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Effect of Some Organic Carboxylic Acids on Nitrification of Urea in a Subtropical Soil

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ABSTRACT

Nitrification inhibition of soil-N and applied fertiliser N is desirable as the accumulation of nitrates in soils in excess of plant needs, leads to enhanced N losses and reduced fertiliser N-use efficiency. The effect of twenty-two different organic carboxylic acids, representing aliphatic (C_1 to C_{18}), di-, chloroacetic, hippuric, benzoic and heterocyclic (furoic, nicotinic and isonicotinic), on nitrification in a semi-arid subtropical sandy loam (Typic Ustrochrepts) soil in a laboratory incubation experiment is reported. The compounds were screened at 10% of applied-N dose and incubated for a period of 21 days at 30°C, maintaining 50% of the water holding capacity of the soil. Most of the test acids were found to be mild inhibitors of nitrification. Nitrification inhibition was the maximum (31.4%) in nicotinic acid treated soil followed by isonicotinic, furoic, palmitic, and stearic acid treated soils (26.6-27.8%). Based on four indicators of inhibitor effectiveness, namely, concentration of ammonium-N and nitrate-N, percent nitrification inhibition, ratio of ammonium-N to nitrate-N, and total mineral N, nicotinic acid showed the highest relative efficiency among all the test acids. A quantitative structure activity relationship (QSAR) model based on nitrification inhibitory activity [Arc sine (% NI)] and various physico-chemical substituent parameters for hydrophobic and steric properties has been developed using multiple linear regression (MLR) analysis technique.

Keywords: Nitrification inhibitors, organic carboxylic acids, urea, nitrification, nitrogen mineralization, QSAR

INTRODUCTION

Worldwide, nitrogen use efficiency (NUE) for cereal production (wheat, corn, rice, barley, sorghum, millet, oat, and rye) is approximately 33 %. The unaccounted 67 % represents a USD15.9 billion annual loss of N fertiliser (assuming fertiliser-soil equilibrium) (Raun and Johnson 1999). The factors contributing to inefficient N-use include ammonia volatilisation, nitrification, denitrification, nitrate-leaching, etc. These processes also contribute to various health and environmental hazards (Prasad and Power 1995). Nitrate leaching into underground waters due to rains and irrigation practices is a subject of global concern. The presence of nitrate concentrations of above 50 mg l^{-1} results in unpotable water, which can lead to

methaemoglobinaemia (blue baby syndrome) in infants (Spalding and Exner 1993). Under anaerobic conditions, nitrates are denitrified to N_2O and N_2. Production of N_2O results in global warming and depletion of the ozone layer in the atmosphere (Prasad 1998). Nitrification seems to be the centre point of inefficient N-use problems and environmental hazards, as denitrification and nitrate leaching are just its consequences. Efforts are, therefore, underway all over the world to retard and regulate the soil microbe driven nitrification phenomenon.

Oxidation of ammonia by *Nitrosomonas europea*, the bacteria that initiates nitrification as a whole, is a two-stage process involving the initial oxidation of ammonia to hydroxylamine by a membrane bound enzyme known as ammonia monooxygenase (AMO). The AMO has a remarkably broad substrate range, accounting for nitrification inhibition by several compounds (McCarty 1999). Subsequently, hydroxylamine is converted into nitrite.

The coal produced polycarboxylic acids (coal acids) are reported to effectively inhibit the nitrification of urea in soil (Patra and Jain 1993). However, no report was traceable in the literature on nitrification inhibitory properties of organic carboxylic acids. Hence, in an ongoing programme on the development of nitrification inhibitors, twenty-two different organic carboxylic acids representing aliphatic (C_1 to C_{18}), di-, chloroacetic, hippuric, benzoic and heterocyclic acids (furoic, nicotinic and isonicotinic) have been evaluated for their nitrification inhibitory activity (Datta *et al.* 2001; Kumar *et al.* 2001; Kumar *et al.* 2002).

Furthermore, a quantitative structure activity relationship (QSAR) model based on nitrification inhibitory activity [Arc sine (% NI)] and various physico-chemical substituent parameters for hydrophobic and steric properties have been developed using multiple linear regression (MLR) analysis to find the major parameters determining the nitrification inhibitory (NI) activity of acids and to design new active molecules.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals were procured from S. D. Fine Chemical Ltd., Mumbai, India and Thomas Baker Ltd., Mumbai, India and used without further purification.

Test Soil

A sandy loam soil (Typic Ustochrepts; 60.8% sand, 20.5% clay and 18.7% silt; pH 8.4 (soil: water 1:2.5); EC at 25°C 0.35 dSm^{-1}; organic carbon 0.26%; and available N 0.6 g kg^{-1} soil) was collected from the Research Farm of the Indian Agricultural Research Institute, New Delhi, India.

