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Evaluation of some nitrification inhibitors at different temperatures under laboratory conditions

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Abstract

Effect of eight compounds on nitrification of the applied $(NH_4)_2 SO_4$ *was studied in two soils incubated at high (35°C) and moderate (16°C) temperatures. The tested compounds included: 1H-benzotriazole; 4-amino-1,2,4-triazole; benzothiazole; 3-methylpyrazole-1-carboxamide; 4-bromo-3-methylpyrazole; pyrazole; lignosulfonic acid, molecular weight 52000, 6% S; and lignosulfonic acid, molecular weight 12000, 2% S. In the absence of inhibitors, nitrification of the applied ammonium was complete within one week at 35°C, whereas it took two to three weeks at 16°C. At 35°C, ATC was the most effective compound causing 44-71% inhibition up to four weeks when applied at 10 mg* kg^{-1}*. The inhibitory effect of ATC increased with increasing application rate to 30 mg* kg^{-1} *(92–94% inhibition for four weeks). Although another compound viz. PZ applied at 10 mg* kg^{-1} *was also effective at 35°C, the inhibitory effect persisted up to three weeks (44-48% inhibition). At 16 °C, six of the test compounds (BTr, ATC, BTh, MPC, BMP and PZ) effectively inhibited nitrification at least up to four weeks. At 16 °C also, ATC was the most effective compound causing 84-90% inhibition for four weeks when applied at 10 mg* kg^{-1}*. The results suggested that ATC can be a potential nitrification inhibitor for agricultural use under summer as well as under winter soil temperatures prevailing in the Indo-Gangetic Plains of the South Asia.*

Keywords: 4-Amino-1,2,4-triazole; 1H-benzotriazole; benzothiazole; lignosulfonic acid; nitrification; pyrazoles

Introduction

Inefficient use of nitrogen fertilizer for crop production not only causes considerable monetary loss to producers and consumers of plant products, but also leads to environmental pollution due to nitrous oxide emission and nitrate leaching (Freney *et al*., 1995). ^{15}N-balance studies have indicated that a substantial proportion of the N fertilizer applied to irrigated croplands in Pakistan is lost; the loss may range from 33% (under wheat; Mahmood *et al*., 1998) to 42% (under cotton; Mahmood *et al*., 2000). Considering a general figure of 30% loss of the applied fertilizer-N, the annual fertilizer-N consumption in Pakistan (3.035 million tons; Anonymous, 2009) and the current urea price (Rs. 1750 per 50 kg), the monetary loss to the National economy is about Rs. 69.4 billion per annum. In addition, considerable monetary loss may occur due to reduced crop yields caused by N deficiency. Therefore, it is imperative to explore appropriate fertilizer N management strategies in order to reduce this loss to the National economy.

Of the two major N loss processes *viz*. ammonia volatilization and denitrification, the latter has been reported as an important N loss mechanism in Pakistani soils particularly under summer crops like maize (Mahmood *et al*., 2005) and cotton (Mahmood *et al*., 2000), and to a lesser extent under winter crops like wheat (Mahmood *et al*., 1999; 2005). Since ammoniacal fertilizers are rapidly nitrified in Pakistani soils due to warm climate, the nitrate thus formed is vulnerable to denitrification loss following flood irrigation (Mahmood *et al*., 2000; Mahmood *et al*., 2005). Maintaining the applied N in ammonium form through the use of nitrification inhibitors (NIs) is a well documented strategy to reduce denitrification loss (Asing *et al*., 2008; Patra *et al*., 2009). Most of the studies on the performance of NIs have been conducted under moderate soil temperatures (Di and Cameron, 2002; Smith *et al*., 2005; Kelliher *et al*., 2008), whereas only little information is available under warm climatic conditions such as those prevailing in the Indo-Gangetic Plains of the South Asia. Recent studies have revealed that the most widely used commercial NI *viz.* nitrapyrin is ineffective due to high summer temperatures in Pakistan (Ali *et al*., 2008). Another commercial NI *viz*. dicyandiamide (DCD) was required in relatively high concentration to effectively inhibit nitrification under high soil temperatures (Ali *et al*., 2009). However, high DCD application rates were phytotoxic and reduced the fertilizer N recovery in plants thus leading to increased fertilizer N loss (Mahmood *et al*., 2011). Therefore, it is imperative to explore compounds

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which could serve as effective NIs under agroclimatic conditions prevailing in Pakistan. The present laboratory study was conducted to evaluate eight compounds for their potential in inhibiting nitrification in soil incubated at high (summer) and moderate (winter) temperatures.

Materials and Methods

The study was carried out using two soils collected from fields which have been under cotton-wheat cropping system for more than 30 years. Soils were collected from Dineywala, Khanewal (30º23′ 17.25″N; 71.52′ 03.71″E; elevation, 437 feet; hereafter referred as KW soil) and from NIAB, Faisalabad (31º23′ 50.25″N; 73.02′ 01.31″E; elevation, 596 feet; hereafter referred as NIAB soil). Soils were air-dried, sieved (<2 mm) and stored at room temperature. Some physicochemical properties of two soils are given in Table 1. Both soils had similar texture (clay-loam) but differed in the organic matter content and thus the water-holding capacity (WHC), which are important factors governing the denitrification loss as well as the persistence/activity of nitrification inhibitors.

