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Natural Nitrification Inhibitors for Augmenting Nitrogen Use Efficiency in Soil-Plant System

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Introduction

In the present scenario of continuous increase of human and animal population, increased and sustainable food and other crop production is limited by the non-availability of cultivable land in many parts of the world. It is essential that highly input efficient management practices be adopted for increasing productivity per unit area per unit time. This calls for sizeable increase in the use of fertilizer, especially nitrogen.

Nitrogen is one of the key inputs for increasing agricultural productivity. Prilled urea is the main source of nitrogen (N) applied to soil, in the Indian subcontinent (Prasad, 1998). Urea, when applied to soil is hydrolysed by urease to form NH_4^+ and is subsequently converted to nitrate (NO_3^-) by the action of nitrifying bacteria. The NO_3^- is subjected to losses either as nitrogen gas or nitrous oxide by the action of denitrifying bacteria and through percolation of soil water. Excessive loss of N due to NO_3^- leaching is a serious problem in light textured soils, especially during rainy season. This results in a very poor recovery of applied N, which seldom exceeds 50%; it is yet low in waterlogged paddies. Therefore, to ensure a continuous and optimal supply of N and to improve fertilizer use efficiency there is a need to regulate its supply by reducing the rate of either urea hydrolysis or nitrification or both. In this context, slow release urea forms such as sulphur coated urea, lac-coated urea, polymer coated urea and urea super granules have been extensively investigated. Similarly, several chemicals which retard hydrolysis have been tried (Gould *et al*, 1986). In order to augment N-use efficiency of crops, several synthetic chemicals such as N-serve (nitrapyrin), dicyandiamide (DCD), AM (2-amino-4-chloro-6-methyl pyrimidine), sodium chlorate, sodium azide, benzene hexachloride etc. have been examined for inhibition of urea hydrolysis or nitrification in soils. Many of these chemicals have been restricted to experimental level because of their high cost, limited availability and adverse influence on beneficial soil microorganisms and above all poor extension and promotional activities for taking the technology to the farmers' level.

Many plant materials such as Karanj (*Pongamia glabra*), neem (*Azadirachta indica*) and tea (*Camellia sinensis*) waste have been reported to inhibit nitrification (Prasad *et al*, 1971, Sahrawat and Parmar, 1975 and Prasad and Power, 1995). However, in spite of encouraging results urea coated with these botanical products has not attracted the attention of the users on a large scale because of the cumbersome process of coating urea and because the materials are not readily available. A number of essential oils and their constituents and the by-products find wide applications in pharmaceutical preparations as antimicrobial agents. Chemical constituents of essential oils such as menthol, menthone, isomonthonone, carvone, thymol, pulegone etc. possess antimicrobial properties (Sivropoulou *et al*, 1995).

Nitrification inhibitors are those compounds that inhibit the first step of nitrification, resulting in a preponderance of ammonium (NH_4^+) over nitrate (NO_3^-) in soil. This affects the persistence of applied N in the soil as well as plant N metabolism and N nutrition. Nitrification inhibitors also affect nitrogen transformation other than nitrification in soil such as ammonium fixation and release, mineralization and immobilization, nitrous oxide production and ammonia volatilization, which affect N persistence in the soil and subsequently availability to the plants.

Apart from many chemically synthesized nitrification inhibitors, some botanicals especially oil seeds and their constituents have been evaluated as sources of chemicals for retarding nitrification and regulating N availability in the soil- plant system (Table-1). Among the edible oil seeds, neem (*Azadirachta indica*), Karanj (*Pongamia glabra*) cakes and their constituents have been traditionally used as admixtures with manure and have since then extensively evaluated for retardation of nitrification in soil (Reddy and Prasad, 1975, Sahrawat and Parmar, 1975, Majumdar, 2002). Similarly, medicinal and aromatic plants products (monoterpene, sesquiterperones, flavonoids) of pyrethrum (*Chrysanthemum sp*), mints (*Mentha sp*), *Artemisia sp* etc. have been evaluated for their urease and nitrification inhibitory properties (Patra *et al*, 2001; Patra *et al* 2002; Kiran and Patra, 2002; Kiran and Patra, 2003; Patra *et al*, 2006).

As compared to chemical urease and nitrification inhibitors those from natural (plant) origin are cheap, safe and eco- friendly in nature. Nevertheless, their availability is sometime a problem due to the fact that they are perennial tree products. Contrary to that the short duration medicinal and aromatic plants are commercially cultivated (Patra *et al* 2001) and yet cheaper than the other botanicals, besides being safe and eco- friendly (Table-2).