Treatments

The test chemicals were evaluated for their nitrification inhibitory activity in laboratory incubation studies at 10% of applied urea-N, along with urea alone as control. The experiments were laid following the randomised complete block

design. One hundred gram of air-dried, finely ground and sieved (10 mesh) soil was taken in 250 mL beakers and mixed with the calculated amount of test chemical (2 mg of test chemical, making, 10% of applied 200 mg urea-N kg^{-1} soil) followed by addition of 20 mg of urea-N in aqueous solution to each treatment. The experiment was conducted in triplicate along with the concomitant controls. The contents of the beakers were mixed thoroughly and incubated at 30°C, at 50% of the water holding capacity of the soil. The moisture content of incubated samples was maintained by adding the required amount of distilled water every alternate day.

Sampling and Estimation of Ammonium, Nitrite and Nitrate-N

Samples were withdrawn after 21 days of incubation and extracted with 1 M sodium sulphate solution. Ammonium, nitrite and nitrate were estimated by indophenol blue, modified Griess-Ilosvay (Keeney and Nelson 1989), and phenol disulphonic acid (Ghosh *et al.* 1983) methods, respectively using Specord 200 (analytikjena AG, Germany) UV-visible spectrophotometer for the analysis.

The ammonium, nitrite and nitrate contents were obtained from standard curves and expressed in mg kg^{-1} soil. Four different parameters, viz. concentration of ammonium-N and nitrate-N, percent nitrification inhibition, ratio of ammonium-N/nitrate-N, and total mineral N, were used as indicators of inhibitor effectiveness. The nitrification rate and percentage nitrification inhibition were calculated as follows (Sahrawat 1980):

Nitrification rate = $\{(NO_3^- + NO_2^-) - N + 100\} / \{(NH_4^+ + NO_3^- + NO_2^-)\text{-}N\}$

Percentage nitrification inhibition =

{(Nitrification rate in control - Nitrification rate in sample) + 100} /

{Nitrification rate in control}

Statistical Treatment

The nitrification inhibition percentage data were statistically analysed following the procedures laid out by Gomez and Gomez (1984). The analysis of variance was computed and treatment means were compared by Critical Difference (CD) at 5% level.

QSAR for Organic Carboxylic Acids as Nitrification Inhibitors

Three primary chemical descriptors for side chain variants viz., hydrophobic parameters like log P and steric parameters like molecular weight (MW) and molar refractivity (MR) were assembled from the literature (Hansch *et al.* 1995), and are listed in Table 1. QSAR analysis was performed using arc sine transformed per cent nitrification inhibition [ASIN (% NI)] as a dependent variable and the three physico-chemical descriptors as independent variables.

The four independent variables (including log MW) were used in constructing the correlation matrix. The mean percentage NI was transformed into sin arc and used as a dependent variable. A perusal of correlation matrix of descriptor variables

TABLE 1
Chemical descriptors for substituents in organic acids (R-COOH) and arc sine transformed % nitrification inhibition [ASIN (%NI)]

R	ASIN (%NI)	MW	Log (MW)	Log P	MR
H	0.40	1.03	0.01	-0.46	1.03
CH_3	0.34	15.05	1.18	0.09	5.65
$ClCH_2$	0.03	49.50	1.69	0.34	10.49
$C_6H_5CONHCH_2$	1.20	134.18	2.13	0.46	39.87
C_2H_5	0.23	29.08	1.46	0.58	10.30
n-C_3H_7	0.52	43.11	1.63	1.07	14.96
n-C_4H_9	6.54	57.13	1.76	1.56	19.59
n-C_5H_{11}	3.55	71.16	1.85	2.05	24.24
n-C_6H_{13}	3.38	85.19	1.93	2.54	28.90
n-C_7H_{15}	8.04	99.22	2.00	3.03	33.55
n-C_8H_{17}	3.73	113.24	2.05	3.52	38.20
n-C_9H_{19}	9.44	127.27	2.10	4.02	42.85
n-$C_{11}H_{23}$	10.60	155.32	2.19	5.00	53.01
n-$C_{15}H_{31}$	15.96	211.43	2.33	6.96	71.54
n-$C_{17}H_{35}$	15.42	239.49	2.38	7.94	80.80
$CH_3(CH_2)_7CH{=}CH(CH_2)_7$	8.33	237.47	2.38	7.73	80.86
HOOC	1.78	45.04	1.65	-1.74	6.93
HOOC-CH_2	4.93	59.06	1.77	-1.25	11.88
C_6H_5	9.20	77.12	1.89	1.87	25.36
2-C_4H_3O	16.02	67.09	1.83	1.00	17.88
3-C_5H_4N	18.29	78.11	1.89	0.69	24.34
4-C_5H_4N	16.13	78.11	1.89	0.69	23.03

TABLE 2
Correlation matrix between the independent variables

	MW	Log MW	Log P	MR
MW	1.000	0.77	0.91	0.99
Log MW	0.77	1.000	0.63	0.74
Log P	0.91	0.63	1.000	0.95
MR	0.99	0.74	0.95	1.000

Correlation is significant at the 0.01 level (2-tailed).

