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Influence of Edaphic Factors on the Mineralization of Neem Oil Coated Urea in Four Indian Soils

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The utility of neem (*Azadirachta indica* A Juss) oil coated urea as a value-added nitrogenous fertilizer has been now widely accepted by Indian farmers and the fertilizer industry. In the present study, the expeller grade (EG) and hexane-extracted (HE) neem oils, the two most common commercial grades, were used to prepare neem oil coated urea (NOCU) of various oil doses, for which mineralization rates were assessed in four soils at three incubation temperatures (20, 27, and 35 °C). Neem oil dose-dependent conservation of ammonium N was observed in NOCU treatments in all of the soils. However, a longer incubation period and a higher soil temperature caused depletion of ammonium N. Overall, the nitrification in NOCU treatment averaged 56.6% against 77.3% for prilled urea in four soils. NOCU prepared from EG neem oil was consistently superior to that derived from hexane-extracted oil. The performance of NOCUs was best in coarse-textured soil and poorest in sodic soil. The nitrification rate (NR) of the NOCUs in the soils followed the order sodic > fine-textured > medium-textured > coarse-textured. The influence of edaphic factors on NR of NOCUs has been highlighted. The utility of the present study in predicting the performance of NOCU in diverse Indian soils was highlighted through the use of algorithms for computation of the optimum neem oil dose that would cause maximum inhibition of nitrification in any soil.

KEYWORDS: ***Azadirachta indica*; edaphic factors; neem oil; mineralization; nitrification**

INTRODUCTION

Urea is a solid source of fertilizer nitrogen (N) high in N content, is easy to handle even in tropical climates, and is used to prepare multinutrient fertilizers by blending with other fertilizer ingredients, such as potassium chloride, diammonium phosphate, and other materials. Between 1973/1974 and 1997/1998, worldwide urea consumption increased from 8.3 million tons of N to 37.6 million tons of N and from 22 to 46% of total world N consumption (*1*). It is now rapidly displacing anhydrous ammonia in the N fertilizer market. It is estimated that precious fossil fuel equivalent to 24 million BTU of natural gas is required to produce 1 ton of urea. Urea suffers from low nitrogen use efficiency (NUE) due to its tendency to lose a substantial portion of the nitrogen values by ammonia volatilization and rapid nitrification leading to nitrate leaching or followed by denitrification. Nitrate leaching leads to groundwater pollution (*2*). The worldwide NUE for cereal production (wheat, corn, rice, barley, sorghum, millet, oat, rye) is approximately 33%. The unaccounted for 67% represents an annual loss of U.S. $15.9 billion (*3*). Rapid nitrification is one of the key factors of inefficient N use, particularly in warmer climates such as India's. Regulation of urea hydrolysis and nitrification in agricultural systems has been one of the major strategies in overcoming these losses (*4*). Neem products have gained global importance owing to their broad-spectrum pesticidal properties (*5*). The nitrification retardation activity of neem products is now well-known (*6*). Our institute has pioneered the discovery and development of neem products as fertilizer urea adjuvants (*7*–*10*).

Neem oil coated urea (NOCU) is perhaps the first indigenously developed urea fertilizer (*11, 12*) that has now been listed under coated urea as per Indian Fertilizer Control Order (1985). At least three Indian industries have now produced over 1.2 million tons of NOCU, which has gained acceptance among farmers (*13, 14*). Singh et al. (*15*) evaluated the NUE of various slow-release urea products on wheat and found that NOCU compared favorably with sulfur- and lac-coated urea. Ramesh and Kotur (*16*) also reported a 7% increase in the NUE over prilled urea (PU) with the application of NOCU in tomato raised on acid sandy loam soil. Furthermore, ammonia volatilization was least with NOCU treatment (*17, 18*). NOCU was also useful in the reduction of nitrous oxide emission (*19, 20*).

Standard specifications for neem oils and NOCU were sought by the Indian Council of Agricultural Research (ICAR) and the Ministry of Chemicals and Fertilizers, government of India. Recently, we reported the most suitable industrial grades of neem oil and the influence of physicochemical properties of

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Chart 1. Physical and Chemical Characteristics of the Four Indian Test Soils

characteristic	soils: fine-textured	medium-textured	coarse-textured	sodic
source	Guntur (Andhra Pradesh)	Ludhiana (Punjab)	Hanumangarh (Rajasthan)	Karnal (Haryana)
class	Vertisols	Entisols	Entisols	Alfisols
typic	Haplusturt	Ustifluvents	Ustic psamments	Natrustalfs
sand (%)	22.8	64.0	82.0	47.0
silt (%)	24.0	18.0	4.0	24.0
clay (%)	53.2	18.0	14.0	29.0
pH (soil/water 1:2)	7.6	8.5	9.3	10.3
EC at 25 °C (dSm^{-1})	1.1	0.37	0.29	8.0
total nitrogen (%)	0.021	0.040	0.025	0.084
organic carbon (%)	0.43	0.64	0.53	0.26
ammonium N (mg/kg of soil)	3.40	2.20	1.90	4.20
nitrite N (mg/kg of soil)	traces	traces	traces	traces
nitrate N (mg/kg of soil)	11.3	16.3	26.3	16.3

neem oils on nitrification inhibitory activity of NOCUs (*21*). We have defined quality standards for industrial grade NOCU (*22*). The diversity of soils in India is large, and therefore an understanding of the mineralization behavior of NOCU in different soils and at various soil temperatures would be useful to predict its performance. Accordingly, the present study was undertaken to evaluate the performance of NOCUs prepared using two industrial grades of neem oils in four soils and at three incubation temperatures.

MATERIALS AND METHODS

Test Soils. The soils were collected from plow layers in the cultivated lands and sieved to 2 mm mesh before use.

The physical and chemical characteristics of the four India test soilds are given in **Chart** 1.

Neem Oils. Expeller grade neem oil was procured from the market, and hexane-extracted neem oil was extracted from neem seed kernel powder (*21*).

Fertilizers used were PU and NOCU.

Coating. Neem oil (20 g) was dissolved in acetone (100 mL) to get a 20% solution. PU (1 kg) was placed in an enamel tray, and the above solution (2.5 mL) was sprayed on the PU using an atomizer to obtain NOCU containing a 500 mg/kg dose of neem oil; the NOCU was mixed thoroughly and air-dried before storage. Similarly, the NOCUs containing higher doses of neem oil, namely, 1000, 2000, and 5000 mg/kg urea N, were prepared by using 5, 10, and 25 mL of above acetonic solution of neem oil.

