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# Nitrification Inhibitors: Classes and Its Use in Nitrification Management

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## Abstract

The explosive expansion of human activity during the last two centuries through industrial and agricultural pursuits has resulted in massive changes in the nitrogen (N) cycle of the planet. Based on the projected population growth and food demand, the N-fertilizer inputs into agricultural systems need to be doubled in the near future which would lead to further increase in the amount of N lost to the environment. If production agriculture continues to move towards high-nitrification agricultural systems with the expansion and intensification of agricultural activities, there is potential for catastrophic consequences to our planet due to the destruction of the ozone layer, global warming, and eutrophication. It is therefore imperative to manage the nitrification in agricultural systems for minimizing N leaks into the environment which are not only a serious economic and energy drain on society but also potentially have long-term ecological and environmental consequences. Currently, more than 60 % of the total N applied to agricultural systems is lost, amounting to an annual economic loss equivalent to US\$17 billion worldwide. Wide substrate range of ammonia monooxygenase (AMO), an important enzyme involved in nitrification, has enabled a range of chemicals or chemical formulations that can be effectively deployed as additives to N fertilizers to regulate nitrification. These chemicals by augmenting the efficiency of N-fertilizer use help us to achieve higher food production for catering the ever increasing population and minimize fertilizer-related pollution of the environment. This paper overviews N transformations in agricultural systems and the salient agrochemicals employed for management of nitrification, the most important transformation, in particular.

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**Keywords**

Agricultural chemicals • Nitrification • Nitrification inhibitors • Soils • Agriculture

**1 Introduction**

Nitrogen (N) is vital for life. It is an essential element for plant growth and reproduction and is one of the most widely distributed elements in nature, with atmosphere as the main reservoir. Of the total naturally available N, 99.96 % is present in the atmosphere. Biosphere contains only 0.005 % out of the remaining 0.04 %. In spite of being present in small proportion in living beings, N is most often the restrictive nutrient for crop production since only a fraction of atmospheric nitrogen is made available to the plants through biological nitrogen fixation. However, the use of chemical nitrogenous fertilizers has resulted in significant increase in crop yields. These fertilizers are one of the key contributors in improving agricultural productivity globally. Ammonium-based fertilizers including urea are the widely used N source for field crops. However, fertilizer N is not an unmixed blessing as the commonly used nitrogenous fertilizers, especially urea, suffer from low nitrogen use efficiency (NUE) and contribute towards environmental pollution and health hazards. Worldwide, the NUE for cereal production (wheat, corn, rice, barley, sorghum, millet, oat, rye etc.) is approximately 33 %. The global annual economic loss due to low NUE is about US\$17 billion (Subbarao et al. 2006; Raun and Johnson 1999; Prasad 1998).

The soil accounts for a small fraction of the lithospheric N. It is, however, the main source of plant-available N. Out of the total soil N, only a small portion is available to plants as ammonium and/or nitrate. Although the majority of plants are capable of using both ammonium-N and nitrate-N, the latter is the predominant form utilized by the plants under arable/terrestrial conditions. This is primarily because of compulsion rather than preference due to rapid

conversion of most of the ammonium-N to nitrate under favorable conditions. Consequently, ammonium-N is available to the plants only for a limited period of time. Plant roots encounter mainly nitrate-N as source of N in the soil.

Nitrification is a key process in managed agricultural ecosystems because the conversion of ammonium to nitrate can lead to substantial loss of agricultural N by leaching and/or denitrification. The fertilizer N loss is of concern because of economic reasons and associated environmental and health hazards. Some of the hazards of excessive use of nitrogenous fertilizers include: (1) “blue baby” syndrome (methemoglobinemia) in infants and ruminants due to nitrate and nitrite in waters and food; (2) gastric cancer, goiter, birth defects, and heart diseases due to nitrites and nitrosamines; (3) respiratory illness due to nitrate, nitrite, and nitric acid in aerosols; (4) eutrophication due to N in surface waters; (5) accumulation of various oxides of nitrogen in the atmosphere contributing to ozone layer destruction, global warming, and acid rain; (6) plant toxicity due to high levels of nitrite and ammonium in soils; and (7) excessive plant growth due to more available N. Therefore, concerted efforts have been and are being made for improving the use efficiency of N fertilizer and plant N uptake (Prasad 1998; Prasad and Power 1995; Azam and Farooq 2003).

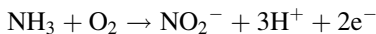
Multidisciplinary approaches followed to increase N use efficiency include: (a) breeding crop varieties with higher fertilizer use efficiency; (b) improved agronomic practices; (c) use of controlled or slow-release fertilizers, urease, and nitrification inhibitors; and (d) supplementation/ integration of fertilizer N with organic manures. These approaches have helped to alleviate the problems arising as a result of fertilizer N use. Fertilizer management through improved formulations, mode and time

of application and placement, etc., has also been found helpful to mitigate some of the problems. Likewise, a large variety of chemicals have been tested as potent inhibitors of specific N transformation process including urea hydrolysis and nitrification. These inhibitors improved the fertilizer N use efficiency along with significant reduction in losses due to ammonia volatilization, denitrification, and nitrate leaching. Nitrification inhibition could lead to: (1) increased rhizospheric microbial activities, (2) enhanced mineralization of native soil N, (3) increased fertilizer N use efficiency, and (4) greater photosynthate partitioning to the rhizosphere, thus enriching the soil with organic matter. It is important, therefore, to develop an understanding of the nitrification process, factors affecting nitrification, methods to regulate the process, and its implications to ecosystem functioning (Abalos et al. 2014).

## 2 The Nitrification Process

Nitrification has been defined as the oxidation of any reduced nitrogen form (organic or inorganic) to nitrate. The microorganisms carry out this oxidation process. Being the only link between reduced and oxidized nitrogen compounds, the nitrification process is of major importance for the nitrogen cycle in aquatic and terrestrial environments. In soils, the nitrification process oxidizes the immobile ammonium to nitrate, a mobile ion.

The biological oxidation of ammonia to nitrate is a two-step process mediated by autotrophic bacteria. It is first oxidized to nitrite and then to nitrate, as follows:



The source of ammonium-N could be soil organic matter (mineralization by soil microorganisms) and/or chemical fertilizers. In the case of soil, organic N is used by the ammonifiers, while chemical fertilizers contain either ammonium as such or its precursors.

The oxidation of reduced organic or inorganic nitrogen to nitrate mediated by heterotrophic organisms has been named heterotrophic nitrification, whereas the oxidation of reduced inorganic nitrogen to nitrate by autotrophic organisms is called autotrophic nitrification.

Several genera and species of ammonium and nitrite-oxidizing heterotrophs including fungi (*Aspergillus flavus*, *Neurospora crassa*, *Penicillium* sp.), actinomycetes (*Streptomyces* sp., *Nocardia* sp.), and bacteria (*Arthrobacter* sp., *Azotobacter* sp., *Pseudomonas fluorescens*, *Aerobacter aerogenes*, *Bacillus megaterium*, *Proteus* sp.) have been reported (Koops et al. 1991; Purkhold et al. 2000; Regan et al. 2002). However, autotrophic nitrifiers are the main organisms responsible for most of the nitrification. Ammonium-oxidizing autotrophs include *Nitrosomonas*, *Nitrosolobus*, and *Nitrosospira*. These organisms have been isolated from a variety of soil environments with ubiquitous distribution. Nitrite produced by the ammonium-oxidizing autotrophs is rapidly oxidized to nitrate by *Nitrobacter* species (Hovanec and Delong 1996).

