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Effect of Neem (*Azadirachta indica* A. Juss.) Materials on Nitrification of Applied Urea in Three Selected Soils of Sri Lanka

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ABSTRACT. *Use of nitrification inhibitors is one measure of increasing the efficiency of N fertilization. Neem cake and its extract have been identified as locally available materials with nitrification inhibitory properties. Laboratory incubation experiments were conducted to investigate whether blending urea with neem materials alters the forms of nitrogen in soils, nitrification and general microbial activity, in Reddish Brown Earth (RBE–Alfisol), Reddish Brown Latosolic (RBL–Ultisol) and Red Yellow Podzolic (RYP–Ultisol) soils of Sri Lanka. The treatments used in these experiments were urea, urea + 20% neem cake, urea + 30% neem cake, urea + 20% neem extract and a control without amendments.*

All neem treatments conserved ammonium ions and reduced nitrate ions compared to urea alone, up to 8 weeks in RBL soil and up to 12 weeks in RBE soil. In contrast, in RYP soil, all neem treatments increased nitrate content and reduced ammonium content up to 6 weeks of incubation. In all soils, rate of nitrification increased with time in all treatments. Nitrification was inhibited by all neem treatments in RBE and RBL soils; however, per cent inhibition of nitrification was reduced over time. In both RBE and RBL soils, neem extract treatment recorded the highest per cent inhibition during second week of incubation. Rate of nitrification was very low in RYP soil, and nitrification was not retarded by any of the neem treatments. This is probably due to the low pH of RYP soil, which is a condition not favourable for nitrification. In all three soils, general microbial activity measured as CO_2 evolution had not been negatively affected by any of the neem treatments, indicating that application of neem cake or extract does not hinder normal microbial activity. Results therefore, indicate the possibility of using neem cake and extract as a means of retarding nitrification, in order to increase the efficiency of nitrogen fertilization.

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INTRODUCTION

The efficiency of fertilizer nitrogen, particularly under conditions of tropical agriculture, rarely exceeds 50%, and is usually 30–40% (De Datta, 1978). Mechanisms of N loss from soil are well established. Three processes appear to be of significance, namely, leaching in light textured soils in areas under heavy precipitation and intensive irrigation, denitrification in submerged soils and volatilisation losses such as ammonia from surface applied urea (Prasad *et al.*, 1971). One process that occurs continuously in almost all agricultural soils, and which frequently frustrates the conservation efforts of agriculturists, is nitrification. There is little doubt that if it was economically feasible to delay the conversion of ammonium to nitrate, this practice might have wide use as a means of decreasing loss of N, since leaching and denitrification losses of fertilizer N occur mainly after its conversion to nitrate form. Inhibition or retardation of nitrification of applied ammonium and amide nitrogen can thus reduce these losses and increase the efficiency of applied nitrogen.

Use of nitrification inhibitors has been identified as one measure to overcome the problems associated with N fertilizer application, such as high loss and low recovery of applied nitrogen due to leaching and denitrification, nitrate accumulation in vegetables and grasses resulting in nitrate toxicity to human and animals, and nitrate accumulation in groundwater due to continuous N fertilizer application. However, commercially available nitrification inhibitors such as N – Serve (2 Chloro – 6 trichloromethyl pyridine), and AM (2 Amino – 4 chloro – 6 methyl pyridimine) are expensive (Trenkal *et al.*, 1982). Therefore, identification of some available materials having nitrification inhibitory properties is essential. Neem (*Azadirachta indica* A. Juss.) materials have been identified as one of the locally available nitrification inhibitors in India (Harishankar and Rathi, 1976; Mishra and Chonkar, 1978; Subbiah and Kothandaraman, 1980; Sahrawat, 1982; Bhardwaj and Singh, 1992; Geethalakshmi and Palaniappan, 1992; Upadhyay and Patel, 1992; Muni *et al.*, 1993; Joseph and Prasad, 1994).

