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ORIGINAL PAPER

Effect of soil-applied calcium carbide and plant derivatives on nitrification inhibition and plant growth promotion

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Abstract The aim of this study was to evaluate the relative performance of three nitrification inhibitors (NIs) viz. calcium carbide (CaC_2), and plant derivatives of *Pongamia glabra* Vent. (karanj) and *Melia azedarach* (dharek) in regulating N transformations, inhibiting nitrification and improving N recovery in soil–plant systems. In the first experiment under laboratory incubation, soil was amended with N fertilizer diammonium phosphate [$(NH_4)_2HPO_4$] at a rate of 200 mg N kg^{-1}, N + CaC_2, N + karanjin, and N + *M. azedarach* and incubated at 22 °C for 56 days period. Changes in total mineral N (TMN), NH_4^+–N and NO_3^-–N were examined during the study. A second experiment was conducted in a glasshouse using pots to evaluate the response of wheat to these amendments. Results indicated that more than 92 % of the NH_4^+ initially present had disappeared from the mineral N pool by the end of incubation. Application of NIs i.e., CaC_2, karanjin, and *M. azedarach* resulted in a significant reduction in the extent of NH_4^+ disappearance by 49, 32, and 13 %, respectively. Accumulation of NO_3^-–N was much higher in N amended soil 57 % compared to 11 % in N + CaC_2, 13 % in N + karanjin, and 18 % in N + *M. azedarach*. Application of NIs significantly increased growth, yield, and N uptake of wheat. The apparent N recovery in N-treated plants was 20 % that was significantly increased to 38, 34, and 37 % with N + CaC_2, N + karanjin, and N + *M. azedarach*, respectively. Among the three NIs tested, CaC_2 and karanjin proved highly effective in inhibiting nitrification and retaining NH_4^+–N in the mineral pool for a longer period.

Keywords Nitrification · Nitrification inhibitor · N recovery · NH_4^+ retention · NO_3^- accumulation · N losses

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Introduction

Nitrogen is an essential nutrient controlling the diversity, dynamics, and functioning of many terrestrial, freshwater, and marine ecosystems. Agricultural ecosystems depend heavily on large inputs of N fertilizer to sustain productivity, as naturally fixed N is seldom adequate for high-production systems (Dinnes et al. 2002; Subbarao et al. 2006a; Abbasi and Khizar 2012). However, N is a highly dynamic and mobile element and significant N losses occur as a result of NO_3^- leaching, denitrification, runoff, NH_3 volatilization and gaseous emissions of N_2O, and NO to the atmosphere (Zaman et al. 2009). These losses of N have long-term adverse ecological and environmental effects contributing to eutrophication, loss of aquatic biodiversity, and increased N_2O emissions (Warneke et al. 2011).

Most of the fertilizer N applied to soils (90 %) for agriculture production is in the form of ammonium (NH_4^+), or NH_4^+-producing compounds. According to IFA report, world apparent ammonia consumption for the Year 2010 was about 121 million tonnes (IFA 2012). Much of this NH_4^+ is usually oxidized quite rapidly to NO_3^-, a highly mobile form of N, providing a much greater potential for N to be lostbeyond the rooting zone (leaching) and into the atmosphere as gaseous molecules (N_2O, NO, and N_2) through denitrification (Subbarao et al. 2006a). It is estimated that nearly 70 % of the applied N from managed ecosystems is lost through nitrification and

subsequent processes (Raun and Johnson 1999; Glass 2003). These losses result in serious environmental consequences and inefficient use of both soil and applied N (Raun and Johnson 1999; Subbarao et al. 2006a).

Controlling the process of nitrification through inhibition or suppression of nitrifiers in soil is perhaps one of the most effective strategies to reduce N losses by NO_3^- leaching and by gaseous N emissions that occur in nitrification (Rodgers 1986; Prasad and Power 1995; Subbarao et al. 2006b). Field evaluations suggested that if nitrification rates are reduced in agricultural systems, plants have more time to take up available N, thereby improving N recovery and uptake and reducing NO_3^- leaching and associated off farm environmental impacts(Rodgers 1986; Subbarao et al. 2006b).

The losses of applied N associated with nitrification could be controlled or reduced by applying nitrification inhibitors (NIs). Rapid nitrification or oxidation of NH_4^+ to NO_3^- in soil catalyzed by microbes had been found to be inhibited by NIs included nitrapyrin (2-chloro-6-tricholoro methyl pyridine), sodium azide, sodium chlorate, dicyandiamide (DCD), ATC (4-amino-1-2-4-triazole), N-serve, and certain other compounds (McCarty 1999; Abbasi et al. 2003; Fangueiro et al. 2009; Khalil et al. 2009; Zaman et al. 2009; Souri 2010; Pereira et al. 2010). The use of these synthetic NIs has been restricted to the academic experimental levels because of high cost, lack of availability, and adverse side effects (Patra and Sukhma 2009).

Apart from these chemically synthesized NIs, nitrification inhibitory properties of some inexpensive compounds like calcium carbide (CaC_2) and several plants materials like Karanji (*Pongamia glabra*), Neem (*Azaddirachta indica*), and tea (*Camellia sinensis*) waste have been evaluated by many researchers and their advantages and disadvantages have been reported (Freney et al. 2000, Kiran and Patra 2003; Majumdar 2002; Abbasi et al. 2011). Among these NIs, acetylene (C_2H_2) has been shown to be a potent inhibitor of nitrification (Freney et al. 2000). However, C_2H_2 is a gas, therefore, it is difficult to apply and maintain at the required concentration in soil to inhibit the oxidation of ammonium. This problem can be overcome by coating calcium carbide with wax (as acetylene is a product of calcium carbide hydrolysis), or by forming a matrix with an insoluble material, to slow down its reaction with water, and produce acetylene in situ in soil (Freney et al. 1992; Smith et al. 1993; Freney et al. 2000).

