

Pine oleoresin: a potential urease inhibitor and coating material for slow-release urea

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In the recent past several attempts were made to increase N use efficiency of urea through coating. In this communication, an attempt has been made to coat urea with pine oleoresin to enhance its N use efficiency. Pine oleoresin is a gum-like substance extracted from pine trees. The crude resin is composed of levopimaric, palustric, l-abietic and neoabietic acids in different proportions. All these acids have antibacterial and antifungal properties. Pine oleoresin acts as a physical barrier around the urea granules thereby reducing the release of N from coated urea; it inhibits urease activity through antibacterial properties, and being acidic in nature, it inhibits volatilization loss by reducing alkaline microsites. A protocol has been developed to coat urea with oleoresin and the coated urea contained 3.82–4.36% pine oleoresin and 44.07–44.31% N. Irrespective of the soil type, urease activity decreased considerably in the pine oleoresin-treated soils compared to control due to acidic and antimicrobial properties of pine oleoresin. The amount of urea extracted at different times from Vertisols fitted to a first-order kinetic equation showed that time required for hydrolysis of 90% of the applied urea markedly increased from 88.56 to 328.94 h in the presence of pine oleoresin. This indicates that pine oleoresin is a potential urease inhibitor. The volatilization loss of pine oleoresin-coated urea from a Vertisol decreased from 16.99% to 10.12% after 240 h. Thus, pine oleoresin-coated urea can be a better substitute for neem-coated urea.

Keywords: Coated urea, pine oleoresin, urease activity, volatilization.

UREA is an important source of nitrogen (N) for crop production. The global consumption of urea was about 6.71 million tonnes during 2008–09 (ref. 1); however, there is concern about the efficiency of using urea-N for agricultural crops because farmers' practices in Asia commonly result in recoveries of < 40% of the applied N due to high N losses². It has been demonstrated that 30–

60% of N is lost in the form of ammonia from the agricultural soils through volatilization^{3,4}. Slowing down the diffusion rate by coating, reducing the urea hydrolysis rate by inhibiting urease activity and inhibiting volatilization loss by reducing the microsite pH through acidifying materials are options of improving the N use efficiency of urea-N⁵. The crude pine oleoresin (POR) has all these three properties which can successfully be utilized for making coated urea for increasing efficiency of urea-N. Previously, a lot of research work was done on the effect of various types of coated urea and urease inhibitors^{6,7}, but high cost and potent risks of ecological damage resulted in their low practical applications⁸.

POR is a gum-like substance extracted from pine tree (*Pinus roxburghii*) which is commercially used for the production of turpentine oil and other products. The annual average production of oleoresin in India is around 30,000 metric tonnes. Three Himalayan states namely, Uttarakhand, Himachal Pradesh, Jammu and Kashmir together produce 90% of the natural resin in India⁹. The crude POR contains about 4–10% water and trash, 12–30% turpentine, 6–10% non-volatile neutral matter and 65–75% resin acids, having empirical formula C₂₀H₃₀O₂. The natural oleoresin is composed of levopimaric acid 22%, palustric acid 11%, l-abietic acid 10% and neoabietic acid 15% (refs 10 and 11). All these acids have antibacterial and antifungal properties¹². Hence an attempt was made to coat urea with POR that provides a physical barrier for slow release of N from coated urea, inhibits urease activity through antibacterial properties and reduces volatilization loss by acidifying alkaline microsites.

A protocol has been developed to coat urea with oleoresin to obtain slow-release urea fertilizer. Water and turpentine were removed from the crude oleoresin by oven-drying. The crude resin was dissolved in an organic solvent and to this urea and methyl red indicator were added. Then the whole content was transferred to a horizontal shaker and shaking operation was continued with maximum speed. Thereafter the whole material was kept in a hot-air oven for hardening and to obtain oleoresin-coated urea. It was found that coated urea contained 3.82–4.36% POR and 44.07–44.31% N. Based on the density of urea (1.32 g cm⁻³) and POR (1.07 g cm⁻³), the calculated thickness of the coating is 11 μm.