Treatments. The experiments were laid out in a complete randomized design with three replicates. Fifty grams of air-dried, finely ground, and sieved (2 mm) soil was taken in 250 mL beakers and mixed with either PU or NOCUs (21.43 mg) to have 200 mg/kg urea N. The contents of beakers were mixed and incubated at 20, 27, and 35 °C, with moisture maintained at a level of 50% of water-holding capacity (*23*) of the soil throughout the study by adding the required amount of distilled water on every alternate day until the completion of the experiment.

Sampling and Estimation of Ammonium, Nitrite, and Nitrate N. Samples (5 g) were withdrawn after 10 and 20 days of incubation and extracted with 1 M sodium sulfate solution (50 mL) on a mechanical shaker for 30 min. Ammonium, nitrite, and nitrate N levels were determined by indophenol blue, modified Griess−Ilosvay, and phenol disulfonic acid methods (*24, 25*), respectively, with a UV−visible spectrophotometer for the analysis.

Because the nitrite N content in the present study was negligible (<0.5 mg/kg), Sahrawat's (*26*) formula is now simplified as

$$\text{nitrification (\%) for a constant period of incubation} = \{(NO_3^{-}\text{-N}) \times 100\}/(NH_4^{+}\text{-N} + NO_3^{-}\text{-N})$$

Statistical Analysis. The data were subjected to analysis of variance (*27*) using the software Statistical Package of Social Services (SPSS version 10.0). The treatment means were compared using critical difference for 5% probability ($CD_{5\%}$).

RESULTS

NOCUs were prepared using EG and HE, two commercial grades of neem oil. The oil dose was varied: 500, 1000, 2000 and 5000 mg/kg of urea. NOCUs thus obtained were screened along with PU in soil incubation studies at three incubation temperatures in four soils. The profiles of ammonium N, nitrite N, and nitrate N were monitored on the 10th and 20th days of incubation.

Mineralization of NOCUs to Ammonium N. The results are presented in **Tables 1** and **2**. Here, we present the ranges of data obtained with various doses of neem oil (from 500 to 5000 mg/kg) in two grades of NOCUs.

Table 1. Mineralization of NOCUs and PU to Ammonium N[a]

treatment (dose; mg/kg)	ammonium N (mg /kg): fine-textured soil, 20 °C, 10D	20D	27 °C, 10D	20D	35 °C, 10D	20D	medium-textured soil, 20 °C, 10D	20D	27 °C, 10D	20D	35 °C, 10D	20D
EG (500)	120.8	73.4	58.0	22.5	27.8	21.1	113.0	84.3	51.0	21.0	13.0	4.0
EG (1000)	125.6	82.8	89.5	37.0	46.0	32.6	131.3	95.0	67.1	26.0	20.7	6.0
EG (2000)	128.6	88.4	104.8	48.6	63.8	34.1	133.0	99.7	81.0	31.0	41.0	11.0
EG (5000)	137.0	93.1	111.4	52.6	66.7	40.1	136.3	112.3	98.0	54.0	63.0	29.0
HE (500)	116.7	68.7	53.6	19.0	28.8	20.3	109.0	88.3	41.0	20.0	10.0	4.0
HE (1000)	123.8	74.7	88.8	38.2	46.7	24.0	128.0	90.7	59.1	23.0	12.7	5.3
HE (2000)	127.2	82.7	89.3	47.8	64.3	34.0	130.1	115.7	74.0	27.0	24.0	8.3
HE (5000)	133.9	90.0	96.3	49.0	67.2	38.9	130.3	117.0	97.0	41.0	61.0	10.3
PU	73.5	41.5	38.9	16.3	22.1	14.7	105.3	84.3	14.0	7.0	4.0	2.0
$CD_{5\%}$	4.0	4.2	6.0	4.2	3.7	3.1	7.3	5.4	2.6	2.3	3.6	1.7

[a] EG, expeller grade neem oil; HE, hexane-extracted neem oil; 10D, 10 days after incubation; 20D, 20 days after incubation; PU, prilled urea.

Table 2. Mineralization of NOCUs and PU to Ammonium N[a]

treatment (dose; mg/kg)	ammonium N (mg /kg)											
	coarse-textured soil						sodic soil					
	20 °C		27 °C		35 °C		20 °C		27 °C		35 °C	
	10D	20D	10D	20D	10D	20D	10D	20D	10D	20D	10D	20D
EG (500)	136.7	110.1	117.9	61.6	69.0	33.9	78.3	36.7	52.3	26.3	10.0	2.0
EG (1000)	142.7	111.7	136.9	82.9	81.4	44.1	98.0	62.0	64.3	38.3	13.0	4.0
EG (2000)	147.3	122.3	142.0	94.1	96.4	56.3	116.7	99.0	60.7	43.3	30.0	6.0
EG (5000)	154.2	125.0	145.3	110.8	113.8	67.5	119.3	105.0	68.7	49.7	33.0	10.0
HE (500)	123.3	91.3	115.1	52.4	55.4	31.5	82.7	27.0	48.7	26.3	6.0	3.0
HE (1000)	138.3	107.5	132.2	73.5	78.7	44.1	102.7	62.7	60.3	37.3	14.3	5.0
HE (2000)	137.0	120.5	139.2	83.8	86.5	55.4	109.0	96.0	69.0	46.3	26.7	8.0
HE (5000)	148.0	126.5	141.8	97.1	99.8	58.7	124.0	92.7	74.7	50.0	31.0	10.0
PU	106.3	71.3	77.8	37.1	46.0	21.1	60.0	32.7	26.7	9.0	4.0	2.0
$CD_{5\%}$	4.4	6.5	6.0	5.0	5.9	4.3	6.5	7.8	3.6	4.8	3.5	1.6

[a] EG, expeller grade neem oil; HE, hexane-extracted neem oil; 10D, 10 days after incubation; 20D, 20 days after incubation; PU, prilled urea.