All nitrifiers are obligate aerobes and hence a restricted nitrification under waterlogged or aquatic environments can be observed. In addition, these microorganisms, especially *Nitrobacter*, are fairly sensitive to acidic pH. As a result nitrification is inhibited in climax ecosystems like forest soils with thick layer of leaf litter and zones of acidic pH. The process of nitrification itself may lead to lowering of pH of the medium due to release of  $\text{H}^+$  as shown in the equation above.

As stated earlier, the autotrophic bacteria mostly carry out the nitrification process. These utilize reduced inorganic nitrogen as energy source and carbon dioxide as carbon source. Hence, this nitrification process may more correctly be called chemolithoautotrophic nitrification. The substrate for the enzyme ammonia monooxygenase (AMO) involved in the first part of the chemolithoautotrophic nitrification process is ammonia (Norton and Stark 2011) rather than ammonium but an acidity-dependent equilibrium always exists between ammonia and

ammonium. It is mediated by two distinct groups of bacteria, ammonia-oxidizing bacteria (AOB), and nitrite-oxidizing bacteria. Both groups are dominated by autotrophic metabolism; however, some nitrite oxidizers may also use organic compounds as carbon sources (mixotrophs) (Norton and Stark 2011).

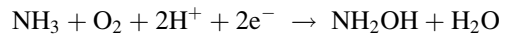
### 3 Chemolithoautotrophic Ammonia-Oxidizing Bacteria (AOB)

The AOB carry out a specific environmental function of oxidation of ammonia to nitrite. All AOB that have been isolated and characterized are gram-negative, obligate aerobic, and obligate chemolithoautotrophs. Together with the nitrite-oxidizing bacteria, they make up the family *Nitroacteraceae*. Five different genera for AOB, namely, *Nitrosomonas*, *Nitrosospira*, *Nitrosococcus*, *Nitrosolobus*, and *Nitrosovibrio* were defined on the basis of classical morphological characteristics (Tomiyama et al. 2001). Most AOB described belong to the  $\beta$ -*Proteobacteria* ( $\beta$ -AOB), but a few marine isolates of *Nitrosococcus*, not known from soil, belong to the  $\gamma$ -*Proteobacteria* ( $\gamma$ -AOB). Recent research based on sequences of 16S rDNA suggests that the  $\beta$ -AOB can be divided into only two major phylogenetic lineages, the *Nitrosospira* and the *Nitrosomonas*. New results showed similar, but not identical, evolutionary relationships of  $\beta$ -AOB when using the 16S rRNA gene or a functional gene (*amo-A*) as marker genes for phylogenetic analysis (Purkhold et al. 2000). Hence, except for a few marine *Nitrosococcus* strains, all known AOB are of monophyletic origin. The high correlation between function and phylogeny is rather unique for AOB when compared to other functional groups of microorganisms in soil, e.g., denitrifiers (Bothe et al. 2000).

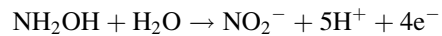
Two key enzymes mediate the ammonia oxidation in AOB – ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) – and both are codependent because

they generate substrate and electrons, respectively, for each other (Bothe et al. 2000).

#### Oxidation of Ammonia to Hydroxylamine (AMO)



#### Oxidation of Hydroxylamine to Nitrite (HAO)



The AMO enzyme consists of three subunits with different sizes (Bothe et al. 2000) – AMO-A, AMO-B, and AMO-C – and mainly the gene (*amo-A*) encoding the A subunit which carry the active site of AMO has been investigated (Purkhold et al. 2000). The AMO enzyme may catalyze co-oxidation of a broad range of substrates (McCarty 1999). Hence, this enzyme has been in focus when exploiting the role of AOB in bioremediation (Duddlestone et al. 2000). Many similarities between AMO of AOB and particulate methane monooxygenase (pMMO) found in methane-oxidizing bacteria have been reported (Bedard and Knowles 1989) and the similarity of nucleotide sequences encoding the enzymes indicate a common evolutionary origin (Holmes et al. 1995).

### 4 Nitrification Inhibition or Regulation

Nitrification being one of the key N cycle processes under most arable situations on land, a need to inhibit nitrification in order to maintain the economy of agroecosystems has been always felt as discussed below.

**Groundwater Pollution** Uncontrolled and excessive nitrification may lead to groundwater contamination with nitrate and nitrite as well as increased concentration of the later in eatables, especially vegetables leading to human health hazards. Nitrate itself is not a threat, while nitrite is definitely a potential health hazard and that too when found in places at a wrong time.

Consumption of water and vegetables containing excessive amounts of nitrate may lead to the production of nitrite in the stomach and the later becomes particularly dangerous for the babies. Methemoglobinemia (blue baby syndrome) may occur in 1-year old babies taking diet with too much nitrate. Methemoglobinemia is the condition in the blood which causes infant cyanosis or blue baby syndrome. Methemoglobin is probably formed in the intestinal tract of an infant when bacteria convert the nitrate ion to nitrite ion. One nitrite molecule then reacts with two molecules of hemoglobin to form methemoglobin. In acid mediums, such as the stomach, the reaction occurs quite rapidly. This altered form of blood protein prevents the blood cells from absorbing oxygen which leads to slow suffocation of the infant which may lead to death. Because of the oxygen deprivation, the infant will often take on a blue or purple tinge in the lips and extremities, hence the name, blue baby syndrome. Other signs of infant methemoglobinemia are gastrointestinal disturbances, such as vomiting and diarrhea; relative absence of distress when severely cyanotic but irritable when mildly cyanotic; and chocolate-brown-colored blood. Stomach and gastrointestinal cancer has also been associated with the concentration of nitrate in potable water. Again, it is nitrite that reacts with amines to form N-nitroso compounds, which are reported to cause stomach cancer. Such an illness may result from consumption of vegetables containing high concentrations of nitrate originating from soil or irrigation water.

In water bodies, however, nitrate and other forms of N may encourage the growth of algae and subsequently the bacteria leading to exhaustion of molecular oxygen, thereby affecting animal life. Indeed, whole ecological balance of water bodies may change due to the so-called eutrophication.

**Nitrous Oxide Production** Nitrous oxide is produced naturally in soils through the microbial processes of nitrification and denitrification. Since 1750, the global atmospheric concentrations of nitrous oxide have risen by

approximately 18 % and are continuing to do so at 0.25 % per annum. This increase is attributed mainly to biospheric processes. Flood irrigation leads to rapid nitrification and denitrification resulting in considerable amounts of atmospheric nitrous oxide emission, which may amount to 35–45 % of the applied N. On the global level, >65 % of the atmospheric nitrous oxide comes from the soil, which is twice the amount produced by burning fossil fuels and four times the amount evolved from the oceans. Being a greenhouse gas, nitrous oxide contributes substantially to the destruction of stratospheric ozone (Azam and Farooq 2003). N<sub>2</sub>O is approximately 300 times more powerful than CO<sub>2</sub> at trapping heat in the atmosphere.