The plant karanji is a source of furanoflavonoid "karanjin" which has been identified as having nitrification inhibitory properties. The nitrification inhibition by karanjin was observed to remain high for a period of approximately 6 weeks, with inhibition ranging between 9 and 76 % for a range of soils studied (Majumdar et al. 2004). Similarly, fruits of dharek (*Melia azedarach*) are poisonous to humans and these are chemically related to Azadirachtin, the primary insecticidal compound in the commercially important neem oil. These compounds are probably antibacterial in action and display insecticidal properties; however, their effects on soil fertility and plant mineral uptake are not clear. In fact, a positive effects of fresh melia ground leaves on growth and mineral composition of plum hybrids during their acclimatization, was observed (Marino et al. 2009). Similarly, Toselli et al. (2010) recently reported that freshly ground melia derivatives did not inhibit nitrification in the soil but, rather, they stimulated mineral N release, N leaf concentration, root N uptake, and peach plant growth.

As compared to synthetic NIs, the natural NIs are eco-friendly, easily available, implying lower cost of production and can be included in the farming systems (Upadhyay et al. 2011). However, the use of these inhibitors for inhibiting nitrification, reducing N losses and increasing NUE in agriculture ecosystem is still not common. Keeping in view, this study was designated to evaluate the relative efficiency of karanjin (*P. glabra* Vent.), dharek (*M. azedarach)* (plant materials), and CaC_2 (a chemical) to inhibit nitrification, improve N recovery in soil–plant system and enhance the growth and yield of wheat. This research has been carried out in the Department of Soil and Environmental Sciences, University of Azad Jammu and Kashmir-Pakistan (Rawalakot), from November 2010 to May 2011.

Materials and methods

Soil sampling/collection

Soil was collected from an arable field located at the research farm, Faculty of Agriculture Rawalakot Azad Jammu and Kashmir. The study area lies between the altitude of 1,800–2,000 m above sea level and latitude 33–36° in the north–east of Pakistan under the foothills of the great Himalayas at Rawalakot district, Poonch division, AJK, Pakistan. The soil in the study site was loam in texture, classified as a Humic Lithic Eutrudepts (Inceptosols). The field was barren at the time of sampling but previously maize and wheat were cultivated during the growing season. Soil samples were collected from the 0 to 15 cm depth at random from 5 different locations and mixed well. The field-moist soil was passed through a 4 mm sieve to eliminate coarse rock and plant material, thoroughly mixed to insure uniformity and stored at 4 °C prior to use. A sub–sample of about 500 g was taken, air dried and passed through 2 mm sieve and used for studying physical and chemical characteristics. The main physio-chemical properties of the soil were determined on dry weight basis were: bulk density: 1.35 g cm^{-1} (intact soil

core method); pH: 7.1; total C: 4.81 g kg^{-1}; total N: 0.89 g kg^{-1}; total mineral N: 8.8 mg kg^{-1}, available P: 7.6 mg kg^{-1} and available K: 87 mg kg^{-1}.

Laboratory incubation

Field-moist soil samples were placed into polyethylene bags and pre-incubated at 22 °C to stabilize the microbial activity. Ten days after pre-incubation, 30 g of soil was weighed and transferred into glass jars of about 100 mL capacity. The initial moisture content of soil was adjusted to a water-filled pore space (WFPS) of approximately 58 % that was maintained throughout the incubation. WFPS was calculated as follows: WFPS = (soil gravimetric water content X bulk density)/[1– (bulk density/particle density)].

There were four N treatments with and without NIs i.e., N without NIs; N + CaC_2, N + karanjin and N + *M. azedarach* and a control (no N); eight sampling times: 0, 7, 14, 21, 28, 35, 42, and 56 days and three replicates. Altogether, a total of 120 experimental units were used at the start of the experiment. An aqueous solution of diammonium phosphate [$(NH_4)_2HPO_4$] i.e., DAP was applied as the N source to all pots except control to supply 200 mg N kg^{-1} soil. A basal dose of 90 mg P_2O_5 kg^{-1} soil as single super phosphate (SSP) and 60 mg K_2O kg^{-1} soil as potassium sulfate was well mixed into the soil.

For the treatments subjected to inhibitors, CaC_2 was obtained in powder form from Marij Scientific Traders, Rawalpindi Pakistan. Seeds/fruits of karanja (*P. glabra* Vent.) and *M. azedarach* were collected locally. Seeds were crushed and seed powder (500 g) was defatted by boiling in hexane (2 L) in a soxhlet apparatus for 24 h. Karanjin and dharek (*M. azedarach*) was precipitated out on cooling the hexane extract in a refrigerator. After 72 h, the precipitate was collected and crystallized in ethanol and its melting point was observed. It was re-crystallized a number of times in ethanol to yield the final product (Majumdar et al. 2004). A solution of karanjin and dharek was prepared by dissolving crystals of karanjin and *M. azedarach* in acetone, and the solution was added to the soil containing DAP (200 mg kg^{-1} soil) at the rate of 20 % of applied N (Majumdar et al. 2004).

The soil was homogeneously mixed with the respective amendments in 100 ml glass pots. The mass of each pot was recorded. Pots were covered with parafilm with 3–4 small holes on the top to allow O_2 exchange. Pots were incubated in an incubator at 22 °C and arranged in a completely randomized design. Soil moisture was checked/adjusted after every 2 days by weighing the pots and adding the required amount of distilled water when the loss was greater than 0.05 g. During this process, care was taken not to disturb the soil either through stirring or shaking.

Soil extraction and analysis

Samples of all the treatments incubated at different timings were analyzed for total mineral nitrogen (TMN) and ammonium-N (NH_4^+–N). Initial concentrations of TMN and NH_4^+–N at day 0 were determined by extracting soil samples with 200 ml of 1 M KCl added directly to the flask immediately after adding each amendment. Thereafter, triplicate samples from each treatment were removed randomly from the incubator at different incubation periods and extracted by shaking for 1 h with 200 ml of 1 M KCl followed by filtration through Whatman's No. 40 filter paper.

The mineral N contents of the extract were determined with steam distillation and the titration method (Keeney and Nelson 1982) using microkjeldahl's distillation apparatus prepared locally. Aliquots (40 ml) of the extracts were pippetted into a distillation flask and steam distillation was carried out after adding MgO for NH_4^+–N determinations while for the determination of TMN, magnesium oxide (MgO) + Deverda's alloy was added. Distillates were collected in 5 ml of boric acid containing bromocresol green/methyl red mixed indicator and titrated against 0.05 M HCl. Nitrate–N was calculated by subtracting NH_4^+–N from TMN. Any NO_2 present would have been included in the NO_3^- fraction.

