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Efficacy of CMP [1-Carboxamide, 3-(5)-Methylpyrazole] and DCD (Dicyandiamide) as Nitrification Inhibitor

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Abstract: Results of pure culture studies indicated that CMP effectively inhibited growth of *Nitrosomonas* at concentration as low as 1 ppm. The soil incubation studies indicated that the inhibitors CMP and DCD were effective in inhibiting nitrification at 10 and 20°C. At 30°C, the inhibition was only 5-8%. Under flooded conditions, CMP effected almost total inhibition of nitrification and prevented build-up of nitrite and nitrate in floodwater. The effect of DCD was lower than that of CMP. The addition of inhibitors resulted in pronounced accumulation of ammonium (NH_4^+) in floodwater, the effect of CMP being greater than DCD. (***Key words: CMP, dicyandiamide, floodwater,*** Nitrosomonas, ***temperature***)

Nitrification inhibitors have been proposed as strategies to retard nitrification and maintain NH_4^+-N for a longer period of time and improve fertilizer-N use efficiency (Prasad & Power 1995). High temperatures and heavy rainfall in the tropics make nitrification inhibitors such as nitrapyrin and DCD ineffective (Rajeswara Rao *et al.* 1990). Krueger *et al.* (1989) based on results of their field studies in Germany, reported CMP as a promising nitrification inhibitor. Very limited data is available on the effectiveness of CMP as a nitrification inhibitor. Laboratory incubation studies were conduced with the following objectives; (i) to find out the effectiveness of CMP *vis-a-vis* DCD on *Nitrosomonas* pure culture, (ii) to find out the effect of temperature on the efficacy of nitrification inhibition by CMP and DCD and (iii) to determine the suitability of CMP for flooded conditions.

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Materials and Methods

Experiment 1: Effect of CMP and DCD on the growth of Nitrosomonas

Nitrosomonas pure culture was obtained from Institute of Botany, Hamburg, Germany and was cultivated in the ATCC - medium 221. Nutrient solution (100 mL) was taken in 300 mL Erlenmeyer flasks and inoculated with the culture and incubated in a dark chamber at 25°C which served as control. To separate set of flasks CMP and DCD were added to obtain concentration of 1, 5 and 10 ppm for CMP and 50 ppm for DCD. The concentration of DCD was decided based on earlier studies at this laboratory (Zacherl & Amberger 1990). Growth of *Nitrosomonas* was measured by estimating the amount of NO_2^- produced using high pressure liquid chromatography (HPLC).

Experiment 2: Influence of temperature on the effectiveness of CMP and DCD

Surface field soil samples (loess brown earths) of Duernast, Freising, Germany having pH 6.8, organic C 1.12% and total N 0.12% was used in the study.

Twenty gram soil samples were placed in 300 mL polyethylene flasks (30 nos.) and moistened to field capacity (20% w/w) and pre-incubated for two weeks at 10, 20 and 30°C. After pre-incubation, the soils were treated with urea @ 100 mg N kg^{-1} soil with or without CMP [5 mg active ingredient (a.i.) kg^{-1} soil] and DCD. In the case of DCD treatment, 10% of the applied N was through DCD and the remainder through urea. A no-N control was also included. The flasks were covered with parafilm provided with a few micropores for aeration and then placed in the respective incubator. DCD was obtained from SKW Trostberg AG, Germany and it contained 66.7% N. CMP (50% a.i.) was obtained from VEB Agrochemisches Piesteritz Cunnersdorf bei Leipzig, Germany. Thirty days after fertilizer application (DAF), the flasks were removed for extraction of mineral-N. Extraction was done by shaking for one hour in an end-over-end shaker with 0.01 *N* $CaCl_2$ (46-48 mL) taking into account the moisture content of the soil. A small portion was filtered and the filtrate was analyzed for NO_2^- and NO_3^- using HPLC (Vilsmeier 1984). Per cent nitrification inhibition was calculated using the formula of Bundy and Bremner (1973): (C-T) × 100/C where C is the amount of NO_2^- + NO_3^- produced in control (urea alone) and T is amount of NO_2^- + NO_3^- produced in the soil treated with CMP or DCD.