Table 1: Some physicochemical characteristics of soils used in experiments

Soil characteristic	Site	
	Khanewal	Faisalabad
FAO Classification	Orthic Solonetz	Haplic Yermosol
USDA Classification	Typic Halothids	Typic Ustocrepts
Series	Sindhelianwali	Hafizabad
Sand (%)	46.6	52.5
Silt (%)	31.2	22.6
Clay (%)	22.2	24.9
Texture	Clay-loam	Clay-loam
Organic matter (%)	1.63	0.85
Maximum water-holding capacity (%)	34.0	30.8
$CaCO_3$ (%)	4.39	1.75
pH (soil:water, 1:1)	8.46	7.90
EC (soil:water, 1:1) ($\mu S\ cm^{-1}$)	1036	380
Total N (%)	0.12	0.08
NH_4^+-N (mg kg^{-1})	6.7	5.7
NO_3^--N (mg kg^{-1})	31.1	27.0
Olsen P (mg kg^{-1})	11.34	14.90
Extractable K (mg kg^{-1})	318	439

Eight compounds were screened under laboratory conditions to test their potential for inhibiting nitrification in soil under high (35°C) and moderate (16°C) temperatures. The selected temperatures correspond to the maximum soil temperatures recorded (at 7 cm depth) in this region under summer crops e.g. cotton (35°C; Mahmood *et al*., 2000) and under winter crops e.g. wheat (16°C; Mahmood *et al*., 1998). The compounds used were of analytical grade and included: 1H-benzotriazole (BTr); 4-amino-1,2,4-triazole (ATC); benzothiazole (BTh); 3-methylpyrazole-1-carboxamide (MPC); 4-bromo-3-methylpyrazole (BMP); pyrazole (PZ); lignosulfonic acid, sodium salt, molecular weight, 52000; 6% S (LS-6S); and lignosulfonic acid, sodium salt, molecular weight, 12000, 2% S (LS-6S). While most of these compounds have already been reported as effective NIs at moderate (25°C) temperature (McCarty and Bremner, 1989), only one (ATC) was found effective at high (35°C) temperature (Aulakh *et al*., 2001). Thirty-gram portions (oven-dry basis) of soil in plastic bottles were moistened to 50% WHC and conditioned for two weeks at room temperature. The soil was then amended with a solution of $(NH_4)_2SO_4$ ± inhibitor to supply NH_4^+-N at 200 mg kg^{-1} and the final soil moisture content equivalent to 60% WHC. Inhibitors were applied at 0, 10 or 30 mg kg^{-1}, except lignosulfonates, which were applied at 0, 50 or 250 mg kg^{-1}. The bottles were loosely capped and incubated either at 35°C or at 16°C. The soil moisture content was maintained at 60% WHC throughout the incubation period. Experiments were carried out in 8 batches; each corresponding to one inhibitor (three application rates), two soil types, two incubation temperatures, and four sampling intervals. At desired sampling interval, bottles were removed in triplicate; all soil (30 g) was extracted with 150 mL of 2*N* KCl (1 h shaking), filtered (Whatman No. 1) and the extract analyzed for mineral-N (NH_4^+-N and NO_3^-+NO_2^--N) using standard Kjeldahl procedure (Keeney and Nelson, 1982). Percent nitrification inhibition at a given sampling interval was calculated by the difference in the % NH_4^+-N nitrified in the control and the inhibitor-treated soils. Data were subjected to an analysis of variance followed by Duncan's multiple range test (Gomez and Gomez, 1984) using COSTAT software. Results are reported as means of three replicates and are based on oven-dry soil weight.

Results

Experiments at 35ºC

In the absence of nitrification inhibitors, nitrification of the applied ammonium was complete within one week in both soils. Benzothiazole (BTh) applied at 10 mg kg^{-1} caused 42–64% inhibition for one week ($P<0.05$); the effect being more pronounced in the NIAB soil than in the KW soil (Table 2). The inhibitory effect of BTh significantly increased (66–71% inhibition) with increasing the application rate from 10 to 30 mg kg^{-1} ($P < 0.05$). However, nitrification was complete in two weeks, except in the NIAB soil where it took three weeks with BTh applied at 30 mg kg^{-1} (Table 2). Of the two triazoles tested, BTr was less effective as compared to ATC

Table 2: Effect of benzothiazole (BTh) on nitrification in Khanewal (KW) and Faisalabad (NIAB) soils incubated at different temperatures[a]

