Mineral N in Soil and Barley Growth as Influenced by Urease and Nitrification Inhibitors

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Abstract

Incubation and pot experiments were conducted in two different soils to study the effect of phenyl mercuric acetate (PMA) and dicyandiamide (DCD) and their mixture on urease activity and nitrification rate. Results showed that an application of PMA alone or its combination with DCD gave higher inhibition of urease enzyme which is reflected positively on barley growth and N uptake. However, PMA had no obvious effect on nitrification. On the other hand, DCD could markedly inhibit nitrification of urea-released NH_4^+ and consequently enhanced plant growth and N uptake over untreated urea, but gave lower than PMA and PMA + DCD treatments.

Keywords: Urease, soil, Urea, nitrification inhibitors

Introduction

Urea fertilizer is the main source of N applied to soil. It is expected that global consumption of urea reaches 41000000 ton (FAO, 2015). Urea in soil is hydrolyzed by urease to form NH_4^+. A part of NH4+ subjected to nitrification process to produce NO_3^- . The NH_4^+ and NO_3^- ions are the main N forms to uptake by plant, but a part of its may be lost by volatilization, leaching, fixing and denitrification as well as increasing environmental pollution.

The strategies of regulating the fate and behavior of urea-N in the soil-plant system focus mainly on the improvement of the fertilization system (Xu *et al*., 2000). One of the most important and applicable strategies is use of inhibitors with urea to improve fertilizer efficiency and reduce environmental pollution.

Urease inhibitors such as NBPT, HQ, PPDA and PMA retarded the hydrolysis of urea in soil and reducing N loss, then improve N uptake. Zhao *et al*. (1992) stated that urease inhibitors not only retarded the hydrolysis of urea but also markedly influenced the behavior of released NH_4^+ in soil afterwards. Nitrification inhibitors delay the convertion of NH_4^+ to NO_3^- and reduce the NO_3^- loss by leaching and/or denitrification. Nitrification inhibitors such as DCD and Nitrapyrin limits N_2O flux from NH_4^+ based fertilizers (Xu *et al*. 2000). Nevertheless, using of nitrification inhibitors might increase NH_3 volatilization by maintaining more NH_4^+ in the soil solution after retarding the nitrification process (Ni *et al*.,2018). Zaman *et al*. (2008) stated that DCD reduced N_2O emission by 37%, but increased NH_3 emission by 29% compared with urea.

A combination of DCD with urease inhibitors could improve the efficiency of the DCD, hence the combination of both inhibitors may be resulted to the retention of N in soil-plant system. Result of Xu *et al*. (2002) indicated that application of DCD and HQ together with

urea can effectively regulate the ratio of NH_4^+-N to (NO_3^- + NO_2^-)-N in soil and weaken the potential of the nitrification-denitrification process. Xu *et al.* (2001) also found that DCD and DCD + HQ can markedly regulate the immobilization-mineralization turnover of fertilizer N in soil.

This paper investigated the influence of urease inhibitor (PMA) and nitrification inhibitor (DCD) and their combination on the fate of urea-N and found the way to regulate N transformations and their effect on barley growth and N uptake.

Materials and Methods

Soil characteristics:

Samples (0-30 cm depth) of a loamy sand soil from AL-Burjusia region and silty clay soil from AL-Qurna region, Basrah province, Iraq were collected. Soil properties were determined according to Page *et al*. (1982) and presented in table 1.

Table (1): Some properties of soils used.

property	unit	Al-Qurna	Al-Burjusia
pH		7.58	8.02
EC	dSm^{-1}	4.40	2.93
$CaCO_3$	$g\ kg^{-1}$	219.19	73.74
CEC	$Cmol^{(+)}\ kg^{-1}$	23.14	4.00
Total N	$g\ kg^{-1}$	0.73	0.06
Organic matter	$g\ kg^{-1}$	15.36	1.26
Urease	$\mu g\ NH_4^+$-N g^{-1} soil $2hrs^{-1}$	5.93	2.20
Texture		Silty clay	Loamy sand

Inhibition experiment design:

Fifty grams air-dried soil, passed through a 2 mm sieve, was put in polyethylene container of 150 cm^3 in volume; urea was added at rates of 0 and 500 $mgNKg^{-1}$soil. Phenyl mercury acetate (PMA) as urease inhibitor, dicyandiamide (DCD) as nitrification inhibitor or PMA + DCD as rate 1:1 were thoroughly mixed with urea at rates of 0.5 and 5.0% of the applied urea (W/W). Control treatment was conducted using urea without inhibitors. Samples were incubated for 7 days in a chamber at 30±2°C with a soil moisture of field capacity at each soil. During the incubated period, the containers were kept open in the chamber and water loss was recovered daily by adding water with a pipette on to the soil surface.