Some uncommon materials like Pyrethrum (*Chrysanthemum sp*) flower waste, mahua (*Brassia latifolia*), castor (*Ricinus communis*) have been found to have nitrification inhibitory properties (Prasad *et al*, 1986). Like wise, linseed (*Linum usitatisimum*) oil (Suri and Datta, 1995) and turmeric (*Curcuma longa*) powder and caster (*Anacardium occidentale*) shell powder have also been found to have nitrification retardation properties. Looking in to the increased use of nitrogenous fertilizer, their loses through different mechanisms, fertilizer mediated environmental problems, enhancement of recovery of N in the soil- plant system is very important. In this context botanical urease and nitrification inhibitors have some major role to play. This paper discusses the advantages and constraints vis-à-vis efficiency of natural product based nitrification inhibitors for retarding N loses in soil-plant system.

Natural nitrification inhibitors and nitrogen use efficiency

Experimentation on neem cake coated- urea for evaluating nitrogen use efficiency in India was initiated by Bains *et al* (1971), who reported a 25-30% increase in N recovery in rice due to use of Neem cake coated urea. Neem cake has since then been evaluated by many workers both in upland and waterlogged condition (Purakayastha *et al*, 1997; Geethalakshmi *et al*, 1998). Vyas *et al* (1981) developed some products by extracting the neem cake with ethanol and marketed as Nimin® (tetranortriterpenoids) and reported an enhancement of apparent N recovery by 25-30% as compared to the uncoated urea. Prasad *et al* (1971) first reported nitrification inhibitory properties of Karanj cake (*Pongamia glabra*). Its chemical fractionation was done by Sahrawat (1981) who reported significant enhancement in N uptake and rice grain protein content (2-14%) over uncoated N source. Prasad *et al* (1986) reported higher nitrogen recovery (20-25% more) with Mahua (*B. latifolia*) and castor (*Ricinus communis*) treated urea as compared to non-treated urea in wheat under calcareous soils of Bihar, India. Suri and Datta (1995) recorded apparent N recovery of 66.9% with linseed (*Sesamum indicum*) oil coated urea compared to 22.2% with uncoated urea. Turmeric powder applied at 100:1 ratio of fertilizer urea and cashew (*A. occidentale*) shell powder at 5:2 ratio could double the fertilizer use efficiency in wheat (Geethalakshmi *et al*, 1998).

Recently some detail studies have been made on nitrification retardation potential of essential oils and their by-products (Patra *et al.*, 2001; Patra *et al.* 2002 ; Kiran and Patra, 2002a; Kiran and Patra, 2002b; Patra *et al.*, 2003; Patra *et al.*, 2006; Patra *et al.*, 2009). It has been observed that apparent nitrogen recovery in mint- wheat- rice cropping system can be augmented by coating urea with mint (*Mentha arvensis*) oil by-products viz DMO (dementholated oil) and pitch (essential oil discard) in the sandy loam soil of Northern Gangetic plains of India (Patra *et al.*, 2003; Patra *et al.*, 2006; Patra *et al.*, 2009). The most significant aspect of the essential oil by-product based nitrification inhibitors is that beside regulating nitrification these help in reducing the volatilization losses of urea-N (Patra *et al.* ,2003)

Reducing fertilizer and crop mediated green house gas emission by natural nitrification inhibitors

Nitrous oxides (N₂O) is one of the major greenhouse gases contributing to global warming and is involved in catalytic destruction of stratospheric ozone layer. Fertilizer application in rice soils leads to increased N₂O emission. Agricultural soils fertilized with nitrogen account for about 81% of the anthropogenic emissions of N₂O to the atmosphere (Iserman, 1994); global scale fertilized soils emit 10-17 Tg N₂O-N per year. Use of nitrification inhibitors could be one of the mitigation strategies of N₂O emissions which can slow down NH₄⁺ oxidation and thereby retard the loss of N₂O during nitrification and denitrification (Aulakh *et al.*, 1984). Mazumdar *et al.* (2000) observed that natural nitrification inhibitors like Neem powder and Nimin® (commercial derivatives of Neem) significantly reduced the N₂O emission from soil transplanted with rice (Table 3). They observed 10-26% inhibition of total N₂O emission by Neem products. In a similar study with wheat, Mazumdar *et al.* (2002), reported the reduction of N₂O emission to the extent of 63, 49, 35 and 39% respectively, with Nimin coated urea, urea+DCD, urea+thiosulphate and Neem coated urea respectively, as compared to control (no inhibitor). Nimin, the product derived from Neem was found promising and had highest N₂O mitigation when applied as coating on urea.

