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RECENT DEVELOPMENTS IN UREASE AND NITRIFICATION INHIBITORS*

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ABSTRACT

The current information on the control of urease activity and nitrification in soil by chemicals is reviewed. A large array of compounds have been proposed as urease and nitrification inhibitors. Compounds capable of retarding nitrification and urease activity are available but their use in practical agriculture is not very popular. The problem appears to be that the soil and environmental conditions that stimulates nitrification and urease activity are also conductive for the instability and ineffectiveness of urease and nitrification inhibitors. For inhibitors to have potential value in practical agriculture, they must be inexpensive in addition to being effective at reasonable rates of application and environmentally safe. Future research is needed to develop compounds/materials to control urease activity and nitrification from resources indigenous to a region or a country.

Urea is the most important nitrogen fertilizer in world agriculture. Its use is steadily increasing and this trend is likely to continue (15, 20, 29). The most important feature of urea is that it is a chemical nitrogen fertilizer whose availability to plants depends very much on the activity of urease enzyme in soil.

In arable soils, urea is rapidly converted to ammonium carbonate by soil urease, which results in several problems encountered in the use of urea as a fertilizer. These include increase in soil pH, ammonia and nitrite concentrations and gaseous losses of N as ammonia and oxides of nitrogen (14, 17, 18, 19, 24, 49, 62, 78). One approach for finding solutions to these problems lies in controlling urea hydrolysis in soils by using chemicals called 'urease inhibitors' that can retard urea hydrolysis and thereby reduce volatile loss of nitrogen as ammonia

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and ... nitrogen, and result in alleviation o... nitrite and ammonia toxicity to young seedlings (49, 62). The ammonium formed from urea in soil is converted to nitrate via nitrite through nitrification. Nitrate, is susceptible to losses through leaching and denitrification. There is considerable interest in conserving nitrogen in the ammonium form by using chemicals called 'nitrification inhibitors' that can retard nitrification and reduce loss of nitrogen in situations where loss of nitrate via leaching and denitrification is severe (49, 62, 76).

The objective of this paper is to review the current information relating to the control of urease activity and nitrification in soil by chemicals. For earlier references on the topics, the reader is referred to the reviews by Sahrawat (62) and Mulvaney and Bremner (49).

UREASE ACTIVITY IN SOILS

The presence of urease in soils was first suggested by Rotini in 1935. However, the poineering work by Conrad (12, 13) left no doubts that urease is responsible for conversion of urea to ammonium in soils treated with urea. Because urea is the most important N fertilizer, urease has received much more research attention than all other soil enzymes combined. The reader is referred to the excellent review by Bremner and Mulvaney (48), which comprehensively covers extensive literature relating to soil urease.

Urease activity in soils generally increases with increase in the substrate concentration. i.e. urea until the amount of urea is sufficient to saturate the enzyme. Results pertaining to the effect of soil water content on urease activity are conflicting. It is generally found that urease activity in soils usually increases with increase in water content upto field capacity but further increase in water content may not influence or may even decrease the activity.

Recently Sahrawat (67) found that urease activity in some Indian semi-arid tropical soils increased with increase in temperature from 10 to 60 or 70°C and then decreased with further increase in temperature upto 100°C where it was nearly completely inhibited (Fig. 1 and 2). Both buffer and non buffer methods of urease assay gave similar trend in results. These results point to the protection of urease in these soils even at high temperatures (60-7...C). Control of urea hydrolysis ... such soil situations in the tropics where soil temperatures are high will pose a few problems in controlling urea hydrolysis by chemicals.

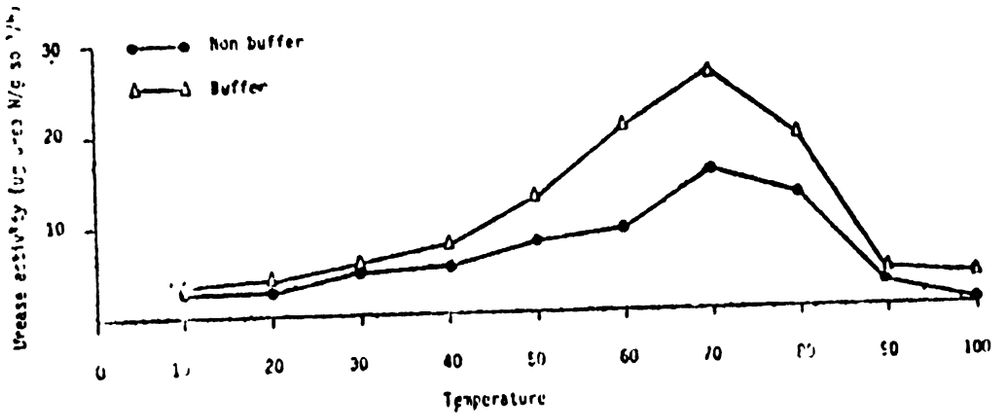

Fig 1. Effect of temperature on urease activity in Alfisol.