Table 3. Nitrate N Content in NOCUs and PU Treatments[a]

treatment (dose; mg/kg)	nitrate N (mg/kg)											
	fine-textured soil						medium-textured soil					
	20 °C		27 °C		35 °C		20 °C		27 °C		35 °C	
	10D	20D	10D	20D	10D	20D	10D	20D	10D	20D	10D	20D
EG (500)	49.0	87.6	120.0	140.9	157.5	160.9	27.7	75.0	134.3	171.0	144.3	181.0
EG (1000)	38.0	80.5	96.4	118.5	135.4	138.5	19.0	58.3	110.0	161.3	130.0	170.3
EG (2000)	29.0	73.7	90.2	108.0	123.1	128.0	15.7	36.7	95.0	150.0	105.0	167.0
EG (5000)	24.0	62.1	81.6	94.0	106.0	112.7	13.0	27.7	46.0	129.0	54.0	129.0
HE (500)	53.4	106.0	128.8	151.0	162.0	168.7	22.3	74.0	141.3	175.0	145.7	185.0
HE (1000)	39.0	98.1	101.0	127.3	136.0	146.0	19.3	58.0	136.0	167.0	134.0	177.0
HE (2000)	32.7	92.9	86.2	118.1	126.0	139.3	18.7	34.7	103.0	151.0	107.0	171.0
HE (5000)	28.9	81.4	82.0	108.0	114.3	133.0	14.3	30.0	51.0	131.3	59.0	141.0
PU	78.8	140.8	155.3	174.7	163.7	187.3	35.0	79.3	161.3	182.0	165.3	189.0
$CD_{5\%}$	4.8	3.7	8.2	6.8	4.8	6.8	3.8	5.8	3.5	5.5	6.3	3.6

[a] EG, expeller grade neem oil; HE, hexane-extracted neem oil; 10D, 10 days after incubation; 20D, 20 days after incubation; PU, prilled urea.

(a) Fine-Textured Soil. Treatments containing expeller grade neem oil NOCUs hereinafter labeled EG-NOCU showed ranges of 120−137, 58−111, and 27−67 mg/kg ammonium N at 20, 27, and 35 °C, respectively, on the 10th day of sampling. The corresponding data for hexane-extracted neem oil NOCU hereinafter labeled HE-NOCU were in the ranges of 116−134, 53−96, and 28−67 mg/kg ammonium N (**Table 1**). On the 20th day, the respective ammonium N contents at 20, 27, and 35 °C for EG-NOCU were in the ranges of 73−93, 22−53, and 21−40, whereas the corresponding data for HE-NOCU were in the ranges of 68−90, 19−49, and 20−39 mg/kg. PU at 20, 27, and 35 °C showed 74, 39, and 22 mg/kg ammonium N on the 10th day of sampling and 42, 16, and 15 mg/kg on the 20th day of sampling, respectively.

(b) Medium-Textured Soil. EG-NOCUs produced in the ranges of 113−136, 51−98, and 13−63 mg/kg ammonium N at 20, 27, and 35 °C, respectively, on the 10th day of sampling. The corresponding data for HE-NOCUs were in the ranges of 109−130, 41−97, and 10−61 mg/kg ammonium N (**Table 1**). On the 20th day, the respective ammonium N contents at 20, 27, and 35 °C for EG-NOCUs were in the ranges of 84−112, 21−54, and 13−63 mg/kg, whereas the corresponding data for HE-NOCUs were in the ranges of 88−117, 20−41, and 4−10 mg/kg, respectively. PU at 20, 27, and 35 °C showed 105, 14, and 4 mg/kg ammonium N on the 10th day of sampling and 84, 14, and 2 mg/kg on the 20th day of sampling, respectively.

(c) Coarse-Textured Soil. EG-NOCUs produced in the ranges of 136−154, 118−145, and 69−114 mg/kg ammonium N at 20, 27, and 35 °C, respectively, on the 10th day of sampling. The corresponding data for HE-NOCUs were in the ranges of 123−148, 115−142, and 55−100 mg/kg ammonium N (**Table 2**). On the 20th day, the respective ammonium N contents at 20, 27, and 35 °C for EG-NOCUs were in the ranges of 110−125, 61−111, and 34−68 mg/kg and for HE-NOCUs were 91−127, 52−97, and 31−59 mg/kg, respectively. PU at 20, 27, and 35 °C showed, respectively, 106, 78, and 46 mg/kg ammonium N on the 10th day of sampling and 71, 37, and 21 mg/kg on the 20th day of sampling.

(d) Sodic Soil. EG-NOCUs produced in the ranges of 78−119, 52−69, and 10−33 mg/kg ammonium N at 20, 27, and 35 °C, respectively, on the 10th day of sampling. The corresponding data for HE-NOCUs were in the ranges of 82−124, 48−75, and 6−31 mg/kg ammonium N (**Table 2**). On the 20th day, the respective ammonium N contents at 20, 27, and 35 °C for EG-NOCUs were in the ranges of 36−105, 26−50, and 2−10 and for HE-NOCUs, they were in the ranges of 27−93, 26−50, and 3−10 mg/kg, respectively. PU at 20, 27, and 35 °C showed 60, 27, and 4 mg/kg ammonium N on the 10th day of sampling and 33, 9, and 2 mg/kg on the 20th day of sampling, respectively.

Nitrate-N Content. The nitrate N content data in different treatments are shown in **Tables 3** and **4**.

(a) Fine-Textured Soil. EG-NOCUs showed ranges of 4−49, 81−120, and 106−158 mg/kg nitrate N at 20, 27, and 35 °C, respectively, on the 10th day of sampling, whereas the corresponding data for HE-NOCUs were in the ranges of 28−53, 82−129, and 114−162 mg/kg nitrate N (**Table 3**). On the 20th day, the

Table 4. Nitrate N Content in NOCUs and PU Treatments[a]

treatment (dose; mg/kg)	nitrate N (mg/kg)											
	coarse-textured soil						sodic soil					
	20 °C		27 °C		35 °C		20 °C		27 °C		35 °C	
	10D	20D	10D	20D	10D	20D	10D	20D	10D	20D	10D	20D
EG (500)	37.0	74.8	33.9	93.4	92.7	142.5	90.0	128.0	125.3	166.7	158.7	186.7
EG (1000)	31.3	70.0	29.6	80.1	79.4	127.2	72.0	121.7	110.0	163.7	140.0	180.3
EG (2000)	30.0	63.3	24.2	61.4	52.0	107.8	25.0	65.7	92.3	158.0	131.7	178.0
EG (5000)	22.0	54.0	23.3	32.2	34.7	88.9	21.0	29.7	74.0	136.7	97.3	173.3
HE (500)	38.7	82.6	38.1	103.1	104.0	149.0	86.0	131.0	128.0	173.7	168.0	189.0
HE (1000)	34.0	73.0	31.3	93.7	93.7	131.7	68.0	110.7	113.0	166.3	151.7	183.0
HE (2000)	32.7	64.7	28.9	65.7	65.7	126.3	26.0	58.7	102.3	164.0	132.3	174.0
HE (5000)	25.7	56.8	25.8	44.1	44.1	100.7	22.3	41.0	80.0	151.7	94.3	172.0
PU	41.0	120.7	85.9	152.0	152.0	172.1	118.0	140.0	166.0	187.0	175.3	191.0
$CD_{5\%}$	2.0	5.7	4.0	4.3	4.2	6.3	4.2	8.7	4.8	6.8	4.5	2.5

[a] EG, expeller grade neem oil; HE, hexane-extracted neem oil; 10D, 10 days after incubation; 20D, 20 days after incubation; PU, prilled urea.