(Table 2) revealed that MW, log P and MR had a strong positive correlation with each other. This is revealed by the very high '*r*' value of log P ($r = 0.91$) and molar refractivity ($r = 0.99$) with respect to MW.

Multiple Linear Regression (MLR) Analysis

Stepwise MLR equation was generated using software package SPSS (Statistical Package for Social Sciences, ver. 10.0).

RESULTS AND DISCUSSION

Results obtained in the *in vitro* soil incubation study are reported in Table 3. Formic, acetic, chloroacetic, hippuric, propanoic, and butanoic acids revealed insignificant effect in increasing ammonium-N content compared to urea alone. The remaining test acid treated soils showed higher contents of ammonium-N, which were significantly higher in palmitic, stearic, furoic, nicotinic and isonicotinic acid treatments (53.3–61.5 ppm) compared to urea alone (5.3 ppm). Heterocyclic acids treated samples showed comparable ammonium-N contents with maximum in isonicotinic acid treatment (61.5 ppm), followed by nicotinic (60.7 ppm) and furoic acid treatment (60.3 ppm). In aliphatic acids, there was no proportional increase in ammonium-N with an increase in number of carbon atoms. Maximum ammonium-N was observed in palmitic acid treatment (58.7 ppm) followed by stearic (53.3 ppm), dodecanoic (36.6 ppm), decanoic (35.2 ppm), octanoic (30.6 ppm), butanoic (24.6 ppm) and malonic (20.0 ppm) acids. Benzoic acid treated soils showed 37.3 ppm ammonium-N.

The nitrite-N remained insignificant in all the samples. In cases of urea alone, palmitic, stearic, benzoic, furoic and isonicotinic acids 4.2, 1.5, 1.4, 4.2, 4.3 and 4.0 ppm, respectively of nitrite-N was observed. In all other treatments, it was non-detectable (<0.5 ppm).

Significantly lesser contents of nitrate-N were observed in dodecanoic, palmitic, stearic, oleic, furoic, nicotinic and isonicotinic acid treatments (120–144 ppm) as compared to urea alone (212 ppm). Pentanoic, octanoic, decanoic, malonic and benzoic acid treated soils also showed lesser nitrate-N content (157–167 ppm) than the control. For the heterocyclic acids, nicotinic acid treated soils showed the lowest minimum nitrate-N content (120.7 ppm) compared to isonicotinic and furoic acids, which showed 143 and 144 ppm nitrate-N, respectively. Among aliphatic acids, minimum nitrate-N was observed in stearic acid (135 ppm) followed by decanoic (157.3 ppm), pentanoic (158.7 ppm), octanoic (161.7 ppm) and malonic (165 ppm) acids. Benzoic acid treated soils showed 166.7 ppm nitrate-N. The rest of the acids revealed insignificant effects in decreasing nitrate-N content.

Most of the test acids have been found to be mild inhibitors of nitrification. Nitrification inhibitory activity of nicotinic acid was the maximum (31.4%) followed by isonicotinic, furoic, palmitic, and stearic acids in the range of 26.6–27.8%. The nitrification inhibitory activities of isonicotinic, furoic, palmitic, and stearic acids are not significantly different as evidenced by CD results. Pentanoic, octanoic, decanoic, dodecanoic, oleic and benzoic acids showed nitrification inhibition in the range of 11.4–18.4%. Nitrification inhibitory activities of octanoic, decanoic, oleic and benzoic acids are not significantly different (CD). The rest of the treatments showed insignificant inhibition of nitrification (<10%).