Temperature (°C)	Soil	BTh applied (mg kg^{-1})	Incubation time (weeks)							
			1		2		3		4	
			NH_4^+	$NO_3^- + NO_2^-$	NH_4^+	$NO_3^- + NO_2^-$	NH_4^+	$NO_3^- + NO_2^-$	NH_4^+	$NO_3^- + NO_2^-$
			Mineral-N (mg kg^{-1})							
35	KW	0	2 g (99)	182 b	3 e (98)	188 cd	5 c (98)	196 c	73 d (96)	181 bcd
		10	86 e (57)	159 c	8 e (96)	220 b	8 c (96)	234 b	4 d (97)	196 abc
		30	134 bc (33)	109 d	3 e (99)	247 a	9 c (96)	235 b	4 d (98)	160 cd
	NIAB	0	1 g (100)	223 a	2 e (99)	260 a	7 c (97)	261 a	7 d (97)	156 d
		10	128 c (36)	114 d	6 e (97)	202 bc	5 c (97)	212 bc	5 d (98)	119 e
		30	143 b (29)	77 e	69 d (66)	124 e	7 c (97)	210 bc	8 d (96)	221 a
16	KW	0	119 d (41)	120 d	7 e (97)	195 cd	3 c (98)	218 bc	6 d (97)	159 cd
		10	189 a (5)	61 ef	154 c (23)	83 f	161 b (19)	87 d	165 c (17)	95 ef
		30	191 a (5)	53 f	170 ab (15)	52 g	182 a (9)	58 e	178 ab (11)	77 f
	NIAB	0	71 f (65)	146 c	6 e (97)	177 d	6 c (97)	207 c	6 d (97)	214 ab
		10	196 a (2)	52 f	163 bc (19)	49 g	183 a (9)	55 e	176 b (12)	68 f
		30	198 a (1)	52 f	177 a (11)	44 g	177 a (11)	47 e	185 a (8)	61 f
		LSD $P<0.05$	9.653	16.069	10.544	21.32	8.291	23.596	7.548	34.794
		$P<0.01$	13.081	21.776	14.288	28.892	11.236	31.976	10.229	47.151

[a] Soil incubated at 60% water-holding capacity with 200 ppm N as $(NH_4)_2SO_4$; figures in parentheses indicate NH_4^+-N nitrified as % of the applied; figures within a column followed by different letter are significantly different by Duncan's multiple range test ($P<0.05$).

Table 3: Effect of 1H-benzotriazole (BTr) on nitrification in Khanewal (KW) and Faisalabad (NIAB) soils incubated at different temperatures[a]

Temperature (°C)	Soil	BTr applied (mg kg^{-1})	Incubation time (weeks)							
			1		2		3		4	
			NH_4^+	NO_3^- + NO_2^-	NH_4^+	NO_3^- + NO_2^-	NH_4^+	NO_3^- + NO_2^-	NH_4^+	NO_3^- + NO_2^-
			Mineral-N (mg kg^{-1})							
35	KW	0	4 h (98)	219 a	3 e (98)	229 a	10 cd (95)	199 b	4 d (98)	230 bc
		10	85 f (58)	163 b	5 e (97)	242 a	12 cd (94)	195 b	5 d (98)	245 b
		30	88 f (56)	155 bc	6 e (97)	223 ab	5 d (98)	266 a	4 d (98)	281 a
	NIAB	0	12 h (94)	208 a	3 e (98)	226 ab	5 cd (97)	109 c	4 d (98)	201 de
		10	119 d (41)	138 d	4 e (98)	187 c	9 cd (96)	205 b	5 d (98)	232 bc
		30	119 d (41)	105 e	25 d (87)	189 c	14 c (93)	203 b	4 d (98)	215 cd
16	KW	0	57 g (72)	145 cd	2 e (99)	208 b	4 d (98)	198 b	4 d (98)	181 e
		10	180 bc (10)	64 f	157 c (22)	78 d	154 b (23)	86 d	136 c (32)	83 f
		30	171 c (15)	59 f	177 b (22)	83 d	155 b (22)	63 de	150 b (25)	66 fg
	NIAB	0	100 e (50)	119 e	28 d (86)	190 c	10 cd (95)	216 b	6 d (97)	198 de
		10	191 a (5)	54 fg	182 b (9)	67 de	176 a (12)	65 de	180 a (10)	58 g
		30	184 ab (8)	43 g	202 a [1]	58 e	181 a (10)	45 e	176 a (12)	45 g
		LSD $P<0.05$	9.267	14.165	10.522	17.434	8.081	21.762	7.965	20.515
		$P<0.01$	12.559	19.197	14.259	23.626	10.952	29.491	10.794	27.801

[a] Soil incubated at 60% water-holding capacity with 200 ppm N as $(NH_4)_2SO_4$; figures in parentheses indicate NH_4^+-N nitrified as % of the applied; figures in brackets indicate % increase in NH_4^+-N due to net mineralization of soil N; figures within a column followed by different letter are significantly different by Duncan's multiple range test ($P<0.05$)