Nitrification and denitrification are the main contributors (Azam et al. 2002) to atmospheric nitrous oxide. However, since the two processes occur simultaneously, it is difficult to ascertain the real contribution of either to the observed nitrous oxide fluxes. Nevertheless, nitrification is reported to make a substantial contribution to the nitrous oxide emission under aerobic conditions. Higher nitrous oxide emissions are often reported from fertilized than unfertilized soils, rates of emission being greatest following application of ammonium or ammonium-forming fertilizers (Azam et al. 2002). In several studies, using isotope methodology and nitrification inhibitors, this increase is attributed to losses of N<sub>2</sub>O occurring during the process of nitrification (Abbasi and Adams 2000). Estimates of the amount of N<sub>2</sub>O resulting from nitrification are variable but generally account for <1 % of the fertilizer N applied. In the case of anhydrous NH<sub>3</sub>, however, the losses may increase to 6–7 %. In most studies, the onset of N<sub>2</sub>O emission is observed very early during the incubation, while nitrification continues for extended periods of time. Williams et al. (1998) reported active nitrification 7–12 days after application of ammonium nitrate, while a flush of N<sub>2</sub>O emission from soil was observed around day 1, followed by a decline. These researches (Abbasi and Adams 2000; Azam et al. 2002; Williams et al. 1998) showed very low molar

ratios of NO to N<sub>2</sub>O and suggested that denitrification was the dominant process involved in N<sub>2</sub>O emission.

Contribution of nitrification to nitrous oxide emissions may be high under the semiarid agroclimatic conditions and with the use of urea as major N fertilizer. Urea is rapidly hydrolyzed followed by a quick nitrification of the resultant ammonium especially under relatively warmer conditions. Thus, nitrification not only contributes to nitrous oxide emissions, but the process of denitrification is fairly well supported by sustained availability of nitrate. In most soils, formation and emissions of nitrous oxide to the atmosphere are enhanced by an increase in available mineral N through increased rates of nitrification and denitrification. Therefore, addition of N in organic or inorganic compounds eventually leads to enhanced N<sub>2</sub>O emissions.

**Nitrification Inhibition and Ecosystem Functioning** Nitrification inhibition and consequent accumulation of ammonium would lead to: (1) increased microbial activities including biological nitrogen fixation, (2) greater photosynthate partitioning to the rhizosphere, (3) enhanced mineralization of native soil N, and (4) increased efficiency of fertilizer N use by plants.

Ammonium is preferred over nitrate as a source of N by microorganisms. As not all organisms possess nitrate reductase to enable them to assimilate nitrate, while almost all of them will be able to assimilate ammonium, so this preference is consequential rather than the reason. In addition, assimilation of nitrate is more energy intensive than ammonium. Hence, sufficient easily oxidizable C will be required for efficient assimilation of nitrate. Studies involving the use of glucose as a C source indeed reveal similar assimilation of both ammonium and nitrate by the soil microorganisms. Nevertheless, the presence of ammonium leads to an enhancement in microbial activities in terms of respiratory response. In experiments aimed at studying

the mineralization of native soil N, ammonium-N is reported to have a significantly higher effect as compared to nitrate-N. This so-called “priming” effect or added nitrogen interaction has been found to increase with the amount of applied N. An indirect effect of chemical fertilizers as well as green manures is their positive influence on the mineralization and plant availability of N from the soil organic reserves.

Most of the plants utilize both the ammonium- and nitrate-N with varied preference for one form over another. However, ammonium as an exclusive source of N may cause growth inhibition in many species, particularly in those grown under arable conditions (Marschner 1999). Under these conditions, nitrification is generally quite rapid and hence deleterious effects of ammonium are avoided. Under saline conditions also, ammonium increases the sensitivity of plants whereas nitrate has been reported to moderate the negative effects of salinity (Khan et al. 1994). However, the plants are bound to face higher concentrations of ammonium under saline conditions because of the inhibitory effects of salts on the process of nitrification. Therefore, nitrification inhibition would be a blessing for arable plants grown on normal agricultural soils, whereas it may be an added problem for those grown on salt-affected lands. Several studies indeed show a positive effect of nitrification inhibitors on plant growth and N use efficiency<sup>4</sup> by decreasing the loss of N through denitrification and nitrate leaching and conservation of the applied N through enhanced immobilization.

The form of N plays a significant role in affecting root growth, rhizodeposition, and the concomitant changes in different rhizospheric microbial functions including root-induced N mineralization. In wheat and maize, root growth may be restricted in ammonium compared to nitrate-fed plants and may be attributed to an increased root respiration, greater allocation of photosynthates to nitrogenous than structural component, and increased export of carbon (probably as amino acids) from root to shoot than that occurring under nitrate nutrition

(Azam and Farooq 2003). In addition, ammonium nutrition leads to a higher rhizodeposition, thereby enlarging the below-ground sink for photosynthates, most probably at the expense of plant tops thereby reducing the biomass yield. However, increase in rhizodeposition due to increased/sustained availability of ammonium may also prove beneficial to plants in terms of increased microbial activities, especially the mineralization of native soil N. In laboratory experiments, a significant increase in the mineralization of soil N has been observed<sup>5</sup> following addition of easily oxidizable C.

It has also been suggested above that mineralization of N from soil organic matter is more intense in the presence of ammonium than nitrate. Jenkinson et al. 1985 attributed this to “pool substitution” whereby the native N stands proxy for the applied N giving the impression of enhanced mineralization of the latter. The fact remains, however, that applied N (especially ammonium) leads to an increase in the availability of soil N. Inhibition of nitrification may therefore lead to a higher mineralization of native soil N thereby augmenting N supplies to plants. In addition, microorganisms responsible for the synthesis of aggregation-adhesion macromolecules may be encouraged by higher availability of carbonaceous materials in the rhizosphere. This will result in better soil structure as well as improved moisture-holding capacity of the soil at the root surface. The latter may help the plants withstand drought stress at least temporarily. Thus, in spite of the negative effects of ammonium, inhibition of nitrification may still exert beneficial effects on plant growth. The negative effects can be overcome to a significant extent by developing plant types more efficient in using ammonium, the so-called ammoniphilic plants. Plants like rice and sugarcane growing under high soil moisture conditions can be considered as ammoniphilic plants. Efforts are needed to engineer arable crops (like wheat) for improved tolerance to ammonium while employing nitrification inhibitors.

Another aspect worth consideration is the susceptibility of nitrate to leaching beyond the effective root zone after being converted to calcium nitrate {Ca (NO<sub>3</sub>)<sub>2</sub>} in the presence of ionic calcium (Ca). Hence, in calcareous soils the conditions are quite conducive to this mode of nitrate escape especially following organic amendment that helps in the release of Ca. The leaching is more pronounced in clayey soils at near neutral pH as negative charge on the clays repels nitrate, thereby facilitating the process of leaching. Hence, not only the use efficiency of nitrate will remain low under these conditions but also N economy of the system will be negatively affected.

