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The effect of neem (*Azadirachta indica* a juss) kernel powder on the release of NH^+_4-N and NO^-_2 + NO^-_3 -N from urea in a savannah ecological zone of Nigeria

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ABSTRACT

An incubation study was conducted to evaluate the potential of powder made from neem (*Azadirachta indica* A Juss) kernel as an alternative nitrification inhibitor for increased utilisation and efficiency of urea fertiliser in soils. Three soil textural classes, namely: loamy sand, sandy clay loam and clay soils in Bauchi State, Nigeria were used for the study. Seven sets of 20 g soil samples were made in triplicate for each soil. Each of the seven sets respectively received seven rates of neem kernel powder (NKP) (i.e. 0, 1, 2, 3, 4, 5 and 6 mg.g^{-1} of soil) and arranged in a completely randomised design for each of the seven incubation periods (0, 3, 7, 15, 30, 45 and 60 days). All the samples received 4 ml of urea solution (50 µg ml^{-1}) and incubated at field capacity and at laboratory temperature (30 ^{0}C ± 2 ^{0}C). At the end of each incubation period, samples were analysed for NH^+_4 -N and (NO^-_2 + NO^-_3)-N. Results of the incubation study revealed that neem kernel powder treatments significantly ($p<0.01$) increased the retention of NH^+_4 -N presumably released through hydrolysis of applied urea by slowing its nitrification. This was observed in all the soils from 7 up to 30 days incubation period. Beyond this period, however, application of NPK ceased to be effective in slowing the nitrification process in loamy sand and sandy clay loam soils but continued to be effective in clay soil up to the end of the study (60 days).

INTRODUCTION

Nitrogen is one of the major nutrients required by plants for successful and sustainable crop production [1]. It plays a vital role in plant metabolism being an essential constituent of diverse types of metabolically active compounds such as flavins, purine and pyrimidine, nucleotides, enzymes, coenzymes and alkaloids [2].

Adequate level of this element is, therefore, essential for proper plant growth. In many tropical agricultural systems, the importance of nitrogen is second only to water [3].

Savannah soils of Nigeria, like most tropical soils, are inherently deficient in total nitrogen as well as organic carbon [4,5]. As one of the major plant nutrients and a key element required for plant growth, deficiency of N could manifest itself in poor yield or total crop failure. Application of mineral fertilisers to these soils has become necessary in order to sustain their productive capacities. Unfortunately, limited supply of mineral fertilisers and their attendant high prices particularly in Nigeria necessitates their judicious use.

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Efficiency of nitrogen fertilizer, particularly under tropical agricultural conditions rarely exceeds 50% and is usually between 30 and 40% [6,7,8]. Nitrogen losses in soils occur mainly through the processes of denitrification, volatilisation and leaching [9,10,11] which deplete a soil of nitrogen fertility. There is, therefore, a need to regulate the rate of depletion of soil nitrogen possibly by slowing down the rate of NO_3^--N formation in order to make it available to crops over longer period. Improved nitrogen fertiliser efficiency will not only help in more food production to meet the demand of the nation's growing population, but will also minimise pollution of surface and ground water which are of prime environmental concern worldwide.

Research efforts had focused on finding chemical products, which selectively inhibit nitrification in order to minimize nitrogen loss in soils. Such chemicals developed include nitrapyrin {2–chloro–6-(trichloromethyl) pyridine} dicyandiamide (DCD or DIDIN), "ST" sulfathiozole, sulphur compounds, furano and furano flavonoid compound, anilines and etridiazol [5-ethoxy-3-(trichloromethyl) }among others. Nitrapyrin {2–chloro–6-(trichloromethyl) pyridine} has been the most studied [12,13,14,15]. Nevertheless, dicyandiamide (DCD or DIDIN) seems to be the most promising inhibitor [16]. These chemicals are, however, expensive and are not environmentally friendly.

With the current thrust on sustainable agriculture and organic farming, the use of natural products has assumed greater practical significance. Neem tree (*Azadirachta indica* A Juss) products have been identified as the most suitable alternative nitrification inhibitors for environmentally safe agricultural development [17,18]. They are biodegradable, cheap and widely available particularly in northern part of Nigeria.