In Sri Lanka, due to high intensity of rainfall and high temperature, loss of applied nitrogen from soil is high. Level of nitrate nitrogen in groundwater used for drinking is well above the limit permitted by World Health Organization in some areas, such as Jaffna peninsula (Nagarajah *et al.*, 1988) and Kalpitiya peninsula (Kuruppuarachchi *et al.*, 1990). This is largely due to the continuous application of high rates of nitrogen fertilizers. However, little research information is available regarding the use of locally available nitrification inhibitors in Sri Lanka.

This study was undertaken to investigate the potential of using neem materials as nitrification inhibitors. Incubation experiments and leaching experiments were conducted to study the effects of blending neem material with urea on forms of soil nitrogen, rate of nitrification, per cent inhibition of nitrification and general microbial activity, in three different soils of Sri Lanka.

MATERIALS AND METHODS

Disturbed soil samples were collected up to a depth of 20 cm from three different locations of Sri Lanka namely, Dodangolla (Ultisol–Reddish Brown Latosolic), Maha Illupallama (Alfisol–Reddish Brown Earth) and Galigammua (Ultisol–Red Yellow Podzolic). They were air–dried and passed through a 2 mm sieve. Selected physical and chemical properties of soils are given in Table 1.

Table 1. Important characteristics of soils used in this study.

Property	Soil		
	RBL	RBE	RYP
Texture: Sand (%)	60.0	68.90	65.10
Silt (%)	14.2	1.60	20.10
Clay (%)	25.8	29.50	14.80
pH 1 : 2.5 soil : water	7.1	7.30	4.90
1 : 2.5 soil : KCl	6.5	6.80	4.40
Cation Exchange Capacity $cmol_c\ kg^{-1}$	19.2	13.50	12.30
Organic matter (%)	1.45	1.15	2.31
Total N ($mg\ kg^{-1}$)	0.09	0.10	0.15
Nitrate N ($mg\ kg^{-1}$)	15.3	8.10	5.40
Ammonium N ($mg\ kg^{-1}$)	10.3	4.90	12.00

Experiment 1

An incubation experiment was conducted to study the effect of applying urea blended with neem cake and alcohol extract of neem cake to soils, on ammonium, nitrate, and nitrite contents of the soils and to assess the

rate of nitrification and per cent inhibition of nitrification. Two hundred grams of each soil was taken for one replicate of each treatment. The neem seed cake used contained 4.9% N, 1.5% P_2O_5 and 1.6% K_2O. Neem extract was obtained from de fatted neem cake by extracting with 95% ethyl alcohol. After removing the solvent, extract was dissolved in acetone and used for these studies. Neem cake and/or extract were thoroughly mixed with urea 2 days prior to incubation, depending on the treatment. Treatments were urea alone (T1), urea + 20% neem cake (T2), urea + 30% neem cake (T3), urea + 20% neem extract (T4) + control without urea or neem (T5). Urea was applied at the rate of 200 mg N kg^{-1} soil. Soil and fertilizer (treated or untreated) were thoroughly mixed and transferred into glass bottles. The moisture content was adjusted to 50% field capacity and maintained at this level throughout the incubation. The bottles were covered with polythene to reduce moisture loss and kept at room temperature. Polythene was punched with two holes to provide aerobic condition. The experiment was carried out in a Complete Randomized Design with three replicates. Ten gram soil samples were drawn at 2, 4, 6, 8, 10 and 12 weeks after incubation and extracted with 1 M KCl and analyzed for ammonium (Keeney and Nelson, 1982), nitrate (Vendrell and Zupanic, 1990) and nitrite (Norwitz and Keliher, 1984) colorimetrically. Per cent inhibition of nitrification was measured according to the method as described by Sahrawat (1982).

Experiment 2

The second incubation experiment was conducted to investigate whether blending neem cake/extract with soils has any effect on the general microbial activity of the soils. The treatments imposed were the same as in Experiment 1. Soil samples (10 g) were treated and placed in glass bottles, to which distilled water (3.5 ml) was added and mixed well. The CO_2 evolved during incubation was trapped in 1 M NaOH in ignition tubes inside the glass bottle. After 2 weeks the contents of each ignition tube was washed into 200 ml beakers and 7.5 ml of 2 M $BaCl_2$ was added and titrated with 0.5 M HCl using phenolphthalein as the indicator. Amount of CO_2 trapped in NaOH was calculated in mg of CO_2/10 g soil. The treatments were arranged in a Complete Randomized Design with three replicates. This experiment was continued up to eight weeks.