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## 5 Management Practices to Reduce or Regulate Nitrification

Various approaches have been suggested to improve the use efficiency of nitrogenous fertilizers. These include the improved agronomic practices, use of coatings, chemical additives, and the various chemical and physical modifications.

**Improved Agronomic Practices** Split application, placement, foliar application, fertigation, etc. are some of the agronomic techniques by which NUE of nitrogenous fertilizers can be increased (Raun and Johnson 1999).

**Controlled or Slow-Release Fertilizers** By using specific fertilizer formulations to release N in synchrony with plant requirement, it should be possible to provide sufficient N in a single application to satisfy the plant's need, yet maintain low concentrations of mineral N in the soil throughout the growing season. If this could be done, losses would be small because of the limited amount of N in the substrate.

Several slow-release forms of N (Shaviv and Mikkelsen 1993) have been suggested. These include:

- *Coated fertilizers*: Soluble urea is coated with an insoluble, slowly permeable but generally biodegradable material to achieve controlled/delayed release of urea-N. Several organic and inorganic coating materials such as sulfur, gypsum, lac, latexes, polyolefins, resins, plastics, polyurethanes, rock phosphates, etc. have been attempted using three types of coating processes, namely, rolling bed, falling curtain, and fluidized bed.
- *Complex organic N compounds with relatively less solubility in water than urea*: This group of compounds consisting of urea-formaldehyde complexes (38 % N), oxamide (30 % N), isobutylidenediurea (IBDU, 30 % N), urea-Z (35 % N), etc., are only slightly soluble in water. The rate of nitrogen release from these compounds depends upon water solubility, microbiological action, and chemical hydrolysis.
- *Urea supergranules (USG)*: The USG consisting of 1–2 discrete urea particles is not so efficient, but its proper deep placement (1 USG for 4 hills at 7–10 cm soil depth with the hole at the placement site closed) makes it efficient.

Many of these fertilizer formulations have been utilized to grow plants in diverse environments. The influence of slow-release forms on levels of soil mineral N and the recovery of fertilizer N have been assessed for upland crops and lowland rice. The use of these formulations has generally decreased the total loss of fertilizer N.

## 5.1 Use of Inhibitors

Fertilizer use efficiency could be greatly increased if the hydrolysis of urea to ammonium by soil urease could be retarded by the use of urease inhibitors or if nitrate accumulation during the cropping phase could be regulated by nitrification inhibitors.

**Urease Inhibitors** These reduce the hydrolysis of urea by inhibiting soil urease activity and thus prevent rapid development of high partial pressure of  $\text{NH}_3$  and high pH of floodwater in rice fields and eventually reduce  $\text{NH}_3$  volatilization losses. A large number of compounds have been tested for their ability to inhibit soil urease but most are ineffective or do not persist in soil. The phosphoroamides, such as phenylphosphorodiamidate (PPD) and N-(*n*-butyl) thiophosphorictriamide (NBPT), have shown promise for limiting the hydrolysis of urea in laboratory and greenhouse studies when used singly or in combination. Relatively few studies have been done on their ability to reduce  $\text{NH}_3$  volatilization and increase grain yield in the field.

Studies using PPD and NBPT as urease inhibitors in flooded rice fields have shown little reduction in  $\text{NH}_3$  loss. The reasons for the failure of PPD in flooded soils seem to be its rapid hydrolysis under the alkaline conditions generated in the floodwater by photosynthetic algae and its decomposition due to the high temperatures reached in the floodwater. The reasons for the failure of NBPT in flooded soils have not been completely explained, but the results of laboratory studies with non-flooded soils suggest that it must be converted to the oxygen analogue to inhibit urease activity. Studies with another thiophosphorictriamide, thiophosphoryl triamide, showed it too to be a relatively weak inhibitor of urease activity. Appreciable inhibition was achieved only after it had been converted to the oxon analogue. These studies indicate that the thiophosphorictriamides do not inhibit urease activity, but that the phosphorictriamides are its potent inhibitors.

Field studies in Thailand show that the activity of PPD can be prolonged, and  $\text{NH}_3$  loss markedly reduced, by controlling the floodwater pH with the algicide terbutryn. In addition, a mixture of NBPT and PPD in the presence of terbutryn was even more effective than PPD alone. It appears that during the time when the



PPD was effective, NBPT was being converted to its oxygen analogue. This inhibited urease activity when PPD lost its capacity to do so. The combined urease inhibitor-algicide treatment reduced ammonia loss from 10 to 0.4 kg N ha<sup>-1</sup> (Freney et al. 2011).

In a laboratory study, cyclohexylphosphoric triamide (CHPT) was found a very effective inhibitor of urease activity and the same was confirmed in a field experiment with flooded rice in Thailand (Freney et al. 2011). The oxon analogue of NBPT, N-(*n*-butyl) phosphoric triamide, was compared with CHPT. The two markedly reduced urea hydrolysis, the CHPT being more effective. Its addition maintained the ammoniacal N concentration of the floodwater below 2 g m<sup>-3</sup> for 11 days, reduced NH<sub>3</sub> loss by 90 %, and increased grain yield. Application of NBPT with urea resulted in increase in cotton yield by 14 % and it was also recommended that NBPT cannot be used in combination with DCD (Kawakami et al. 2012). Whereas, in maize crop, NBPT did not significantly increase the grain yield and it was also concluded that effectiveness of NBPT + DCD combination is influenced by management practices (Sanz-Cobena et al. 2012).

**Nitrification Inhibitors** The nitrification inhibitors (NIs) decrease the availability of nitrate and consequently its vulnerability to escape mechanisms. A lot of work has been reported on the ways to retard/inhibit the rate of nitrification not only to reduce fertilizer N losses (Prasad and Power 1995) but also to prolong the persistence of fertilizer N in ammoniacal form (Prasad and Power 1995; McCarty 1999). Since ammonia or ammonium-producing compounds are the main source of fertilizer N, maintenance of the applied N in the ammonium form should mean that less N is lost by denitrification. One mechanism of maintaining added N as ammonium is to use a nitrification inhibitor with the fertilizer.

Numerous substances have been tested for their ability to inhibit nitrification (Table 1), and several of these have been patented. Only a limited

**Table 1** Some commercial and extensively tested synthetic nitrification inhibitors

Nitrapyrin [2-chloro-6-(trichloromethyl)-pyridine]
2-Amino-4-chloro-6-methylpyrimidine (AM)
Dicyandiamide (DCD)
Etridiazole (5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole)
N-(2,5-dichlorophenyl succinamic acid) (DCS)
Potassium azide (KN <sub>3</sub> )
3-Amino-1,2,4 triazole (ATC)
Thiourea (TU)
MBT (2-mercaptobenzothiazole)
2-Ethynyl pyridine
MPC (3-methylpyrazole-1-carboxamide)
ST (2-sulfanilamidothiazole)
Carbon disulfide
3-Mercapto-1,2,4-triazole
Sodium diethyldithiocarbamate
Acetylene
Gaseous hydrocarbon, e.g., methane, ethane, and ethylene
Ammonium thiosulfate
Thiophosphoryl triamide
4-Mesyl benzotrichloride
4-Nitrobenzotrichloride
Guanyl thiourea
2,4-Diamino-6-trichloromethyl triazine
Potassium trithiocarbonate
Sodium thiocarbonate
2-Amino-4-methyl-6-trichloromethyltriazine (MAST)
2-Benzothiazole sulfone morpholine
3,4-Dimethylpyrazole phosphate

number of chemicals are available commercially for use in agriculture. These include 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin), sulfathiazole, dicyandiamide, 2-amino-4-chloro-6-methyl pyrimidine, 2-mercaptobenzothiazole, thiourea, and 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole (terrazole). Unfortunately, most of these compounds have limited usefulness. For example, the most commonly used nitrification inhibitor, nitrapyrin, is seldom effective because of sorption on soil colloids, hydrolysis to 6-chloropicolinic acid, and loss by volatilization.