Although there are numerous literatures on the use of neem products as pesticide, little information is available on their application as nitrification inhibitor especially in Nigeria. The aim of the study therefore is to exploit the potential of this natural product as a nitrification inhibitor.

MATERIALS AND METHODS

Soils

The soils used for this study were collected from three locations in Bauchi state, which fall within guinea savannah ecological zone of the country and lies between latitudes $9^0 30^1$-$12^0 30^1$ N and longitudes $8^0 42^1$-$11^0 50^1$ E.

Soil Sampling and Preparation

Surface (0-15 cm) soil samples (60 kg soil) were collected from each of the three locations. Using grid method, sampling locations were identified and each location was divided into six sections from where soil samples were collected. The soil samples were thoroughly mixed to get a composite sample before air-drying. From the composite samples, sub-samples were taken, crushed (except for loamy sand), sieved using a 2-mm stainless steel sieve and used for analysis and incubation studies. Some physico-chemical properties of the soils of each location are presented in Table 1.

Treatments

For each site, seven sets of 20 g sample in plastic container (about 100 cm^3) were made in triplicate. All the seven sets in triplicate respectively received seven rates of Neem Kernel Powder (NKP) treatment in the following order: 0, 1, 2, 3, 4, 5 and 6 $mg.g^{-1}$ of soil. The NKP -soil mixtures were arranged in a completely randomised block design and

subjected to varying incubation periods. In total, seven incubation periods (0, 3, 7, 15, 30, 45 and 60 days) were used. The cover of each container was perforated (five small holes) for necessary aeration.

Each of the treated soil samples was thoroughly mixed by shaking on a mechanical shaker for one hour to achieve maximum blending of NKP with the soil. All the treated samples received 4 ml of urea solution (50 µg/ml), which corresponded to application rate of 100 kgN/ha and were watered to field capacity

The individual weight of each sample was determined at field capacity and the samples so prepared were aerobically incubated at laboratory temperature (30^0 C ± 2^0 C). All the samples were weighed at 3 days interval through out the incubation period and maintained at field capacity by adding a few drops of distilled water where necessary.

Analysis of samples for NH^+_4-N and ($N0^-_2$ + $N0^-_3$)-N

At the end of each incubation period, a total of 21 samples representing each treatment in triplicate per soil, were drawn and analysed for NH^+_4-N and ($N0^-_2$ + $N0^-_3$)-N by micro-Kjeldahl distillation method [19]. It involved leaching the soil samples with 1M KCL solution by shaking on mechanical shaker for 1 hour The leachates were filtered and distilled. Distillates collected on 2% boric acid solution were titrated against 0.25 M H_2SO_4 to determine NH_4^+-N. Magnesium oxide powder and devaadey alloy were added on to the residue and further distilled. Distillates collected on fresh 2% boric acid were titrated against 0.25 M H_2SO_4 to determine ($N0^-_2$ + $N0^-_3$)-N. Because of the large number of samples (63) involved, three drops of toluene were added to each leachate and stored in a refrigerator to avoid microbial activity prior to analysis.

Statistical Analysis

The data collected were subjected to Analysis of Variance (ANOVA) using Minitab Package. Main effects and interaction among treatments were compared, using the Least Significant Difference (LSD) procedure.

RESULTS AND DISCUSSION

Data on the amounts of ammonium-N (NH^+_4-N) and nitrite + nitrate-N ($N0^-_2$+ $N0^-_3$)-N retained by the soils at various incubation periods are presented in Tables II and III, respectively, while (small w) the effects of NKP treatments and incubation periods on the amounts of NH_4^+-N and $N0^-_2$ + $N0^-_3$-N retained by the three soils are presented in Tables IV and V, respectively. Table VI shows means of sum squares (from ANOVA Table) for the effect of neem kernel powder on NH_4^+-N and $N0^-_2$ + $N0^-_3$-N retained by the soils during incubation.