The inhibition of nitrification by NIs was calculated as reported by Sahrawat (1996) and Majumdar et al. (2001):

$$\text{Inhibition of nitrification } (\%) = \frac{N_N - N_I}{N_N} \times 100$$

where N_N = nitrified N, i.e., NO_3^-–N as percent of total mineral N, i.e., (NH_4^+–N + NO_3^-–N) in soil amended with N alone; N_I = nitrified N, i.e., NO_3^-–N as percent of total mineral N, i.e., (NH_4^+–N + NO_3^-–N) in soil amended with N combined with an inhibitor.

Nitrified N (%) was calculated at different stages of the incubation according to Majumdar et al. (2001):

$$\text{Nitrified N } (\%) = \frac{NO_3^- - N}{\text{Total mineral N } \left(NH_4^+ - N + NO_3^- - N\right)} \times 100$$

To calculate nitrified N by the above formula, initial soil NO_3^-–N and total mineral N at day 0 and the concentration in the control were subtracted from their respective content.

Wheat growth and N-uptake

A separate experiment was conducted in pots in the greenhouse at the Faculty of Agriculture, Rawalakot Azad Jammu and Kashmir. Wheat (*Triticum aestivum* L.) variety

"Inqalab-91" was used as a test crop. Thoroughly cleaned earthen pots of 38 cm height and 18 cm width were used in the experiment. The same soil used in the laboratory incubation experiment was also used in this experiment after sieving to <4 mm. Each pot contained approximately 12 kg soil. There were six N treatments with and without NIs and a control, with three replicates, comprising 21 pots in total. The treatments included: T_1 = control T_2 = N alone; T_3 = N + CaC_2 at the rate 15 mg kg^{-1}; T_4 = N + CaC_2 at the rate 30 mg kg^{-1}; T_5 = N + CaC_2 at the rate 45 mg kg^{-1}; T_6 = N + karanjin, and T_7 = N + *M. azedarach*. An aqueous solution of diammonium phosphate [$(NH_4)_2HPO_4$] i.e., DAP was applied as N source to all pots except control to supply 200 mg N kg^{-1} soil. A 200 mg l^{-1} solution of karanjin and *M. azedarach* was prepared by dissolving crystals of karanjin and dharek in acetone and the solution was added to the soil containing DAP (200 mg kg^{-1} soil) at the rate of 20 % of applied N (Majumdar et al. 2004). A basal dose of 90 mg P_2O_5 kg^{-1} soil as SSP and 60 mg K_2O kg^{-1} soil as potassium sulfate was well mixed into the soil before sowing. The pots were arranged in a completely randomized design.

The soil was moistened with water and maintained at 58 % water-filled pore space. In each pot, eight seeds were sown at a depth of about 4 cm on November 06, 2010. Pots were kept under shade to reduce evapo-transpiration during the course of germination. After complete germination, plants were thinned to six plants per pot. All pots were equally irrigated when needed. Two plants from each treatment were sampled at Vn, milking and maturity stages. At each sampling time data were collected for shoot length, root length, shoot dry weight, and root dry weight. At maturity, number of grains per spike, 1 000-grain weight and grain yield per plant was also recorded. Bulked plant parts (shoot + leave) were washed, and cleaned. The cleaned plant parts and grains were oven dried at 70 °C for 48 h. For analysis of the N content in grain and straw, oven-dried plant material (grain and straw) from each plot were ground separately with a grinder (Polymix PX-MFC 90D; Switzerland) to pass through a 2 mm-mesh sieve. The N contents in samples were determined by the Kjeldahl method (Jackson 1962).

Statistical analysis

All data were statistically analyzed by multifactorial analysis of variance (ANOVA) using the software package Statgraphics (1992). Least significant differences (LSD) are given to indicate significant variations between the values of either treatments or time intervals. Confidence values (*P*) are given in the text for the significance between treatments, manures, time interval and their interactions. A probability level of ≤0.05 was considered significant.

Results and discussion

Changes in NH_4^+–N

The NH_4^+–N concentration in the un-amended control was quite low (<11 mg kg^{-1}) at the start (day 0), continued to decrease with time and was negligible by the end of incubation (day 56) (Fig. 1). The low N concentration in

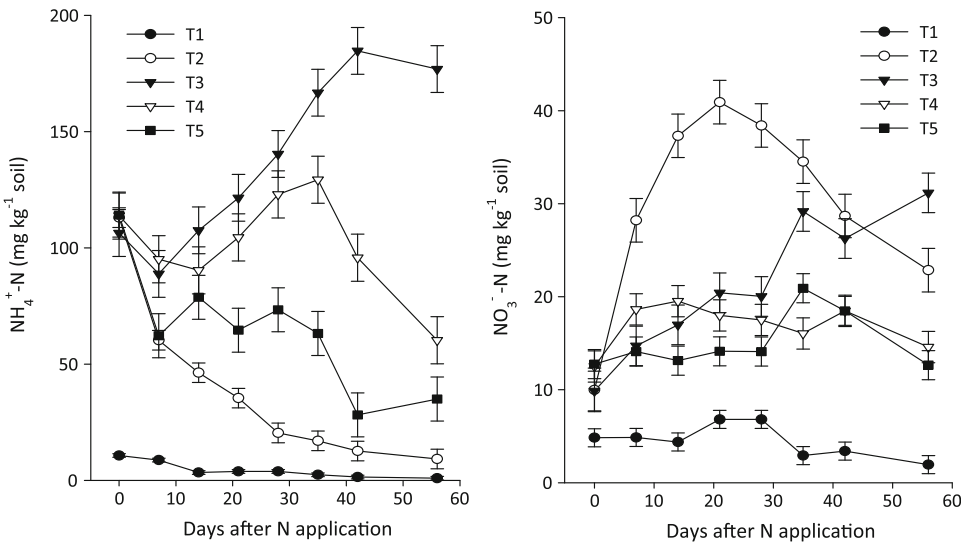

Fig. 1 Changes in the concentration of NH_4^+–N and accumulation of NO_3^-–N in a soil collected from an arable filed, amended with fertilizer N with and without nitrification inhibitors and incubated at 22 °C under controlled laboratory conditions. The *legends* indicate: T_1 = control; T_2 = N without nitrification inhibitors (NIs); T_3 = N + CaC_2; T_4 = N + karanjin; T_5 = N + *M. azedarach*

the control soil throughout the incubation indicated low mineralization which was unusual because the soil collected from an arable field cultivated for crop production. These results were in contrast to our previous study, where the available N of an arable control soil was 39 mg kg^{-1} during a 50 day incubation (Abbasi et al. 2011).