*Nitrapyrin* Nitrapyrin [2-chloro-6-(trichloromethyl)-pyridine, **I**] was developed by

Dow Chemical Company. It is marketed under the trade name “N-Serve 24 nitrogen stabilizer” (a.i. 240 g L<sup>-1</sup>) and “N-Serve 24E nitrogen stabilizer” (a.i. 240 g L<sup>-1</sup>). The rates of application advised by Dow Chemical Company for band and row placement are 1.125–1.25 L ha<sup>-1</sup> of N-Serve 24E for cotton, maize, sugar beet, sorghum, and wheat and 4.50–6.75 L ha<sup>-1</sup> for potatoes before or after planting or sowing. For broadcasting, the rate of application has to be increased considerably. When granulated fertilizer is used, it can be applied at 0.2–1.0 % of the amount of fertilizer N (Kawakami et al. 2012). Because of its high vapor pressure, nitrapyrin cannot be granulated with solid-N fertilizer like urea without loss of the inhibitor during processing, storing, and handling. Nitrapyrin sometimes shows poor activity due to sorption on soil colloids, hydrolysis to 6-picolinic acid, and loss by volatilization.

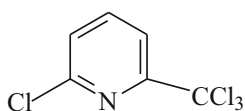
**AM** 2-Amino-4-chloro-6-methylpyrimidine (**II**) is another well-known nitrification inhibitor developed by Toyo Kaotsu Industries Inc. (now Mitsui Toatsu) of Japan. Pure AM is a white crystalline substance (mp, 182 °C) and is soluble in water but unlike nitrapyrin; it is relatively insoluble in organic solvents. AM is less volatile and less effective than nitrapyrin. AM is effective (Prasad and Power 1995) when applied at 5–6 kg ha<sup>-1</sup>.

**Etridiazole** 5-Ethoxy-3-trichloromethyl-1, 2, 4-thiadiazole (Terrazole, Etridiazole, Dwell, **III**) is an effective nitrification inhibitor developed by Olin Corporation, Baltimore, USA. This product is available as a wettable powder or technical grade liquid with 35 % and 95 % a.i., respectively. As a coating on ammonium sulfate and urea, terrazole 95 % a.i. is used up to 1.5 % by weight. The recommended rates of compound for crops like potatoes, sugar beet, lettuce, and onions are 0.6–1 kg ha<sup>-1</sup> (Slangen and Kerkhoff 1984). Besides this compound, some other thiadiazoles are also known to inhibit ammonia oxidation. Among them 3, 4-dichloro-1, 2, -5-thiadiazole is noteworthy.

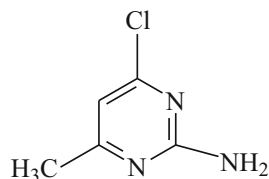
**Dicyandiamide (DCD, IV)** It has been developed both as a slow-release nitrogen source as well as nitrification inhibitor (Slangen and Kerkhoff 1984). In Japan, it is added to mixed fertilizer and a product urea-form plus containing 10 % by weight of DCD is produced. A fertilizer containing urea and DCD in a 4: 1 ratio is commercially available in West Germany. DCD is toxic to plants, but the effect differs with plant species. This compound is effective over a period of 1–3 months. When applied at 10–15 % of applied nitrogen, it remains active for a period of 2 months. Cost of production of DCD is lower than the corresponding cost of nitrapyrin and etridiazole. It has the advantage of completely decomposing in soil to CO<sub>2</sub> and NH<sub>4</sub><sup>+</sup> over several weeks and thereby acts as a high analysis (66.7 % N) slow-release N fertilizer. Compounds containing ammonium sulfate and dicyandiamide are available in granulated and coated form from Sueddeutsche Kalkstickstoff-Werke AG, Trostberg, Germany, and Sisco Corporation, Japan, and recommended as slow-release fertilizers. Increase in NUE of urea by DCD in field studies on different crops has been reported by various workers (Ma et al. 2013). In a recent study, DCD was found highly effective in reducing N<sub>2</sub>O emissions by 58–63 % in a dairy pasture (Ball et al. 2012).

**CMP** [1-Carbamoyl-3-methylpyrazole, **V**] has been found to be an effective nitrification inhibitor (McCarty 1999). Under flooded conditions, CMP affected almost total inhibition of nitrification and prevented buildup of nitrite and nitrate in floodwater. In pure culture studies, CMP inhibited the growth of *Nitrosomonas* at concentrations as low as 1 ppm.

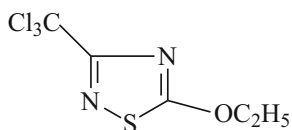
**ST** A group of thiazoles such as sulfathiazoles and especially 2-sulfanilamidothiazole (ST, **VI**) were introduced as nitrification inhibitors by Mitsui Toatsu Chemicals Inc., Japan. ST is more stable than AM and can be formulated with both acidic and basic fertilizers. It is apparently more volatile than AM. ST is commercially used in Japan on a limited scale.



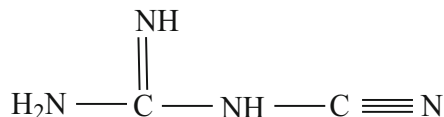
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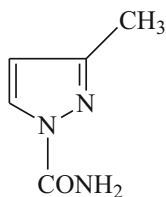
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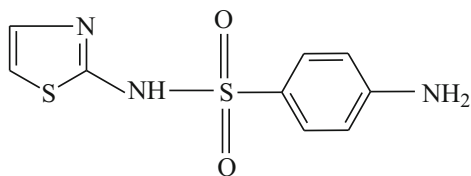
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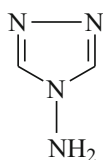
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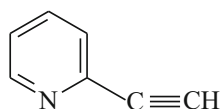
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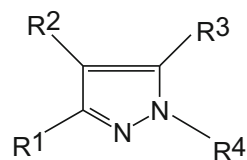
(VI)



(VII)



(VIII)



(IX)

Some commercial nitrification inhibitors

*ATC* Many triazoles particularly 1, 2, 4-triazoles are reported to have nitrification inhibition activity. Among these, 4-amino-1, 2, 4- triazole (*ATC*, **VII**) is the most potent inhibitor but not as effective as nitrapyrin. *ATC* was produced

from formic acid and hydrazine by Ishihara Industries, Japan. It completely checks nitrification of urea for four weeks at a concentration of 5 % by weight of urea.