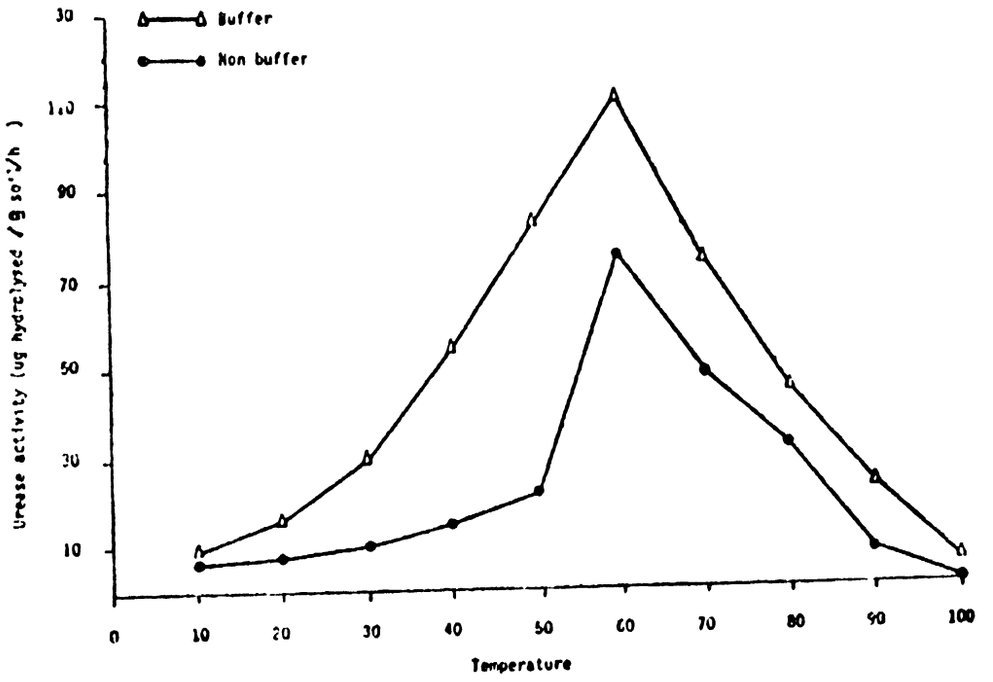

Fig. 2. Effect of temperature on urease activity in Vertisol.

CONTROL OF UREA HYDROLYSIS IN SOILS

Many compounds have been proposed to retard urea hydroylsis in soil (Table 1). In a recent study, Martens and Bremner (40) found that phenylphosphorodiamidate (PPD) was effective in retarding urea hydrolysis to varying degrees in 15 diverse soils. The inhibitory effect of PPD on urea hydrolysis

Table 1. [illegible] compounds proposed as urease inhibitors

Class of compounds/materials	References
1. Mono and poly hydric phenols quinones, anthroquinones and benzoquinones.	Quastel (1933); Bremner and Douglas (1971), Bundy and Bremner (1973b); Mishra and Flaig (1979), Mulvaney and Bremner (1978), Mishra et al. (1980).
2. Phosphoro amides specially phenylphosphorodiamidate (PPD)	Matzel et al. (1978); Vlek et al. (1980); Byrnes et al. (1983); Kampfe (1983); Martens and Bremner (1984).
3. Hydroxamates	Bremner and Douglas (1971); Gale and Atkins (1969); Kumaki et al. (1972); Pugh and Waid (1969 a, b), Waid (1975).
4. Phenyl ureas and substituted ureas.	Kistiakowsky and Shaw (1953); Shaw and Raval (1961).
5. Heterocyclic mercaptans	Gould et al. (1978).
6. Antimetabolite compounds	Mulvaney and Bremner (1977).
7. Metallic compounds	Hughes and Welch (1970); Bremner and Douglas (1971); Toren and Burger (1968); Tabatabai (1977).
8. Non edible oil seed cakes and their constituents, organic manures, residues and other plants products	Balasubramanian et al. (1972); Fernando and Roberts (1976).
9. Miscellaneous compounds and materials (chelating compounds, solvents, biuret, pesticides etc.).	Cervelli et al. (1975, 1976); Lethbridge and Burns (1976); Sahrawat (1977); Sahrawat (1979 a, b, 1980a); Bremner and Douglas (1971).

