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EFFECT OF 3,5-DIMETHYLPYRAZOLE AND NITRAPYRIN ON NITRIFICATION UNDER HIGH SOIL TEMPERATURE

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Abstract

Experiments were conducted under laboratory conditions to elucidate the effect of 2 nitrification inhibitors viz., 3,5-dimethylpyrazole (DMP) and nitrapyrin on nitrification of $(NH_4)_2SO_4$ in soil incubated at 35°C. When these inhibitors were applied at rates generally recommended for agricultural use (DMP at 0.225–0.45 mg kg^{-1}; nitrapyrin at 0.25–0.50 mg kg^{-1}), almost all NH_4^+-N disappeared within a week in the soil treated with DMP, whereas it took 2 weeks in the presence of nitrapyrin. When DMP application rate was increased in the range of 0.225–3.6 mg kg^{-1}, NH_4^+-N disappeared within 2 weeks in the soil receiving DMP upto 0.90 mg kg^{-1}, whereas it took 3 weeks with DMP applied at 1.8–3.6 mg kg^{-1}. In another experiment, nitrapyrin application rate was increased in the range of 1.04–52 mg kg^{-1} using a commercial product viz., N-Serve 24 (22.2% active ingredient). In contrast to the unamended control where all NH_4^+-N disappeared within a week, nitrification was delayed particularly with higher concentrations of nitrapyrin and only 50 and 8 % of the NH_4^+-N was nitrified in 4 weeks with nitrapyrin applied at 8.32 and 26 mg kg^{-1}, respectively. Results suggested that DMP and nitrapyrin applied at rates generally recommended for agricultural use under moderate climate may not be effective under high summer temperatures prevailing in Pakistan. Application of high concentrations of these chemicals to achieve the desired effects under warm agro-climate may not be economically feasible. Besides, higher concentrations of these nitrification inhibitors can be phytotoxic or adversely affect the activities of soil microflora.

Introduction

The nitrification process plays a pivotal role in nitrogen cycling in the ecosystem since through this process ammonium (NH_4^+) released from the native soil N pool and from NH_4^+-based fertilizers is transformed into NO_3^-. Besides being available for the plant uptake, the NO_3^- thus produced is susceptible to losses *via* leaching and denitrification. Nitrogen (N) loss through denitrification is particularly high in irrigated crops like cotton, maize and rice due to warm summer temperatures (Freney, 1997; Mahmood *et al*., 2000, 2005)**.** Loss of N from soil-plant systems not only represents a monetary loss to producers and consumers of plant products, but may also have environmental implications like atmospheric pollution due to N oxides and deterioration of the groundwater quality as a result of NO_3^- leaching.

Ammonium-based fertilizers are the most widely used source of N for crop production and keeping the applied fertilizer N in NH_4^+ form by using nitrification inhibitors (NIs) is a well documented strategy for reducing N loss and to minimize negative environmental impacts of the fertilizer-use (Zerulla *et al*., 2000; Weiske *et al*., 2001). Three compounds have been commercialized as NIs for agricultural use including (i) nitrapyrin

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(2-chloro-6-trichloromethyl-pyridin, trade name N-Serve), (ii) dicyandiamide or DCD (trade name Didin, Alzon or and Ensan), and (iii) more recently DMPP (a pyrazole derivative, 3,4-dimethylpyrazole phosphate; trade name ENTEC) (Zerulla *et al*., 2000; Weiske *et al*., 2001). However, performance of NIs is highly variable in different agro-ecosystems as their persistence and activity is strongly influenced by various environmental factors. Adsorption of NIs on soil colloids can particularly reduce their effectiveness in inhibiting nitrification in soil, and this is well documented for the most widely used inhibitor *viz*., nitrapyrin (Keeney, 1983). In addition, increasing temperatures significantly reduce the persistence of these inhibitors in soil and this is well established for DCD and DMPP (Irigoyen *et al*., 2003; Di & Cameron, 2004).

Most of the studies regarding the performance of NIs have been reported from temperate regions characterized by mild agro-climate, whereas little information exists under semiarid subtropics where performance of NIs may be questionable due to high soil temperatures under summer crops (Bhupinderpal-Singh *et al*., 1993; Aulakh *et al*., 2001). Objective of the present study was to evaluate under laboratory conditions the effect of two NIs viz., 3,5-dimethylpyrazole (DMP, a pyrazole derivative like DMPP) and nitrapyrin on nitrification in soil incubated at 35°C.