*Pyridines* Among the various pyridines tested, 2-ethynylpyridine (**VIII**) and nitrapyrin (**I**) are the most potent inhibitors of nitrification, but other compounds also possess this activity. Among those, 2-chloropyridine, 2, 6-dichloropyridine, and 6-chloro-2-picoline significantly inhibited ammonia oxidation in soil, whereas compounds containing carboxylic group (6-chloropicolinic acid) had little effect (Ball et al. 2012). In a wheat crop, chlorinated pyridine performed better than DCD in yield enhancement and reduction in N<sub>2</sub>O emission (McCarty and Bremner 1989).

### 3, 4-Dimethylpyrazole Phosphate (DMPP, IX)

It is a new nitrification inhibitor with highly favorable toxicological and ecotoxicological properties and shows several distinct advantages compared to the currently used nitrification inhibitors. Application rates of 0.5–1.5 kg ha<sup>-1</sup> are sufficient to achieve optimal nitrification inhibition. It can significantly reduce nitrate leaching, without being liable to leaching itself (Zerulla et al. 2001). Significant reduction in N<sub>2</sub>O production was observed when DMPP was used as NI (Menendez et al. 2012).

## 5.2 Indigenous Nitrification Inhibitors

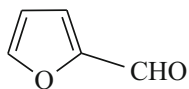
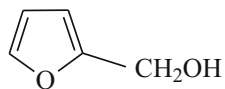
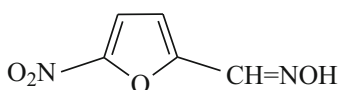
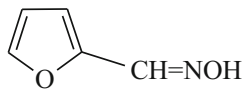
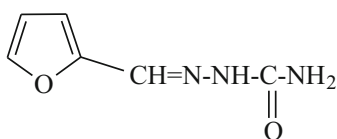
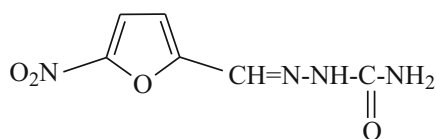
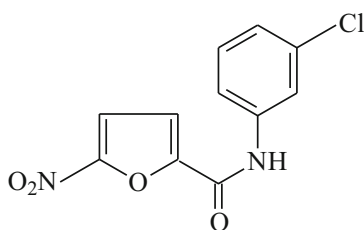
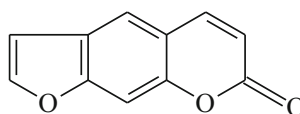
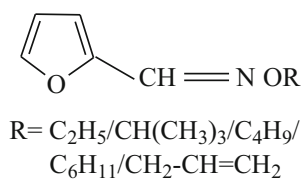
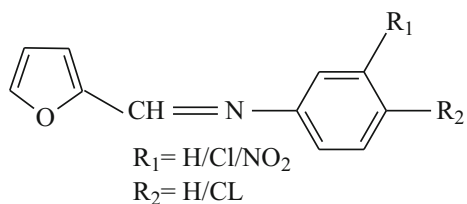
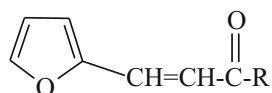
*Furan Derivatives* Sahrawat and Mukerjee (1977) after observing the effect of furan ring on nitrification inhibition screened some furano compounds like furfural (**X**) and furfural alcohol (**XI**) for possible effect on nitrification. Twenty to 30 % concentrations of these compounds matched 5 % karanjin (the major furanoflavonoid constituent of *Pongamia glabra*) in nitrification inhibition during 45–60 days. The inhibition of nitrification decreased after 45 days, while karanjin remained effective even after this period. Furfural alcohol was a better inhibitor of

nitrification of ammonium sulfate than that of urea.

Kuzvintzwa et al. (1984) tested derivatives of furfural along with a natural furanocoumarin – psoralen – for nitrification inhibition in laboratory incubation studies. 5-Nitrofurfural oxime (**XII**), furfural oxime (**XIII**), and furfural semicarbazone (**XIV**) were the most effective followed by 5-nitrofurfural semicarbazone (**XV**), 5'-nitro-3-chloro-2-furanilide (**XVI**), and psoralen (**XVII**). Only 5-nitrofurfural oxime approached nitrapyrin in effectiveness. The nitro derivatives tended to become general bactericides and became effective against *Nitrobacter* species also, thereby causing accumulation of nitrite nitrogen. 3-Chlorofuranilide and furfural oxime caused very little accumulation of nitrite even at the highest concentration (15 %, N-basis).

Datta et al. (2001) examined three series of furfural derivatives, namely, N-O-furfural oxime ethers (**XVIII**), furfural Schiff bases (**XIX**), and furfural chalcones (**XX**), as possible nitrification inhibitors in laboratory incubation study. Furfural oxime ethers and Schiff bases showed potential activity, but furfural chalcones were only mildly active. N-O-Ethyl furfural oxime among the oxime ethers and furfurylidine-4-chloroaniline among Schiff bases performed the best. These two compounds showed more than 50 % nitrification on the 45th day at 5 % dose as compared to 73 % by nitrapyrin.

Activity of the ethers decreased with increase in N-O-alkyl chain length and introduction of chlorine in phenyl ring of furfurylidene anilines increased the activity of Schiff base. Schiff bases derived from 2, 4/2, 6-dichlorobenzaldehyde and 2/3/4-fluoroaniline were also reported as potent nitrification inhibitors (Aggarwal et al. 2009).

**X****XI****XII****XIII****XIV****XV****XVI****XVII****XVIII****XIX**

R = CH<sub>3</sub>/C<sub>6</sub>H<sub>5</sub>/4-OH-C<sub>6</sub>H<sub>5</sub>

**XX**

Furfural derivatives (X-XX) with nitrification inhibitory property

**Acetylenic Compounds** Acetylene was first found to inhibit ammonia oxidation in pure cultures of *Nitrosomonas europaea* and then established as a potent inhibitor of nitrification (McCarty 1999). However, because it is a gas, there are problems in introducing it into the soil in field and sustaining its availability during the growing period at a concentration required to limit nitrification. The problem has been overcome by the use of calcium carbide coated with layers of wax and shellac to provide a slow-release source of acetylene. Addition of wax-coated calcium carbide to the fertilized soil has reduced nitrification and increased yield, or recovery of N, in irrigated wheat, maize, cotton, and flooded rice (Banerjee et al. 1990).

Another way of overcoming the problem of applying gaseous acetylene is to use substituted acetylenes such as 2-ethynylpyridine or phenyl acetylene, which are liquids at ambient temperatures. These two compounds have proved as effective inhibitors in laboratory studies. The use of 2-ethynylpyridine in irrigated cotton has resulted in greatly increased recovery of applied N (Frenay et al. 2011).