Effects of Neem Kernel Powder Application on Release of NH^+_4-N and $N0^-_2$ + $N0^-_3$-N during Incubation

At 0-day incubation, there was insufficient time for urease activity to take place and also for the applied neem kernel powder to have a meaningful effect on the urease enzyme. The releases of NH^+_4-N and $N0^-_2$ + $N0^-_3$-N and thus urea hydrolysis were therefore not affected by the treatment at this period. At 3-day incubation, however, there was sufficient time for urease activity and thus hydrolysis of urea to take place with consequent release of NH^+_4-N into the soil. The urease inhibitory effect of the neem kernel powder was also observed as samples from the control, released more NH^+_4-N than the neem treated soil samples. Previous researchers made similar observation with neem products [20,21] and

subjected to varying incubation periods. In total, seven incubation periods (0, 3, 7, 15, 30, 45 and 60 days} were used. The cover of each container was perforated (five small holes) for necessary aeration.

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Effects of Neem Kernel Powder Application on Release of NH^+_4-N and $N0^-_2$ + $N0^-_3$-N during Incubation

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from use of Agrotail N, a commercial *urease* inhibitor [22].

The hydrolysis of the applied urea might have been completed at the end of 7-day incubation and presumably the released NH^+_4-N would become substrate for nitrifying bacteria. In all the soils, samples where high levels (≥4 mg NKP/g) neem kernel powder was applied, produced higher amounts of NH_4^+-N than those with lower levels. This signified that, at higher levels of neem treatment (≥4 mg NKP/g), the activities of the nitrifying bacteria would have been negatively affected, leading to the inhibition of NH_4^+-N nitrification. Thus the resultant higher NH^+_4-N content in samples into which higher levels of NKP were applied. This finding is in line with other works [23,24,25,25,26,27,28,29]. The ($N0^-_2$ + $N0^-_3$)-N contents were, however, not correspondingly affected by the level of neem powder treatment, as was the case with NH^+_4-N. The NH^+_4-N contents of the three soils were by far less than what was recorded at 3-day incubation. However, the percentage reductions in NH^+_4-N, varied with soils. Loamy sand soil recorded the highest reduction constituting about 85% of what was recorded at 3-day incubation period, while sandy clay loam and clay soils recorded reductions of 76.88% and 47%, respectively. Differences in texture, organic matter content and cation exchange capacity, which all contribute greatly in nutrient retention could be responsible for variation in the N reduction between the soils [30]. Reductions in NH^+_4-N content in the soils could also be attributed to volatilization, as NH^+_3 smell was heavily perceived in the incubation environment. Immobilization by soil microorganism might also contribute to the NH^+_4-N reduction.

The nitrification inhibitory effect of the neem kernel powder appeared to have continued between 15 and 30-day incubation in all the soils. Samples with higher levels (4 and 5 mg NKP/g) neem treatment recorded higher NH^+_4-N content than those with lower levels. Beyond 30-day incubation period however, the effect of neem treatment on NH^+_4-N content had remarkably declined in the light-textured soils (loamy sand and sandy clay loam), but still significantly affected NH^+_4-N content in clay soil. More favorable condition for decomposition (especially aeration) in the lighter soils than the heavy clay soil, might have assisted in the degradation of the neem kernel powder and subsequent attenuation of its action. Our results confirm other reports [31,32]. This could explain why neem kernel powder ceased to be effective beyond 30-day in the lighter soils. On the other hand, the relatively higher organic matter content in clay soil (Table I) might have, through its sorption activity, protected the neem kernel powder from degradation and subsequent attenuation of its action in that soil. These could further justify why higher dosages of neem treatment would be needed by lighter than the heavy-textured clay soil to attain high dry matter and grain yields. The sorption of nitrification inhibitors by organic matter was also advanced among the reasons why inhibitors stay longer in soils rich in organic matter [33].

In all the soils, over the entire incubation period, progressive decrease in NH_4^+-N content with increase in incubation period was observed. Reductions in NH_4^+-N content were higher in samples with lower than higher levels of neem kernel treatment presumably due to nitrification inhibitory action of the neem kernel powder. The decrease in NH^+_4-N content was drastic between 3 and 15-day incubation period. This may be the critical period for intervention aimed at enhancing the use efficiency of applied urea especially in the loamy sand and sandy clay loam soils.