On day 7 after incubation, three replicates of each treatment were taken out to assay urease activity and nitrification rate. For urease activity, 5g sub-sample of soil, treated with 0.2 ml tulene, 9 ml of THAM and 1 ml of 0.20M urea and incubated for 2 hrs at 37°C. Then a mixture of KCl-Ag_2SO_4 was added. Ammonium (NH_4^+-N) was measured by steam distillation (Tabatabi and Bremner, 1972).

For nitrification rate, 10 g of incubated soil was extracted with 50 ml of 2*M* KCl solution for 30 min. The NO_2^--N and NO_3^--N concentrations in the extract were determined using steam distillation method described by Bremner and Edwards (1965).

The % urease inhibition was calculated as follows (Bremner *et al*., 1991):

$$(C-T)/C \times 100$$

Where: T: NH_4^+-N resulted in samples treated with inhibitors.

C: NH_4^+-N resulted in control treatment.

The % nitrification inhibition was calculated as follows (Bremner and McCarty, 1988):

$$(C-T)/C \times 100$$

Where T: NO_3^--N + NO_2^--N resulted in samples treated with inhibitors.
C: NO_3^--N + NO_2^--N resulted in control treatment.

Pots experiment design:

Three kilograms air-dried soil, passed through a 4 mm sieve, was put in pots and urea was added at rates of 0, 50, 100 and 150 $KgNha^{-1}$. PMA, DCD or PMA + DCD were thoroughly mixed with urea at rates of 0.5 and 5.0% of applied urea (W/W). The mixture of urea and the inhibitor was incorporated into the surface soil layer of pot. Control treatment was conducted using urea without inhibitor. Each treatment was replicated three times. Barley seeds were grown in the pots in mid of Nov. and pots were put in greenhouse. After 60 days, shoots were harvested, oven-dried at 70°C and weighing to obtain shoot dry weight. Sub-sample of dry shoot was digested by a mixture of $HClO_4$ + H_2SO_4 (Cresser and Parcons,1979), then N concentration was measured by steam distillation (Bremner, 1970). To calculate N uptake in shoot, shoot dry weight was multiplied by N concentration.

Statistical analysis:

All the obtained data of main effects and their interactions were subjected to analysis of variance (ANOVA) under CRD design using GenStat Procedure Library Release PL 15 Programme. The significant differences were obtained using Revised LSD at propability of ≤ 0.01 among the means.

Results and Discussion

Inhibition experiment:

Tables 2 and 3 shows that the PMA and PMA + DCD treatments had a much lower concentrations of NH_4^+-N in soil compared with control treatment at the two soils used. The percents of inhibition were arranged 18.63 – 29.67% and 34.68 – 58.18 for AL-Qurna soil and AL-Burjusia soil, respectively (figs 1 and 2). However, treating soil with DCD alone resulting in a significant large concentration of NH_4^+-N compared with control treatment, for the two soils (tables 2 and 3). The influence of PMA alone or incombination with DCD on hydrolysis of the applied urea was clear at short period (7 days) of fertilization. Chen *et al*.

(1998) showed that a combined application of the urease inhibitor HQ and the nitrification inhibitor DCD retarded the hydrolysis of urea. The combination of PMA with DCD gave the largest NH_4^+-N concentration in soil as compared with PMA alone especially in AL-Burjusia soil, that means under the experiment conditions, there was a synergestic effect of PMA and DCD on the retention of exchangeable NH_4^+ in soil after fertilization. For addition of DCD

Table 2: Recoveries of NH_4^+-N and NO^-3-N in AL-Qurna soil received urea amended with PMA and DCD. (means followed by different letters are significantly different at 0.01 level; capital letters for main effect and small letters for interactions)

trt.	NH_4^+-N			NO_3^--N		
	0.5%	5.0%	mean	0.5%	5.0%	mean
PMA	98.00a	86.14a	92.07A	45.11b	44.00b	44.55C
DCD	136.25c	141.56c	138.90B	21.75a	15.75a	18.75A
PMA+DCD	99.66a	91.91a	95.78A	37.50b	35.50b	36.50B
Cont.		122.50b			47.35b	
mean	114.10B	110.52A		37.90B	28.50A	