Materials and Methods

The soil used in this study was a sandy-clay loam (Hafizabad Series; Haplic Yermosol; Anon., 1966) collected from the plough layer of a cotton field at the NIAB campus. It contained: total organic C, 1.2%; total nitrogen, 0.06%; pH (saturation paste), 7.9; EC (saturation extract), 0.06 S m^{-1} maximum water-holding capacity (WHC), 35.5%; bulk density, 1.5 g cm^{-3}; porosity, 44.4%; sand, 57.8%; silt, 22.7% and clay, 19.5%. The soil was air-died, sieved (<2 mm) and stored at room temperature.

Nitrification inhibitors used in this study included 3,5-dimethylpyrazole (DMP; as pure active ingredient, Acros Organics) and nitrapyrin either as the pure active ingredient i.e., 2-chloro-6-(trichloromethyl) pyridine, or as a commercial preparation viz., N-Serve 24 (Dow Agro-Sciences) that contains 22.2% active ingredient. In experiments with DMP, stock solution of the inhibitor was prepared in distilled water, whereas for experiment with nitrapyrin as pure active ingredient, the stock was prepared in acetone; in all nitrapyrin treatments as well as the control, the final concentration of acetone in soil was 1 µl g^{-1}. In experiment utilizing N-Serve-24, the stock was prepared by mixing the inhibitor in soil at 78 µl g^{-1} and portions of this soil were used to achieve the desired inhibitor levels in soil.

Incubations were carried out in plastic vials (capacity 200 ml) using 100 g soil. The soil was moistened to 60% WHC with a solution containing $(NH_4)_2SO_4$ to provide NH_4^+-N at 200 mg kg^{-1}. The bottles were covered with Parafilm that was perforated for gaseous exchange. The treated soil was kept at 4°C (18 h) to allow uniform distribution of moisture and then incubated at 35±1°C. Beakers filled with water were placed in the incubator to avoid the soil from desiccation. The incubation temperature of 35°C was selected because this is close to the maximum soil temperatures recorded under summer crops in Pakistan (Mahmood *et al*., 1998, 2000). Sufficient bottles were kept for each treatment to sacrifice triplicate bottles at each sampling interval for the analysis of soil mineral N.

Soil mineral N (NH_4^+, NO_3^-, and NO_2^-) was determined by a micro-Kjeldahl method (Keeney & Nelson, 1982). Twenty g of moist soil was shaken for 1 h with 100 ml of 2N KCl and filtered (Whatman No. 1). For NH_4^+-N, 50 ml of the extract was steam-distilled (5 min.) after adding 0.2 g MgO. The distillate collected in boric acid-mixed indicator solution was titrated with 0.002–0.005N H_2SO_4 to give NH_4^+-N. After removal of NH_4^+ from the extract, 0.2 g Devarda's alloy was mixed and the sample distilled again. The distillate was titrated with standard H_2SO_4 to give NO_3^-+NO_2^- content. Nitrite-N of the soil extract was also measured separately using modified Griess-Ilosway method (Keeney & Nelson, 1982). To 5 ml of the soil extract, 1 ml of 0.5% sulfanilamide solution was added. After 5 min., 1 ml of N-(1-Naphthyl) ethylenediamine dihydrochloride solution was added and left for 20 min. Optical density was measured at 540 nm. For the determination of dry weight, soils were dried at 105°C for 24 h.

Data were subjected to an analysis of variance followed by Duncan's multiple range test (Gomez & Gomez, 1984). Results are reported as means of three replicates and are expressed on the soil dry weight basis.

