

Use of urea coated with natural products to inhibit urea hydrolysis and nitrification in soil

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Abstract Laboratory incubation experiments were conducted with uncoated urea or urea coated with dementholized oil (DMO), pitch (the mint oil discard), terpenes (the products of menthol mint oil), or dicyandiamide (DCD) to study the retardation of urea hydrolysis and nitrification in soil. Two levels (0.5 and 1 %) of coating were tested. Urea was applied at a rate of 200 mg kg⁻¹ of dry weight of soil. The urea hydrolysis and nitrification processes were inhibited by all three natural products. All the three natural products viz., DMO, terpenes, and pitch significantly retarded urease activity of soil.

Keywords Essential oil · Nitrification inhibitors · Urease inhibitors · Urea N

Introduction

It is well established that the use of fertilizers is necessary for crop yield, but it can cause environmental problems such as increase of nitrate concentration in the groundwater, contribution to the formation of acid rain, ozone layer depletion due to release of nitrous oxides by denitrification, etc. Average estimates indicate that recovery of applied urea by kharif crops in India is 30–50%, but it can be as low as 20% in lowland rice because it can be lost through different

processes (Katyal et al. 1985; Patra et al. 2000). By reducing these N losses in the field, it is possible to reduce rate of application and avoid N-pollution of the environment (Patra et al. 2002).

Fertilizer urea, when applied to soil, is hydrolysed by urease to NH₄⁺ which is then oxidized to NO₃⁻ which can be leached or denitrified. To ensure a continuous and optimal supply of N, and to decrease losses, chemicals that retard either urea hydrolysis, or nitrification or both have been extensively tested. In this context, slow-release urea forms such as sulfur-coated urea, lac-coated urea, polymer-coated urea, and urea super granules have been extensively investigated (Prasad et al. 1971; Prasad 1998); urease inhibitors retarding urea hydrolysis have been also studied (Gould et al. 1986). In order to improve nitrogen use efficiency of crops, several synthetic chemicals such as N-serve (nitrpyrin), DCD (dicyandiamide), CS₂ (carbon disulphide), sodium chlorate, BHC (benzene hexachloride) etc. have been examined for inhibition of urea hydrolysis or nitrification or both in soils (Zaman et al. 2008). However, the use of many of these chemicals has been restricted to academic experimental studies because of high cost, lack of availability, and adverse effects on soil microflora (Purakayastha 1997).

Some natural products can inhibit nitrification such as Karanjin from Karanj (*Pongamia glabra*), tea waste containing tannins, and neem (*Azadirachta indica*; Prasad et al. 1971; Sahrawat and Parmar 1975; Sahrawat 1989). Another important discovery was the nitrification-inhibitory properties of Neem-cake-coated urea which has been used by Indian farmers (Prasad 1998).

A number of essential oils such as mint (*Mentha* sp.) oil find wide application as an antiseptic agent in

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pharmaceutical preparations. Chemical constituents such as menthol, carvone, thymol, terpene, pulegone present in mint oil and other essential oils have antimicrobial properties (Kiran and Patra 2002a, 2002b; Patra et al. 2001, 2002). *Mentha spicata* oil was as effective as DCD in retarding nitrification and increasing N use efficiency (Patra et al. 2002). During the last 4–5 years, several laboratory, greenhouse, and field experiments were conducted to evaluate the inhibition of nitrification and urea hydrolysis by such products (Patra et al. 2001, 2002, Kiran et al. 2003; Kiran and Patra 2003; Patra et al. 2006). Since many of the chemical inhibitors are expensive, not easily available and have significant adverse effect on beneficial soil microorganism, it is needed to find cheap natural products as urease and nitrification inhibitors (Prasad 1998).

The scope of the present investigation was to study the effects of natural essential oil by-products such as dementholized oil (DMO), and pitch (essential oil discard), and terpenes (the product of menthol mint oil) on nitrification and urease activity. The effects of these substances were compared with those of dicyandiamide (DCD). All these materials were used as urea-coating materials.

Materials and methods

Soil, inhibitors, and laboratory incubation

The soil (sandy loam, Entisol, Ustifluvent) used in this experiment has the following properties: pH 8.50, EC 0.40 dSm⁻¹; organic C 4.60 g kg⁻¹ soil; available N (alkaline permanganate extractable) 168 kg ha⁻¹; available P (0.50 M NaHCO₃ extractable) 12.8 kg ha⁻¹; and available K (1 N NH₄OAc extractable) 107 kg ha⁻¹.