The NH_4^+-N/NO_3^--N ratio, which is considered a better and more sensitive

TABLE 3

Effect of acids (R-COOH, 1-22) on nitrification of urea in an incubated soil

Inhibitor acid	R	NH_4^+-N*[a]	NO_2^--N*[a]	NO_3^--N*[a]	TMN*[a]	A/N*[a]	% NI*
None	-	5.3	4.2	212.0	221.5	0.025	-
Formic	H	6.3	ND	193.0	199.3	0.033	0.7
Acetic	CH_3	6.2	ND	212.9	219.1	0.029	0.6
Chloro acetic	$ClCH_2$	4.0	ND	186.7	190.7	0.021	0.06
Hippuric	$C_6H_5CONHCH_2$	9.0	ND	188.0	197	0.048	2.1
Propanoic	C_2H_5	6.4	ND	217.0	223.4	0.029	0.4
Butanoic	n-C_3H_7	7.4	ND	214.4	221.8	0.035	0.9
Pentanoic	n-C_4H_9	24.6	ND	158.7	183.3	0.155	11.4
Hexanoic	n-C_5H_{11}	17.3	ND	186.6	203.9	0.093	6.2
Heptanoic	n-C_6H_{13}	16.3	ND	182.7	199	0.089	5.9
Octanoic	n-C_7H_{15}	30.6	ND	161.7	192.3	0.189	14.0
Nonanoic	n-C_8H_{17}	17.3	ND	178.7	196	0.097	6.5
Decanoic	n-C_9H_{19}	35.2	ND	157.3	192.5	0.224	16.4
Dodecanoic	n-$C_{11}H_{23}$	36.6	ND	144.0	180.6	0.254	18.4
Palmitic	n-$C_{15}H_{31}$	58.7	1.5	142.7	202.9	0.411	27.5
Stearic	n-$C_{17}H_{35}$	53.3	1.4	135.0	189.7	0.395	26.6
Oleic	$CH_3(CH_2)_7CH{=}CH(CH_2)_7$	27.3	ND	135.7	163	0.201	14.5
Oxalic	HOOC	10.7	ND	184.0	194.7	0.058	3.1
Malonic	$HOOC\text{-}CH_2$	20.0	ND	165.0	185	0.121	8.6
Benzoic	C_6H_5	37.3	4.2	166.7	208.2	0.224	16.0
Furoic	2-C_4H_3O	60.3	4.3	144.0	208.6	0.419	27.6
Nicotinic	3-C_5H_4N	60.7	ND	120.7	181.4	0.503	31.4
Isonicotinic	4-C_5H_4N	61.5	4.0	143.0	208.5	0.430	27.8
CD (P=0.05)	-	3.48	0.33	12.2	12.4	0.033	2.4

*The values are means of three replicates; a = all values are in ppm; TMN = Total mineral N; A/N = NH_4^+-N/NO_3^--N ratio; % NI = percent nitrification inhibition; ND = Non detectable (<0.5ppm)

indicator of inhibitor effectiveness as it is independent of the application rate of N, method of application, spatial variability (Hauck 1984) revealed that nicotinic acid is most effective having the highest ratio followed by isonicotinic, furoic, palmitic, and stearic acids. All these acids have 16-20 times higher ratio than the urea alone treatment. Pentanoic, octanoic, oleic, decanoic, benzoic and dodecanoic acids also have 6–10 times higher ratio than urea alone.

The total mineral N in all the treatments did not show appreciable differences with an exceptionally low value (163 ppm) in the oleic acid treated soil compared to urea alone (212.5 ppm). The total mineral N in all the remaining treatments ranged from 180–224 ppm, with a gradual difference among them.

General Comparison of NI Activity

Based on four indicators of inhibitor effectiveness, namely, concentration of ammonium-N and nitrate-N, percent nitrification inhibition, ratio of ammonium-

N and nitrate-N, and total mineral N, the relative efficiency of heterocyclic acids as nitrification inhibitors is higher than others with the highest being for nicotinic acid. The relative efficiency for isonicotinic and furoic acid was comparable. The highest relative efficiency among the remaining test acids was for palmitic acid followed by stearic, dodecanoic and decanoic acids.

QSAR for Organic Carboxylic Acids as Nitrification Inhibitors

The following quantitative structure activity relationship (QSAR) model based on nitrification inhibitory activity [arc sine (% NI)] and various physico-chemical substituent parameters for hydrophobic and steric properties were developed using multiple linear regression (MLR) analysis technique:

$$\text{ASIN (\% NI)} = 0.049\ \text{MW}\ (\pm 0.018) + 2.4\ (\pm 2.0) \quad (1)$$
$$\text{with } n = 22,\ r = 0.53,\ r^2 = 0.28;\ s = 5.35;\ F_{1,20} = 7.75.$$

In Equation 1, n stands for number of compounds, r the correlation coefficient, s the standard error of estimate, F the ratio of correlation ($p < 0.05$) respectively. The figures in parentheses are 95% confidence interval for the respective constant. Equation 1 was highly significant and accounted for 98.9% variation in nitrification inhibitory activity. It anchored the molecular weight (MW) as the principal descriptor variable, having a r value of 0.53. Thus, NI can be explained by the positive contribution of MW. The equation indicates that a higher bulk or steric factor of substituents is the dominating factor in deciding the NI activity. The substituents chosen must satisfy the criterion of higher bulk in the side chain of different acids tested which could facilitate the development of more potent analogues.

CONCLUSION

The results elucidate that acids with heterocyclic moieties, ex. nicotinic, and higher bulk, ex. palmitic, are better inhibitors of nitrification in soil. QSAR revealed that NI activity depended on the steric factor of acid substituents. This observation could be utilised to design and synthesise more active nitrification inhibitors.

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