RESULTS AND DISCUSSION

Figures 1 and 2 show the effect of blending neem with urea on ammonium and nitrate contents of RBE soils at 2, 4, 6, 8, 10 and 12 weeks after incubation. Soils in all neem treatments had a higher ammonium content and a lower nitrate content at all time intervals, compared to the treatment

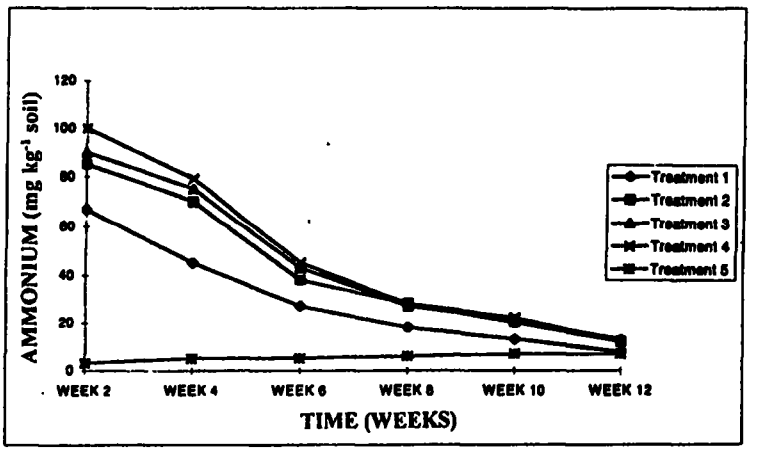

Figure 1. **Effect of blending neem with urea on ammonium content in RBE soil.**
[Note: See text for description of the treatments].

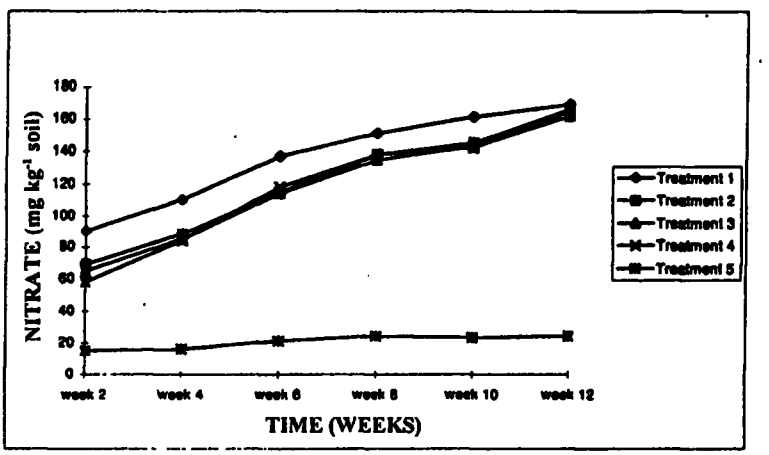

Figure 2. **Effect of blending neem with urea on nitrate content in RBE soil.**
[Note: See text for description of the treatments].

where urea was applied without neem materials (T1). Neem extract treatment (T4) recorded the highest ammonium content and the lowest nitrate content at early stages of incubation, compared to neem cake treatments (T2 and T3). Similarly, soils of 30% neem cake treatment (T3) had comparatively higher ammonium contents and lower nitrate contents up to four weeks, than soils of 20% neem cake treatment (T2). However, after six weeks the difference among neem treatments disappeared. Even though nitrite content was also found to be affected by treatments, the highest recorded value was 0.51 mg kg^{-1} soil, therefore it needs no emphasis.

Figures 3 and 4 illustrate the effects of blending neem with urea on ammonium and nitrate contents of RBL soil during the experimental period. Conservation of ammonium ions and reduction of nitrate ions by neem treatments were effective up to 8 weeks of incubation. At two weeks of incubation, soil treated with neem extract showed comparatively higher ammonium contents than other neem treatments, while treatment with 30% neem cake had higher ammonium than 20% neem cake during second week of incubation. During other time intervals, there was little difference in ammonium content among neem treatments. There was no significant difference among neem treatments in nitrate content during second and sixth weeks of incubation. However, at four and eight weeks of incubation neem extract recorded less nitrate content than other treatments.