The NH_4^+–N concentration of N treated soils (with and without NIs) was between 97 and 110 mg N kg^{-1} at day 0, with the difference between the N treatments being non-significant (Fig. 1). Despite adding 200 mg NH_4^+–N per kg soil, only about 106 mg (mean) of the added N was recovered as NH_4^+–N just after N application (day 0). Under similar experimental conditions, Souri (2008) also reported less NH_4^+–N recovery than the applied N and explained that the N unaccounted for may be due to NH_4^+ fixation by clay minerals, volatilization as NH_3, or inefficient extraction of NH_4^+, which has been done with 1 M KCl. The concentration of KCl used for extraction was 1 M that might be a major factor for inefficient extraction of NH_4^+.

In the soil to which N was added (without NIs), initial NH_4^+ concentration significantly ($P \leq 0.05$) decreased with time and very little NH_4^+ was left in the mineral N pool by the end of incubation (9.2 mg kg^{-1} soil) (Fig. 1). Results showed that 96 % of the applied N had disappeared over a period of 56 days. During this NH_4^+ disappearance, concentrations of NO_3^-–N increased to a maximum of 41 mg of NO_3^-–N kg^{-1} at day 21 (Fig. 1), indicating that nitrification was also occurring. However, the buildup of NO_3–N was lower than the rate of NH_4^+ depletion. The pattern of changes in NH_4^+–N and NO_3^-–N observed here was almost similar to that observed in our previous study where more than 58 % of applied N had been disappeared over a period of 50 days and only 21 % of depleted N was accumulated as NO_3^-–N (Abbasi et al. 2011). We had earlier concluded that under the experimental conditions reported here nitrification–denitrification occurred simultaneously (Abbasi and Adams 2000a, b). Produced NO_3^- can simply be diffused into adjacent microsites of denitrification, therefore, a substantial accumulation of NO_3–N would not be expected where both nitrification and denitrification occur simultaneously (Abbasi and Adams 1998). Remde and Conrad (1991) reported that depletion of NH_4^+ in the soil was apparently not balanced by the production of NO_2 and NO_3^-, and this discrepancy may be due to the simultaneous nitrification and denitrification. However, the soil incubated under aerobic conditions with moisture content of about 60 % WFPS, denitrification may not be considered the only loss mechanism of N unaccounted for. Therefore, further investigations are suggested to explore the routes of N losses occurred even under controlled conditions without crop uptake and leaching loss.

A significant reduction in NH_4^+-N concentration was also observed in soils treated with NIs i.e., karanjin and *M. azedarach* but the rate and amount of NH_4^+ depletion was slower than the soil treated with N (without NIs). For example, only about 9.2 mg kg^{-1} of NH_4^+ was left in the mineral N pool of N treated soil by the end of incubation, while 60.3, and 35.0 mg kg^{-1} of NH_4^+-N was persisted and retained until the end in the soil treated with N + karanjin and N + *M. azedarach*, respectively (Fig. 2). The NH_4^+–N concentration in the treatment receiving N + karanjin showed temporary increase and decrease between days 14 and 42. The reasons for the development of this trend are

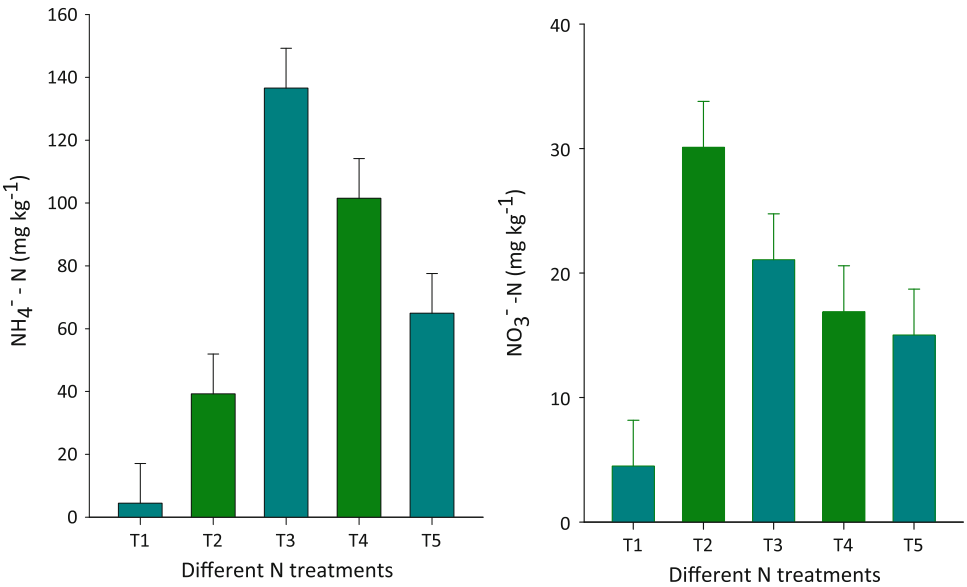

Fig. 2 Over all changes (average over 56 days incubation) in NH_4^+-N and accumulation/inhibition of NO_3^-–N in a soil collected from an arable filed, amended with fertilizer N with and without nitrification inhibitors and incubated at 22 °C under controlled laboratory conditions. The symbols on *X-axis* indicate: T_1 = Control; T_2 = N without nitrification inhibitors (NIs); T_3 = N + CaC_2; T_4 = N + karanjin; T_5 = N + *M. azedarach*

Table 1 Net rates of nitrification (NO_3^-–N mg kg^{-1} day^{-1}) in soil amended with N (200 mg N kg^{-1} soil) with and without nitrification inhibitors over 56 days of laboratory incubation