Table 5. Nitrification of NOCUs and PU Treatments[a]

treatment (dose; mg/kg)	nitrification (%)											
	fine-textured soil						medium-textured soil					
	20 °C		27 °C		35 °C		20 °C		27 °C		35 °C	
	10D	20D	10D	20D	10D	20D	10D	20D	10D	20D	10D	20D
EG (500)	28.8	54.4	67.4	86.2	85.0	88.4	19.7	47.1	72.5	89.1	91.7	97.8
EG (1000)	23.2	49.3	51.9	76.2	74.6	80.9	12.6	38.0	62.1	86.1	86.3	96.6
EG (2000)	18.4	45.4	46.3	69.0	65.9	79.0	10.5	26.9	54.0	82.9	71.9	93.8
EG (5000)	14.9	40.0	42.3	64.1	61.4	73.8	8.7	19.8	31.9	70.5	46.2	81.6
HE (500)	31.4	60.7	70.6	88.8	84.9	89.2	17.0	45.6	77.5	89.7	93.6	97.9
HE (1000)	23.9	56.8	53.2	76.9	74.4	85.9	13.0	39.0	69.7	87.9	91.4	97.1
HE (2000)	20.5	52.9	49.1	71.2	66.2	80.4	12.5	23.1	58.2	84.8	81.7	95.4
HE (5000)	17.7	47.5	46.0	68.8	63.0	77.4	9.9	20.4	34.5	76.2	49.2	93.2
PU	51.7	77.2	80.0	91.5	88.1	92.7	24.9	48.5	92.0	96.3	97.7	99.0
$CD_{5\%}$	2.0	1.7	2.8	1.9	1.7	1.7	2.1	4.3	3.1	4.4	2.4	1.0

[a] EG, expeller grade neem oil; HE, hexane-extracted neem oil; 10D, 10 days after incubation; 20D, 20 days after incubation; PU, prilled urea.

Table 6. Nitrification of NOCUs and PU Treatments[a]

treatment (dose; mg/kg)	nitrification (%)											
	coarse-textured soil						sodic soil					
	20 °C		27 °C		35 °C		20 °C		27 °C		35 °C	
	10D	20D	10D	20D	10D	20D	10D	20D	10D	20D	10D	20D
EG (500)	21.3	40.5	22.3	60.2	57.4	80.8	53.5	77.6	70.6	86.4	94.1	98.9
EG (1000)	18.0	38.5	17.8	49.2	49.4	74.3	42.4	66.2	63.2	81.0	91.5	97.8
EG (2000)	16.9	34.1	14.5	39.5	35.1	65.6	17.6	40.0	60.4	78.5	81.4	96.7
EG (5000)	12.5	30.2	13.8	22.5	23.3	56.8	15.0	22.0	51.9	73.3	74.7	94.6
HE (500)	23.8	47.5	24.9	66.2	65.2	82.5	51.0	82.9	72.4	86.8	96.6	98.5
HE (1000)	19.7	40.5	19.1	56.1	54.4	74.9	39.8	63.9	65.2	81.7	91.4	97.3
HE (2000)	19.2	34.9	17.2	43.9	43.2	69.5	19.4	37.9	59.7	78.0	83.3	95.6
HE (5000)	14.8	30.9	15.4	31.2	30.6	63.2	15.2	30.6	51.7	75.2	75.3	94.5
PU	27.9	62.9	52.5	80.4	76.8	89.1	66.3	81.1	86.1	95.4	97.8	99.0
$CD_{5\%}$	1.9	2.6	2.2	2.4	2.2	2.5	2.9	4.1	1.7	1.7	1.8	0.8

[a] EG, expeller grade neem oil; HE, hexane-extracted neem oil; 10D, 10 days after incubation; 20D, 20 days after incubation; PU, prilled urea.

respective nitrate N contents at 20, 27, and 35 °C for EG-NOCUs were 62−88, 94−141, and 112−161 mg/kg and for HE-NOCUs were 81−106, 108−151, and 133−169 mg/kg, respectively. PU treatments recorded ranges of 79, 155, and 164 mg/kg nitrate N on the 10th day of sampling at 20, 27, and 35 °C, respectively, and the corresponding values on the 20th day were 141, 175, and 187 mg/kg.

(b) Medium-Textured Soil. EG-NOCUs gave ranges of 13−28, 46−134, and 54−144 mg/kg nitrate N at 20, 27, and 35 °C, respectively, on the 10th day of sampling, whereas the corresponding data for HE-NOCUs were 14−22, 51−141, and 59−146 mg/kg nitrate N (**Table 3**). On the 20th day, the respective nitrate N contents at 20, 27, and 35 °C for EG-NOCUs were 27−75, 129−171, and 129−181 mg/kg and for HE-NOCUs were 30−74, 131−175, and 141−185 mg/kg, respectively. PU showed 35, 161, and 165 mg/kg nitrate N on the 10th day of sampling at 20, 27, and 35 °C, respectively, and the corresponding values on the 20th day were 79, 182, and 189 mg/kg.