(Tables 3 & 4). In the KW soil, the inhibitory effect of BTr persisted only for one week (40–42% inhibition at both concentrations; Table 3). In the NIAB soil, BTr was more effective (53% inhibition after one week at both concentrations) than in the KW soil ($P<0.05$). In the NIAB soil, the inhibitory effect subsided within two weeks with BTr applied at 10 mg kg^{-1} whereas 11% inhibition was still recorded with BTr applied at 30 mg kg^{-1} (Table 3). The other triazole *viz.* ATC was most effective in delaying nitrification. When applied at 10 mg kg^{-1}, ATC caused 42–68% inhibition up to four weeks (Table 4); the effect being more pronounced in the NIAB soil than in the KW soil ($P<0.05$). Increasing the ATC application rate to 30 mg kg^{-1} almost completely retarded nitrification in both soils (Table 4). Although unsubstituted pyrazole (PZ) was less effective as compared to ATC, it was more effective than the two PZ derivatives *viz*. MPC and BMP. In both soils, PZ applied at 10 mg kg^{-1} caused 44–48% inhibition up to three weeks; it was much more effective at 30 mg kg^{-1} causing 79–84% inhibition for four weeks (Table 5). In the NIAB soil while MPC applied at 10 mg kg^{-1} effectively inhibited nitrification up to two weeks ($P<0.05$), it was not effective in the KW soil (Table 6). At higher application rate (30 mg kg^{-1}) MPC delayed nitrification for four weeks; the inhibitory effect being significantly higher in the NIAB soil than in the KW soil (58% vs. 21% inhibition; $P<0.05$). With BMP applied at 10 mg kg^{-1}, 18–23% inhibition was recorded up to two weeks (Table 7). The inhibitory effect of BMP increased with increasing application rate to 30 mg kg^{-1} (43–49% inhibition after two weeks; $P<0.05$). Whereas in the KW soil the inhibitory effect of BMP subsided after three weeks, it still showed 23% inhibition in the NIAB soil when applied at 30 mg kg^{-1} (Table 7). Both types of lignosulfonic acids were ineffective in inhibiting nitrification, which was almost complete within one week in all treatments (Table 8).

Experiments at 16ºC

In the absence of nitrification inhibitors, most of the ammonium disappeared in 2–3 weeks. Although, BTh and BTr were ineffective at 35°C, both compounds effectively inhibited nitrification up to four weeks at 16°C. When applied at 10 mg kg^{-1}, BTh caused 80–85% inhibition (Table 2), whereas BTr caused 66–87% inhibition (Table 3). The inhibitory effect of BTh and BTr was significantly higher in the NIAB soil as compared to the KW soil ($P<0.05$). As observed at 35°C, ATC was also most effective at 16°C and caused 84–90% inhibition up to four weeks when applied at 10 mg kg^{-1} (Table 4). As observed with BTh and BTr, the inhibitory effect of ATC was higher in the NIAB soil as compared to the KW soil ($P<0.05$). All the tested pyrazoles (PZ, MPC and BPM) applied at 10 mg kg^{-1} effectively inhibited nitrification at 16°C at least for four weeks. The inhibition ranged from 80–88% with PZ (Table 5), 78–89% with MPC (Table 6) and 80–83% with BMP (Table 7). The two soils did not differ with respect to the inhibitory effect of BMP or PZ, whereas MPC was more effective in the NIAB soil than in the KW soil ($P<0.05$). As observed at 35°C, the two lignosulfonic acids were ineffective also at 16°C (Table 8).

Discussion

Although NIs have shown their potential in reducing denitrification loss and N_2O emission, such studies mostly pertain to moderate climate (Smith *et al*., 2005; Di *et al*., 2007; Kelliher *et al*., 2008). Due to increased volatilization and/or microbial decomposition, the activity of commercial NIs *viz.* nitrapyrin, dicyandiamide (DCD) and 3,4-dimethylepyrazole phosphate (DMPP) is greatly reduced with increasing soil temperatures (Keeney, 1983; Rajbanshi *et al*., 1992; Puttanna *et al*., 1999; Irigoyen *et al*., 2003; Di and Cameron, 2004; Kelliher *et al*., 2008; Ali *et al*., 2008). In a subtropical soil incubated at 16°C for 30 days, while DCD applied at 5 mg kg^{-1} inhibited nitrification by 89%, the inhibition at 33°C was only 38% with DCD applied at 15 mg kg^{-1} (Mahmood *et al*., 2011). In the present study too, while activity of some inhibitors (PZ and ATC) was significantly reduced at higher temperature, other compounds were almost ineffective. Besides, the potential of almost all NIs was higher in the NIAB soil than in the KW soil, which is attributable to relatively higher organic matter content of the KW soil. Increasing soil organic matter content is known to reduce the potential of various NIs including those employed in the present study (McCarty and Bremner, 1989; Callender and Davis, 2003).

In the present study, only two of the tested inhibitors *viz.* ATC and PZ were effective at high as well as at moderate temperature, whereas the other four inhibitors *viz.* BTh, BTr, BMP and MPC were effective only at moderate temperature. Results of our experiments carried out at moderate temperature are in agreement with those of earlier studies carried out at a temperature range of 22–25°C (McCarty and Bremner, 1989; Callender and Davis, 2003). Of the 45 heterocyclic N compounds tested at 25°C, McCarty and Bremner (1989) concluded that in addition to substituted pyrazoles and thiadiazoles, unsubstituted heterocyclic N compounds containing two adjacent N atoms (e.g. PZ and ATC) are potential nitrification inhibitors. Besides conventional NIs, the paper mill waste 'lignin sulfonate' has also been reported to inhibit nitrification (Azam and Malik, 1985). However, both types of lignosulfonates tested in the present study were found ineffective at both temperatures.