Experiment 3: Effectiveness of CMP and DCD under flooded conditions

Hundred gram soil samples were weighed in 500 mL polyethylene flasks and were flooded to a depth of 2 cm with deionized water and preincubated for two weeks at 30°C. After preincubation, the floodwater was carefully siphoned out and the soils treated with urea solution (100 mg N kg^{-1} soil) with or without CMP and DCD (as in Expt. 2). The flasks were then placed in the incubator. After three days, the flasks were removed and the soils re-flooded to a depth of 2 cm (simulating farmers practice of reflooding the fields 3-4 days after fertilizer application). Triplicate flasks of each treatment were removed at 3, 6, 9, 12, 15 and 30 DAF for extraction of mineral N. Floodwater was carefully siphoned out, the volume recorded and filtered. Then a few drops of toluene was added to prevent microbial activity. The samples were deep frozen till analysis. The NH_4^+-N was estimated by NH_3 specific electrodes and NO_2^- + NO_3^- using HPLC. The soils were thoroughly mixed and 20 g portions of wet soil were taken and extracted with 0.01 *N* $CaCl_2$ as described in Expt. 2. Ammonium was estimated using NH_3 specific ion electrode (Orion 95-12). All data on wet soil analysis were converted to oven-dry weight basis after accounting for moisture content. Nitrite and nitrate in soil were detected only on third and sixth day and were less than 0.4 ppm; so values are not included. Data in experiment 3 were statistically analysed after (log x + 1) transformation and treatment means separated out using the DMRT (Gomez & Gomez 1984).

Results and Discussion

Experiment 1: Effect of CMP and DCD on the growth of Nitrosomonas europaea

Table 1. Effect of nitrification inhibitors CMP and DCD on the growth of pure culture *Nitrosomonas*

	Days				
	3	6	9	15	30
	mg NO_2^--N/2				
Control	0.41±0.03*	0.59±0.03	3.81±0.13	27.8±1.80	71.0±4.50
DCD 50 ppm	0.09±0.008	0.15±0.02	0.19±0.03	1.05±0.28	2.21±0.54
CMP 1 ppm	-	0.34±0.03	0.37±0.04	0.56±0.02	0.54±0.02
CMP 5 ppm	-	0.24±0.007	0.24±0.016	0.25±0.012	0.24±0.004
CMP 10 ppm	-	0.24±0.01	0.24±0.02	0.26±0.018	0.25±0.008

* Standard error

Data on the growth of *N. europaea* in pure culture is given in table 1. By applying nitrification inhibitors CMP and DCD, the growth was effectively inhibited. At the end of 30 days, the inhibition by CMP was almost total with concentration as low as 1 ppm. Per cent reduction for DCD (50 ppm) was about 97%. Zacherl and Amberger (1990) also reported growth of pure cultures to be completely suppressed with nitrification inhibitors nitrapyrin and thiourea. The increasing NO_2^- content indicating increased growth of *Nitrosomonas*, towards the later part of the incubation suggest that DCD has merely a bacteriostatic effect further substantiating the findings of Zacherl and Amberger (1990).

Experiment 2: Influence of temperature on effectiveness of CMP and DCD

Data on per cent nitrification inhibition of CMP and DCD at the end of 30 days of incubation at 10, 20 and 30°C is given in table 2. At 10°C, DCD had higher per cent inhibition compared to CMP. At 20°C, CMP and DCD were equally effective. When the soil was incubated at 30°C, inhibition was hardly 5-8%. Decline in the inhibitory effects of DCD with increasing temperature, due to rapid decomposition of DCD at elevated temperatures, has been observed earlier (McCarty & Bremner 1989). Walter *et al.* (1986) reported CMP to strongly inhibit nitrification compared to nitrapyrin at an incubation temperature of 20°C. Our present results indicate decline in nitrification inhibition of CMP at elevated temperature, however, the reason is not known at present.