**Sulfur Compounds** A broad range of S-containing compounds including thiosulfates, thiocarbamates, xanthates, S-containing amino acids, and several pesticides including fungicides inhibit nitrification. Specific compounds include: S-benzyl isothiuronium salts (Kumar et al. 2004; Bhatia et al. 2010), carbon disulfide (CS<sub>2</sub>), thiourea, allyl thiourea, guanyl thiourea, 2-mercaptobenzothiazole, 3-mercapto-1, 2, 4-triazole, thioacetamide, sodium diethyl-dithiocarbamate, sodium thiocarbonate, thiosemicarbazide, thiocarbohydrazide, diphenylthiocarbazon, dithiocarbamate, s-ethyl dipropyl thiocarbamate, ethylene-bis-dithiocarbamate, and N-methyl dithiocarbamate.

**Heterocyclic Compounds** Several strong inhibitors of ammonia oxidation in soil can be classified by their heterocyclic ring structures. This class of compounds includes some of the

more potent inhibitors of nitrification in soil, namely, nitrapyrin, etridiazole, 2-ethynyl pyridine, 4-amino-1, 2, 4-triazole, 3-methylpyrazole-1-carboxamide and recently reported furan derivatives (Datta et al. 2001), 1, 3, 4-oxa/thiadiazoles (Kumar et al. 2005; Saha et al. 2010), 3, 4-dimethyl pyrazole phosphate (Zerulla et al. 2001), and naphthyridine derivatives (Aggarwal et al. 2010). The heterocycles involved in general are: furan, pyrazole, pyridine, pyridazine, benzotriazole, 1, 2, 4-triazole, thiadiazoles, 1, 3, 5-triazines, and s-tetrazines. Several of the heterocyclic N compounds found to inhibit ammonia oxidation are structurally similar in that they contain chloro (Cl) and/or trichloromethyl (CCl<sub>3</sub>) groups substituted on carbon atom(s) adjacent to a ring N [e.g., nitrapyrin {2-chloro-5-(trichloromethyl) pyridine}, etridiazole (5-ethoxy-3-trichloromethyl-1, 2, 4-thiadiazole), 2-chloropyridine, 2,6-dichloropyridine, 6-chloro-2-picoline, and 3,4-dichloro-1,3,4-thiadiazole].

**Inhibitors of Natural Origin** Synthetic nitrification inhibitors, though expensive, can efficiently inhibit nitrification. Certain allelochemicals released by plants are also reported to have an inhibitory effect. Rice postulated that because inhibition of nitrification results in conservation of both energy and nitrogen, vegetation in late succession or climax ecosystems contains plants that release allelochemicals that inhibit nitrification in soil (Rice 1984). Some natural products from neem (*Azadirachta indica*, A. Juss), karanja (*Pongamia glabra*, Vent.), mint (*Mentha spicata*, *Mentha arvensis* L.), and mahua (*Madhuca longifolia*, L.) are reported to inhibit the activity of nitrifiers (Sahrawat and Parmar 1975; Prasad et al. 1993; Prasad et al. 2002; Saxena et al. 1999; Kumar et al. 2007, 2008, 2010, 2011; Sahrawat 1982; Majumdar 2008; Patra and Chand 2009; Opoku et al. 2014). Among them, neem-based products like Nimin, Neemex, and Neem Gold-A are commercially available in the Indian market.

Nonedible oilseeds like neem (*Azadirachta indica* A. Juss.), karanja (*Pongamia glabra* Vent.), and mahua (*Madhuca indica*, *M. latifolia*) have been extensively studied for nitrification inhibition properties. Oil cakes in general and nonedible cakes in particular have been known to possess certain minor nonfatty biologically active constituents which make them unsuitable for human consumption. These oilseeds have been traditionally used as slow nutrient release manures or in admixture with manures to regulate the nutrient release.

**Neem** The utility of neem cake in improving the nitrogen use efficiency of prilled urea in different crops has been exhaustively demonstrated (Sahrawat and Parmar 1975; Prasad et al. 1993). Due to the poor shelf life of neem cake, its industrial production did not merit attention (Prasad et al. 2002). Neem oil-coated urea (NOCU) on the other hand was found to be more suited (Saxena et al. 1999; Kumar et al. 2007, 2008, 2010, 2011). Its efficacy has been demonstrated at two-fertilizer plants in India, viz., KRIBHCO, Hazira, and Shriram Fertilizers and Chemicals, Kota. M/s National Fertilizer Ltd., Panipat, have claimed independently produced neem oil-coated urea. The production of NOCU has been grown to over 2.0 million tonnes per year and neem-coated urea is manufactured by: (1) National Fertilizers Ltd., (2) Shriram Fertilisers and Chemicals Limited, (3) Indo Gulf Fertilisers, (4) Tata Chemicals Ltd, (5) Chambal Fertilisers and Chemicals Ltd, and (6) Mangalore Chemicals and Fertilizers Ltd as per Ministry of Agriculture Notification No: S.O. No. 2073 (E) dated August 10, 2009 (Agricoop 2009).

**Karanja** A number of physiologically active furanoflavonoids (Sahrawat 1982; Sahrawat et al. 1974; Majumdar 2008, 2002) are found in seeds, bark, and leaves of the tree *Pongamia glabra* Vent. The hot ethanol extract of the defatted seeds (applied at 20 and 30 % of applied N) had maximum nitrification inhibitory activity

followed by bark extract (prepared with 40:60, v/v, mixture of petroleum ether and acetone), and leaves had negligible effect. The alcohol extract of seeds was effective in retarding nitrification for 60 days when applied at 20 % of the fertilizer nitrogen dose. The percentage inhibition of nitrification of urea in soil was 47–55 % even after 45 days of application of seed extract.

Karanjin, present in karanja seeds, is a potent inhibitor of nitrification. The inhibition of nitrification of urea or ammonium sulfate was around 43 % after 8 weeks of incubation with its dose of 5 % of applied fertilizer nitrogen. Comparative evaluation of karanjin and three commercial inhibitors (nitrapyrin, AM, and DCD) when applied in a sandy loam soil at 5 mg kg<sup>-1</sup> of soil reduced the nitrification rate as:

**Nitrapyrin > Karanjin > AM > DCD**

The furan ring of karanjin was responsible for nitrification inhibition property. This information led to the study of several furan derivatives (Sahrawat and Mukherjee 1977; Kuzvinzwa et al. 1984; Datta et al. 2001) as nitrification inhibitors (described above the synthetic compounds).

**Mahua** Seed cake and extracts of *Bassia latifolia* Roxb., *Madhuca indica* J.F. Gmel, and *Madhuca latifolia* L. are known to possess nitrification inhibition property. The seed cake and extracts contain saponins, responsible for nitrification inhibition. In an incubation study employing clay loam soil, the inhibitory effect of mahua cake extract persisted only for 20 days (Slangen and Kerckhoff 1984).

**Miscellaneous** Many other plants and plant products from *Citrullus colocynthis* Schrad., Sal (*Shorea robusta*), *Eucalyptus globosus*, *Ricinus communis*, *Acacia catechu*, *Calotropis gigantea*, *Onosma hispidum*, *Mentha arvensis*, *Mentha spicata*, *Artemisia annua*, *Chrysanthemum cinerariifolium*, *Tagetes erecta*, *Catharanthus roseus*, *Ricinus communis* L., turmeric powder, tea waste, and cashew shell powder have been

reported to show varying degree of nitrification inhibition.