A preliminary test was conducted to study the dissolution of urea from POR coated urea. Urea and oleoresin-coated urea were taken in separate test tubes to which soil extract was added to imitate the native soil microbial and enzymatic conditions and kept for 10 min without shaking. Next, a drop of 0.05 M NaOH and few drops of phenolphthalein indicator were added. The uncoated urea dissolved completely and the colour of the entire solution remained pink, whereas a clear demarcation of pink and white layers at the bottom indicates acidic nature of oleoresin-coated urea. Also, after the release of urea the coating material floated at the top and the pink colour

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disappeared because of the acidic nature of the resin (Figure 1).

Volatilization loss of $\text{NH}_4\text{-N}$ was measured using a force draft technique¹³. About 1 kg capacity sealed plastic boxes were filled with 500 g sieved Vertisol (pH 7.91) and the soil was treated with 400 mg uncoated urea (total N = 184 mg/pot) and 400 mg coated urea (total N = 178.64 mg N/pot). The closed dynamic (aerobic), air-flow system contained an air-exchange chamber (500 ml plastic box) and a trapping flask (250 ml Erlenmeyer flask). The stopper on each flask was fitted with an inlet and outlet facility. The inlet was linked to an air pump and the outlet was connected by polyethylene tubing to the trapping flasks containing 25 ml 2% boric acid to absorb the volatilized ammonia. Measurements were made after 48, 96, 168 and 240 h. The loss of $\text{NH}_4\text{-N}$ after different periods from coated and uncoated urea was calculated.

Stock solutions of 2000, 3000, 4000 and 5000 ppm POR were prepared using organic solvent and 5 ml of these stock solutions was added to each 100 g soil to supply 100, 150, 200 and 250 ppm of POR; control soil was maintained by only adding 5 ml of organic solvent. Thus 5 g treated soil (in triplicate) was mixed with 5 ml urea solution (containing 2000 ppm urea) and incubated at $37 \pm 2^\circ\text{C}$ for 5, 24, 48, 72 and 96 h. After the desired

incubation periods, unhydrolysed urea was extracted using 2 M KCl solution containing 5 ppm phenyl mercuric acetate¹⁴. Urea was estimated by the diacetyl monoxime method¹⁵. The amount of urea extracted at different times was fitted to first-order kinetic equation as described below.

$$\ln \frac{C_0}{C} = Kt,$$

where K is the first-order rate constant (h^{-1}), t the time (h), C_0 the initial concentration of urea (mg urea kg^{-1} soil) and C is the concentration of urea at time t .

The amount of N volatilized from urea and POR-coated urea treated Vertisol at different time intervals and the percentage of loss are summarized in Table 1. The total amount of $\text{NH}_4\text{-N}$ volatilized from normal urea and POR-coated urea after 240 h was 31.278 and 18.079 mg N pot^{-1} respectively, and the observed reduction in volatilization loss was 40.43% under POR-coated urea pretreated soil (Table 1). The acidic nature of POR might have reduced the microsite pH around the urea granules and thereby NH_3 loss through volatilization was reduced. Also, the antimicrobial properties of POR might have reduced the rate of hydrolysis of urea to $(\text{NH}_4)_2\text{CO}_3$ and subsequently to NH_4OH and CO_2 . The results thus show that POR has a potential to reduce N loss by volatilization from urea. Similar results were reported by several workers^{16,17} when urea was coated with biodegradable polymers and micronutrients.

The pH of aqueous solutions that contain pine oleoresins (0, 100, 200, 300, 400 and 500 ppm) was recorded as 7.54, 5.46, 4.56, 4.31, 4.18 and 4.10 respectively. The average urease activity ($\text{mg kg}^{-1} \text{h}^{-1}$) decreased considerably from 151.8 to 62.4 in Vertisol, 163.2 to 60.8 in Inceptisol, 143.5 to 50.4 in Aridisol and 121.2 to 44.6 in Alfisol when 250 ppm POR was applied to the soil (Table 2). The urea hydrolysis was found to follow first-order reaction and based on the value of first-order rate constant ($K = 0.026$, $R^2 = 0.963$), the time required for 90% hydrolysis of applied urea (2000 mg g^{-1} soil) was 88.56 h (Table 3). Several workers have also reported that urea hydrolysis in the soil follows first-order reaction¹⁸⁻²⁰. However, the time required for 90% hydrolysis of applied urea (2000 mg g^{-1} soil) was markedly increased in the presence of POR (250 ppm) to 328.94 h. Even at the end of the incubation period (96 h), considerable amount of urea remained unhydrolysed in the soil due to pretreatment with POR. This indicates that POR is a potential urease inhibitor. The urease inhibitory effect of POR is due to its acidity and antimicrobial properties controlling the population of urease-producing microorganisms in the soil. Moreover, POR may contain phenolic compounds and aromatic ketones which could bind with the urease enzymes, thereby reducing the enzyme-substrate reaction rate⁶. Similarly, it has been reported that the pine needle