Although, ATC is also known to inhibit nitrification at moderately high temperature (30°C; Bundy and Bremner, 1974), its inhibitory effect has rarely been reported at

Table 4: Effect of 4-amino-1,2,4-triazole (ATC) on nitrification in Khanewal (KW) and Faisalabad (NIAB) soils incubated at different temperatures[a]

Temperature (ºC)	Soil	ATC applied (mg kg^{-1})	Incubation time (weeks)							
			1		2		3		4	
			NH_4^+	$NO_3^- + NO_2^-$	NH_4^+	$NO_3^- + NO_2^-$	NH_4^+	$NO_3^- + NO_2^-$	NH_4^+	$NO_3^- + NO_2^-$
			Mineral-N (mg kg^{-1})							
35	KW	0	3 e (98)	223 a	8 e (96)	253 a	7 f (97)	212 c	3 f (98)	230 a
		10	178 bc (11)	63 c	162 d (19)	85 c	134 e (33)	126 d	88 e (56)	184 c
		30	187 abc (7)	55 c	194 b (3)	60 d	194 c (3)	67 f	188 b (6)	72 e
	NIAB	0	5 e (97)	203 a	8 e (96)	212 b	7 f (97)	253 a	6 f (97)	219 ab
		10	160 c (20)	54 c	177 cd (12)	62 d	171 d (15)	85 e	141 d (29)	109 d
		30	192 abc (4)	50 c	195 bc (3)	33 e	194 c (3)	45 g	206 a [3]	60 f
16	KW	0	108 d (46)	136 b	15 e (93)	225 b	9 f (96)	236 b	3 f (98)	212 b
		10	179 bc (10)	49 c	216 a [8]	46 de	198 bc (1)	45 g	172 c (14)	49 fg
		30	231 a [15]	55 c	202 ab [1]	16 f	205 bc [3]	51 g	191 b (5)	44 g
	NIAB	0	116 d (42)	126 b	16 e (92)	213 b	6 f (97)	224 bc	6 f (97)	217 ab
		10	216 ab [8]	61 c	213 ab [7]	37 e	221 a [11]	49 g	214 a [7]	39 g
		30	223 ab [11]	41 c	210 ab [5]	36 e	208 b [4]	43 g	210 a [5]	38 g
		LSD $P<0.05$	41.168	32.977	18.982	15.087	12.135	15.705	9.713	12.632
		$P<0.01$	58.72	44.69	26.997	21.326	16.444	21.283	13.163	17.118

[a] Soil incubated at 60% water-holding capacity with 200 ppm N as $(NH_4)_2SO_4$; figures in parentheses indicate NH_4^+-N nitrified as % of the applied; figures in brackets indicate % increase in NH_4^+-N due to net mineralization of soil N; figures within a column followed by different letter are significantly different by Duncan's multiple range test ($P<0.05$)

Table 5: Effect of pyrazole (PZ) on nitrification in Khanewal (KW) and Faisalabad (NIAB) soils incubated at different temperatures[a]

Temperature (°C)	Soil	PZ applied (mg kg^{-1})	Incubation time (weeks) 1 NH_4^+	1 $NO_3^- + NO_2^-$	2 NH_4^+	2 $NO_3^- + NO_2^-$	3 NH_4^+	3 $NO_3^- + NO_2^-$	4 NH_4^+	4 $NO_3^- + NO_2^-$
			Mineral-N (mg kg^{-1})							
35	KW	0	9 d (95)	206 b	7 d (97)	254 a	8 c (96)	246 a	16 d (92)	299 a
		10	197 a (2)	46 ef	180 a (10)	77 c	104 b (48)	38 ef	14 d (93)	290 a
		30	198 a (1)	47 ef	187 a (6)	59 cde	192 a (4)	32 f	185 b (8)	82 d
	NIAB	0	5 d (98)	231 a	16 d (92)	203 b	8 c (96)	232 a	8 d (96)	260 b
		10	185 ab (8)	58 e	108 c (46)	69 cd	96 b (52)	103 b	11 d (94)	288 a
		30	219 a [10]	42 fg	143 b (29)	59 cde	157 a (21)	41 ef	166 c (17)	67 de
16	KW	0	126 c (37)	114 c	26 d (87)	222 b	7 c (97)	244 a	21 d (89)	281 ab
		10	197 a (2)	35 fg	192 a (4)	51 def	188 a (6)	57 cd	203 a [1]	117 c
		30	195 a (2)	35 fg	198 a (1)	56 de	194 a (3)	59 c	215 a [7]	80 de
	NIAB	0	152 b (26)	93 d	12 d (94)	206 b	5 c (97)	239 a	22 d (89)	266 b
		10	211 a [6]	43 fg	189 a (5)	39 ef	181 a (10)	52 cde	219 a [9]	69 de
		30	205 a [3]	31 g	198 a (1)	34 f	187 a (7)	44 def	220 a [10]	59 e
		LSD $P<0.05$	35.512	11.187	24.525	18.74	33.06	13.073	15.945	20.333
		$P<0.01$	48.125	15.161	33.235	25.397	44.802	17.716	21.609	27.555

[a] Soil incubated at 60% water-holding capacity with 200 ppm N as $(NH_4)_2SO_4$; figures in parentheses indicate NH_4^+-N nitrified as % of the applied; figures in brackets indicate % increase in NH_4^+-N due to net mineralization of soil N; figures within a column followed by different letter are significantly different by Duncan's multiple

Table 6: Effect of 3-methylpyrazole-1-carboxamide (MPC) on nitrification in Khanewal (KW) and Faisalabad (NIAB) soils incubated at different temperatures[a]

