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EFFECT OF DICYANDIAMIDE ON MICROBIAL ACTIVITY IN THE RHIZOSPHERE AND BULK SOILS UNDER COTTON

REHMAT ALI[1], JAVED IQBAL[2], UM-E-ROBAB[1] AND TARIQ MAHMOOD[1*]

[1]Nuclear Institute for Agriculture & Biology, PO Box 128, Jhang Road, Faisalabad-38000, Pakistan.
[2]School of Biological Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan.

Abstract

Study was conducted under greenhouse conditions to elucidate the effects of dicyandiamide (DCD, a nitrification inhibitor) on microbial activity in the root-zone and bulk soils under cotton fertilized with two levels (60 and 120 mg kg^{-1}) of urea-N. Dicyandiamide applied @ 15–30 mg kg^{-1} effectively inhibited nitrification at relatively high soil temperatures (19.5–33°C) prevailing during the four-week experiment period by the end of which 25–46% of the mineral N was found in NH_4^+ form in DCD-treated pots. Application of DCD caused a minor leaf tip necrosis but without negative effects on the biomass and N yields. Averaged across treatments, microbial activity (aerobic and anaerobic soil respiration, denitrification potential, microbial biomass carrying capacity and dehydrogenase activity) was highest in the root-zone soil followed by planted-bulk and unplanted soils. Averaged across soil types, microbial activity parameters generally showed higher values in the DCD-treated soil. The stimulatory effect of DCD was more pronounced at lower N application rate, and was consistently observed in the root-zone, planted bulk and unplanted soils. At higher N application rate, however, DCD had no effect on the microbial activity of the unplanted soil. Results suggested that relatively high concentrations of DCD required to inhibit nitrification under warm climates may not be phytotoxic but may have significant implications in soil microbial processes.

Introduction

Dicyandiamide (DCD) is an effective nitrification inhibitor and widely used in agriculture for improving fertilizer N efficiency (Vilsmeier, 1991; Aulakh *et al*., 2001; Gioacchini *et al*., 2002; Di & Cameron, 2004; Chaves *et al*., 2006). Besides, DCD may serve as N source for plants as it contains 67% N that becomes plant-available following its mineralization (Reider & Michaud, 1980). The mineralization of DCD in soil is strongly influenced by soil temperature (Rajbanshi *et al*., 1992), availability of organic carbon (Reddy, 1964) and Fe^{3+} hydroxides (Amberger & Vilsmeier, 1979). Although DCD can improve fertilizer N efficiency, it is well known to have phytotoxic effects including leaf tip and margin chlorosis and necrosis, and a reduction in the biomass yield (Sommer & Rossig 1978; Maftoun & Sheibany, 1979; Reeves & Touchton, 1986; Macadam *et al*., 2003). However, severity of these symptoms varies with plant species and with the DCD application rate (Reeves & Toughton, 1986). Dicyandiamide applied at 67-72 mg kg^{-1} is reported to reduce root and shoot growth of cotton (Reeves & Toughton, 1986).

Studies pertaining to DCD have been mostly conducted at relatively moderate soil temperature, whereas only few deal with the behaviour of DCD under soil temperatures ≥30°C (Rajbanshi *et al*., 1992; Aulakh *et al*., 2001) that are common during the summer season in semiarid subtropics. Besides, most of the literature on the use of DCD and other