(c) Coarse-Textured Soil. EG-NOCUs gave 22−37, 23−34, and 35−93 mg/kg nitrate N at 20, 27, and 35 °C, respectively, on the 10th day of sampling, whereas the corresponding data for HE-NOCUs were 25−39, 25−38, and 44−104 mg/kg nitrate N (**Table 4**). On the 20th day, the respective nitrate N contents

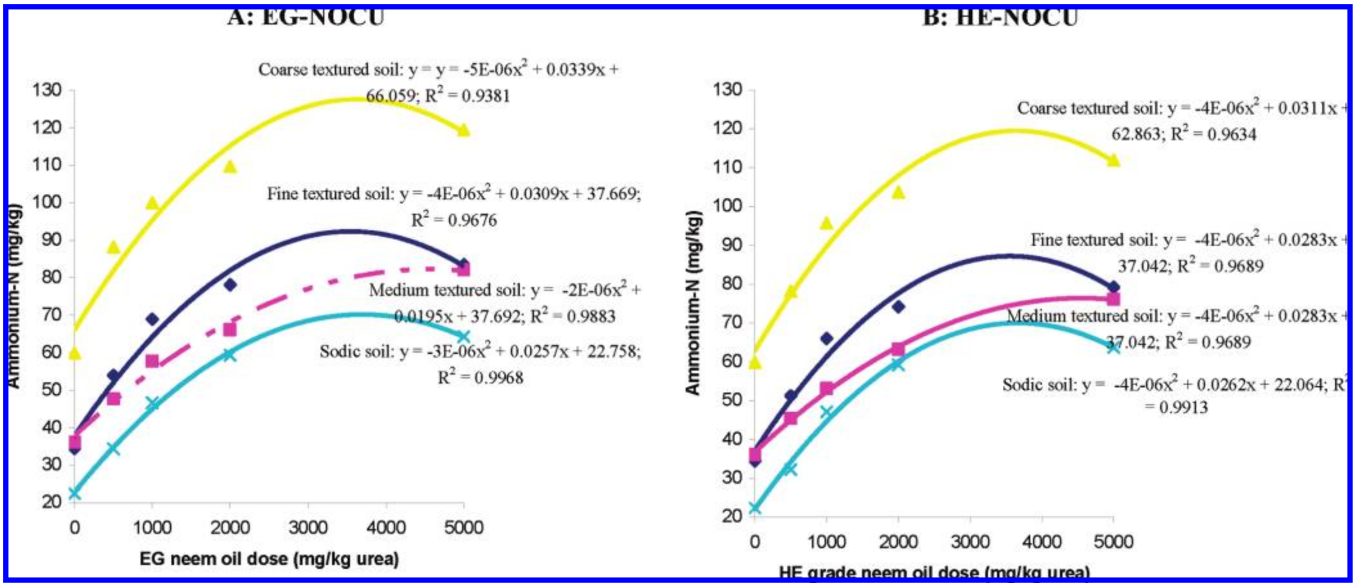

Figure 1. Effect of neem oil dose in NOCUs on ammonium N conservation in soils.

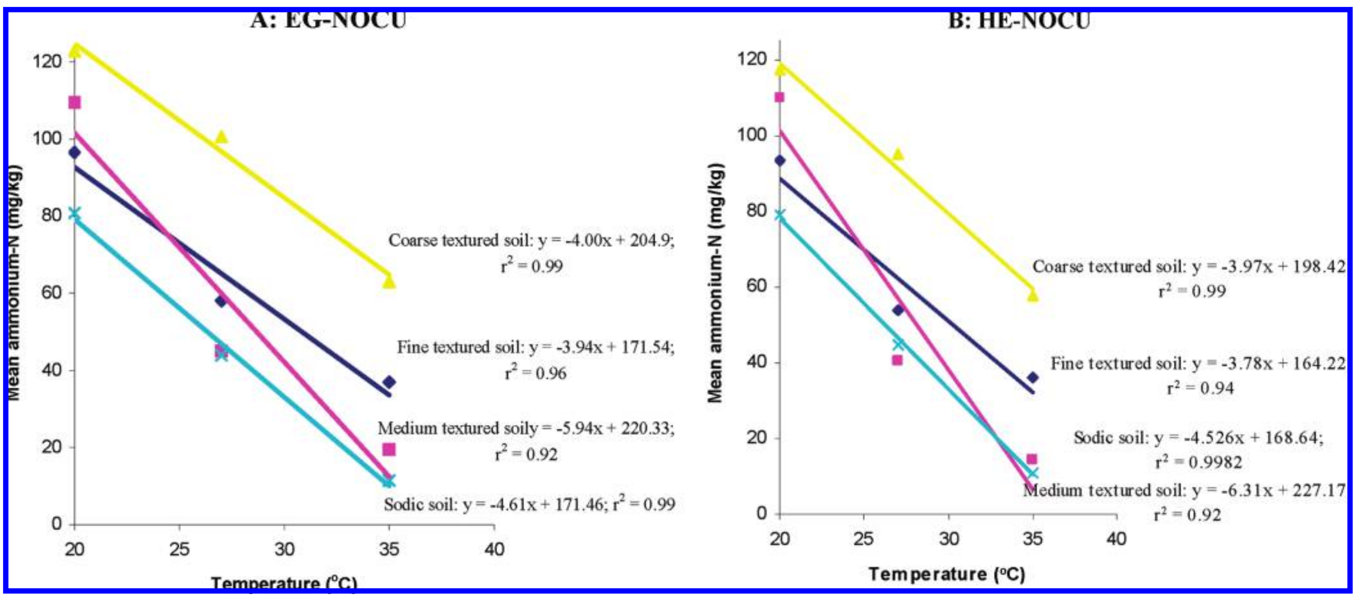

Figure 2. Effect of soil incubation temperature on mineralization of NOCU in soils.