Treatments	NO_3^-–N (mg kg^{-1} day^{-1} soil)							
	Days after N application							
	7	14	21	28	35	42	56	LSD ($P \leq 0.05$)
T_1	0.00	−0.03	0.09	0.07	−0.05	−0.03	−0.05	0.01
T_2	2.61	1.95	1.47	1.02	0.70	0.45	0.23	0.23
T_3	0.69	0.51	0.50	0.36	0.55	0.39	0.38	0.14
T_4	0.88	0.50	0.26	0.18	0.10	0.14	0.04	0.09
T_5	0.19	0.03	0.07	0.05	0.23	0.14	0.00	0.06
LSD ($P \leq 0.05$)	0.46	0.27	0.21	0.16	0.12	0.13	0.13	

Treatments effect (average over 0–56 days; mg kg^{-1} soil)					
T_1	T_2	T_3	T_4	T_5	LSD ($P \leq 0.05$)
0.00	1.20	0.48	0.30	0.10	0.174

Timings effect (average over T_1–T_5; mg kg^{-1} soil)							
7	14	21	28	35	42	56	LSD ($P \leq 0.05$)
0.87	0.59	0.48	0.34	0.31	0.22	0.12	0.116

T_1, control; T_2, N without nitrification inhibitors (NIs); T_3, N+ CaC_2; T_4, N+ karanjin; T_5, N+ *M. azedarach*

not known. The response of NH_4^+ turnover to soil with N + CaC_2 was quite different and except day 7, concentration of NH_4^+ was significantly increased throughout the incubation (Fig. 1). However, in response to the total N applied (200 mg kg^{-1}), about 12 % of NH_4^+ had been unaccounted for by the end of incubation. These results indicated that CaC_2 and karanjin were highly effective in retaining NH_4^+–N in mineral pool at higher concentration and for longer period. It appeared that addition of NIs inhibited nitrification and consequently denitrification by restricting the supply of NO_3^- to the denitrifying organisms (Souri 2010; Pereira et al. 2010; Abbasi et al. 2011), resulting in relatively higher concentration of NH_4^+ in these treatments. However, it is likely to mention that under arable soil deficient in organic matter, denitrification and N_2O may not be the sole cause of NH_4^+ depletion. It is important to explore other possible loss routes of added NH_4^+ in the soil.

Results indicated that instead of decreasing NH_4^+ to the initial value recorded at day 0, concentration of NH_4^+ in N + CaC_2 treated soil was significantly increased over time (Fig. 1). The tendency of NH_4^+ turnover observed here was in accordance with the previous studies. For example, when CaC_2 was applied with urea N, the NH_4^+ concentration in soil remained at high level for 36 days and increased from 30 mg kg^{-1} to about 38 mg kg^{-1} (Keerthisinghe et al. 1993). Similarly, in another experiment the concentration of NH_4^+ was increased by 27 and 34 % when CaC_2 was applied to urea N (Keerthisinghe et al. 1996). However, the mechanism involved in CaC_2 action in increasing NH_4^+ level is not understood. It may possible that CaC_2 application to a soil resulted in the priming of mineralization of residual soil organic matter.

Accumulation of NO_3^-–N

The concentration of NO_3^-–N in the control without N was low (<5 mg kg^{-1}), remained consistent till day 42 and thereafter further decreased (Fig. 1). The NO_3^-–N in N amended soil (without NIs) increased ($P \leq 0.05$) during incubation to a maximum concentration of 40.9 mg kg^{-1} on day 21. The concentration remained stable until day 35 and thereafter significantly decreased to a minimum of 23 mg kg^{-1} by the end of the 56 days. The extent and rate of NO_3^-–N accumulation in NIs treated soils was much lower compared to N treated soil (without NIs). For example, the concentrations of NO_3^-–N in N + CaC_2, N + karanjin and N + *M. azedarach* ranged between 15 and 31, 15 and 20 and 13 and 21 mg kg^{-1}, respectively compared to the 23 and 41 mg kg^{-1} in N treated soil

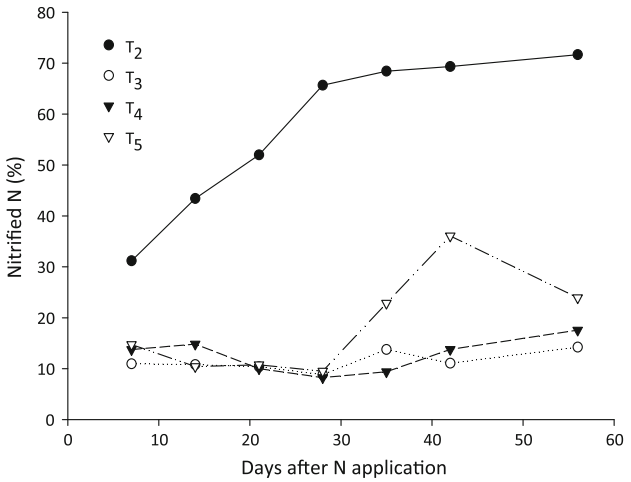

Fig. 3 The amount of NO_3^-–N nitrified (%) as percent of total mineral N affected by NIs during 56 days incubation. The *legends* indicate: T_1 = Control; T_2 = N without nitrification inhibitors (NIs); T_3 = N + CaC_2; T_4 = N + karanjin; T_5 = N + *M. azedarach*

without NIs. Averaged across different timings, accumulation of NO_3^- in the treatments receiving N, N + CaC_2, N + karanjin and N + *M. azedarac* was 30, 21, 17, and 15 mg kg^{-1}, respectively (Fig. 2). These values indicated that NIs included CaC_2, karanjin and *M. azedarach* were able to inhibit nitrification by 69, 67, and 57 %, respectively.

Net rates of nitrification showed a similar trend to that observed for NO_3^-–N accumulation. Net rates of nitrification for N amended soil without NIs were significantly higher ($P \leq 0.05$) than the rates recorded for soils with NIs (Table 1). Comparative analysis indicated that net rates of nitrification for N amended soil (without NIs) ranged between 1.02 and 2.61 mg kg^{-1} day^{-1} between 7 and 28 day with a maximum rate in the initial stage of incubation (day 7). Thereafter, rates declined to a minimum of 0.23 mg kg^{-1} day^{-1} on day 56. In contrast, net rates of nitrification in soil with NIs were much lower and the highest rates of 0.88 mg kg^{-1} day^{-1} recorded on day 7 for the N + karanjin treatment. The average net rates of nitrification in soil with N, N + CaC_2, N + karanjin and N + *M. azedarach* were 1.20, 0.48, 0.30 and 0.10 mg kg^{-1} day^{-1}, respectively showing a substantial inhibition of nitrification by NIs.