Results and Discussion

In all experiments, transient accumulation of NO_2^--N was negligible (<0.2 mg kg^{-1}); therefore, only data of NH_4^+- and NO_3^--N have been considered. In experiments with 3,5-Dimethylpyrazole (DMP), nitrification of the applied NH_4^+-N was complete within a week in the control as well as in the DMP-treated soil but without showing proportionate increase in NO_3^--N (Table 1). After 3 weeks, a deficit of 62–74% in the total mineral N was recorded in the DMP-treated soil as compared to the control that showed a 36% deficit (Table 1). However, after 4 weeks, this deficit decreased to 12–15% without showing treatment effects. In another experiment, the concentration of DMP was increased up to 3.6 mg kg^{-1}. After 1 week, all NH_4^+-N disappeared in treatments receiving DMP in the range of 0–0.45 mg kg^{-1}, whereas nitrification was 18–73% lower with DMP applied in the range of 0.675–3.6 mg kg^{-1} (Table 2). However, by the end of 2 weeks, a 45% inhibition still persisted in soil treated with DMP at 3.6 mg kg^{-1} though it was offset during the next week. After 4 weeks, the total mineral-N balance showed a 51–74% deficit in treatments receiving 0–0.675 mg DMP kg^{-1} (Table 2). At higher DMP application rates (i.e. 0.90–3.6 mg kg^{-1}), the deficit in mineral-N balance was much less (9–29%) and by the end of 4-week incubation, the mineral-N content in these treatments was not significantly different than that present at the start of incubation (Table 2).

In experiment using pure active ingredient of nitrapyrin, 76 and 21% of NH_4^+-N disappeared in 1 week in treatments receiving nitrapyrin at 0.25 and 0.50 mg kg^{-1}, respectively, whereas almost all (97%) NH_4^+-N disappeared in the control (p<0.05; Table 3). However, the inhibitory effect of nitrapyrin was almost subsided by the end of 2 weeks. During the 4th week, re-mineralization caused an accumulation of NH_4^+-N in the range of 14–27 mg kg^{-1}, the amount being significantly higher in treatment receiving nitrapyrin at 0.50 mg kg^{-1} (p<0.05). Although most of the NH_4^+-N disappeared within 2 weeks, NO_3^--N did not show a proportionate increase. Instead, a significant decrease was recorded in the nitrapyrin-treated soil during the 3rd week; by the end of 4-week incubation, a 31–53% deficit in the mineral-N was recorded without any treatment effect (Table 3). In another experiment carried out with a commercial nitrapyrin product *viz*., N-Serve 24, the inhibitor application rate was further increased in the range of 1.04–52 mg kg^{-1} active ingredient (Table 4). The magnitude and duration of the inhibitory effect increased with the nitrapyrin application rate. Nitrapyrin applied in the range of 4.16–10.4 mg kg^{-1} caused a 10–25%

Table 1. Changes in mineral nitrogen of soil amended with 200 mg N kg^{-1} as $(NH_4)_2SO_4$ and 0–0.45 mg kg^{-1} of 3,5-dimethylpyrazole (DMP).

Time (weeks)	DMP applied (mg kg^{-1})			Time mean
	0	0.225	0.45	
	---------- NH_4^+-N (mg kg^{-1}) ----------			
0	202 a[a]	202 a	202 a	202 A[b]
1	8 (97)[c] b	4 (99) b	5 (97) b	6 (98) BC
2	0 (100) b	9 (98) b	4 (98) b	4 (99) BC
3	0 (100) b	0 (100) b	0 (100) b	0 (100) C
4	10 (96) b	11 (98) b	11 (96) b	11 (96) B
Treatment mean[b]	44 NS	45 NS	45 NS	
	---------- NO_3^--N (mg kg^{-1}) ----------			
0	11 g[a]	11 g	11 g	11 D[b]
1	144 abcd	134 cd	139 abcd	139 B
2	114 de	144 abcd	151 abcd	136 B
3	137 bcd	81 ef	55 f	91 C
4	178 a	176 ab	170 abc	174 A
Treatment mean[b]	117 NS	109 NS	105 NS	
	---------- NH_4^++NO_3^--N (mg kg^{-1}) ----------			
0	213 a[a]	213 a	213 a	213 A[b]
1	152 bcd	137 cd	144 bcd	145 C
2	114 de	153 bcd	155 bcd	141 C
3	137 cd	81 ef	55 f	91 D
4	188 ab	187 ab	180 abc	185 B
Treatment mean[b]	161 NS	154 NS	150 NS	

[a]All values are mean of 3 replicates; For each nitrogen fraction, figures followed by different letter (*lower case*) are significantly different at $p<0.05$ (Duncan's multiple range test).

[b]For each nitrogen fraction, time or treatment means followed by different letter (*upper case*) are significantly different at $p<0.05$ (Duncan's multiple range test).

