and N uptake in three oranges of rubber tree at 90 DAF. Leaves got an advantage more than stem and root. Therefore, data in dry matter, N total and N uptake of leaf were always higher than the ones of root and stem. N uptake in leaf was highest at 164.30mg/pot equal 94.93% of N uptake in plant. Other parts were range from 10.43%-14.64%.

Thus, amount of N derived from fertilizer mainly used for producing leaves. Because of this, rubber tree also considers as copious N supply when they fall.

Table 3: Dry matter, N total and N uptake of rubber tree at 90 DAF

Ingredient	Dry matter	N total	N uptake	
	(g/pot)	(%)	(g/pot)	% in plant
Root	24.55	0.93	22.83	10.43
Stem	27.16	1.18	32.05	14.64
Leaf	69.21	2.37	164.03	94.93
Total	**128.98**		**218.91**	

The distribution of N derived from fertilizer among plant parts is of interest not only for its relevance to estimates fertilizer use which are based on tracer ^{15}N stable isotope but also because variation among plant parts may shed light on metabolic processes within the plant [5]**.** Basing on % $^{15}Na.e$ in the parts Ndff and fertilizer N utilization were calculated. As can be seen in the table 4, there were big differences of $^{15}Na.e$, Ndff and Fertilizer N utilization in the tree parts. $^{15}Na.e$ of stem and leaf at 0.19% were higher than the one in root at 0.16%. Similarly, amount of Ndff also was highest in leaf at 0.331 g/pot. In other parts this parameter substantial decreased, stem was at 0.037% less than fivefold and root was 0.037g/pot less than nearly ninefold. Total amount of N derived from fertilizer was 0.432g/pot and it means fertilizer N utilization was 21.09%. Ndff was mainly affected by: soil nutrient status, pH value and temperature, soil water content. Result from research of H.C.Guo et al. indicated that urea fertilizer utilization of rubber tree in China was 30.12%. The result in our experiment was obviously lower.

Table 4: $^{15}Na.e$, N dff and Fertilizer N utilization

Ingredient	% $^{15}Na.e$	Ndff (%)	Amount of Ndff (g/pot)	Fertilizer N utilization (%)
Root	0.16	1.65	0.037	1.88
Stem	0.19	1.96	0.063	3.14
Leaf	0.19	1.96	0.331	16.06
Total		**5.57**	**0.432**	**21.09**

3.2 N delivered from fertilizer in soil depth

Result of N total of soil (table 3) reflected that there was insignificant between %N total of soil layers range from 0.029%-0.031%. It means that experiment soil – haplic arisol was malnutrition. Hence, nutrition supplement plays an important role for rubber tree in young stage. According to International Plant Nutrition Institute.

There are five major processes that happen to applied fertilizer: (i) It is taken up by the crop; (ii) It reacts with soil minerals and organic matter to become part of the soil reserve; (iii) It reacts with soil minerals and organic matter to become part of the soil reserve; (iv) It can leach from the root zone with water; (vi) It can be lost to the atmosphere as a gas and (vii) It can move from the field through soil erosion and water runoff. Moreover, transformation of nitrogen in soil depends on soil temperature, moisture, pH and microorganism [6].

In this experiment, urea containing ^{15}N was fertilized 10cm from root zone and 5m from soil surface to minimizing N fertilizer loses. Table 4 and table 5 show the trend of % $^{15}Na.e$ and Ndff of ^{15}N fertilizer in different soil depth. In general, at layer 0-20cm, % $^{15}Na.e$ dramatically reduced following period of time 0,924% in 15DAF to 0.15% in 90DAF. In contrast, at layer 20cm-40cm, the increase of %$^{15}Na.e$ and Ndff occurred quickly by time with 90DAF six fold higher than 30DAF. It can be seen that there was a clear correlation between time and movement downward of ^{15}N fertilizer. After fertilizing, urea was hydrolyzed to produce NH_4^+ and this ion then immediately combined with negative ion in soil. Consequently, if cation exchange in soil slow means low soil NH_4^+, N fertilizer penetrates less [8]. The penetrate process was fasten only when NH_4^+ was transferred to NO_3^- by soil microorganism. Because NO_3 has negative charged then it should not be fix by particles and organic matter in soil. Hence, it easily absorbed deeper levels [7]

Table 5: % $^{15}Na.e$ at difference period of time and soil layer

Soil depth	% $^{15}Na.e$			
	15DAF	30DAF	60DAF	90DAF
0-20cm	0.924	0.499	0.392	0.15
20cm-40cm	ND	0.003	0.04	0.07
CV%			14.97	10.03

Note: ND – None detection

Table 6*:* Amount of ^{15}N derived from fertilizer at 0-20cm và 20cm-40cm

Soil depth	Amount of ^{15}N derived from fertilizer (gN/pot)			
	15DAF	30DAF	60DAF	90DAF
0-20cm	1.171	0.853	0.657	0.395
20cm-40cm	0	0.027	0.134	0.195
Total	**1.171**	**0.878**	**0.791**	**0.590**
CV%			10.23	5.42

Data in table 7 shows that amount of N fertilizer in pot trial of rubber trait RRIV 214 in haplic acrisol was low at 21.09% while loses occupied very high at 44,75%. It can be explained because the experiment was in rainy season. With pot trial, we lack method to prevent the pots from flooded condition if it suddenly heavy rained. However, at the end of the study 90 days, N fertilizer had residue in soil at 25%. It reflects that N did keep much in haplic acrisol soil.

Table 7: N fertilizer balance of rubber tree (2gN/pot)

Distribution of N fertilizer	Amount of N fertilizer (mg/pot)	Compare to N fertilizer input (%)
Plant used	0.422	21.09
Residue in soil	0.590	29.50
Loses	0.825	41,25
Total	**1816,86**	**91.84**

4. Conclusion

This experiment was conducted in the rainy season therefore it was needed to repeat in sunny season for comparison. Based on the result, it can be include that Ndff of leaf always dominated

However, the recommended nutrient rates (2gN/pot) had huge loses and less residue in haplic acrisol soil. More studies are needed to optimize nutrient rate and timing of fertilizer application to achieve the twin goals of plant use N fertilizer improvement and decrease loses in rubber growing in Vietnam.

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