Table 3: Recoveries of NH_4^+-N and NO^-_3-N in AL-Burjusia soil received urea amended with PMA and DCD. (means followed by different letters are significantly different at 0.01 level; capital letters for main effect and small letters for interactions)

trt.	NH_4^+-N			NO_3^--N		
	0.5%	5.0%	mean	0.5%	5.0%	mean
PMA	59.05c	40.25a	49.65A	13.14	13.45	13.30A
DCD	103.25d	122.50e	112.87C	6.03	4.14	5.08A
PMA+DCD	62.86c	50.30a	56.58B	11.13	10.13	10.63A
Cont.		96.25d			14.00a	
mean	80.35B	77.32A		11.07	10.43	

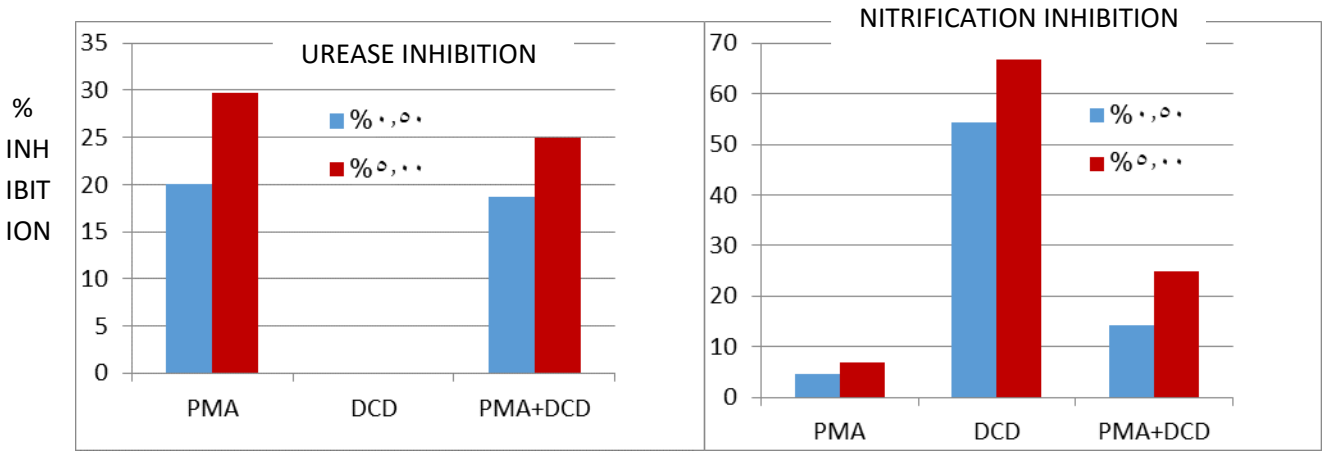

Fig 1: (%) inhibition of urease and nitrification in Al-Qurna soil received urea amended with PMA and DCD. (means followed by different letters are significantly different at 0.01 level).