Prilled urea (46% N) was coated with the natural products or with DCD using castor (*Ricinus communis*) oil as the fixative material. Therefore, urea granules were first coated with castor oil (1.00%, v/w basis), air dried for 24 h and then coated with DMO, Pitch, terpenes, or DCD at two rates (0.50% and 1.00% on v/w basis).

Then, coated uncoated granules which contained 200 mg N kg⁻¹ were used in the experiment which included ten treatments, four coating materials applied at two levels of coating (0.5 and 1.0%), uncoated urea and the control (no N and no coating material). The urea was applied at a rate of 200 mg kg⁻¹ soil. The statistical design followed a completely randomized design (CRD) with three replicates.

The collected soils samples were mixed, homogenized, sieved (<2 mm), and pre-incubated in airtight containers at 5°C in the presence of moist soda-lime for

5 days (Chand et al. 2004). Soil (100 g) was placed in plastic containers and treated with urea granules or left untreated (control). The mixture soil urea was thoroughly mixed and incubated at 60% of maximum water holding capacity and at 25±2°C for 3 weeks.

Analysis

Soil samples were removed at 1, 2, 3, 7, 14, and 21 days and extracted with 1 M Na₂SO₄-phenyl mercuric acetate (PMA) for urea-N, NH₄⁺-N and NO₃⁻-N analysis as described by Douglas and Bremner (1970), Yuen and Pollard (1952), and Sims and Jackson (1977), respectively.

The effect of the used products on urease activity was studied by incubating soil with urea (200 mg N kg⁻¹ soil) coated with the four urease/nitrification inhibitors or with uncoated urea at 25±2 °C for 4 h. The unhydrolysed urea was determined colorimetrically as described by Douglas and Bremner (1970).

Chromatographic (GC) analysis

By-products of dementholized oil (DMO) and terpenes were determined by gas chromatography using a Nucon make, microprocessor-controlled GLC model No. 5765

Table 1 Major constituents of by-products dementholized oil (DMO) and terpenes used for coating urea

Constituent	(%)
DMO (<i>M. arvensis</i> cv. Shivalik)	
Menthone	20.00
Isomenthone	12.14
L-menthol	24.55
Neomenthol	1.14
Methyl acetate	3.49
1,8-Cineol	2.90
Isomenthol	1.97
α-Pinene	2.08
β-Pinene	2.58
Terpenes (<i>Mentha</i>)	
Limonene	28.21
Menthone	16.15
Isomenthone	3.69
L-menthol	3.65
Isomenthol	1.43
Neomenthol	0.67
Methyl acetate	0.92
α-Pinene	0.39
β-Pinene	1.46

Table 2 Influence of natural and synthetic nitrification inhibitors on the fate of urea added to soil under laboratory condition (urea applied at 200 mg N kg⁻¹ soil)

Treatments	Forms of N (mg kg ⁻¹ soil)																		
	Day 1			Day 2			Day 3			Day 7			Day 14			Day 21			
	Urea	NH ₄ ⁺ N	NO ₃ ⁻ N	Urea	NH ₄ ⁺ N	NO ₃ ⁻ N	Urea	NH ₄ ⁺ N	NO ₃ ⁻ N	Urea	NH ₄ ⁺ N	NO ₃ ⁻ N	Urea	NH ₄ ⁺ N	NO ₃ ⁻ N	Urea	NH ₄ ⁺ N	NO ₃ ⁻ N	
Control (no N inhibitors)	0.00	5.08	67.4	0.00	2.00	73.5	0.00	8.41	89.5	0.00	2.88	69.3	0.00	1.11	70.0	0.00	0.00	0.00	73.0
Urea alone (no coating materials)	14.5	42.5	99.0	0.00	26.2	188	0.00	16.70	182	0.00	10.30	155	0.00	6.90	136	0.00	0.00	0.00	109
Urea + DCD																			
0.50% ^a	26.0	79.5	71.0	0.00	38.0	122	0.00	23.20	144	0.00	15.29	95	0.00	8.90	135	0.00	0.00	3.66	92
1.00%	26.8	89.5	60.2	0.00	48.5	104	0.00	32.3	156	0.00	18.29	92	0.00	9.80	120	0.00	0.00	2.17	77
Urea + DMO																			
0.50%	38.8	64.2	73.9	0.00	35.0	124	0.00	20.10	155	0.00	13.2	109	0.00	3.20	121	0.00	0.00	1.52	93
1.00%	44.8	72.3	61.0	0.00	38.0	105	0.00	23.5	144	0.00	13.8	101	0.00	3.80	122	0.00	0.00	2.38	80
Urea + pitch																			
0.50%	30.1	65.9	58.2	8.00	28.0	113	0.00	18.90	138	0.00	14.55	106	0.00	4.50	128	0.00	0.00	2.62	91
1.00%	38.0	74.0	56.1	9.45	43.0	108	0.00	32.80	132	0.00	12.70	99	0.00	2.70	132	0.00	0.00	3.00	85
Urea + Terpenes																			
0.50%	34.5	62.0	66.5	16.8	36.0	158	0.00	20.30	130	0.00	12.3	108	0.00	2.30	120	0.00	0.00	2.07	93
1.00%	52.0	72.0	58.0	18.2	35.0	106	0.00	30.10	132	0.00	12.9	101	0.00	2.90	122	0.00	0.00	2.00	87
LSD (P=0.05)	3.98	8.2	7.3	–	1.23	10.3	–	3.85	7.88	–	–	6.25	–	–	15.2	–	–	0.88	11.9