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## 6 Biological Nitrification Inhibitor: Concept

Biological nitrification inhibition is a rhizospheric process where different class of compounds released by plant roots act as nitrification inhibitor (Subbarao et al. 2013). It was hypothesized that it can improve N uptake due to its inhibitory effects on nitrification by improving  $NUE_{\text{agronomic}}$  mostly contributed by the improvement in crop N uptake.

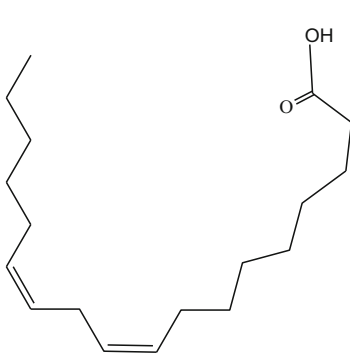
Primary productivity is positively impacted in the tropical savannas dominated by native African grasses such as *Hyparrhenia diplandra* which appear to have a significant ability to suppress nitrification (Boudsocq et al. 2009).

Recent studies by Boudsocq et al. (2011) reported the role of biological nitrification inhibition in controlling nitrification in temperate and tropical grasslands and contrasting preferences for  $NH_4^+$  or  $NO_3^-$  between two plant species. The ability of one species to control nitrification (i.e., to stimulate or inhibit) could enhance their ability to compete for mineral N with other species. This is consistent with the results of the studies suggesting that biological nitrification inhibition strongly affects plant invasions (Hawkes et al. 2005; Rossiter-Rachor et al. 2009).

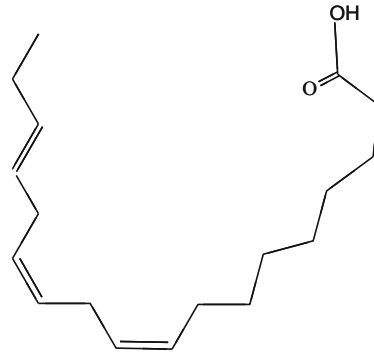
Several compounds belonging to different chemical groups have been successfully isolated and identified from plant tissue or root exudates using bioassay-guided purification approaches and are reported to be biological nitrification inhibitor (Subbarao et al. 2013). The identified compounds from the aerial parts of *Brachiaria humidicola* are the unsaturated free fatty acids, linoleic acid (XXI), and  $\alpha$ -linolenic acid (XXII). They are relatively weak inhibitors of nitrification. In root tissues of *B. humidicola*, two phenyl propanoids, methyl-p-coumarate (XXIII) and methyl ferulate (XXIV), were identified as major biological nitrification inhibitor (Gopalakrishnan et al. 2007). From root exudates of hydroponically grown sorghum, a phenylpropanoid, methyl 3-(4-hydroxyphenyl) propionate (XXV) has been identified as the biological nitrification inhibitor (Zakir et al. 2008). Bachialactone, a cyclic diterpene, was identified from the root exudates of *B. humidicola* (Subbarao et al. 2013). Further, sorgoleone, a p-benzoquinone exuded from sorghum roots, has a strong inhibitory effect on *Nitrosomonas* sp. and contributes significantly to nitrification inhibition capacity in sorghum.

In a recent review (Subbarao et al. 2013), karanjin was also included as the biological nitrification inhibitor. It seems that there is an overlap of compounds known to be biological nitrification inhibitors. Some compounds are secondary metabolites produced and stored in the different parts of plant, and the compounds released as root exudates.

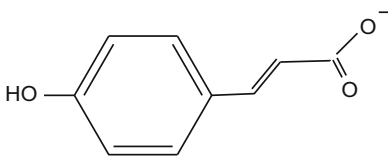




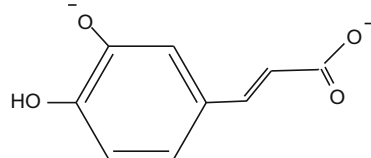
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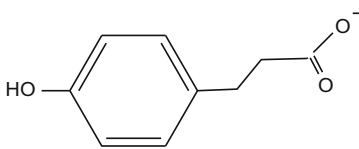
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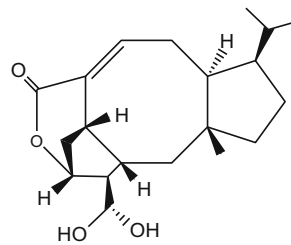
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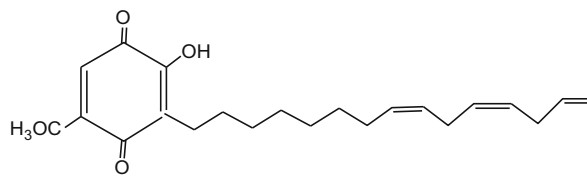
XXIV



XXV



XXVI



XXVII

Structures of biological nitrification inhibitors

**Conclusion**

Several nitrification inhibitors have been reported in literature from time to time. Most of these are not fully satisfactory due to one or more of the following disadvantages:

- Complicated synthesis and related preparation steps

- High volatility, leading to low persistence and high losses into the atmosphere unless introduced in soil using technically complicated processes (e.g., by probe)
- High toxicity or ecotoxicity

- Low stability against hydrolysis, reducing the duration of action in soil and the shelf life
- High application rates
- Requirement of further modification of the active molecule
- Addition of costly formulants
- High cost

Therefore, an ideal nitrification inhibitor is still elusive. It needs to be simple, safe, efficient, persistent, specific, and economical in use. It implies that the nitrification inhibitor should have specificity to nitrifying bacteria responsible for conversion of ammonium to nitrite. Inhibition of *Nitrobacter* is not desirable as it leads to accumulation of nitrite. The inhibitor should be nontoxic to other soil organisms, fish, mammals, and crops and be safe to the environment. It should be effective throughout the nitrogen-soil interaction zone and be sufficiently persistent in action so that nitrification is inhibited for an adequate period of time. Furthermore, it should be a low cost additive to the fertilizer.

A major consideration during the selection of nitrification inhibitors (NI) is their high effectiveness at the lowest possible application rate with a minimum of undesirable side effects. The availability of an inhibitor at effective concentration is essential. This can be achieved by coating fertilizer granules with the inhibitor or by incorporating it into granules (Slangen and Kerckhoff 1984). The aim of both the approaches is to ensure an intimate and uniform interaction of the substrate with the inhibitor. The application of an effective concentration of an NI to soil, together with N fertilizer, is a difficult task since it involves different crops, forms, and rates of N application. It leads to different concentrations of NI reaching the nitrifiers, particularly if N is applied as granules.

While application of chemical fertilizers to agricultural crops has resulted in tremendous increase in yield, problems arising due to escape to the environment of different nitrogen species, especially  $N_2O$ , nitrite, and nitrate, have raised serious economic and environmental concerns. Of the different

processes responsible for these, nitrification and denitrification are of prime importance. Hence, efforts have to be made to regulate the process of nitrification (major source of different N species) as a means to enhancing the use efficiency of N, decreasing environmental/economic concerns, and optimizing the functioning of agroecosystems. Use of nitrification inhibitors has been helpful in mitigating the negative effects of fertilizer application. However, continued efforts need to be made for finding more efficient and environment-friendly products to suit the ever-changing agroclimatic conditions.

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