Temperature (°C)	Soil	MPC applied (mg kg^{-1})	Incubation time (weeks)							
			1		2		3		4	
			NH_4^+	$NO_3^- + NO_2^-$	NH_4^+	$NO_3^- + NO_2^-$	NH_4^+	$NO_3^- + NO_2^-$	NH_4^+	$NO_3^- + NO_2^-$
			Mineral-N (mg kg^{-1})							
35	KW	0	1 g (100)	194 b	5 g (98)	205 bc	2 e (99)	235 ab	7 f (97)	230 a
		10	110 f (45)	127 c	7 g (97)	202 bc	5 e (97)	248 a	9 f (96)	174 c
		30	175 cd (12)	69 ef	127 d (37)	107 d	90 d (55)	163 d	49 e (76)	169 cd
	NIAB	0	3 g (99)	223 a	4 g (98)	238 a	2 e (99)	222 b	5 f (97)	155 d
		10	132 e (34)	97 d	58 f (71)	187 c	4 e (98)	222 b	7 f (96)	155 d
		30	184 bc (8)	49 fgh	153 c (23)	71 e	142 c (29)	88 e	123 d (39)	100 f
16	KW	0	133 e (33)	88 de	12 g (94)	219 b	5 e (98)	249 a	3 f (99)	124 e
		10	177 cd (12)	60 fgh	176 b (12)	67 e	162 b (19)	62 f	158 c (21)	73 g
		30	197 ab (2)	42 gh	193 a (4)	40 f	196 a (2)	40 g	194 a (3)	53 h
	NIAB	0	164 d (18)	63 fg	117 e (42)	93 d	6 e (97)	187 c	8 f (96)	196 b
		10	189 abc (5)	45 gh	170 b (15)	36 f	164 b (18)	52 fg	185 b (7)	57 gh
		30	202 a [1]	39 h	189 a (6)	31 f	202 a [1]	40 g	199 a (0)	41 h
		LSD $P<0.05$	16.479	19.27	9.746	16.932	7.535	13.959	7.906	17.107
		$P<0.01$	22.332	26.114	13.208	22.946	10.212	18.917	10.714	23.183

[a] Soil incubated at 60% water-holding capacity with 200 ppm N as $(NH_4)_2SO_4$; figures in parentheses indicate NH_4^+-N nitrified as % of the applied; figures in brackets indicate % increase in NH_4^+-N due to net mineralization of soil N; figures within a column followed by different letter are significantly different by Duncan's multiple range test ($P<0.05$)

Table 7: **Effect of 4-bromo-3-methylpyrazole (BMP) on nitrification in Khanewal (KW) and Faisalabad (NIAB) soils incubated at different temperatures**[a]

Temperature (°C)	Soil	BMP applied (mg kg^{-1})	Incubation time (weeks) 1		2		3		4	
			NH_4^+	$NO_3^- + NO_2^-$	NH_4^+	$NO_3^- + NO_2^-$	NH_4^+	$NO_3^- + NO_2^-$	NH_4^+	$NO_3^- + NO_2^-$
			Mineral-N (mg kg^{-1})							
35	KW	0	9 f (95)	272 a	7 h (97)	258 a	8 e (96)	246 a	13 de (93)	239 bc
		10	132 e (34)	150 c	51 f (74)	222 c	9 e (95)	238 ab	19 cd (90)	250 b
		30	159 c (21)	116 e	92 e (54)	193 de	5 e (97)	251 a	23 c (89)	292 a
	NIAB	0	11 f (94)	243 b	10 h (95)	203 d	8 e (96)	239 ab	12 de (94)	251 b
		10	137 e (31)	105 f	47 f (77)	178 e	22 d (89)	112 c	13 de (94)	242 bc
		30	172 b (14)	93 g	108 d (46)	109 g	55 c (73)	73 de	12 de (94)	237 bc
16	KW	0	140 de (30)	135 d	26 g (87)	241 b	8 e (96)	244 a	4 e (98)	222 c
		10	223 a [12]	91 g	181 c (10)	100 gh	171 b (15)	81 d	163 b (18)	100 d
		30	229 a [15]	75 h	189 b (6)	86 hi	187 a (7)	82 d	189 a (6)	87 de
	NIAB	0	152 cd (24)	93 g	100 de (50)	156 f	8 e (96)	220 b	6 e (97)	227 bc
		10	218 a [9]	59 i	206 a [3]	74 i	174 b (13)	70 de	172 b (14)	80 de
		30	226 a [13]	36 j	194 ab (3)	53 j	171 b (15)	56 e	188 a (6)	67 e
		LSD $P<0.05$	12.198	9.055	12.624	15.206	10.741	20.836	8.325	24.184
		$P<0.01$	16.531	12.271	17.108	20.607	14.555	28.237	11.282	32.774

[a] Soil incubated at 60% water-holding capacity with 200 ppm N as $(NH_4)_2SO_4$; figures in parentheses indicate NH_4^+-N nitrified as % of the applied; figures in brackets indicate % increase in NH_4^+-N due to net mineralization of soil N; figures within a column followed by different letter are significantly different by Duncan's multiple range test ($P<0.05$)

Table 8: Effect of two lignosulfonic acids (LS-6S and LS-2S) on nitrification in Khanewal (KW) and Faisalabad (NIAB) soils incubated at different temperatures[a]