Each value represents the mean of three measurements

DCD Dicyandiamide, DMO dementholated oil, Pitch essential oil discard

^aCoating expressed as weight/weight basis of inhibitor and urea

with a 10'×1/8" FFAP column diameter filled with 80/100 chromosorb WAW, and a FID detector. The other conditions of the GLC were initial column temperature 100°C, final column temperature 220°C with a rise of 4°C/min. Injector and detector temperature was 225°C and the inlet pressure of the carrier gas N₂ was 30 psi. Chromatography of pitch could not be done due to its coal-tar-like texture. The chemical composition of natural essential oil by-products is given in Table 1.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and least significant difference (LSD) were calculated by using T-method (Sakal and Rholf 1981).

Results and discussion

All used inhibitors significantly reduced the urea-N hydrolysis and their effect differed markedly with the two levels applied (Table 2). Most of the urea coated with different materials was hydrolysed in the first 2 days of incubation; only terpenes and pitch-coated urea-treated soil showed residual urea. Urea hydrolysis inhibition and, consequently, reduced ammonia volatilization by natural products like neem cake and mustard cake have been reported by Purakayastha (1997). Dicyandiamide did not inhibit urea hydrolysis.

All the coating materials significantly retarded the nitrification in soil in comparison to the uncoated urea (Table 2). Nitrification inhibition was more pronounced at 1.0% level of coating but the ranking order of the different forms changed with level of coating. At 0.5% level of coating, the ranking order was: Pitch>DCD>DMO>terpenes; whereas, at 1.0% level of coating the order was: DCD>DMO>Pitch>terpenes. Nitrification inhibition by the coating materials may be due to the sensitiveness of nitrifiers to these products as it has been shown for plant products rich in phenols and alkaloids, which act as antibacterial substances (Olsen and Reiners 1983). Nitrification, however, increased with all treatments by increasing in the incubation period.

The NH₄⁺-N concentration was higher in the dicyandiamide treated soils and in soils treated with other coating materials, than in the soil amended with uncoated urea. High accumulation of NH₄⁺ in soil is not desirable as it makes the microsite soil pH high and the edaphic condition congenial for ammonia volatilization (Prakasa Rao and Puttanna 1987).

All three natural products significantly retarded urease activity at both the levels of coating; and the

Table 3 Influence of nitrification inhibitors on soil-urease activity under controlled conditions

Treatments	Urease activity (μg NH ₄ ⁺ -N released g ⁻¹ soil h ⁻¹)
Control (no N and no inhibitor)	30.62
Urea alone (no coating material)	79.80
Urea + DCD	
0.50%	75.60
1.00%	68.84
Urea + DMO	
0.50%	56.64
1.00%	57.00
Urea + pitch	
0.50%	62.40
1.00%	59.04
Urea + terpenes	
0.50%	67.80
1.00%	52.44
LSD (P=0.05)	10.88

Urea was applied at 200 mg N kg⁻¹ soil. Each value represents the mean of three measurements

highest inhibition was at the highest level of coating (Table 3). Inhibition of urease activity at 0.5% ranked as: DMO>pitch>terpenes>DCD. Trends were different at 1.0% level of coating and it was in the decreasing order: terpenes>DMO>pitch>DCD. The phenolic compounds of tea waste can substantially retard N mineralization in soil by inhibiting microbial activity (Sivapalan and Fernando 1985); this inhibition may be responsible for the decrease in urease and nitrifying activity. The natural products were more inhibitory than DCD.

Thus, different coating materials used viz., pitch, dementholized oil, and terpenes are potential retardants of urease activity and nitrification unlike dicyandiamide which is a potential retardant of nitrification but has little effect on the urea hydrolysis. The compounds of the used by-product (Table 1) responsible for such retardation need further investigation. Among the three mint oil by-products used, pitch, being cheapest and having a semisolid consistency, is most effective to be used as a coating material.

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