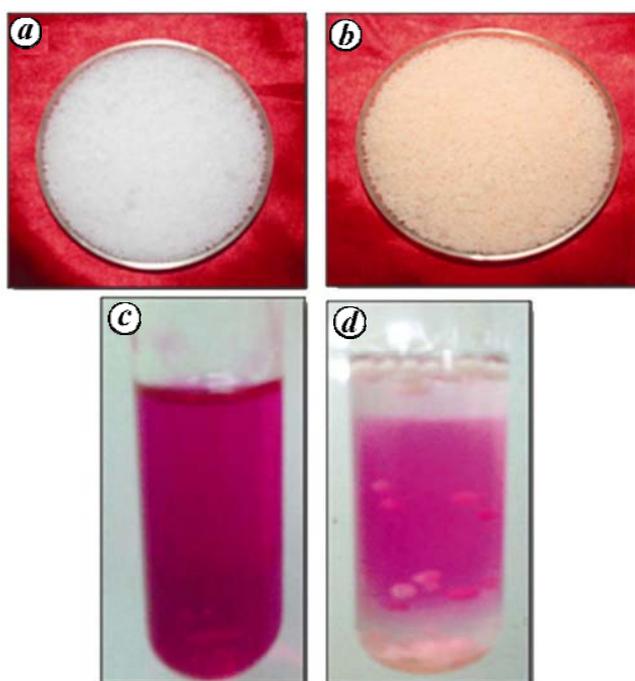


Figure 1. Dissolution of urea from pine oleoresin-coated urea **a**, Normal urea; **b**, Oleoresin-coated urea; **c**, Urea in soil solution when NaOH and phenolphthalein indicator were added resulting in pink colouration; **d**, Oleoresin-coated urea in soil solution when NaOH and phenolphthalein indicator were added, showing a clear demarcation of pink and white layers at the bottom of the tube. Also, after the release of urea the coating material floated at the top and the pink colour disappeared because of the acidic nature of the resin.

Table 1. Volatilization loss of N from normal urea and pine oleoresin (POR)-coated urea

Treatment	NH ₄ -N volatilized (mg N/pot) after				Volatilization loss (%)
	48 h	96 h	168 h	240 h	
Normal urea	6.436	17.634	26.148	31.278	16.99
POR-coated urea	3.108	10.626	14.462	18.079	10.12

Table 2. Effect of soil application of pine oleoresin (POR) on urease activity (mg kg⁻¹ h⁻¹)

Treatment	Vertisol (Bhopal)	Inceptisol (Ludhiana)	Aridisol (Jodhpur)	Alfisol (Betul)
Control	151.8	163.2	143.5	121.2
100 ppm POR	107.4	129.6	97.4	91.8
150 ppm POR	96.6	83.4	77.6	60.2
200 ppm POR	78.8	70.2	63.2	56.9
250 ppm POR	62.4	60.8	50.4	44.6
CD (<i>P</i> = 0.05)	14.26	15.34	11.87	13.43

Table 3. Effect of soil application of pine oleoresin (POR) on kinetics of urea hydrolysis rate in Vertisols

Treatment	Urea extracted (mg kg ⁻¹) after different times (h)					First-order rate constant <i>k</i> (h ⁻¹)	Time required for 90% hydrolysis of urea (h)
	5	24	48	72	96		
Control	1241	1005	605	331	114	0.026 (<i>R</i> ² = 0.963)	88.56
100 ppm POR	1463	1276	883	613	487	0.014 (<i>R</i> ² = 0.975)	164.47
150 ppm POR	1517	1318	1161	852	637	0.010 (<i>R</i> ² = 0.959)	230.25
200 ppm POR	1606	1473	1283	1005	723	0.009 (<i>R</i> ² = 0.959)	255.84
250 ppm POR	1688	1554	1291	1107	948	0.007 (<i>R</i> ² = 0.975)	328.94
CD (<i>P</i> = 0.05)	71.3	68.9	81.1	125.1	87.3		

and chinaberry seed extracts decrease the urease activity and urea hydrolysis rate in a sandy soil²⁰. Thus, application of POR is equally effective in inhibiting urease activity and hence is able to delay the hydrolysis of applied urea in the soil.