Source : Adapted from Shrawat (1980 a).

increased with the amount of PPD added and decreased markedly with time and increase in temperature from 10 to 40°C. The effectiveness of PPD to retard urea hydrolysis was considerably lower at 30°C and 40°C than at lower temperatures and this fact should be borne in mind while developing compounds or materials for use as urease inhibitors in the tropics. It was found that the ability of PPD to retard urea hydrolysis was significantly correlated with organic C ($r = -0.68^{**}$), total N ($r = -0.74^{**}$), CEC ($r = -0.65^{**}$), sand ($r = 0.66^{**}$), clay ($r = -0.64^{**}$) and surface area ($r = -0.60^{*}$) but was not significantly correlated with pH, silt content, urease activity or $CaCO_3$ equivalent. Multiple regression analyses showed that the effectiveness of PPD to retard urea hydrolysis in soils increased with decrease in soil organic matter content. Similarly, the effectiveness of p-benzoquinone (PBQ) and hydroquinone (HQ) for retarding urea hydrolysis in 25 diverse surface soils was affected by organic matter content, CEC, sand, silt and clay content, surface area and urease activity of soils. The general conclusion was that the effectiveness of PBQ and HQ for retardation of urea hydrolysis in soils increased with decrease in soil organic matter content (Mulvaney and Bremner) (48).

In addition to soil properties, perhaps temperature is the most important factor that greatly affects the effectiveness of urease inhibitors for retarding urea hydrolysis.

Interest in the use of urease inhibitors stems from the finding that retardation of urea hydrolysis reduces the problems such as volatile loss of ammonia associated with the rapid hydrolysis and concomitant rise in soil pH, specially in light-textured poorly buffered soils (49, 62). For example, Bundy and Bremner (7) showed that the effective urease inhibitors such as substituted p-benzoquinones reduced the volatile loss of ammonia from 62.8% (in the control) to 0.1% from a sandy soil fertilized with urea. Comprehensive review by Mulvaney and Bremner (49) suggests that several inorganic and organic compounds previously isolated from microorganisms or plants have the ability to reduce the gaseous loss of urea N as ammonia from soils when they are applied in admixture with urea.

Earlier work covered by a U.S. Patent (see Mulvaney and Bremner) (49) suggested that ammonia volatilization loss from urea-treated soils can be reduced by addition antimetabolic compounds. The compounds when added to soils retard urea hydrolysis not by inhibiting urease activity but by inhibiting the production of urease by microorganisms. However, subsequent study with these antimetabolites clearly showed that these compounds (pyridine-3-sulfonic acid, desthiobiotin and oxythiamine chloride) neither retarded urea hydrolysis nor reduced the volatile loss of ammonia from a sandy soil fertilized with urea. Hydroquinone was however, found to be very effective in retarding urea hydrolysis as well as in reducing ammonia volatilization loss from the soil (47).

NITRIFICATION AND ITS CONTROL

Among the factors that affect nitrification in soils, pH, moisture regime, temperature and oxygen supply are impor-

tant (1, 22). Numerous compounds have been proposed for retarding nitrification in soils. The aim of this paper is to cite a few examples on control of nitrification by compounds recently proposed with special reference to indigenous compounds/materials because the more usual literature on nitrification inhibitors are covered by several reviews (25, 30, 32, 43, 49, 50, 51, 52, 62, 65, 76).