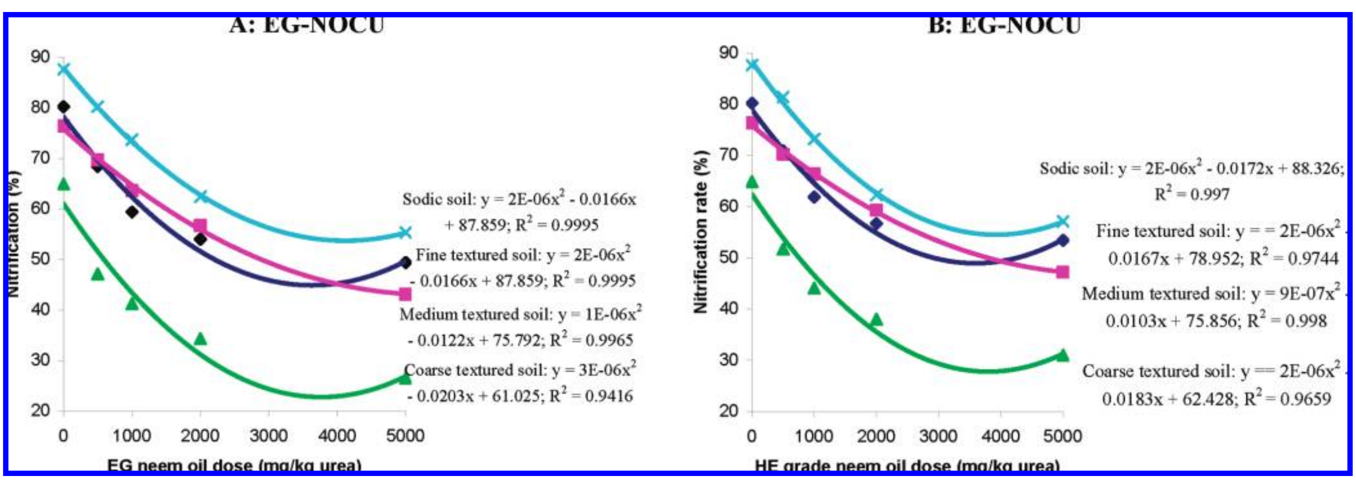

Figure 3. Effect of neem oil dose on nitrification of NOCUs in soils.

at 20, 27, and 35 °C for EG-NOCUs were 54−75, 32−93, and 89−143 mg/kg and for HE-NOCUs were 56−83, 44−103, and 100−149 mg/kg, respectively. PU showed 41, 86, and 152 mg/kg nitrate N on the 10th day of sampling at 20, 27, and 35 °C, respectively, and the corresponding data on the 20th day were 121, 152, and 172 mg/kg.

(d) Sodic Soil. EG-NOCUs treatments gave 21−90, 74−125, and 97−159 mg/kg nitrate N at 20, 27, and 35 °C, respectively, on the 10th day of sampling, whereas the corresponding data for HE-NOCUs were 22−86, 80−128, and 94−168 mg/kg nitrate N (**Table 4**). On the 20th day of sampling, the respective nitrate N contents at 20, 27, and 35 °C for EG-NOCUs were 29−128, 136−167, and 173−187 mg/kg. The corresponding values for HE-NOCUs were 41−131, 151−174, and 172−189 mg/kg, respectively. PU gave 118, 166, and 175 mg/kg nitrate N on the 10th day of sampling at 20, 27, and 35 °C, respectively, and the corresponding values on the 20th day were 140, 187, and 191 mg/kg.

Nitrification Rates (NR). The NRs in different treatments are given in **Tables 5** and **6**.

(a) Fine-Textured Soil. The NRs for EG-NOCUs at 20, 27, and 35 °C were in the ranges of 14.9−28.8, 42.3−67.4, and 61.4−85.0% conversion of added N, respectively, on the 10th day of sampling, whereas for HE-NOCUs the values were 17.7−31.4, 46.0−70.6, and 63.0−84.9% (**Table 5**). On the 20th day, the NRs were 40.0−54.4, 64.1−86.2, and 73.8−88.4 for EG-NOCUs and 47.5−60.7, 68.8−88.8, and 77.4−89.2%, respectively, for HE-NOCUs. PU showed 51.7, 80.0, and 88.1% on the 10th day of sampling and 77.5, 91.5, and 92.7% NRs on the 20th day of sampling at 20, 27, and 35 °C, respectively.

(b) Medium-Textured Soil. The NRs for EG-NOCUs at 20, 27, and 35 °C were 8.7−19.7, 31.9−72.5, and 46.2−91.7%, whereas HE-NOCUs showed 9.9−17.0, 34.5−77.5, and 49.2−93.6%, respectively, on the 10th day of sampling (**Table 5**). On the 20th day, the NRs were 19.8−47.1, 70.5−89.1, and 81.6−97.8% for EG-NOCUs and 20.4−45.6, 76.2−89.7, and 93.2−97.9% for HE-NOCUs, respectively. PU showed 24.9, 92.0, and 97.7% and 48.5, 96.3, and 99.0% NRs, respectively, on the 10th and 20th days of sampling.

(c) Coarse-Textured Soil. The NRs at 20, 27, and 35 °C were in the respective ranges of 12.5−21.3, 13.8−22.3, and 23.3−57.4% for EG-NOCUs and 14.8−23.8, 15.4−24.9, and 30.6−65.2% for HE-NOCUs on the 10th day of sampling (**Table 6**). On the 20th day, the corresponding data were in the ranges of 30.2−40.5, 22.5−60.2, and 56.8−80.8% for EG-NOCUs and 30.9−47.5, 31.2−66.2, and 63.2−82.5% for HE-NOCUs, respectively. PU showed 27.9, 52.5, and 76.8% on the 10th day of sampling and 62.9, 80.4, and 89.1% on the 20th day of sampling at 20, 27, and 35 °C, respectively.

(d) Sodic Soil. The NRs for EG-NOCUs at 20, 27, and 35 °C were in the respective ranges of 15.0−53.5, 51.9−70.6, and 74.7−94.1% on the 10th day of sampling, and the corresponding data for HE-NOCUs were 15.2−51.0, 51.7−72.4, and 75.3−96.6% (**Table 6**). On the 20th day, the NRs were 22.0−77.6, 73.3−86.4, and 94.6−98.9% for EG-NOCUs and 30.6−82.9, 75.2−86.8, and 94.5−98.5% for HE-NOCUs, respectively. PU showed 66.3, 86.1, and 97.8% on the 10th day of sampling and 81.1, 95.4, and 99.0% nitrification on the 20th day of sampling at 20, 27, and 35 °C, respectively.

DISCUSSION

Mineralization of NOCUs and PU to Ammonium N. *(a) Effect of Oil Dose.* The grand means of NH_4-N concentrations in four soils due to all treatments of NOCUs were obtained by combining the means due to variation in temperature regimens and periods of observations from the data in **Tables 1** and **2**. These grand means were plotted against oil dose for each class of NOCU as shown in **Figure 1**. The data for PU were combined as zero oil dose in the above plots. It is seen that the conservation of ammonium N followed a quadratic relationship with very high R^2 values. A perusal of the trend equations [where y is the NH_4-N concentration in mg/kg of soil and x is the oil dose (mg/kg of urea)] reveals that the conservations of NH_4-N in both NOCU treatments were very similar with various maxima. To derive x_{max}, the oil dose that would cause maximum accumulation of ammonium N, dy/dx is set to zero. The x_{max} value for EG-NOCU was calculated as 3390 mg/kg urea in coarse-textured soil. One can extend this calculation for other soils and HE-NOCU.