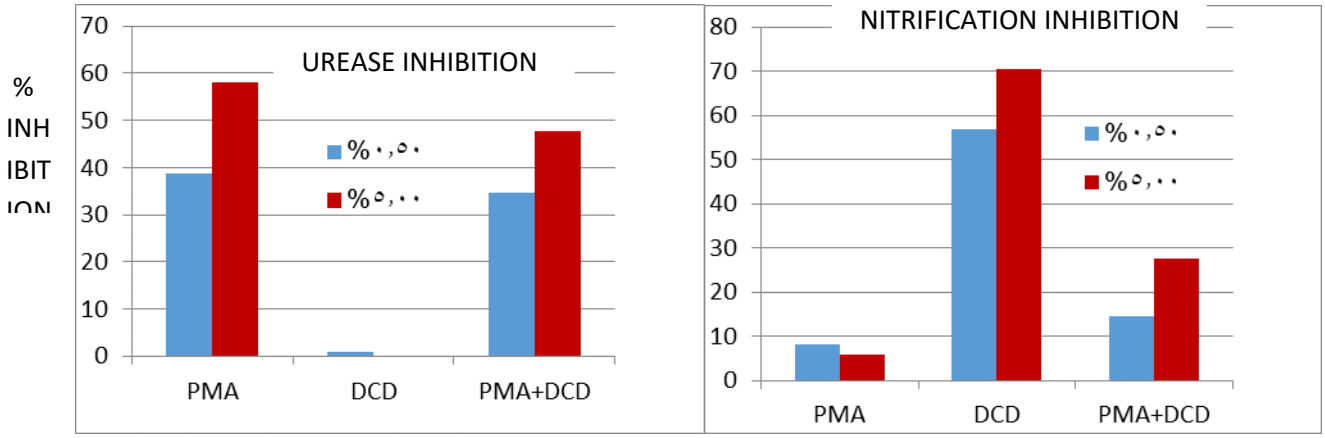

Fig 2: (%) inhibition of urease and nitrification in Al-Burjusia soil received urea amended with PMA and DCD. (means followed by different letters are significantly different at 0.01 level).

alone, the higher amount of NH_4^+-N was found in soil, which can attributed to the effectiviness of DCD to inhibit the oxidation of NH_4^+-N soil immediately following the hydrolysis of urea (Xu *et al*, 2002).

Addition of inhibitor(s) at rate of 5.0% was more effective to change NH_4^+-N concentrations than the rate of 0.5% (tables 2 and 3). At PMA and PMA + DCD treatments, the concentrations of NH_4^+ -N were reduced at percents of 10 and 26% for AL-Qurna soil and AL-Burjusia soil, respectively. At DCD treatment, the concentrations of NH_4^+ -N were increased at percents of 4 and 16% for AL-Qurna soil and AL-Burjusia soil, respectively.

The inhibitory effect of PMA was lower in AL-Qurna soil, which had the highest organic C and clay contents (figs 1 and 2). Similar results were obtained by Abdulkareem and AL-Amiri (2008) and Yaseen (2011) who found that the inhibition of urease was lower in heavy

soil with high content of organic C compared with the light soil. Moreno *et al*. (2001) stated that the inhibitory effect of Cd was reduced in soils with high content of organic C because of immobilization of Cd in organic matter.

In comparison with control treatment, the soil treated with inhibitor(s) had a smaller concentrations of NO_3^--N with the smallest values in the DCD treatmens (tables 2 and 3). Presence of PMA alone or incombination with DCD reduced the inhibitory effect of nitrification as compared with DCD treatment. Therefore, under the experimental conditions, there was a little effect of PMA on nitrification in the two soils. The inhibition percents of PMA were 5.69 and 6.93% for AL-Qurna and AL-Burjusia soils, respectively. At different experimental conditions (inhibitor concentration, soil type, soil moisture …. etc) PMA may be influence nitrification potential. In our study, the little effect of PMA was probably related to the decrease in the NH_4^+-N content released due to urease inhibition (tables 2 and 3).

Increasing inhibitor concentration from 0.5 to 5.0% decreased the NO_3^--N concenctrations in soil and consequently increased nitrification inhibition (figs 1 and 2). For DCD treatment the persent of nitrification inhibition increased from 54.31 to 66.66% at AL-Qurna soil and from 56.92 to 70.42% at AL-Burjusia soil when the concentration increased from 0.5 to 5.0% . Similar finding was observed by Watson *et al*. (1994).

In comparison with urease inhibition, nitrification inhibition was not differ with different soils which ranged 4.52-66.66% for AL-Qurna soil and 5.68-70.42% for AL-Burjasia soil (figs 1 and 2).

Shoot dry weight of barley plant:

The shoot dry weight of barley plants was significantly higher ($p \leq 0.01$) in the PMA and PMA + DCD treatments than in the other two treatments. However, there is no significant differences between the two treatments (tables 4 and 5). Furtheremore, addition of DCD alone gave significant differences compared with control. The increasing percents of PMA, DCD and PMA+DCD treatments as comparecl with control were 149.31, 69.86 and 152.05% at AL-Qurna soil and were 123.80, 49.20 and 325.39% at AL-Burjusia soil, respectively. Du to application of PMA, the NH_3 volatilization was reduced as a reselt to slow release of N from urea (tables 2 and 3) and consequently increased the amount of available N in soil which affect the dry weight of plant positively.