Bundy and Bremner (6) suggested that % inhibition of nitrification in soils treated with nitrifiable nitrogen source can be calculated from the following equation.

$$\% \text{ inhibition of nitrification} = \frac{(NO_2^- + NO_3^-) \text{ in control} - (NO_2^- + NO_3^-) - N \text{ in treatment}}{(NO_2^- + NO_3^-) - N \text{ in control}} \times 100$$

Subsequently, Sahrawat (63) suggested that criterion used by Bundy and Bremner (6) was applicable in situations where control soil samples contained no appreciable amounts of $NH_4^+ - N$ and that the following equations are preferable when soil samples contained appreciable amount of $NH_4^+ - N$ because they provide a better criterion for computing per cent inhibition of nitrification:

$$\% \text{ inhibition of nitrification} = \frac{\text{Nitrification rate in control} - \text{nitrification rate in treatment}}{\text{Nitrification rate in control}} \times 100$$

$$\text{Where, nitrification rate, } = \frac{(NO_2^- + NO_3^-) - N}{(NH_4^+ + NO_2^- + NO_3^-) - N} \times 100$$

Literature surveys indicate that out of numerous compounds proposed, nitrapyrin (2-chloro-6-(trichloromethyl pyridine) (a Dow Chemical product see Goring (26, 27) is the most effective [illegible].

The various results that have been obtained with nitrification inhibitors, could perhaps be explained by the divergent soil and environmental conditions where they have been tested. The nitrification inhibitors are more effective in light textured soils and their effectiveness decreases with increase in organic matter and clay content of soils (6, 7, 26, 27, 31). Similarly, soil pH and fertilizer source also affect inhibition of nitrification. In tropical soils where nitrification is faster due to warmer temperatures, these conditions may also be responsible for the degradation and lack of effectiveness of the inhibitors in these situations.

Work done in India has shown that non-edible oil seed cakes and their constitutes particularly those of neem (Azadirachta indica L.) and Karanja (Pongamia glabra Vent.) have the ability to retard nitrification. For example, Mishra et al. (46) found that increasing amounts of neem seed cake powder decreased the number of nitrite forming microorganisms in soil. The highest concentration (2% on C content basis) decreased the nitrite formers for 21 days though the lower concentrations were effective for 3 to 14 days. Sahrawat and Parmar (71) showed that alcohol extract of neem seed cake was effective in retarding the nitrification of ammonium from urea or ammonium sulfate. Reddy and Prasad (56) found that neem seed cake (20% of urea W/W) and acetone extract of coaltar were effective in retarding nitrification of urea in a sandy clay loam soil upto 2 weeks. However, these materials were found to be considerably inferior to sulphathiazole and nitrapyrin in retarding nitrification.

Detailed study of karanja and its constituents (for review see Sahrawat) (64) have shown that extracts of karanja seed and bark and karanjin, a furanoflavonoid from karanja seed have poteitial for inhibiting nitrification in soils. The details of preparing these materials/compounds are summarised in Table 2. For example, Sahrawat et al. (72) found that karanja seed bark extracts inhibited nitrification of urea upto 45 days when they were added at 30% of urea N applied in a sandly loam soil.

Sahrawat (65) evaluated the comparative effectiveness of three patented nitrification inhibitors, nitrapyrin, AM. (2-amino-4-chloro-6-methyl pyrimidine) and DCD (dicyandiamide) with karanjin to retard nitrification of urea in a sandy clay loam soil and found that when added at 5 ppm of soil, the effectiveness of the inhibitors decreased in the following order: nitrapyrin > karanjin > AM > DCD. In another study, Sahrawat (66) found that alcohol extracts of neem and karanja seed cake when added at 30% of urea N gave comparable performance with that of karanjin added at 5% of urea N in inhibiting nitrification of urea in a sandy clay loam soil upto 45 days but karanjin was superior inhibitor at 60 days.

Table 2 : Constituents of Karanja (**Pongamia glabra**) tested as nitrification inhibitors

Particulars of the Constituent	Description of the Constituents	References
1	2	3
Karanja seed cake	Ground karanja seeds are defatted by extraction with boiling petroleum ether and the seed cake used for evaluation.	Singh (1966)
Karanja leaves	The Leaves are dried and ground before use.	Sahrawat et al. (1974)
Karanja seed extract	The ground seeds are first defatted with petroleum ether and the residue (cake) is then extracted with boiling ethanol (95%), solvent removed to obtain alcohol extract, which is used for testing without further purification.	Sahrawat et al. (1974)
Karanja bark extract	Fresh bark of the tree is ground and extracted with 40 : 60 (v/v) mixture of petroleum ether : acetone, solvent removed to obtain the extract.	Sahrawat et al. (1974)
Karanjin, a furano-flavonoid from karanja seed	Karanjin, a crystalline solid with molecular formula $C_{16}H_{12}O_4$ and chemically 3-methoxy furano-2, 3, 7, 8-flavone is prepared from karanja seeds.	Sahrawat (1973) Sahrawat and Mukerjee (1977)