The extent of nitrified N (NO_3^-–N) as percent of total mineral N [NH_4^+–N + NO_3^-–N] was much higher in N treated soil without NIs (average 57 %) compared to N + CaC_2 (11 %), N + karanjin (13 %) and N + *M. azedarach* (18 %) (Fig. 3), confirmed the inhibitory effects of tested NIs on nitrification. Percent inhibition of nitrification was calculated to find out the effectiveness of three inhibitors used in the study. The percent inhibition of nitrification by CaC_2, karanjin and *M. azedarach* ranged between 69, 67, and 57 %, respectively indicating CaC_2 and karanjin were superior to *M. azedarach*.

Several previous studies clearly indicated nitrification inhibitory properties of CaC_2 (Freney et al. 1992; Freney et al. 2000; Keerthisinghe et al. 1993, 1996). The inhibitory effect of CaC_2 is attributed to its capacity to release acetylene which is considered a potent nitrification inhibitor (Smith et al. 1993). The effect of karanjin reported here is in accordance with that reported earlier by Majumdar (2002) under laboratory conditions where karanjin inhibited nitrification by 62–75 % and mitigated N_2O emission by 92–96 %. In another experiment conducted under laboratory incubation, nitrification inhibition by karanjin ranged between 9 and 76 %, remained high for a period of approximately 6 weeks and decreased with time (Majumdar et al. 2004). Similarly, in this study, the plant product *M. azedarach* inhibited nitrification by 57 %. However, the potential of *M. azedarach* to retain NH_4^+ in mineral N pool and its persistence to inhibit nitrification for longer period was lower when compared with CaC_2 and karanjin. In contrast to our findings, Toselli et al. (2010) reported that *M. azedarach* did not inhibit soil NO_3^-–N, rather stimulated the release of mineral N.

Recovery of applied N in soil

Effects of NIs on changes in TMN indicated that applied N started to disappear shortly after application (Table 2). By the end of incubation, 70 % of the applied N had been depleted from the mineral N pool. In the soils amended with NIs, the extent and rate of depletion was much lower and the loss of applied N from N + CaC_2, N + karanjin and N + *M. azedarach* was 26, 45, and 64 %, respectively.

With regard to soil N recovery, CaC_2 proved highly effective in increasing N recovery of applied N followed by karanjin while *M. azedarach* was found less effective (Table 3). When N was applied without NIs, the ANR was 30 %. Addition of the NIs CaC_2, and karanjin substantially increased of N by 74, and 55 %, respectively, while the ANR by *M. azedarach* was 36 %, close to that of the N only treatment. Thus, of the total amount of N applied, 6–44 % more N was recovered when NIs were added to the soil. Keerthisinghe et al. (1993) found 84 % recovery of applied N from a CaC_2 treatment compared with 43 % for the nitrapyrin and control treatments. A 46 % greater recovery of applied N was recorded in soil–plant system when CaC_2 was applied to irrigated wheat (Freney et al. 1993). Apparent N recovery of soil amended with neem seedcake (a plant material) was 83 % compared with 63 % for soil amended with urea N without NIs (Abbasi et al. 2011). Kiran and Patra (2003) used essential oil bearing

Table 2 Changes in the total mineral N (NH_4^+–N + NO_3^-N) in soil amended with N (200 mg N kg^{-1} soil) with and without nitrification inhibitors over 56 days of laboratory incubation

Treatments	Total mineral N (NH_4^+–N + NO_3^-–N) mg kg^{-1} soil								LSD ($P \leq 0.05$)
	Days after N application								
	0	7	14	21	28	35	42	56	
T_1	15.5	13.6	7.8	10.7	10.7	5.3	4.9	2.9	1.63
T_2	123.0	88.5	83.6	76.3	58.8	41.3	51.5	32.1	10.55
T_3	113.3	103.5	124.4	141.9	160.4	195.9	211.0	208.1	20.10
T_4	126.4	113.8	109.9	122.5	140.5	145.3	114.2	74.9	21.32
T_5	126.9	76.3	91.9	78.8	87.5	84.1	46.7	36.5	9.96
LSD ($P \leq 0.05$)	14.54	23.49	13.72	11.82	14.51	13.79	13.56	14.79	

Treatments effect (average over 0–56 days)				
T_1	T_2	T_3	T_4	T_5	LSD ($P \leq 0.05$)
8.9	69.4	157.3	118.4	80.0	21.4

Timings effect (average over T_1–T_5)								
0	7	14	21	28	35	42	56	LSD ($P \leq 0.05$)
101.0	79.1	83.5	86.0	91.6	94.4	85.7	73.1	6.15

T_1, control; T_2, N without nitrification inhibitors (NIs); T_3, N+ CaC_2; T_4, N+ karanjin; T_5, N+ *M. azedarach*

Table 3 Fate and apparent recovery of N (at the rate of 200 mg N kg^{-1} soil) applied to soil with and without nitrification inhibitors over 56 days of laboratory incubation

Treatments	TMN (mg kg$-^1$ soil, mean of 56 days)	TMN recovered from applied N (mg kg$-^1$ soil)	N unaccounted for (mg kg^{-1} soil)	ANR (%)	Increase in N recovery due to NI (%)
T_1	8.9	–	–	–	–
T_2	69.4	60	140	30	–
T_3	157.3	148	52	74	145
T_4	118.4	109	91	55	81
T_5	80.0	71	129	36	17

T_1, control; T_2, N without NIs; T_3, N + CaC_2; T_4, N + karanjin; T_5, N + *M. azedarach*

TMN total mineral nitrogen, *ANR* apparent N recovery, *NI* nitrification inhibitors

plants *M. spicata*, *Artemisia* and synthetic inhibitor, DCD, and reported that the ANR by plant material was the highest (75.1 %) with *M. spicata* oil followed by *Artemisia* oil (62.8 %), DCD (46.2 %) and urea without NIs (35.5 %).