[c]Figures in parentheses indicate NH_4^+-N disappearance as % of the initial content.

Table 2. Changes in mineral nitrogen in soil amended with 200 mg N kg^{-1} as $(NH_4)_2SO_4$ and 0–3.6 mg kg^{-1} of 3,5-dimethylpyrazole (DMP).

Time (weeks)	DMP applied (mg kg^{-1})							Time mean
	0	0.225	0.45	0.675	0.90	1.8	3.6	
	NH_4^+-N (mg kg^{-1})							
0	195 a[a]	195 a	195 a	195 a	195 a	195 a	195 a	195 A[b]
1	0 (100)[c] h	0 (100) h	0 (100) h	37 (82) f	76 (61) e	130 (34) c	143 (27) b	55 (72) B
2	0 (100) h	2 (99) h	14 (96) gh	7 (95) h	7 (97) h	24 (88) g	89 (55) d	20 (90) C
3	0 (100) h	0 (100) h	3 (99) h	8 (96) h	0 (100) h	2 (99) h	0 (100) h	2 (99) D
4	2 (98) h	4 (99) h	3 (99) h	8 (96) h	14 (94) gh	2 (99) h	10 (95) h	6 (97) D
Treatment mean	39 E[b]	40 E	43 E	51 D	58 C	71 B	87 A	
	NO_3^--N (mg kg^{-1})							
0	16 i[a]	16 i	16 i	16 i	16 i	16 i	16 i	16 D[b]
1	96 efgh	73 gh	128 abcdef	138 abcde	123 abcdefg	75 efg	52 hi	98 C
2	170 ab	154 abcd	123 abcdefg	154 abcd	116 bcdefg	160 abc	116 bcdefg	142 A
3	132 abcde	138 bcde	170 a	98 efgh	103 defgh	91 efgh	78 fgh	116 B
4	101 defg	52 hi	98 efgh	110 cdefg	137 abcde	157 abc	152 abcd	115 B
Treatment mean	103 AB[b]	87 AB	107 A	103 AB	99 AB	100 AB	83 B	
	$NH_4^+ + NO_3^-$-N (mg kg^{-1})							
0	211 a[a]	211 a	211 a	211 a	211 a	211 a	211 a	211 A[b]
1	96 ghijk	73 jk	128 defghij	175 abcde	195 ab	204 a	195 ab	152 B
2	170 abcde	156 abcdefg	137 bcdefghi	161 abcdef	124 defghij	184 abcd	205 a	162 B
3	132 cdefghij	138 bcdefghi	173 abcde	105 fghijk	103 fghijk	93 hijk	78 ijk	118 C
4	103 fghijk	56 k	101 fghijk	118 efghij	151 abcdefgh	193 abc	162 abcdef	126 C
Treatment mean	142 CD[b]	127 D	150 BCD	154 ABC	157 ABC	177 A	170 AB	

[a]All values are mean of 3 replicates; For each mineral N fraction, figures followed by different letter (lower case) are significantly different at $p<0.05$ (Duncan's multiple range test).

[b]Time or treatment means followed by different letter (upper case) are significantly different at $p<0.05$ (Duncan's multiple range test).

[c]Figures in parentheses indicate NH_4^+-N disappearance as % of the initial content.

Table 3. Changes in mineral nitrogen of soil amended with 200 mg N kg^{-1} as $(NH_4)_2SO_4$ and 0–0.5 mg kg^{-1} of nitrapyrin (pure active ingredient).

Time (weeks)	Nitrapyrin applied (mg kg^{-1})			Time mean
	0	0.25	0.5	
	---------- NH_4^+-N (mg kg^{-1}) ----------			
0	171 a[a]	171 a	171 a	171 A[b]
1	6 (97)[c] e	41 (76) c	136 (21) b	61 (65) B
2	7 (96) e	11 (94) e	17 (89) de	12 (93) D
3	8 (95) e	16 (90) de	10 (94) e	12 (93) D
4	16 (91) e	14 (92) e	27 (84) d	19 (89) C
Treatment mean[b]	42 C[b]	51 B	72 A	
	---------- NO_3^--N (mg kg^{-1}) ----------			
0	32 f[a]	32 f	32 f	32 C[b]
1	116 bcd	136 ab	95 de	115 A
2	75 e	158 a	118 bcd	117 A
3	127 bc	28 f	44 f	66 B
4	99 cde	82 e	114 bcd	98 A
Treatment mean[b]	90 NS	87 NS	80 NS	
	---------- NH_4^++NO_3^--N (mg kg^{-1}) ----------			
0	203 b[a]	203 b	203 b	203 A[b]
1	119 de	177 bc	231 a	175 B
2	82 f	170 c	135 d	129 C
3	136 d	44 g	54 g	78 D
4	115 de	96 ef	141 d	117 C
Treatment mean[b]	131 B	138 B	153 A	