Temperature (°C)	Soil	LS applied (mg kg^{-1})	LS-6S				LS-2S			
			Incubation time (weeks)				Incubation time (weeks)			
			1		2		1		2	
			NH_4^+	NO_3^- + NO_2^-	NH_4^+	NO_3^- + NO_2^-	NH_4^+	NO_3^- + NO_2^-	NH_4^+	NO_3^- + NO_2^-
			Mineral-N (mg kg^{-1})							
35	KW	0	15 de (93)	114 d	5 cd (98)	26 f	8 e (96)	170 cd	6 bc (97)	66 ab
		50	15 de (93)	86 d	10 ab (95)	66 cde	2 e (99)	156 d	6 bc (97)	50 b
		250	4 f (98)	84 d	12 a (94)	92 bc	9 e (95)	112 e	10 b (95)	49 b
	NIAB	0	6 ef (97)	194 ab	12 a (94)	47 def	3 e (98)	194 b	5 bc (97)	61 b
		50	4 f (98)	202 ab	3 d (98)	128 a	4 e (98)	227 a	6 bc (97)	77 ab
		250	8 ef (96)	199 ab	7 bcd (97)	110 ab	3 e (98)	235 a	8 bc (96)	77 ab
16	KW	0	20 d (90)	185 ab	4 cd (98)	45 def	52 c (74)	185 bc	2 c (99)	51 b
		50	22 d (89)	180 ab	6 cd (97)	45 def	52 c (74)	90 f	10 b (95)	70 ab
		250	59 b (71)	208 a	5 cd (98)	43 ef	60 b (70)	58 g	7 bc (96)	79 ab
	NIAB	0	41 c (80)	123 cd	13 a (93)	58 de	35 d (83)	29 e	7 b (97)	97 a
		50	72 a (64)	112 d	8 bc (96)	75 cd	48 c (76)	85 f	11 b (95)	76 ab
		250	74 a (63)	159 bc	7 bcd (97)	50 def	94 a (53)	54 g	27 a (86)	77 ab
		LSD $P<0.05$	9.273	40.961	3.65	27.068	7.272	18.802	5.325	30.209
		$P<0.01$	12.566	55.51	4.946	36.681	9.855	25.48	7.216	40.939

[a] Soil incubated at 60% water-holding capacity with 200 ppm N as $(NH_4)_2SO_4$; figures in parentheses indicate NH_4^+-N nitrified as % of the applied; figures within a column followed by different letter are significantly different by Duncan's multiple range test ($P<0.05$)

higher temperatures such as those prevailing during the summer season in the Indo-Gangetic Plains. In a subtropical soil incubated at 35°C under aerobic conditions, ATC applied at 10 mg kg^{-1} caused 84% inhibition in nitrification up to twenty days (Aulakh *et al*., 2001), whereas with the same ATC concentration applied in the present study, we observed 84–90% inhibition at least for four weeks.

Conclusions

Results of the present study indicated that ATC can be a potential nitrification inhibitor at high as well as at moderate soil temperatures, at least under upland conditions. However, the effective ATC concentration in the present study (i.e. 10 mg kg^{-1}) is equivalent to about 22 kg ha^{-1}, which is prohibitively expensive for field application. Alternatively, instead of applying in bulk soil, incorporating ATC in the fertilizer granules could reduce its application rate as reported for other NIs e.g. DMPP (Zerulla *et al*., 2001). Our preliminary work on this aspect has indeed indicated that incorporating ATC in urea granules could substantially reduce the inhibitor application rate without compromising its potential for inhibiting nitrification (unpublished data).

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References

Ali, R., J. Iqbal, G.R. Tahir and T. Mahmood. 2008. Effect of 3,5-dimethylpyrazole and nitrapyrin on nitrification under high soil temperature. *Pakistan Journal of Botany* 40: 1053–1062.

Ali, R., J. Iqbal, U. Robab and T. Mahmood. 2009. Effect of dicyandiamide on soil microbial activity under cotton. *Pakistan Journal of Botany* 41: 1401–1409.

Anonymous. 2009. *Agricultural Statistics of Pakistan 2008-2009*. Government of Pakistan, Ministry of Food and Agriculture (Economic Wing), Islamabad.

Asing, J., S. Saggar, J. Singh and N.S. Bolan. 2008. Assessment of nitrogen losses from urea and an organic manure with and without nitrification inhibitor, dicyandiamide, applied to lettuce under glasshouse conditions. *Australian Journal of Soil Research* 46: 535–541.

Azam, F. and K.A. Malik. 1985. Effect of lignin sulphonates on nitrogen transformations. p. 261-267. *In: Nitrogen and the Environment*. K.A. Malik, SHM Naqvi and MIH Aleem (eds.). Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan.

Aulakh, M.S., Kuldip-Singh and J. Doran. 2001. Effects of 4-amino 1,2,4-triazole, dicyandiamide and encapsulated calcium carbide on nitrification inhibition in a subtropical soil under upland and flooded conditions. *Biology and Fertility of Soils* 33: 258–263.

Bundy, L.G. and J.M. Bremner. 1974. Effects of nitrification inhibitors on transformations of urea nitrogen in soils. *Soil Biology and Biochemistry* 6: 369-376.

Callender, T. and L.C. Davis. 2003. Nitrification inhibition using benzotriazoles. *Journal of Hazardous Substances Research* 4: 1-16.