Wheat growth, plant N uptake and N-recovery

The response of growth and yield characteristics of wheat varied in response to the type of NI treatments (Table 4). Shoot length, shoot dry weight, number of leaves and root dry weight were significantly ($P \leq 0.05$) higher in soil amended with N + *M. azedarach* (T_7) compared with soil amended with N only (T_2). Karanjin and CaC_2 did not result in any significant effect on most of the growth characteristics. Use of the *M. azedarach* extract as a nitrification inhibitor has seldom been studied. However, recently Toselli et al. (2010) reported increased productivity in peach trees after the application of *M. azedarach* fruit and leaves to the soil. The yield and yield traits were significantly higher in the soil amended with CaC_2 compared with the soil amended with N only. In general, the number of grains $spike^{-1}$, 1 000-seed weight and seed yield $plant^{-1}$ following NIs application increased between 39 and 52 %, 12 and 26 %, and 12 and 18 %, respectively, compared to N only. The increase in growth and yield attributes due to NIs may be attributed to better utilization of N by the crop as a result of decreases in N

Table 4 Effect of nitrification inhibitors i.e., calcium carbide, karanjin and *M. azedarach* applied with N fertilizer on growth and yield contributing parameters of wheat (average of three repeats) grown in pots under greenhouse conditions

Treatments	Shoot length (cm)	Shoot dry weight (g)	No. of Leaves plant^{-1}	Root length (cm)	Root dry weight (g)	No. of grains spike^{-1}	1,000-grain weight (g)	Grain yield plant^{-1} (g)
T_1	23.8c	0.51c	5.2d	7.6c	0.07c	11.0d	14.2d	0.28e
T_2	53.7b	1.17b	14.3bc	12.0ab	0.23b	26.2c	22.7c	1.77d
T_3	54.7b	1.23b	13.0c	12.1ab	0.25ab	33.5ab	29.5a	2.28a
T_4	56.1ab	1.41ab	15.8ab	12.4a	0.27ab	32.7b	30.1a	1.86 cd
T_5	56.1ab	1.274b	15.8ab	10.8b	0.24ab	37.6a	25.8bc	2.11ab
T6	59.7a	1.32b	14.0bc	11.6ab	0.23b	31.5b	25.4bc	1.73d
T_7	59.1a	1.61a	17.7a	11.8ab	0.30a	32.3b	28.7ab	1.99bc
LSD ($P \leq 0.05$)	3.99	0.28	2.13	1.41	0.06	4.36	3.6	0.19

T_1, control; T_2, N without nitrification inhibitors (NIs); T_3, N + CaC_2 at the rate of 15 mg kg^{-1}; T_4, N + CaC_2 at the rate of 30 mg kg^{-1}; T_5, N + CaC_2 at the rate of 45 mg kg^{-1}; T_6, N + karanjin; T_7, N + *M. azedarach*

losses. Yaseen et al. (2006) reported that application of CaC_2 with N fertilizer significantly increased the number of tillers (up to 45.5 %), straw (up to 32.8 %), and grain yield (up to 37.3 %) of wheat over the N fertilizer alone. Similarly, a 37 % increase in green pod yield of Okra was observed after CaC_2 application equivalent to 60 kg ha^{-1} along with N fertilizer (Kashif et al. 2008). Karanjin has also been reported to increase grain and straw yield, total N uptake and grain protein content of rice (Sahrawat 1996).

Results indicated that N concentration and N uptake in plant shoot and grains were significantly increased in the treatments supplemented with NIs (Fig. 4). The relative increase in shoot and grain N concentration due to NIs was between 23 and 40 %, and 52 and 73 %, respectively over N fertilizer only. Similarly, the relative increase in shoot and grains N uptake due to NIs was between 41 and 76 %, and 66 and 87 %, respectively. Response of total N uptake to different amendments is presented in Fig. 5. The total N uptake in N fertilizer treatment was 46 mg N plant^{-1} that significantly ($P \leq 0.05$) increased to 94, 75, 74 mg N plant^{-1} (mean 81 mg N plant^{-1}) with CaC_2, 73 and 79 mg N plant^{-1} with karanjin and *M. azedarach*, respectively. Application of CaC_2 at the rate of 30 mg kg^{-1} soil exhibited the highest N uptake of 94 mg N plant^{-1}.

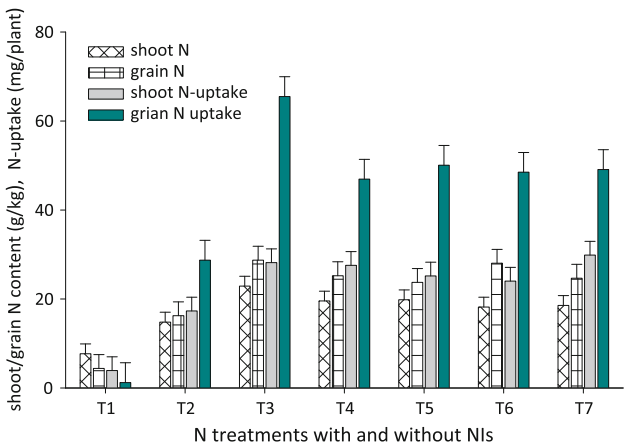

Fig. 4 Effect of nitrification inhibitors i.e., calcium carbide, karanjin and *M. azedarach* applied with N fertilizer on shoot and grain N contents and N uptake of wheat (average of three repeats) grown in pots under greenhouse conditions. The symbols on *X-axis* indicate: T_1 = control; T_2 = N without nitrification inhibitors (NIs); T_3 = N + CaC_2 at the rate of 15 mg kg^{-1}; T_4 = N + CaC_2 at the rate of 30 mg kg^{-1}; T_5 = N + CaC_2 at the rate of 45 mg kg^{-1}; T_6 = N + karanjin; T_7 = N + *M. azedarach*

The N concentration in shoots and grains of wheat was significantly increased with NIs compared to N application alone (Fig. 4). The relative increase in shoot and grain N concentration due to NIs was between 23 and 40 %, and 52 and 73 %, respectively over N fertilizer without NIs. Among the three NIs tested, CaC_2 showed the highest response. Application of lower rate of CaC_2 i.e., 15 mg kg^{-1} showed significantly higher N concentration both in shoot and grains of wheat.