Addition of DCD affect the transformations of applied urea and N recovery in soil which produces an a bundance of NH_4^+-N and NO_3^--N in soil solution readly to uptake by plant. Xu *et al*.(2001) stated that under the well-drained conditions, DCD could markedly inhibit nitrification of urea-released NH^+, with beneficial effects on the conservation and re-utilization of soil urea N. At PMA and DCD treatments, plant still absorb the needed amount of N derived from urea and this event may be achieved by delaying N availability in soil. Application of PMA and DCD together with urea can effectively regulate the ratio of NH_4^+-N to NO_3^--N in soil (Xu *et al*. 2000) as well as regulate the immobilization-mineralization turnover of fertilizer N in soil (Xu *et al*.,2001).

Nitrogen plays an important role for chlorophyll formation, protein and enzymes synthesis, and stimulates the production of auxin which enhance cell division and consequently increasing plant biomass (Barker and Bryson, 2007).

The results of tables 4 and 5 indicated that there was an increase in shoot dry weight with increasing the level of N at the two soils. The differences were significant among N levels, except that of the levels 100 and 150 Kg N ha^{-1} at AL-Qurna soil. This result is similar to AL-Malaky and Abdulkareem(2019).

On respecting of inhibitor(s) concentration, data of tables 4 and 5 clearly indicated that increasing the concentration of inhibitor(s) from 0.5 to 5.0%

Table 4: Shoot dry weight, N concentration and N-uptake of barley grown in AL-Qurna soil received urea amended with PMA and DCD. (means followed by different letters are siqnificantly different at 0.01 level; capital letters for main effect and small letters for interactions)

	inhibitor conc. 0.5%				inhibitor conc. 5.0%				
trt.	0	50	100	150	0	50	100	150	mean
	(Kg N ha^{-1})				(Kg N ha^{-1})				
	Shoot dry weight (g pot^{-1})								
PMA	0.63	1.40	2.03	2.10	0.63	2.11	2.81	2.92	1.82C
DCD	0.63	1.00	1.19	1.35	0.63	1.30	1.90	1.95	1.24B
PMA+DCD	0.63	1.43	2.00	2.19	0.63	2.00	2.89	2.95	1.84C
Cont.	0.63	0.69	0.78	0.83	0.63	0.69	0.78	0.83	0.73A
mean	0.63A	1.13B	1.50C	1.61C	0.63A	1.52C	2.09D	2.16D	
	N conc. in shoot (g Kg^{-1})								
PMA	12.03	19.35	26.04	29.43	12.03	20.01	28.33	29.62	22.10C
DCD	12.03	20.13	23.04	24.15	12.03	20.02	28.31	28.88	21.07B
PMA+DCD	12.03	20.06	26.42	28.86	12.03	20.45	29.05	29.45	22.29C
Cont.	12.03	19.41	20.01	20.03	12.03	19.41	20.01	20.03	17.87A
mean	12.03A	19.73B	23.86C	25.61D	12.03A	19.97B	26.42D	26.99D	
	N uptake in shoot (mg pot^{-1})								
PMA	7.56a	26.81e	42.84g	61.40j	7.56a	42.23g	79.38k	86.42i	44.27C
DCD	7.56a	20.13d	27.43	32.58f	7.56a	26.03e	54.45hi	56.29i	29.00B
PMA+DCD	7.56a	28.68e	52.92h	63.36j	7.56a	40.96g	86.74	86.74i	46.81D
Cont.	7.56a	13.40b	15.57bc	16.62c	7.56a	13.40b	15.57bc	16.62c	13.28A
mean	7.56A	22.23B	34.69D	43.49E	7.56A	30.65C	59.03F	61.51F	

Table 5: Shoot dry weight, N concentration and N-uptake of barley grown in AL-Burjusia soil received urea amended with PMA and DCD. (means followed by different letters are siqnificantly different at 0.01 level; capital letters for main effect and small letters for interactions)