[a]All values are mean of 3 replicates; For each nitrogen fraction, figures followed by different letter (*lower case*) are significantly different at $p<0.05$ (Duncan's multiple range test).

[b]For each nitrogen fraction, time or treatment means followed by different letter (*upper case*) are significantly different at $p<0.05$ (Duncan's multiple range test).

[c]Figures in parentheses indicate NH_4^+-N disappearance as % of the initial content.

Table 4. Changes in mineral nitrogen in soil amended with 200 mg N kg^{-1} as $(NH_4)_2SO_4$ and 0–52 mg kg^{-1} nitrapyrin as N-Serve 24.

Time (weeks)	Nitrapyrin applied (mg kg^{-1})								Time mean
	0	1.04	2.08	4.16	8.32	10.4	26.0	52.0	
	NH_4^+-N (mg kg^{-1})								
0	222 a[a]	222 a	222 a	222 a	222 a	222 a	222 a	222 a	222 A[b]
1	5 (98)[c] lm	20 (91) l	134 (40) l	179 (19) ef	182 (18) def	211 (6) ab	198 (11) bcd	197 (11) bcd	141 (36) B
2	4 (99) lm	5 (98) lm	51 (76) k	101 (55) j	173 (22) f	192 (13) cde	198 (10) bcd	206 (7) abc	116 (48) C
3	0 (100) m	0 (100) m	12 (95) lm	10 (96) lm	140 (37) hi	155 (30) gh	183 (17) def	197 (11) bcd	87 (61) D
4	0 (100) m	3 (99) lm	4 (98) lm	9 (96) lm	112 (50) j	167 (25) fg	204 (8) abc	196 (12) bcde	87 (61) D
Treatment mean	46 F[b]	50 F	85 E	104 D	166 C	189 B	201 A	203 A	
	NO_3^--N (mg kg^{-1})								
0	30 k[a]	30 k	30 k	30 k	30 k	30 k	30 k	30 k	30 D[b]
1	180 de	169 ef	113 h	64 ij	45 jk	49 jk	40 jk	43 jk	88 C
2	238 b	227 bc	203 cd	147 fg	64 ij	48 kj	48 jk	43 jk	127 B
3	186 de	183 de	179 de	192 de	106 h	78 I	45 jk	41 jk	126 B
4	220 bc	235 b	263 a	225 bc	142 g	114 h	60 ijk	48 jk	163 A
Treatment mean	171 A[b]	169 A	157 B	132 C	77 D	64 E	44 F	41 F	
	NH_4^++ NO_3^--N (mg kg^{-1})								
0	251 bcd[a]	251 bcd	251 bcd	251 bcd	251 bcd[a]	251 bcd	251 bcd	251 bcd	251 A[b]
1	185 g	183 g	247 bcd	243 bcde	227 de	261 abc	238 bcde	239 bcde	228 B
2	242 bcde	233 de	253 bcd	248 bcd	237 cde	240 bcde	246 bcde	249 bcd	244 A
3	186 g	183 g	191 g	201 fg	246 bcde	232 de	228 de	238 bcde	213 C
4	220 ef	238 bcde	233 de	235 cde	254 bcd	282 a	264 ab	244 bcde	246 A
Treatment mean	217 C[b]	218 C	235 B	236 B	243 AB	253 A	245 AB	244 AB	

[a]All values are mean of 3 replicates; For each mineral N fraction, figures followed by different letter (lower case) are significantly different at $P<0.05$ (Duncan's multiple range test).

[b]Time or treatment means followed by different letter (upper case) are significantly different at $P<0.05$ (Duncan's multiple range test).