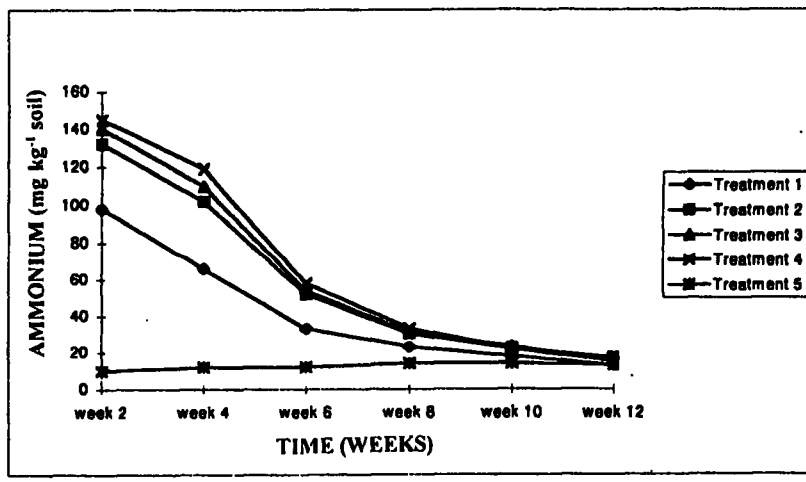

Figure 3. **Effect of blending neem with urea on ammonium content in RBL soil.**
[Note: See text for description of the treatments].

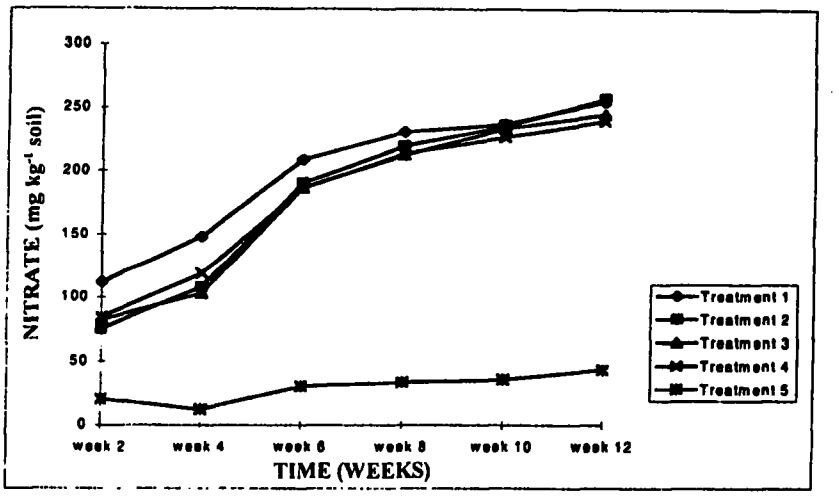

Figure 4. **Effect of blending neem with urea on nitrate content in RBL soil.**
[Note: See text for description of the treatments].

In RYP soil, unlike in other soils, neem treatments neither conserved the ammonium nor reduced the nitrate content at any time interval. In contrast, all neem treatments increased nitrate contents and reduced ammonium contents up to six weeks of incubation. In all treatments, nitrite was not detectable up to six weeks. Even beyond that, the recorded maximum nitrite value was 0.044 mg kg^{-1} soil.