	inhibitor conc. 0.5%				inhibitor conc. 5.0%				
trt.	0	50	100	150	0	50	100	150	mean
	(Kg N ha^{-1})				(Kg N ha^{-1})				
	Shoot dry weight (g pot^{-1})								
PMA	0.50a	0.88de	1.22g	1.90l	0.50a	1.63k	2.01m	2.70o	1.41C
DCD	0.50a	0.73c	0.97ef	1.00f	0.50a	1.00f	1.34i	1.48j	0.94B
PMA+DCD	0.50a	0.84de	1.27h	1.95lm	0.50a	1.65k	2.00m	2.68n	1.42C
Cont.	0.50a	0.60b	0.64b	0.79c	0.50a	0.60b	0.64b	0.79d	0.63A
mean	0.50A	0.76B	1.02C	1.41E	0.50A	1.22D	1.49E	1.91F	
	N conc. in shoot (g Kg^{-1})								
PMA	11.01	18.64	19.00	22.34	11.01	20.01	21.11	24.85	18.49B
DCD	11.01	18.99	20.16	21.03	11.01	20.32	23.02	23.08	18.57B
PMA+DCD	11.01	18.92	20.06	22.40	11.01	20.73	21.03	24.96	18.76B
Cont.	11.01	18.03	19.34	20.00	11.01	18.03	19.34	20.00	17.09A
mean	11.01A	18.64B	19.64C	21.44D	11.01A	19.77C	21.12D	23.22E	
	N uptake in shoot (mg pot^{-1})								
PMA	5.47	18.27	24.49	38.02	5.47	32.61	42.34	48.11	26.84C
DCD	5.47	15.79	19.57	23.09	5.47	20.34	30.80	33.70	19.27B
PMA+DCD	5.47	17.65	28.05	39.02	5.47	34.20	42.10	46.73	27.33C
Cont.	5.47	9.74	12.34	15.72	5.47	9.74	12.34	15.34	10.77A
mean	5.47A	15.36B	21.11BC	28.96DE	5.47A	24.22CD	31.89EF	35.97F	

increased shoot dry weight at all treatments. This is true for the two soils used. This result can attribut to the higher inhibition of urease and nitrification at concentration of 5.0% (figs 1 and 2) resulting less loss of N and more regulation of NH_4^+-N and/or NO_3^--N in soil.

Higher shoot dry weights were obtained at AL-Qurna soil compared with AL-Burjusia soil (tables 4 and 5). The mean values were 1.40 and 1.10 g pot^{-1} for AL-Qurna and AL-Burjusia soils, respectively. Despite the higher urease inhibition in AL-Burjusia soil (figs 1 and 2), but the presence of higher amounts of NH_4^+-N and NO_3^--N (tables 2 and 3) as well as initial properties of AL-Qurna soil may give an explanation for the higher growth of barley plant in this soil.

N concentration and uptake of barley plant:

N concentration and uptake of PMA and PMA+DCD treatments were significantly higher that these of DCD and control treatments with no significant differences between PMA and PMA+DCD treatments (tables 4 and 5). These defferences were applicable to the two soils. The highest plant growth at treatments PMA and PMA+DCD and presence of large amounts of NH_4^+-N and NO_3^--N in soil may justify the highest N uptake. These results were in

agreement with Abdulkareem and AL-Amiri (2008) who obtained a higher N uptake by tomato plant received urea treated with PMA as compared with untreated urea. Results of Abdulkareem (2006) also indicated a higher N uptake at DCD treatment comparing with control.

Data presented in tables (4 and 5) showed that increasing N rates lead to an increase in N concentration and uptake of barley at the two soils. The mean values of N uptake were 7.56, 26.44, 46.86 and 52.5 mg pot^{-1} for AL-Qurna soil and 7.56, 19.79, 26.50 and 32.46 mg pot^{-1} for AL-Burjusia soil at rates of 0, 50, 100 and 150 Kg N ha^{-1}, respectively.

Increasing inhibitor(s) concentration significantly increased N concentration and uptake for all treatments at the two soils. The higher inhibition rates (urease or nitrification) at 5.0% concentration can resulte a sufficient amount of NH_4^+-N and NO_3^--N in balanced ratio, and this enables the plant to use excess of urea-N.

Higher values of N concentration and uptake were recorded in plant grown in AL-Qurna soil in related to their counterparts of AL-Burjusia soil. Similae results have been reported by Abdulkareem and AL-Amiri (2008) who pointed out that N uptake of tomato plant grown in silt loam soil was significant higher than plant grown in loamy sand soil.

In conclusion, PMA alone or with DCD gave a much larger recovery of soil urea N with the smallest NH_4^+-N and largest NO_3^--N concentrations due to urease inhibition. However, the influence of PMA on nitrification is not clear under the experimental condition. DCD could markedly inhibit nitrification of urea-released NH_4^+. The higher the urease or nitrification inhibition, the higher the growth and N uptake of barley plants. That was true, except for respecting of soil type which the plant growth and N uptake related to total amount of inorganic N and soil initial properties. Further field studies on a local conditions associated with economic benefit are required to use PMA and DCD as practical potential.

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