Under laboratory incubation study, Mazumdar (2002) evaluated nitrification and N₂O-N emission in soil treated with DCD (a chemical nitrification inhibitor) and a natural nitrification inhibitor Karanjin (a furanoflavonoid, obtained from Karanj- *Poagmia glabra* seeds). Application of Karanjin resulted in higher mitigation of total NO₃-N emission (92-96%) as compared to DCD (60-71%). Similar observations were made by Patra *et al.* (2009) while working on natural essential oil by-product coated urea in Gangetic Alluvial soils of Central India. Nitrification inhibitors can indirectly help mitigate CH₄ emission through regulation of N transformation in soil. Application of ammonium fertilizers and nitrification inhibitors in aerobic crop fields may reduce the potential consumption of CH₄, formation and emission, due to their positive influence on plant growth and the consequent increase of C input to the soil from plant residues and root exudates. Ammonium may stimulate the growth of methanogens when C is used as a substrate by these microbes and thus it may indirectly increase CH₄ production in the soil. Nitrification inhibitors reduce the N₂O emissions from soil and increase N use efficiency and crop yield. Question arises whether the magnitude of N₂O mitigation can offset the impact of global warming of decreased CH₄ consumption by soil or not. Mazumzar and Mitra (2004) studied effect of urea and urea mixed with different doses of two nitrification inhibitors viz DCD and Karanjin, on methane consumption in an alluvial (inceptisol) soil under rice – wheat

cropping system. Methane consumption rate was found to be negatively correlated with soil NH_4^+ and positively with $\text{NO}_2^- + \text{NO}_3^-$ contents. Mean CH_4 consumption rate, as well as total CH_4 consumption, was lower on the addition of Karanjin due to slower nitrification and higher conservation of NH_4 released from applied urea. Addition of urea led to a 17% reduction of total CH_4 consumption, while urea combined with Karanjin and DCD had 50-64% and 19-34% reduction, respectively. Karanjin was reported to be more effective nitrification inhibitor than DCD, regulating CH_4 consumption. Results from such experiments indicate that an effort to increase N use efficiency and reduce environmental degradation by applying nitrification inhibitors to agricultural soils may lead to a reduction in CH_4 consumption by aerobic soils and consequently may increase CH_4 emissions. However, the estimation of CH_4 consumption varies under different climatic and land management practices *vis-à-vis* fertilizer and nitrification inhibitor application.

Natural nitrification inhibitors *vis-à-vis* ammonia volatilization losses

Retardation of nitrification in soils results in accumulation of ammonium and enhances pH of soils, which are conducive to ammonia volatilization. Urea which is commonly used as the major source of nitrogen is most prone to gaseous losses through volatilization. Urease (urea amidohydrolase) is an enzyme that catalyses the hydrolysis of urea to ammonium carbonate and is therefore, important in the process of rapid urea hydrolysis leading to appreciable losses of N through volatilization. Some of the natural product based nitrification inhibitors, have been reported to have urease inhibitory properties (Purakayastha *et al*, 1997; Kiran and Patra 2002a; Kiran and Patra 2002b; Patra *et al* 2003; Patra *et al* 2009). Purakayastha *et al* (1997) reported that both Neem and mustard cake significantly retarded NH_3 volatilization in urea fertilized waterlogged paddy soils, of different physico-chemical characteristics; loss of $\text{NH}_3\text{-N}$ in a Typic Haplustert under paddy was estimated to be in a range of 1.70-6.70 % with Neem cake coated urea at different plant growth stages. The corresponding values with uncoated urea was 6.5-21.30% Patra *et al* (2003, 2006, 2009) reported both urease and nitrification inhibitors properties in *Mentha spicata* oil, dementholized oil (DMO) and the essential and discard, pitch and Nimin®.

Natural nitrification inhibitors *vis-à-vis* crop productivity

Use of nitrification inhibitors regulates the losses of N in soil, increase apparent N utilization efficiency and therefore, positively influence the crop yield. In India experiments have been conducted on major crops viz rice (*Oryza sativa*), corn (*Zea mays*), wheat (*Triticum aestivum*), potato (*Solanum tuberosum*), cotton (*Gossypium hirsutum*), Japanese mint (*Mentha arvensis*) etc. In early seventies some work on influence of Neem cake, Karanj cake coated urea on N use efficiency and crop yield has been initiated at IARI, New Delhi, India (Bains *et al* 1971, Sahrawat, 1982). The results of many studies indicated a significant increase in yield of rice and wheat with the application of natural product coated urea. Similar results have been reported subsequently by many researchers (Jain *et al*, 1980; Patra *et al* 2003; Kiran *et al* 2003; Patra *et al* 2006). Neem cake coated urea increased grain yield of wheat by 4-12% over uncoated urea in a multilocation trial conducted at Kanpur, Hisar and Pusa in India (Mishra *et al*, 1991). Nitrification inhibitors are reported to increase grain yield of wheat in the Pacific

Northwest and Southern Mid-West of United States. However, the data from other parts of the world is limited.