Table 2. Influence of temperature on the per cent nitrification inhibition of fertilizer-N by CMP and DCD at the end of 30 days

	10°C	20°C	30°C
CMP	48.8	62.6	5.5
DCD	59.5	55.9	8.0

Experiment 3: Effectiveness of CMP and DCD under flooded conditions

Soil Ammonium-N

CMP treated soils had significantly higher NH_4^+-N at 12 and 15 DAF, as compared to urea alone (Fig. 1) but was at par with urea + DCD treatment. Urea alone and urea + DCD treatments

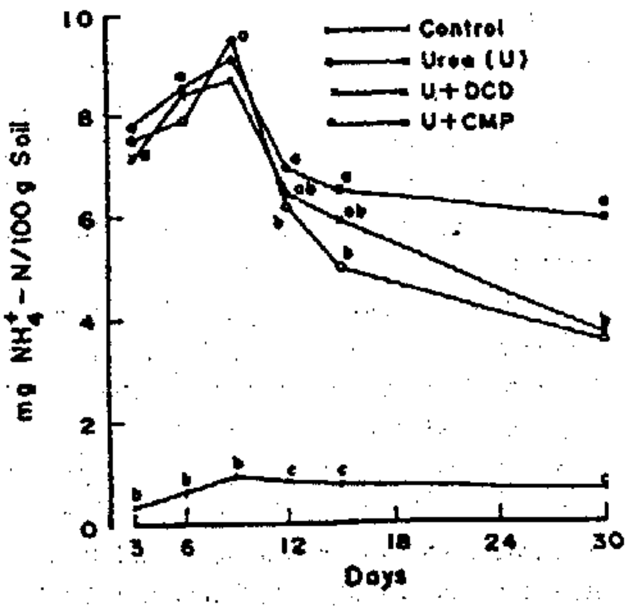

Fig. 1. Effect of CMP and DCD addition on soil NH_4^+-N following addition of 10 mg urea - N 100 g^{-1} soil (values followed by same letter are not statistically significant, P = 0.05)

did not differ significantly. At the end of 30 days, urea + CMP treatment had significantly higher NH_4^+ concentration over urea and urea + DCD. Nitrification inhibitors have been found to maintain higher levels of NH_4^+-N for a prolonged period. Walter *et al.* (1986) also reported higher NH_4^+-N in soils amended with urea + CMP under aerobic conditions.

Floodwater NH_4^+-N

Six DAF (first sampling after flooding), 12.7-17.4% of applied N was recovered as NH_4^+-N, from the floodwater as compared to only 0.1-9.6% at 30 DAF (Fig. 2). Significantly higher amounts of NH_4^+-N were recorded with CMP addition throughout; except 6 DAF when it was at par with urea + DCD.

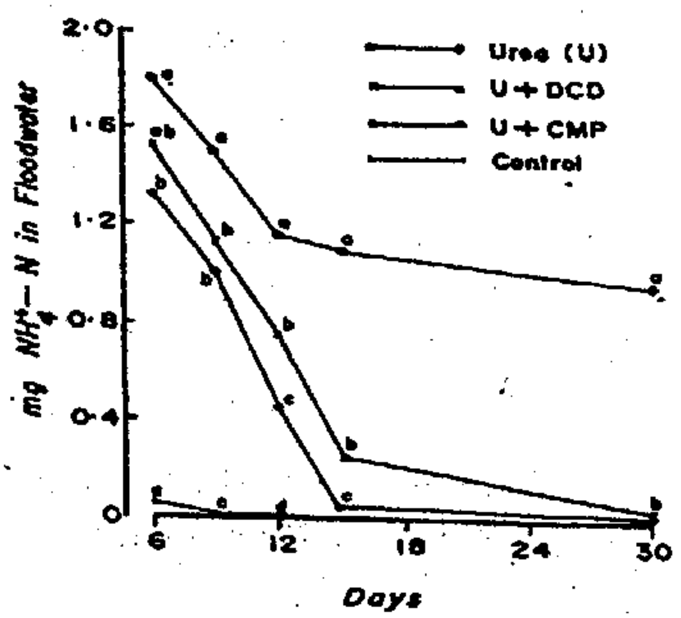

Fig.2. Flood water NH_4^+ - N as influenced by CMP and DCD addition

At the end of 30 days, no significant differences were observed between control, urea and urea + DCD treatments, whereas urea + CMP still had 0.96 mg NH_4^+-N in floodwater. CMP and DCD addition delayed the disappearance of NH_4^+-N in floodwater evidently due to their nitrification inhibiting property. Chauhan and Mishra (1989) observed higher NH_4^+-N in floodwater with nitrification inhibitor amended urea materials.