Conservation of ammonium content and reduction of nitrate formation by blending neem cake with urea as observed in the present study in RBE and RBL soils, have been reported by several workers (Mishra *et al.*, 1975; Muthuswamy *et al.*, 1975; Reddy and Prasad, 1976; Mishra and Chonkar, 1978; Subbiah and Kothandaraman, 1980; Muni *et al.*, 1993; Joseph and Prasad, 1994). The same effect has been recorded with neem extract treated urea (Sahrawat and Parmer, 1975; Sahrawat, 1982). In contrast, a commercial preparation of neem extract, which was obtained from neem oil, when coated with urea gave no accumulation of ammonium in soil, while reducing nitrate content (Khandelwel *et al.*, 1977). No reports are available, supporting the results of RYP soil. However, the available information revealed that all the soils studied had neutral or alkaline pH values, whereas RYP soil was acidic. Therefore, pH may have played a role in this aspect. It is well documented that nitrification is inhibited at low pH levels (Keen and Prosser, 1987; Boer *et al.*, 1992; Hayatsu, 1993).

Rate of nitrification and per cent inhibition of nitrification by neem treatments in RBE, RBL and RYP soils are presented in Table 2. In both RBE and RBL soils, rate of nitrification increased with time, while per cent inhibition of nitrification decreased with time, in all neem treatments. In both these soils, neem extract treatment (T4) showed the highest per cent inhibition of nitrification. The RBE treated with neem extract blended urea recorded the

Table 2. Effect of blending urea with neem on nitrification rate and per cent inhibition of nitrification in RBE, RBL and RYP soils.

Soil and Treatment	Period of incubation (weeks)											
	2		4		6		8		10		12	
	NR	PIN	NR	PIN	NR	PIN	NR	PIN	NR	PIN	NR	PIN
RBE Soil												
T1	57	-	71	-	84	-	89	-	92	-	95	-
T2	45	21	56	21	75	11	83	07	92	03	93	02
T3	40	30	53	25	73	13	83	07	87	-	92	03
T4	37	52	52	27	72	14	84	06	87	-	93	02
RBL Soil												
T1	53	-	69	-	86	-	91	-	93	-	95	-
T2	36	32	54	22	79	08	88	03	92	01	94	01
T3	37	30	51	26	78	10	87	04	91	02	94	01
T4	34	36	48	30	76	12	87	04	91	02	94	01
RYP Soil												
T1	08	-	17	-	31	-	40	-	48	-	58	-
T2	10	-	19	-	31	-	46	-	55	-	63	-
T3	09	-	17	-	34	-	47	-	54	-	62	-
T4	08	-	17	-	34	-	47	-	53	-	61	-

NR – Nitrification rate
PIN – Per cent inhibition of nitrogen
T1-T4 – See text for description of the treatments.

highest inhibition of 37% during 2 weeks after incubation. The RBE treated with 20% neem cake blended urea (T2) gave the lowest nitrification inhibition of 21% at similar time interval. Treatment with 30% neem cake blended urea (T3) gave a 30% inhibition of nitrification. In RBL soils, 20% neem cake treatment (T2), 30% neem cake treatment (T3) and neem extract treatment (T4) recorded 32%, 30% and 36% nitrification inhibition, respectively. Nitrification retardation due to blending of neem products with urea had been reported by several workers. Urea blended with alcohol extract of neem cake (Sahrawat and Parmer, 1975; Khandelwal *et al.*, 1977; Sahrawat, 1982), urea blended with neem cake powder under laboratory conditions (Mishra *et al.*, 1975; Reddy and Prasad, 1976) and neem cake blended urea under field conditions (Subbiah and Kothandaraman, 1980; Geethalakshmi and Palaniappan, 1992; Joseph and Prasad, 1993) have been found to retard nitrification.

In the RYP soil, inhibition of nitrification was not observed with any of the neem treatments. In addition, rate of nitrification was very low in this soil, compared to other two soils (Table 2). Low rate of nitrification in RYP soil is probably due to the acidic nature, which agrees with findings of several workers (Sarathchandra, 1978; Keen and Prosser, 1987; Boer *et al.*, 1992). The lowest limit for nitrification in soils has been generally considered to be around pH 4.5 (Dancer *et al.*, 1973; Gilmour, 1984), which is only slightly below the pH of the RYP soil. Even though inhibition of nitrification was not observed in this study in RYP soil, Krishnapillai (1979) observed inhibition of nitrification in RYP soil, when urea was mixed with tea fluff (waste tea).