Table 7. Rates of Depletion of Ammonium N from the 10th to the 20th Day of Incubation in NOCUs and PU

source of urea	rate of depletion (%) of ammonium N[a] fine-textured soil	medium-textured soil	coarse-textured soil	sodic soil	mean
EG-NOCU	42.0	39.6	31.2	35.2	37.0
HE-NOCU	43.3	37.2	32.5	38.0	37.8
PU	46.1	24.3	43.7	51.8	41.5
$CD_{5\%}$	1.7	1.5	2.1	1.9	0.9

[a] $\{([NH_4\text{-}N]_{10th\ day} - [NH_4\text{-}N]_{20th\ day})/[NH_4\text{-}N]_{20th\ day}\} \times 100$, where $[NH_4\text{-}N]_n$ is the mean concentration averaged over the data (**Tables 1** and **2**) for four oil doses and three temperature regimens for each class of urea and soil for the day of observation.

Table 8. Nitrification during the Second Half (20th Day) of Incubation in Various Treatments

source of urea	mean nitrification (%) in soils[a] fine-textured	medium-textured	coarse-textured	sodic
EG-NOCU	18.9	21.9	24.2	16.4
HE-NOCU	20.5	20.2	24.4	16.8
PU	13.8	9.8	25.1	8.4
CD (5%)	0.6	0.7	0.6	0.6

[a] The difference in the means of data (**Tables 5** and **6**) averaged over four oil doses and three incubation temperatures for each day of observation for each urea source and soil.

(b) Effect of Soil Temperature Regimen. The grand means that were computed by combining mean data for five oil doses including PU data and two periods of observations for each temperature regimen from **Tables 1** and **2** are plotted against soil incubation temperature for each class of NOCU (**Figure 2**). Linear graphs were obtained for each soil with high r^2 values and established the inverse relationship between these two parameters. It shows that nitrification was enhanced by an increase in soil temperature. Two factors could contribute to this phenomenon. First, it is known that the optimum temperature for native nitrifiers in a tropical environment is 35 °C (*28*). Second, negative temperature influence on the efficacy of nitrification inhibitors is known, as the degradation of the active chemicals is likely to occur at the higher temperature (*29, 30*).

From the slopes of the lines, one could deduce that depletion of ammonium N ions was highest in medium-textured soils followed by sodic soil in both NOCU treatments. The depletion rates were on par in the other two soils. The depletion of NH_4-N from HE-NOCU was more rapid than from EG-NOCU treatments in medium-textured soils. It appears that this soil is conducive to N mineralization of urea.

(c) Effect of Period of Incubation. It was of interest to know whether NOCU slowed the process of nitrification in comparison to PU. The grand means of NH_4-N concentration at the 10th and 20th days of incubation were calculated from the individual means arising from four oil doses and three incubation temperatures for each soil and for each class of urea for a particular period of

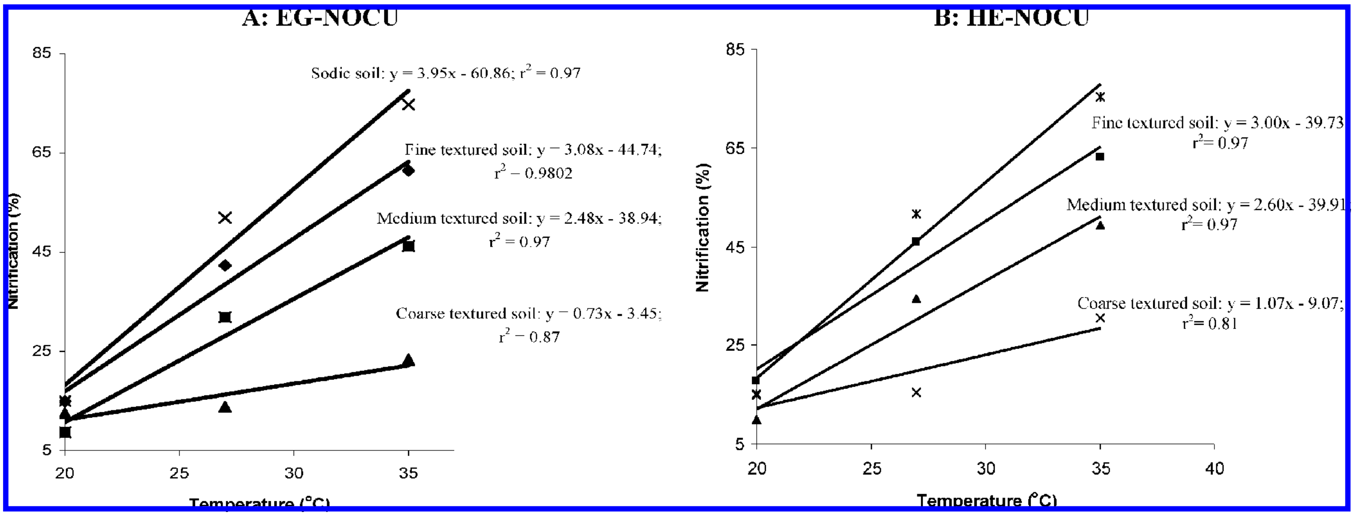

Figure 4. Effect of incubation temperature on nitrification of NOCUs coated with neem oil (5000 ppm) on the 10th day of incubation in soils.

Table 9. Correlation Coefficients of NRs of NOCUs with Edaphic Factors of Four Soils

edaphic factor	NR of NOCUs coated with	
	EG oil	HE oil
pH	0.113	0.042
EC (dSm^{-1})	0.772	0.732
sand (%)	−0.709	−0.758
silt (%)	0.941	0.963
clay (%)	0.511	0.569
organic carbon (%)	−0.677	−0.662

observation (from **Tables 1** and **2**). From the grand means so obtained, the rate of depletion was derived as per the formula shown in the footnote of **Table 7**. It is evident from **Table 7** that the net mineralization rate is slowed due to coating of PU with neem oil to the extent of 16.4% in sodic soil when the treatment was EG-NOCU. Overall, NOCU irrespective of the class and soils slowed the rate of depletion of NH_4-N by about 4%. However, in medium-textured soil, the rate of depletion was higher in NOCUs than in PU treatments. This soil was characteristically different from other soils with relatively higher organic C and lower EC values. Organic C is known to promote microbial growth, which in turn can degrade the active principles of neem oil. Furthermore, a spurt in the proliferation of nitrifiers following suppressed initial phase is also possible. Salinity is also reported to retard nitrification (*31*).