CONCLUSION

The results of this study have demonstrated that neem kernel powder has the beneficial effect of slowing down the processes of urea hydrolysis and nitrification of NH^{+}_{4} to $N0^{-}_{2}$ + $N0^{-}_{3}$ which would in turn, increase the use efficiency of the applied urea by extending the period of N availability to plants. The resultant effects of all these would be increase in crop yield. It would also eliminate the need for second dose of urea application to plants.

Table I **Some physical and chemical properties of the soils used for the study**

Parameter	Textural class			Mean (± SD)
	Loamy sand	Sandy clay loam	Clay	
Particle-size distribution (%): Sand	70.40	54.40	26.40	50.4
Silt	4.0	12.0	26.20	14.07
Clay	25.60	33.60	47.40	35.53
pH in H2O (1:1)	6.48	6.38	5.55	6.14
pH in CaCl2 (1:2)	4.55	4.60	4.03	4.39
Total N (g/kg)	0.70	0.97	1.49	1.05
Organic C (g/kg)	2.65	4.32	9.38	5.45
Available P (mg/kg)	3.66	7.15	12.56	7.79
Exchangeable bases (cmol/kg) :				
Na	0.03	0.02	0.04	0.03
K	0.02	0.03	0.03	0.027
Ca	0.28	0.30	0.42	0.33
Mg	0.089	0.04	0.04	0.056
Exchangeable acidity (cmol/kg): (H +Al)	0.08	0.10	0.40	0.19

Table II **Average NH^{+}_{4}-N (µg/g) content of soils at various incubation periods as affected by the levels of neem kernel powder**

Soil	Neem kernel powder (mg/g)	Incubation periods (in days)						
		0	3	7	15	30	45	60
Loamy sand	0	22.75	288.75	41.42	15.17	11.09	10.50	9.63
	1	23.33	227.50	29.92	17.50	11.84	9.92	9.34
	2	18.08	232.74	25.67	14.58	11.08	9.92	9.63
	3	15.25	169.17	30.62	21.67	12.25	10.50	9.92
	4	17.50	185.50	32.67	28.00	12.25	9.63	9.04
	5	19.25	177.33	35.00	41.42	29.17	9.63	9.04
	6	20.42	174.13	32.67	35.00	29.17	9.34	9.34
Sandy clay loam	0	25.08	302.00	63.00	43.17	33.25	28.58	25.67
	1	24.50	266.08	61.21	39.08	32.08	26.83	26.25
	2	25.67	274.17	64.46	38.50	30.33	25.67	22.75
	3	25.67	257.25	61.83	56.00	29.17	24.49	23.92
	4	28.88	278.83	68.25	65.95	31.50	26.23	23.92
	5	30.63	283.50	66.25	66.50	42.58	23.90	25.08
	6	25.05	292.25	67.67	65.92	42.00	25.07	25.67
Clay	0	26.25	501.08	252.58	217.58	168.58	135.33	121.33
	1	22.75	469.00	245.00	201.25	164.50	137.67	126.58
	2	22.17	457.92	247.33	201.83	149.92	127.75	123.67
	3	23.33	467.42	240.33	201.25	161.00	137.08	124.83
	4	23.92	463.92	266.58	215.25	165.67	140.00	127.17
	5	23.92	499.33	279.42	254.92	170.92	144.08	128.92
	6	25.08	499.92	262.50	306.83	161.00	141.75	129.50

Table III **Average $N0^{-}_{2}$ and $N0^{-}_{3}$-N (µg/g) content of soils at various incubation periods as affected by the levels of neem kernel powder**