*Corresponding author E.mail: tmahmood114@yahoo.com

nitrification inhibitors deals with their effectiveness in keeping the applied N fertilizer in NH_4^+ form and relatively less attention has been paid to their effects on the overall microbial activity in soil, particularly in the rhizosphere. Priming effect of DCD leading to increased mineralization of soil organic matter is well documented (Prasad & Power, 1995; Gioacchini *et al*., 2002; Chaves *et al*., 2006) and is attributed to NH_4^+, which being the predominant N source (e.g. in DCD-treated soils) is utilized preferably by microbes as compared to NO_3^- (Recous *et al*., 1990; Rangle-Castro & Taylor, 2002). Only limited information exists on the effect of N source on the belowground carbon partitioning in plants, particularly root exudation and subsequent effects on the rhizosphere microbial activity. Ammonium compared to NO_3^- nutrition of plants is known to stimulate root sugar exudation in hydroponics (Mahmood *et al*., 2002); the phenomenon proposed as an important mechanism through which plants feed the root-associated N_2 fixing soil microbes. In the soil-grown wheat and maize plants, which were supplied with either NH_4^+ or NO_3^- as N source, microbial activity in the rhizosphere was significantly higher under NH_4^+ compared to NO_3^- nutrition (Mahmood *et al*., 2005); the stimulatory effect was attributed mainly to the increased root sugar exudation under NH_4^+ nutrition. In that study, however, the effect of DCD *per se* was not discernable since the inhibitor was applied to both NH_4^+- and NO_3^--treated soils in order to keep the applied N in NH_4^+ form (in the NH_4^+-treated soil) and to balance the side effects of DCD (in NO_3^--treated soil). Since increased C availability is also known to stimulate mineralization of DCD (Reddy, 1964), persistence and activity of DCD in the rhizosphere may be different than that observed in bulk soils. However, comparative effects of DCD on microbial activity in the rhizosphere and bulk soils have not been reported earlier. The present study was conducted to examine the effects of DCD on microbial activity in the root-zone, planted-bulk and unplanted soils under cotton plants grown in pots with two urea-N levels.

Materials and Methods

The soil used in this study was a sandy-clay loam (Hafizabad Series; Haplic Yermosol; Anonymous, 1966) collected from the plough layer of a cotton field. It contained: total organic C, 0.54%; 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%. Five kg soil in plastic pots (20×20 cm, inner diameter×depth) received a basal dose of K (as K_2SO_4) and P (as single superphosphate), each applied at 50 mg kg^{-1}. The pots were irrigated with tap water (60% WHC) and after 24-h equilibration the soil was mixed and sown with cotton (*Gossypium hirsutum* L. var CM-499; 10 seeds pot^{-1}). Fifteen days after germination, the plant population was reduced to four pot^{-1} and treatments applied. There were eight treatments (replicated four times) comprising two urea-N levels (60 and 120 mg kg^{-1}), each with four levels of DCD (0, 7.5, 15 and 30 mg kg^{-1}). In a preliminary laboratory study employing various nitrification inhibitors, only DCD applied at high concentrations (15–30 mg kg^{-1}) was found effective in retarding nitrification at high soil temperatures (33°C) that prevail during the cotton-growing season in this region (unpublished data). To evaluate the efficacy of DCD in retarding nitrification of the applied urea-N, an unplanted set of pots with similar treatments was also kept under same conditions as the planted pots. The soil moisture was maintained at 60% WHC throughout the experiment.

Four weeks after treatment application, plants were harvested by cutting shoots 2-cm above the soil surface. The root-zone soil was sampled by inserting a core sampler (10×150 mm, inner diameter×depth) around the tap root. In this way, four root-zone soil cores were extracted from each pot and were pooled together to represent one replicate. Rest of the soil of the planted pots represented as the planted-bulk soil. Visible roots were manually removed, and all soils (root-zone, planted-bulk and unplanted) were sieved (<2 mm) and stored overnight at 4°C before measuring soil microbial activity parameters viz., aerobic and anaerobic respiration, denitrification capacity, microbial biomass carrying capacity and dehydrogenase activity. Roots and shoots were dried at 60°C for 48 h, weighed and ground (<0.5 mm) before analyzing for the total N.