Nitrification Rates in Various Treatments. *(a) Effect of Oil Dose.* Grand means for both NOCUs were computed from the means listed in **Tables 5** and **6** by pooling data over three soil incubation temperatures and two periods of observations for each soil. The means for PU were taken as zero oil dose data for each class of NOCU. The grand means were plotted against oil dose for each class of NOCU (**Figure 3**). It is observed that the NR followed a quadratic relationship with very high R^2 values similar to the behavior in **Figure 1**. The trend equations [where y is the % NR and x is the oil dose (mg/kg of urea)] shown in **Figure 3** reveal that NRs in both NOCU treatments were very similar with various maxima of the curves. The maximum oil dose (x_{max}) in EG-NOCU to cause minimum nitrification (that is, to cause maximum inhibition of nitrification) is computed to be 3383 mg/kg of urea for coarse-textured soil from dy/dx data. This is in agreement with an earlier calculation made from the depletion of NH_4-N data.

(b) Effect of Period of Incubation. It was of interest to know whether NOCU slowed the process of nitrification in comparison to PU. Earlier, we carried out the test from the data of depletion of NH_4-N concentration between two time intervals. We have repeated this test using mean NR data between two time intervals, obtained from **Tables 5** and **6** by pooling mean data over oil doses and incubation temperatures, and the same for the second half of the incubation period is presented in **Table 8**. It is conspicuous that the NR picked up acceleration from the initial slow pace in NOCU treatments compared to PU in all soils except coarse-textured soils in which the NR values for all sources of urea were on par. It is inferred that the active ingredients in neem oil controlling nitrifier population possibly started to degrade to less active metabolites. It may be mentioned here that the constituents of neem oil such as azadirachtin are highly biodegradable (*32*). In the absence of retarding principles, the nitrifiers could reach their threshold population between the 10th and 20th days of incubation. A wide range in the lag phase of nitrifiers among different soils was observed, implying that the nitrification inhibition may vary depending on the length of time the experiment is run (*33*).

(c) Effect of Soil Temperature Regimen. Linear plots of NR data (computed by pooling the data for two periods of incubation) versus soil incubation temperature for both classes

Table 10. Overall Mineralization Performance of NOCUs and PU in Different Soils

fertilizer	soils: mean ammonium N (mg/kg)					mean nitrate-N (mg/kg)					mean nitrification (%)				
	fine	medium	coarse	sodic	mean	fine	medium	coarse	sodic	mean	fine	medium	coarse	sodic	mean
EG-NOCU	71.1	63.4	104.3	51.1	72.5	98.2	97.9	61.9	117.7	93.9	57.8	58.3	37.2	67.9	55.3
HE-NOCU	67.7	59.5	97.4	50.6	68.8	106.7	101.9	68.9	120.3	99.5	60.7	60.8	41.2	68.5	57.8
NOCU	69.4	61.5	100.9	50.9	70.6	102.5	99.9	65.4	119.0	96.7	59.3	59.6	39.2	68.2	56.6
PU	34.5	36.1	59.9	22.4	38.2	150.1	135.3	120.6	162.9	142.2	80.2	76.4	64.9	87.6	77.3
$CD_{5\%}$	0.8	0.8	0.9	0.9	0.9	1.0	0.8	0.7	1.1	0.9	0.4	0.4	0.5	0.4	0.4

of NOCU with the same oil dose (5000 ppm of urea) were obtained as shown in **Figure 4**. It is seen that EG-NOCU treatment slowed the NR more than HE-NOCU. It was slowest in the coarse-textured soil in both cases. For rapid assay of promising candidates of nitrification inhibitors or slow-release nitrogen fertilizers, coarse-textured soil at 35 °C and 10 days of incubation appears to be the best choice.

Influence of Edaphic Factors. The NRs of NOCUs were least at the highest test dose of neem oil (5000 ppm) over three test temperatures in four test soils (**Figure 4**). From the slopes of the trend lines, it is observed that the NR of the NOCUs followed the order sodic > fine-textured > medium-textured > coarse-textured. Using these slopes, the influence of edaphic factors such as pH, EC, organic C, and texture on NR was correlated as shown in the **Table 9**. It is seen that very high positive correlations were obtained with silt and EC, but the organic carbon and sand fractions gave negative correlations. The high correlation of the silt fraction with NR implies the positive influence of clay plus organic complexes, also known as soil protective capacity (*34*). However, more evidence is required to confirm this hypothesis. The availability of NH_4^+ to nitrifiers in soil depends on ammonium fixation capacity of clay minerals and the presence of other competing cations on the exchange sites, making nitrification in the soil a rather complex process (*35, 36*). Strong et al. (*37*) found that clay and sand (but not silt) were often observed to have a significant influence on nitrification. In soils kept continuously moist, clay content had a negative relationship with nitrification, but this relationship was positive in soils that had been dried and rewetted. In our study, the moisture regimen was maintained by periodic adjustment and the positive correlation observed possibly implies the alternate wetting and drying cycle. The soil pH showed very low positive correlation on NR. According to Strong et al. (*37*), soil pH often seemed to be a dependent rather than an independent variable in relation to nitrification.

Overall Performance of NOCUs. To assess the overall performance of NOCUs, grand means of NH_4-N, NO_3-N, and NR (%) averaged over all variables, namely, oil doses, incubation temperatures, and two days of observations, are computed for each soil in **Table 10**. It is observed that NOCU respectively produced 70.6 and 65.4 mg/kg of NH_4-N and NO_3-N, whereas the corresponding data for PU were 38.2 and 120.6 mg/kg of soil. This was amply reflected in a lower NR value of 56.6% as against 77.3% for PU. A perusal of the data shows that EG-NOCU was consistently superior to HE-NOCU. The performance of NOCUs was best in coarse-textured soil and poorest in sodic soil.

We could demonstrate the prediction of the optimum neem oil dose that would cause maximum inhibitions of nitrification in one soil. Thus, the present study would be a good primer for developing useful algorithms on the performance of NOCUs in diverse Indian soils.

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