Results indicated that N uptake in plant shoot and grains was significantly increased in the treatments supplemented with NIs (Fig. 4). The relative increase in shoot and grains N uptake due to NIs was between 41 and 76 %, and 66 and 87 %, respectively over the N treatment without NIs. Similarly, response of total N uptake to different amendments is presented in Fig. 5. The total N uptake in N fertilizer treatment was 46 mg N plant^{-1} that significantly ($P \leq 0.05$) increased to 94, 75, 74 mg N plant^{-1} (mean 81 mg N plant^{-1}) with CaC_2, 73 and 79 mg N plant^{-1} with karanjin and *M. azedarach*, respectively. The relative increase in N uptake due to CaC_2, karanjin, and

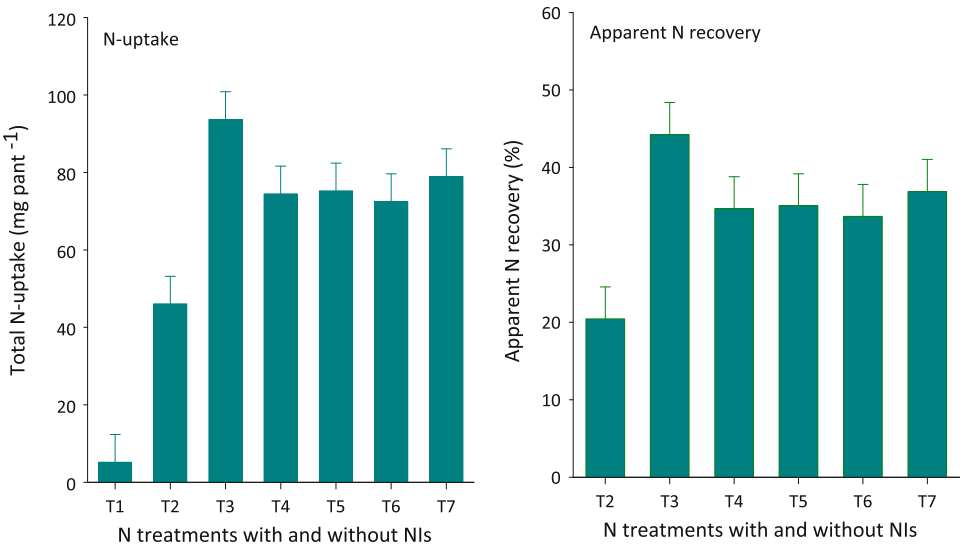

Fig. 5 Effect of nitrification inhibitors i.e., calcium carbide, karanjin and *M. azedarach* applied with N fertilizer on total N uptake and apparent N recovery by wheat grown in pots under greenhouse conditions. The symbols on *X-axis* indicate: T_1 = control; T_2 = N without nitrification inhibitors (NIs); T_3 = N + CaC_2 at the rate of 15 mg kg^{-1}; T_4 = N + CaC_2 at the rate of 30 mg kg^{-1}; T_5 = N + CaC_2 at the rate of 45 mg kg^{-1}; T_6 = N + karanjin; T_7 = N + *M. azedarach*

M. azedarach over N fertilizer was 76, 59, and 72 %, respectively. Application of CaC_2 at the rate of 30 mg kg^{-1} soil exhibited the highest N uptake of 94 mg N $plant^{-1}$.

Mahmood et al. (2007) reported a 14 and 24 % increase in total N uptake (over the N only treatment) in wheat due to the application of 30 and 45 mg kg^{-1} CaC_2, respectively. Freney et al. (1992) reported that CaC_2 when applied with N fertilizer limited NH_4^+ oxidation and prevented N loss thereby increasing accumulation of N both in shoot and grains of wheat. Application of *M. azedarach* to Peach increased shoot N concentration by 24 and 33 % over N treatment applied alone (Toselli et al. 2010).

Results indicated that the plants supplemented with N + NIs recovered more N than those which received N only (Fig. 5). The apparent N recovery (ANR) of N amended plants was 20 % which was significantly increased to 44, 35, 35 % (mean 38 %) with CaC_2, 34 and 37 % with karanjin and *M. azedarach*, respectively. The increase in the recovery of applied N due to NIs may be due to the inhibition of nitrification (less chance of N losses) thereby retaining NH_4^+–N in mineral pool that may be utilized by the plants for longer period. Our results are in agreement with the previous findings (Freney et al. 1992; Kiran and Patra 2003; Mahmood et al. 2007). Yaseen et al. (2006) reported that application of encapsulated CaC_2 resulted in greater N use efficiency (NUE) (up to 61.1 %) by both wheat and cotton crops than that observed with the same rates of N fertilizer alone.

Conclusion

The present study exhibited higher rate of NH_4^+–N disappearance from the mineral N pool over a short period of time under moisture and temperature conditions (temperature 22 °C; 58 % WFPS) which normally exist in the field during kharif (summer) season. These findings suggested that N use efficiency and N recovery is critical in soil–plant systems because of the possible losses and N disappearance from the system. The applied N in this study was nitrified rapidly with 70 % of the total mineral N converted to NO_3^-–N within 56 days of incubation. This accumulated NO_3^- is real concern for the environment, N recovery and crop productivity. Results of this study demonstrated that NIs calcium carbide, karanjin and *M. azedarach* when applied with N fertilizer DAP, significantly reduced NO_3^- accumulation and maintained NH_4^+ in the mineral pool at higher concentration and for a longer period. By delaying and decreasing the extent of NH_4^+ oxidation, NIs were able to increase N recovery in soil–plant systems possibly because of reducing N losses associated with nitrification. Calcium carbide and karanjin were found effective in reducing NO_3^- accumulation and increasing N recovery in soil while *M. azedarach* was equally effective in plant growth promoting ability. On the basis of these results, it is imperative to exploit and introduce NIs especially those which are efficient, cost-effective, and suitable for both tropical and temperate production systems. Since this study was conducted in the laboratory scale, the benefits found here need to be further investigated in field scale experiments to assess the N use efficiency and N balances of these NIs in soil–crop systems.

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