A detailed study of structure-nitrification inhibition activity with karanjin, a furanoflavonoid from karanja seeds, has established that the furan ring in the molecule is essential for the activity of karanjin (58, 64, 69). It was found that structural analogs of karanjin (karanjketone, karanjonol) where furan ring was present in the molecule showed varying degrees of inhibition of nitrification while in the case of dihydrokaranjin, were furan ring was saturated by hydrogenation, showed no activity (Table 3). Follow up studies with a number of compounds having the furan group attached to either alkyl or aryl ring showed that this group imparts nitrification inhibitory activity to compounds to varying degrees (38, 70) (Table 4).

Table 3 : Effects of karanj in [illegible] its structural analogs on inhibition of nitrification in a sandy loam soil[a].

Compound	Furan ring in the molecule present of absent	% inhibition of nitrification at 15 days
Karanjin	Present	47
Karanj ketone	Present	44
Karanjonol	Present	28
Dihydro karanjin	Absent	0

[a]Soil samples were incubated under aerobic conditions after adding 200 ppm of urea N and 5% (of N added) of the compounds specified.

Table 4 : Effects of furfural and furfuryl alcohol on nitrification in a sandy clay loam fertilized with urea[a]

Treatment	Nitrification rate (%) after days		
	15	30	45
Urea	19	45	74
Urea + furfural	6	15	68
Urea + fufuryl alcohol	13	27	67

[a]Soil samples were treated with 200 ppm of urea N and with test compounds at the rate of 10% of N added and incubated at 30 C under aerobic conditions.

Further research is needed to develop nitrification inhibitors from indigenous materials such as non-edible oil seed cakes (64, 74, 75, 80) and plant products.

EFFECTS OF NITRIFICATION RETARDATION ON GASEOUS LOSS OF NITROGEN

Cornforth and Chasney (16) and Bundy and Bremner (8) found that nitrification inhibitors enhanced volatile loss of ammonia from urea-fertilized soils specially when urea was surface applied. Field evaluation of these results in the presence of growing plants needs to be investigated. Some recent studies have also shown that retardation of nitrification reduces nitrous oxide evolution from soils fertilized with nitrifiable nitrogen sources (3). McElhannon and Mills (42) found that nitrapyrin significantly reduced nitrous oxide emissions from soils fertilized with ammonium or nitrate form of nitrogen and planted with sweet corn in a 2-year field study. Probably nitrapyrin reduced N_2O evolution associated with both nitrification of ammonium and denitrification of nitrate. Nitrapyrin is reported to reduce nitrogen loss by denitrification in situations where readily oxidizable carbon substrate is available; for example in

... in a living plant or when nitrapyrin is applied to the nitrogen fertilizer band as opposed to broadcast application (42).

In addition to the above effects, nitrification inhibitors may also enhance immobilization of ammonium nitrogen. For example, Juma and Paul (34) found that 4-amino-1, 2, 4-triazole (ACT), nitrification inhibitor enhanced immobilization of ^{15}N as well as affected its subsequent release from non-exchangeable ammonium and microbial biomass.

CONCLUSIONS

Despite a great deal of research particularly on nitrification inhibitors, only a few compounds have been adopted for agricultural use. For example, nitrapyrin is registered and approved for use in the United States and dicyandiamide is produced and marketed for use in West Germany. The main problems seem to be (i) the high cost involved in the development and registration of effective urease and nitrification inhibitors, (ii) the economics of their use particularly in low input agriculture and (iii) the fact that they have given variable results. This should not, however, deter research efforts in developing effective urease and nitrification inhibitors from indigenous resources so that their use is economical.

Some of the Indian work, where non edible oil seed cakes (particularly neem) has been found useful in increasing the efficiency of fertilizer N in some situations cannot be wholly attributed to nitrification inhibition because immobilization cannot be discounted in situations, where these carbonaceous materials are added in rather high amounts. Use of nitrification inhibitors should be confined to specific situations where they could reduce loss of nitrified N by leaching and denitrification and to crops whose metabolism is not affected by high ammonium nitrogen (68).

Research on urease inhibitors is gaining momentum because of widespread use of urea as N fertilizer and the need to find solutions to some problems associated with the use of urea particularly ammonia toxicity to plants, germinating seeds and young seedlings and volatile loss of ammonia. However, the advantage accruing from urease inhibition could be offset by leaching of urea in some situations.

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