[c]Figures in parentheses indicate NH_4^+-N disappearance as % of the initial content.

inhibition during first 2 weeks; all the NH_4^+-N was nitrified in 4 weeks at nitrapyrin application rates up to 4.16 mg kg^{-1} (Table 4). The inhibitory effect of nitrapyrin was much higher at application rates of 8.32, 10.4, 26 and 52 mg kg^{-1}, producing 50, 75, 100 and 100% inhibition during 4 weeks. With 26 and 52 mg kg^{-1} nitrapyrin, the inhibitory effect persisted as long as 12 weeks producing a 49% and 79% inhibition, respectively (data beyond 4 weeks not reported). After 4-week incubation, a 12% deficit in the total mineral-N balance was recorded in the control and a 12% gain in 10.4 mg kg^{-1} treatment ($p<0.05$), whereas other concentrations had no effect (Table 4).

After 18-h equilibration at 4°C (i.e., the time-zero of incubation at 35°C), the NH_4^+-N content was generally lower than the sum of added NH_4^+-N (200 mg kg^{-1}) and that already present in soil. This deficit occurring during the 18-h equilibration is attributable to the sorption of NH_4^+-N on negatively charged exchange sites or its entrapment in the interlayer of the clay fraction of the soil. However, this deficit varied in different batch experiments most probably due to differences in the content of soluble organic C, which is known to play a significant role in sorption and desorption of NH_4^+ (Fernando *et al*., 2005). Results of the present study are consistent with those of previous field studies conducted on the same soil under maize (Mahmood *et al*., 1998, 2005) or cotton (Mahmood *et al*., 2000) where nitrification of applied N fertilizer was complete within a week.

The deficit in the total mineral-N balance observed during incubation may partly be attributed to microbial immobilization, and losses through NH_3 volatilization and denitrification. Although, the observed deficit in NO_3^--N balance seems to be less likely due to denitrification (since the soils were aerobic), the possibility of denitrification can not be completely ruled out. The fact that denitrification can occur in well-structured aerobic soils, has been reasonably explained by the microsite concept (Crasswell & Martin, 1975; Firestone, 1982). Under present experimental conditions, the high temperature might have been conducive for the development of anoxic microsites within otherwise bulk aerobic soil. Microbial immobilization of NH_4^+- as well as of NO_3^--N in the presence of easily available C is well documented (Azam & Malik, 1985; Azam *et al*., 1988). Since the soil was air-dried and stored prior to use, a considerable increase in the carbon availability might be expected. Therefore, microbial immobilization of NH_4^+- and NO_3^--N appears to be an important mechanism responsible for the observed deficit in mineral-N.

Conclusions

3,5-dimethylpyrazole, when applied at 0.225–0.45 mg kg^{-1}, was not effective at 35°C, whereas increasing its application rate to 3.6 mg kg^{-1} caused a 45% inhibition up to 2 weeks. Increasing the DMP application rate in the range of 0.9–3.6 mg kg^{-1} also improved the mineral-N balance relative to the control and lower application rates. The most widely used nitrification inhibitor *viz*., nitrapyrin also was not effective at 35°C when applied in the range of 0.25–0.50 mg kg^{-1} (0.56–1.12 kg ha^{-1}), the rates generally recommended for agricultural use under moderate temperatures (Touchton & Boswell 1980). However, increasing the nitrapyrin application rate to 8.32 mg kg^{-1} caused a 50% reduction in nitrification up to 4 weeks. These results signify that when applied at rates generally recommended for moderate agro-climates, DMP and nitrapyrin are ineffective in inhibiting nitrification at high soil temperatures such as those prevailing under summer crops in Pakistan. A 16-fold increase in the application rate of DMP could not improve its efficiency beyond 2 weeks. On the other hand, an 8-fold increase in the nitrapyrin

application rate caused about 50% inhibition till 4 weeks, and with 26–52 fold higher application rates, the inhibitory effect of nitrapyrin persisted as long as 12 weeks producing a 49% and 79% inhibition, respectively. However, such high application rates of the tested nitrification inhibitors may not be economically feasible. Moreover, very high concentrations of these inhibitors might be phytotoxic, or adversely affect the activities of soil microflora. Therefore, more studies are desirable to search for compounds which could effectively inhibit nitrification at high soil temperatures that prevail during the summer season in this region.

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