Floodwater NO_2^- + NO_3^--N

Urea + CMP treatment had the least amount of NO_2^- + NO_3^- except at 6 DAF, when differences among the treatments were not significant (Fig. 3). Nitrate content increased with urea alone and urea + DCD treatments up to 15 DAF followed by a decline possibly due to denitrification. The disappearance of NO_3^- from a flooded soil system is the simplest indicator of denitrification (Hauck 1979). Generally, urea alone had the highest build-up of NO_2^- + NO_3^-. Such high build-up of NO_2^- and NO_3^- in the absence of nitrification inhibitors have been reported earlier (Simpson *et al.* 1984). According to our results, DCD was not very effective in inhibiting nitrification under flooded conditions as evident from the fast disappearance of NH_4^+ (Fig. 2) and the relatively high build-up of nitrate in floodwater (Fig. 3). Simpson *et al.* (1985) also reported the inefficacy of DCD under flooded conditions. CMP effected an almost total inhibition under flooded conditions. The nitrification inhibition under aerobic conditions, at similar temperatures (30°C), was only 5.5%. At present the reason for this difference is not known. It is possible that CMP may be decomposing relatively slowly under flooded conditions as compared to aerated soils. Amberger and Vilsmeier (1979) found DCD to decompose at a slower rate with increasing soil moisture content.

Our results indicate that CMP and DCD are effective nitrification inhibitors at the low range of temperatures (10 and 20°C) as compared to 30°C. CMP holds promise for use in the flooded paddies.

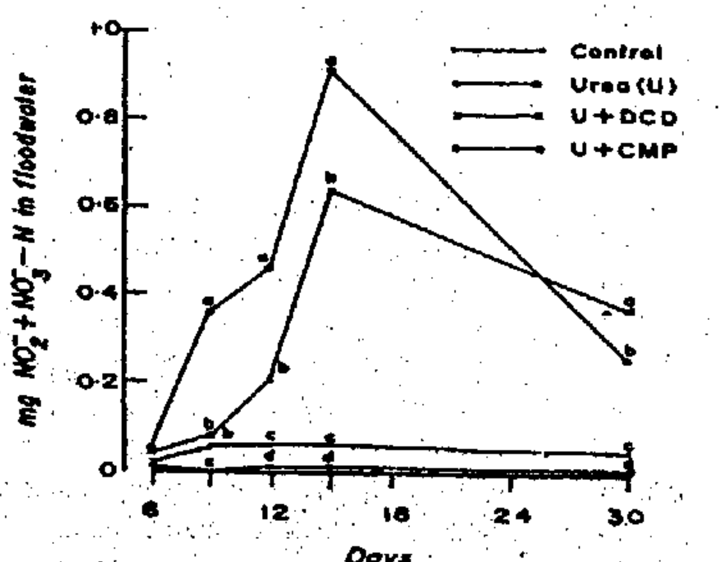

Fig.3. Effect on nitrification inhibitors CMP and DCD on nitrate content in floodwater

However, field scale studies are necessary to further evaluate the efficacy of this compound.

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Effect of K and Na Applied in Different Proportions on the Growth, Yield and Nutrient Content of Cassava (*Manihot esculenta* Crantz.)

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Abstract: Field experiments were conducted during 1991-1994 to study the effect of K and Na applied in different proportions on the growth, yield and nutrient content of cassava grown on a Rhodic Haplustox. Weight of plants was maximum when the proportion of K and Na applied was 50:50 as muriate of potash and sodium chloride (common salt), respectively. Application of Na alone significantly reduced plant weight. Highest number of roots, root volume and tuber yield were observed in plants receiving 50% K as muriate of potash and 50% Na as common salt. Proportionate decrease in number of roots and root volume was obtained when the proportion of Na exceeded 50%. Lower yields at 25 and 75% levels indicated that there was a synergistic effect in the combination of K and Na at 50% level. Content of N and P was more in Na-supplied treatments and K in full K treatment in the early stages of growth. Absorption of N and P decreased and K increased in Na-added treatments towards the later stages of growth. Na