Di, H.J. and K.C. Cameron. 2002. The use of nitrification inhibitor, dicyandiamide (DCD), to reduce nitrate leaching and nitrous oxide emission in a simulated grazed and irrigated grassland. *Soil Use and Management* 18: 395-403.

Di, H.J. and K.C. Cameron. 2004. Effect of temperature and application rate of a nitrification inhibitor, dicyandiamide (DCD), on nitrification rate and microbial biomass in a grazed pasture soil. *Australian Journal of Soil Research* 42: 927-932.

Di, H.J., K.C. Cameron and R.R. Sherlock. 2007. Comparison of the effectiveness of a nitrification inhibitor, dicyandiamide, in reducing nitrous oxide emissions in four different soils under different climatic and management conditions. *Soil Use and Management* 23: 1-9

Freney, J.R., M.B. Peoples and A.R. Mosier. 1995. Efficient use of fertilizer nitrogen by crops. Food & Fertilizer Technology Center, Extension Bulletin 414, 13 pp.

Gomez, K.A. and A.A. Gomez. 1984. *Statistical Procedures for Agricultural Research*. Wiley, Singapore.

Irigoyen, I., J. Muro, M. Azpilikueta, P. Aparicio-Tejo, and C. Lamsfus. 2003. Ammonium oxidation kinetics in the presence of nitrification inhibitors DCD and DMPP at various temperatures. *Australian Journal of Soil Research* 41: 1177–1183.

Keeney, D.R. and D.W. Nelson. 1982. Nitrogen – inorganic forms. p. 643-698. *In:* Methods of Soil Analysis. A.L. Page, R.H. Miller and D.R. Keeney (eds.). Agronomy monograph no. 9, vol 2, 2nd Ed. American Society of Agronomy, Madison, WI,

Keeney, D.R. 1983. Factor affecting the persistence and bioactivity of nitrification inhibitors. p. 33-46. *In:* Nitrification Inhibitors – Potentials and Limitations.

J.J. Meisinger, G.W. Randall and M.L. Vitosh (eds.). ASA Special Publication No. 38, American Society of Agronomy, Madison, WI.

Kelliher, F.M., T.J. Clough, H. Clark, G. Rys and J.R. Sedcole. 2008. The temperature dependence of dicyandiamide (DCD) degradation in soils: A data analysis. *Soil Biology and Biochemistry* 40: 1878–1882.

MacCarty, G.W. and J.M. Bremner. 1989. Inhibition of nitrification in soil by heterocyclic nitrogen compounds. *Biology and Fertility of Soils* 8: 204–211.

Mahmood, T., K.A. Malik, S.R.A. Shamsi and M.I. Sajjad. 1998. Denitrification and total N losses from an irrigated sandy-clay loam under maize-wheat cropping system. *Plant and Soil* 199: 239–250.

Mahmood, T., R. Ali, F. Azam and K.A. Malik. 1999. Comparison of two versions of the acetylene inhibition/soil core method for measuring denitrification loss from an irrigated wheat field. *Biology and Fertility of Soils* 29: 328–331.

Mahmood, T., R. Ali, M.I. Sajjad, M.B. Chaudhri, G.R. Tahir and F. Azam. 2000. Denitrification and total fertilizer N losses from an irrigated cotton field. *Biology and Fertility of Soils* 31:270–278.

Mahmood, T., R. Ali, K.A. Malik, Z. Aslam and S. Ali. 2005. Seasonal pattern of denitrification under an irrigated wheat-maize cropping system fertilized with urea and farmyard manure in different combinations. *Biology and Fertility of Soils* 42: 1-9.

Mahmood, T., R. Ali, Z. Latif and W. Ishaque. 2011. Dicyandiamide increases the fertilizer-N loss from an alkaline calcareous soil treated with ^{15}N-labelled urea under warm climate and under different crops. *Biology and Fertility of Soils* 47:619–631.

Patra, D.D., U. Kiran, S. Chand and M. Anwar. 2009. Use of urea coated with natural products to inhibit urea hydrolysis and nitrification in soil. *Biology and Fertility of Soils* 45: 617–621.

Puttanna, K., N.M. Nanje Gowda and E.V.S. Prakasa Rao. 1999. Effect of concentration, temperature, moisture, liming and organic matter on the efficacy of the nitrification inhibitors benzotriazole, o-nitrophenol, m-nitroaniline and dicyandiamide. *Nutrient Cycling in Agroecosystems* 54: 251–257.

Rajbanshi, S.S., G. Benckiser and J.C.G. Ottow. 1992. Mineralization kinetics and utilization as an N source of dicyandiamide (DCD) in soil. *Naturwissenschaften* 79: 26–27.

Smith, L.C., R.M. Monaghan, S.F. Ledgard and W.D. Catto. 2005. The effectiveness of different nitrification inhibitor formulations in limiting nitrate accumulation in a Southland pastoral soil. *New Zealand Journal of Agricultural Research* 48: 517–529.

Zerulla, W., T. Barth, J. Dressel, K. Erhardt, K. Horchler-von-Loquenghien, G. Pasda, M. Radle and A.H. Wissemeier. 2001. 3,4 Dimethylpyrazole phosphate – a new nitrification inhibitor for agriculture and horticulture. *Biology and Fertility of Soils* 34:79–84.