For measuring aerobic soil respiration, 10-g portions of the moist soil were taken in 100-ml serum vials, vials sealed with silicone rubber septa and incubated at 30°C. After 12, 24, 36 and 48 h, the headspace was analyzed for CO_2-C by gas chromatography using a thermal-conductivity detector (TCD). For denitrification potential and anaerobic soil respiration, 10-gram portions of the moist soil were taken in 100-ml serum vials and treated with 10 ml of KNO_3 solution to provide NO_3^- level of approximately 200 mg N kg^{-1}. After sealing with silicone rubber septa, the vials were evacuated and flushed with O_2-free N_2 three times. The headspace was then replaced with 5% acid-washed C_2H_2 and the vials incubated at 30°C. After 12, 24, 36 and 48 h, the contents in the vials were vigorously shaken by hand and the headspace analyzed for N_2O (denitrification potential) and CO_2 (anaerobic soil respiration) by gas chromatography using a TCD. The N_2O data were corrected for that dissolved in the solution phase using Bunsen absorption coefficients (Moraghan & Buresh, 1977). Microbial biomass carrying capacity of the soil (Groffman & Tiedje, 1989) was determined by chloroform-fumigation incubation technique (Jenkinson & Powelson, 1976). Ten-g portions of the moist soil in 100-ml serum vials were fumigated with ethanol-free $CHCl_3$ for 24 h at 25°C. The $CHCl_3$ vapours were removed by repeated evacuation, the soils reinoculated with 0.1 g fresh soil, vials sealed with rubber septa and incubated at 30°C. After 10 days, the headspace was analyzed for CO_2-C by gas chromatography employing TCD. Microbial biomass carrying capacity was calculated by dividing the amount of CO_2-C evolved from the fumigated soil by a k_c factor of 0.45 (Jenkinson & Ladd, 1981). Soil dehydrogenase activity was determined by triphenyl formazan method as described by Casida *et al*., (1964). Total N content of plant samples was determined by a micro-Kjeldahl method (Bremner & Mulvaney, 1982), whereas the soil mineral-N was measured by a micro-Kjeldahl method after extracting 10-g soil with 50 ml of 2*N* KCl (Keeney & Nelson, 1982).

Data were subjected to an analysis of variance followed by DMRT (Duncan's multiple range test) as described by Gomez & Gomez (1984). Results are reported on the basis of soil/plant dry weight and as mean of four replicates.

Results

The soil temperature in pots of 5 cm depth during the four-week treatment period was relatively high and varied from 19.5–33°C (average 26.8°C). In unplanted pots without DCD, nitrification of the applied urea-N was almost complete within four weeks (Table 1). The inhibitory effect of DCD on nitrification was more pronounced in treatments receiving higher urea-N level where after four weeks, 9, 23 and 46% of the mineral N was still present as NH_4^+ in treatments receiving DCD at 7.5, 15 and 30 mg kg^{-1}, respectively (p<0.05; Table 1). At the lower N application rate, however, the inhibitory effect of DCD persisted only in treatment receiving highest DCD concentration (30 mg kg^{-1}) where 25% of the mineral N was found in the NH_4^+ fraction after four weeks.

At the lower N application rate, DCD did not affect the root and shoot dry matter yields, nor did it show significant effects on N concentration or uptake (Table 1). However, at the higher N application rate, root biomass was significantly higher with DCD applied at 30 mg kg^{-1}, whereas shoot biomass also increased due to DCD applied at 15–30 mg kg^{-1} ($p<0.05$; Table 1). At the higher N application rate, DCD applied at 15 mg kg^{-1} significantly increased the shoot N concentration and uptake ($p<0.05$; Table 1).

In the absence of DCD, increasing the N application rate from 60 to 120 mg kg^{-1} generally had no effect on the soil microbial activity except that it increased the anaerobic soil respiration of the planted-bulk and unplanted soils, and the dehydrogenase activity of the unplanted soil. The effect of DCD varied with its concentration, N application rate and the soil type. Averaged across treatments, aerobic and anaerobic soil respiration, denitrification potential, microbial biomass carrying capacity and dehydrogenase activity showed highest values for the root-zone soil, followed by the planted-bulk and unplanted soils ($p<0.05$; Table 2, soil type means). Averaged across soil types (treatment means, Table 2), microbial activity parameters showed higher values in the DCD-treated soil; the stimulatory effect being more pronounced and consistent in treatments receiving 60 mg kg^{-1} of urea-N.