POR is equally effective in inhibiting urease enzyme activity in the soil due to its antimicrobial properties, acidic nature and the presence of phenolic and aromatic ketone compounds that bind with the urease enzymes. The urea coated with POR dissolves slowly because it acts as a physical barrier for diffusion of urea. The decrease in urea microsite pH in the presence of POR also facilitates the inhibition of urease activity and reduces the volatilization loss of NH₃ from urea. Thus, POR can be used as a urease inhibitor in the soil and POR-coated urea can be a better substitute for neem-coated urea.

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Subsurface sediment characteristics of the Great Rann of Kachchh, western India based on preliminary evaluation of textural analysis of two continuous sediment cores

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The present communication describes the lithological characteristics of two drill cores raised from the Great Rann of Kachchh based on physical characteristics, grain size distribution, textural characteristics and X-ray radiographs. The two cores were raised from the southern fringe of the salt-encrusted region, one to the north of Dhordo (~60 m depth) in the central part of the Great Rann basin and the second from the northeast of Berada (~51 m depth) in the southern Banni plain from the southern marginal part of the

basin. Based on textural characteristics, the Dhordo core is subdivided into 16 litho units and the Berada core is subdivided into 13 litho units. Based on the AMS date of $17,700 \pm 86$ cal years BP obtained from Dhordo core at a depth of 60.13 m and $9,515 \pm 61$ cal years BP obtained from the basal part of the marine sequence in the Berada core at 38.88 m depth, we infer that the central part of the Great Rann basin was submerged by a shallow sea by ~18 ka BP, whereas the marginal parts, including the Banni plain were completely submerged by ~10 ka BP.

Keywords: Great Rann, Kachchh, lithological characteristics, textural analysis, sediment cores, subsurface stratigraphy.

THE Great Rann of Kachchh is an E–W trending sub-basin which is bounded by the Nagar Parkar Fault (NPF) in the north and the Kachchh Mainland Fault (KMF) in the south¹ and comprises almost half of the area of the seismically active Kachchh palaeorift basin (Figure 1). Geomorphologically, the rann (meaning saline wasteland in local dialect) comprises a flat hyper saline terrain that opens up to the Arabian Sea in the west. Previous workers have described it as 'intriguing' to 'without any counterpart in the world'^{2,3}. Based on geomorphological characteristics, it is generally believed that the inherently saline sediments of the Great Rann basin were deposited during the Holocene in an embayed gulf with the surrounding raised land masses forming the source of the sediments^{3–5}. Historical accounts suggest that the rann was occupied by a shallow navigable sea⁶, which is confirmed by the presence of several archaeological sites belonging to the Harrapan civilization, including the port town of Dholavira located on the Khadir Island^{5–8}. Presently also, a large part of the Rann surface gets inundated by storm tides from the west and the rest by annual monsoon precipitation³. The Great Rann comprises four geomorphic units – the Bet zone, linear trench zone, Banni plain and the Great Barren zone³. Amongst these, the Banni plain by virtue of its higher elevation is not affected by marine inundation^{9,10}. Recent studies have documented raised marine terraces along the margins of the rocky islands of Khadir, Bhanjada and Kuar bet that suggest uninterrupted deposition in the Rann up to ~500 years BP (refs 11, 12). The Rann has also been studied in terms of the geomorphic changes attributed to recurrent seismic activity, including the 1819 Allah bund and the 2001 Bhuj earthquakes^{13,14}. However, no precise information exists about the subsurface stratigraphy and geological evolution of the Great Rann, which can be attributed to the harsh, inhospitable conditions, difficult terrain and lack of exposure.

To understand the geological evolution of the Great Rann and to delineate the palaeoenvironmental changes, two shallow continuous sediment cores were raised (Figure 1). One core of ~60 m depth was raised from the

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