Soil	Neem kernel powder (mg/g)	Incubation periods (in days)						
		0	3	7	15	30	45	60
Loamy sand	0	33.83	18.66	5.83	2.92	2.63	1.75	1.75
	1	33.25	9.92	7.00	2.92	2.34	2.04	1.75
	2	29.17	11.08	5.25	2.33	2.35	1.75	1.75
	3	22.75	7.58	3.50	2.33	2.92	1.75	1.75
	4	23.33	8.17	6.42	3.50	1.75	2.04	1.75
	5	23.33	8.17	5.83	3.50	2.92	1.75	1.75
	6	23.33	6.42	4.08	2.33	2.05	1.75	1.75
Sandy clay loam	0	29.17	7.00	4.38	3.50	2.92	2.33	1.75
	1	27.42	4.08	4.08	2.92	3.21	1+.75	1.75
	2	30.33	4.67	4.08	2.92	3.06	1.75	1.75
	3	29.75	4.67	4.08	4.67	1.75	1.75	1.75
	4	38.50	4.67	4.67	2.33	2.34	1.75	1.75
	5	36.50	6.42	3.50	2.33	2.34	1.75	1.75
	6	31.50	4.67	3.50	2.33	2.34	1.75	1.75
Clay	0	30.92	7.00	4.67	3.50	2.92	2.05	1.75
	1	30.33	6.42	5.25	3.17	2.63	1.75	1.75
	2	31.50	5.83	4.67	3.50	3.21	1.75	1.75
	3	30.33	6.42	4.67	3.50	2.92	1.75	1.75
	4	32.08	5.83	4.08	4.08	2.04	2.34	1.75
	5	29.75	5.83	4.08	4.08	2.34	2.63	1.75
	6	30.63	5.83	4.38	2.92	2.34	2.63	1.75

Table IV **Effect of neem kernel powder (mg/g) and incubation period (days) on NH^{+}_{4}-N (µg/g) content in soils**

Treatment	NH^{+}_{4}-N (µg/g) content in soils from		
	Loamy sand	Sandy clay loam	Clay
Neem kernel powder (mg/g of soil)			
0	57.04	74.39	203.25
1	47.05	68.72	195.25
2	45.96	68.79	190.08
3	38.48	68.33	193.61
4	42.08	74.80	207.50
5	45.83	79.21	214.50
6	44.29	77.66	218.08
Significance	**	**	**
LSD (0.01)	6.43	7.062	10.15
Incubation period (days)			
0	19.51	29.50	23.92
3	207.88	279.15	486.94
7	32.56	64.67	256.25
15	24.76	53.59	228.42
30	16.69	34.42	163.08
45	9.92	25.82	137.67
60	9.42	24.75	126.00
Significance	**	**	**
LSD (0.01)	6.43	7.06	10.15

** Significant at 0.01 level of probability ($P<0.01$)

Table V **Effect of neem kernel powder (mg/g) and incubation period (days) on NO^{-}_{2} + NO^{-}_{3}-N (µg/g) contents soils**

Treatment	$NO_2^- + NO_3^-$ -N (µg/g) content in soil from		
	Loamy sand	Sandy clay loam	Clay
Neem kernel powder (mg/g of soil)			
0	9.63	37.38	7.54
1	8.46b	5.17	7.33
2	7.67	4.04	7.46
3	6.08	3.00	7.33
4	6.71	2.56	7.46
5	6.75	1.83	7.21
6	5.96	1.75	7.21
Significance	**	**	NS
LSD (0.01)	1.20	2.49	–
Incubation period (days)			
0	27.0	37.36	30.79
3	10.0	5.17	6.17
7	5.42	4.04	4.54
15	2.83	3.00	3.54
30	2.42	2.56	2.63
45	1.83	1.83	2.12
60	1.75	1.75	1.75
Significance	**	**	**
LSD (0.01)	1.20	2.4	0.85

** Significant at 0.01 level of probability ($P<0.01$)

Table VI **Analysis of variance, ANOVA (mean sum of squares) for the effects of neem kernel powder on NH^{+}_{4} and (NO^{-}_{2} + NO^{-}_{3})-N contents of soils during incubation**

Source of variation	DF	Mean sum of squares	
		NH_4^+-N	(NO_2^-+NO_3^-)-N
Inc. days	6	640012**	7663.54**
Treat	6	2367**	64.51**
Soil	2	1040791**	36.07**
Inc. days x Treat	36	909**	57.02**
Inc. days x Soil	12	47677**	151.75**
Soil x Treat	12	759**	82.55**
Soil x Treat x Inc. days	72	474**	77.47**
Error	294	95	1.51
Total	**440**		

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