Conclusions

Fertilizer N is one of the key inputs in augmenting agricultural productivity and realizing the maximum yield potential of a crop variety. It is ample clear that only a part of the applied N is being accumulated by the crops and a part of the remaining amount remains in soil, leaving the major part exposed to various N loss mechanisms ie denitrification leaching, volatilization etc. This adds to the fertilizer mediated environmental pollution. Control of urea transformation reactions that occur in soil for better utilization of its N by crops has been an active area of soil chemistry-biology research. Several synthetic chemicals have been proposed as urease and nitrification inhibitors for retarding urea hydrolysis and nitrification thereby alleviating N losses. However, the main constraint in their large scale use is difficult availability, expensive nature and long persistence resulting in significant adverse side effects. Many botanicals have great promise as nitrification inhibitors because of their easy availability and eco-friendly and inexpensive nature. In this context, the mint (*Mentha sp*) essential oil and their by-products have a major role to play. India is at the moment the largest producer of mint oil. The farmers of north Indian plains and Himalayan foot hills have already adopted this crop as most potential cash crop. There is a scope for large scale use of mint oil by-product coated urea as a slow release N fertilizer to obtain the following advantages: i) higher income by the farmers, ii) immense employment opportunities in the rural sector , iii) saving on fertilizer consumption (the fertilizer thus saved could be used for covering more areas which are under sub-optimal supply of the costly fossil fuel based input, there by increasing agricultural productivity), and v) opening of new vistas in fertilizer industry by production of slow release urea formulation.

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Table 1: Plant products used as nitrification inhibitors

Materials/ Source/ Compounds	Reference
Neem (<i>Azadirachta indica</i> Juss) cake and its isolates	Reddey and Prasad 1975; Subbiah, 1979
Nimin, Neem oil based triterpene vegetable tannin, waste tea	Vyas <i>et al</i> , 1981; Majumdar <i>et al</i> , 2000; Fernando and Robert,1976; Krishnapillai, 1979
<i>Citrullus colosynthis</i> cake	Jain <i>et al</i> , 1980
Brassis latifolia cake	Mago and Totawat, 1989, Muthuswamy <i>et al</i> 1975
Karanj (<i>Pongamia glabra</i> Vent) seed cake and its isolates	Ahmed <i>et al</i> 1978; Sahrawat, 1981b; Sahrawat 1982
Karanjin, a furano- flavonoids from Karanj seeds	Sahrawat 1981b, Sahrawat 1981a
Mint essential oil	Patra <i>et al</i> , 2001, 2002
Medicinal and aromatic plant materials	Kiran and Patra, 2003
<i>Artemisia annua</i> leaves	Patra <i>et al</i> , 2002
Natural essential oil by-products	Kiran <i>et al</i> , 2003

Table 2: Some natural botanicals vis-a-vis dicyandiamide as nitrification inhibitors: relative cost, availability and agro-ecological safety.

Urea coated material	Cost of coating material Rs/ Mg urea	Quantity of Material required (%)	Resource of coating Material	Resource availability/ sustainability	Ecological Safety	Reference
Dicyandiamide	>4,000	1-20	Chemical synthesis	Unlimited	Persistent	Prasad and Power, 1995, Prasad, 1981
Neem cake	>1,000	10-60	Plantation	Limited	Biodegradable	Thomas and Prasad 1983
Other Neem Products	1,000	1-5	Plantation	Limited	Biodegradable	Vyas <i>et al</i> , 1993
Karanj (oil and other products)	1,000	20-30	Plantation	Limited	Biodegradable	Sahrawart <i>et al</i> , 1974 Sahrawat, 1981 Majumdar, <i>et al</i> , 2001
Dementholated mint oil	700	0.5-1.0	Seasonal crops	As desired	Biodegradable	Patra <i>et al</i> , 2001
Pitch (Mint oil discard)	300	0.5-1.0	Seasonal crops	As desired	Biodegradable	Patra <i>et al</i> , 2006

Table 3: Total nitrous oxide emission (mean \pm SE) with fertilizer urea and different nitrification inhibitors from a rice field in India (during 70 days)

Treatment	Total emission g N₂O-N ha⁻¹
Control	34.3 \pm 3.1
Urea alone	59.3 \pm 3.8
Urea + DCD	49.0 \pm 7.5
Nimin Coated Urea	57.0 \pm 4.9
Neem Coated urea	53.2 \pm 3.6

Source: Mazumdar *et al* (2000)