The general microbial activity, measured as CO_2 evolution of each soil treated with different neem treatments is presented in Table 3. In RBE soil, during the second week of incubation, significant difference in microbial activity among treatments was observed. The 30% neem cake treatment (T3) recorded the highest microbial activity, although there was no significant difference between 20% neem cake treatment (T2) and 30% neem cake treatment (T3). Both neem cake treatments showed significantly higher microbial activity than neem extract treatment (T4), urea alone (T1) and control (T5). Beyond 2 weeks, there was no significant difference in microbial activity among the different treatments. Santhi *et al.* (1985), also observed that neem products have no adverse effects on population of heterotrophic bacteria, whereas total microbial count was increased by neem products mixed with urea up to 15 days after application while retarding nitrifying bacteria. Shattuk and Alexander (1963) also reported similar findings with N Serve.

Table 3. Effect of blending urea with neem on microbial activity in soils.

Soil and Treatment	(Microbial activity - mg CO_2/10 g soil) Incubation period (weeks)					
	2	4	6	8	10	12
RBE Soil						
T1	15.17 c	9.68 a	14.50 a	11.50 a	18.50 a	20.23 a
T2	17.53 ab	11.20 a	14.57 a	10.90 a	18.93 a	19.60 a
T3	18.55 a	10.31 a	10.31 a	11.03 a	18.57 a	19.67 a
T4	16.35 bc	11.97 a	11.97 a	11.80 a	18.50 a	19.50 a
T5	15.84 c	7.27 a	7.27 a	11.07 a	18.50 a	17.30 a
RBL Soil						
T1	17.50 ab	10.37 a	17.36 a	13.37 a	21.67 a	20.70 a
T2	19.20 a	10.87 a	16.60 a	12.90 a	20.40 a	20.37 a
T3	19.27 a	10.41 a	16.40 a	12.67 a	21.17 a	21.17 a
T4	17.83 ab	11.57 a	16.60 a	12.71 a	21.00 a	20.73 a
T5	16.23 b	9.23 a	14.47 a	13.43 a	19.97 a	20.20 a
RYP Soil						
T5	15.53 a	8.17 a	16.43 a	18.17 a	7.80 a	13.00 a
T2	16.67 a	7.83 a	16.33 a	18.66 a	7.30 a	21.83 a
T3	15.43 a	7.10 a	16.20 a	18.40 a	7.87 a	13.60 a
T4	15.80 a	8.37 a	15.67 a	18.00 a	8.07 a	11.43 a
T5	15.77 a	8.86 a	16.47 a	18.80 a	8.30 a	12.60 a

See text for description of the treatments.

Values denoted by the same letter within a column are not significantly different by the Duncan's New Multiple Range Test at P=0.05.

In RBL soil, there was no significant difference in general microbial activity among the neem treatments and treatment with urea alone, at two weeks of incubation. Neem cake treatments recorded significantly higher microbial activity than the control treatment. Similar to RBE soil, there was no significant difference in microbial activity among treatments beyond 2 weeks. However, in RYP soil, there was no significant difference in microbial activity among treatments at any of the time intervals tested.

CONCLUSIONS

All neem treatments namely, urea + 20% neem cake (T2), urea + 30% neem cake (T3) and urea + 20% neem extract (T4) conserved ammonium ions and reduced nitrate ions compared to urea alone treatment (T1), up to 8 weeks in RBL soil and up to 12 weeks in RBE soil. Neem treatments differed in this aspect, mostly during the early stages of incubation. In most instances, whenever differences among neem treatments were observed, the neem extract treatment (T4) had a greater effect than the neem cake treatments. Between neem cake treatments, 30% neem cake (T3) had a higher effect than 20% neem cake (T2). On the other hand, in the RYP soil, neem treatment neither increased ammonium content nor reduced nitrate content at any time interval.

In all soils, rate of nitrification increased with time. In both RBE and RBL soils, nitrification was inhibited by neem treatments, however, with increasing time, per cent inhibition of nitrification was reduced. RBE and RBL soils treated with neem extract blended urea recorded the highest per cent nitrification inhibition during second week of incubation. Rate of nitrification was very poor in RYP soil. In all three soils, the general microbial activity measured as CO_2 evolution was not affected by neem treatments.

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