At the lower N application rate, DCD applied at 15–30 mg kg^{-1} increased aerobic respiration (26–31% increase), denitrification potential (19–30% increase), microbial biomass carrying capacity (28–29% increase) and dehydrogenase activity (8–17% increase) of the root-zone soil ($p<0.05$), whereas anaerobic soil respiration also increased (12% increase) with DCD applied at 7.5 mg kg^{-1}. The stimulatory effect of DCD was also recorded in the planted-bulk and unplanted soils ($p<0.05$), except that microbial biomass carrying capacity of the planted-bulk and denitrification potential of the unplanted soils were not affected. In the unplanted soil, the stimulatory effect of DCD (where observed) was generally of the same magnitude (for anaerobic soil respiration and microbial biomass carrying capacity) or even higher (for aerobic soil respiration and dehydrogenase activity) than that observed for the root-zone or the planted-bulk soils.

At higher N application rate also, at least one concentration of DCD (mostly 15 mg kg^{-1}) significantly increased the microbial activity in the root-zone soil, except that denitrification potential was not affected. Increasing the N application rate from 60 to 120 mg kg^{-1} greatly modified the effect of DCD in the planted-bulk and the unplanted soils. For the planted-bulk soil, the stimulatory effect of DCD was only observed on aerobic respiration (33% increase), whereas dehydrogenase activity was even suppressed ($p<0.05$). For the unplanted soil, DCD applied at 30 mg kg^{-1} even decreased the anaerobic soil respiration (29% decrease; $p<0.05$), whereas other parameters were not affected.

Discussion

Dicyandiamide effectively inhibited nitrification during the four-week treatment period, and in unplanted pots treated with DCD at 30 mg kg^{-1} as much as 25% (in low-N treatment) to 46% (high-N treatment) of the mineral N existed in NH_4^+ form. We may not rule out the contribution of DCD-N itself toward the soil NH_4^+ since the high soil temperatures during the experimental period could have caused substantial mineralization of DCD. Present results indicate that at high soil temperatures, such as those prevailing during the summer season in semiarid subtropics, relatively higher concentrations of DCD would be required for effective inhibition of nitrification. Despite the high DCD concentrations used in the present study, the only visible phytoxicity symptom was a minor leaf-tip necrosis in all DCD-treated cotton plants but without showing negative effects on the biomass and N yields. In some treatments, DCD even stimulated the cotton

plant growth as well as the N uptake. The lack of the injurious effects of DCD on cotton plant growth in the present study may be due to relatively short period of exposure to DCD because of the high soil temperature that was conducive for its mineralization, which probably started soon after its application. These results signify that under hot summer climates such as those prevailing in the Indo-Pakistan subcontinent, the high DCD application rates (e.g., 30 mg kg^{-1} or ~ 67 kg ha^{-1} in the present study) required to achieve effective inhibition of nitrification in cotton fields may not be phytotoxic as compared to relatively mild or cool climates which prolong the half-life of DCD (Di & Cameron, 2004). Besides, keeping in view the increased root sugar exudation by plant roots under NH_4^+ nutrition (Mahmood *et al*., 2002) and the increased mineralization of DCD in the presence of available C (Reddy, 1964), it is envisaged that conditions in the rhizosphere of DCD-treated plants particularly favoured the mineralization of DCD thus alleviating its injurious effects on the cotton plant growth.

Much higher microbial activity observed in the root-zone and the planted-bulk than unplanted soils is a common phenomenon and attributable to the root-derived C (Priha *et al*., 1999; Mahmood *et al*., 1997; Mahmood *et al*., 2005). In the present study, almost all microbial activity parameters generally showed higher values in treatments receiving DCD, particularly at the lower N application rate. At the lower urea application rate, the stimulatory effect of DCD was generally consistent in the root-zone, planted-bulk and unplanted soils, whereas at higher N application rate, it was totally absent in the unplanted soil. The increased microbial activity in the DCD-treated unplanted soil is attributable to the abundance of NH_4^+-N and the preferential use of NH_4^+ by soil microbes. However, the stimulated microbial activity in the unplanted soil only at the lower urea-N application (i.e. N-limiting conditions) indicates the role of DCD-N that was made available to soil microbes following its mineralization. In the root-zone and the planted-bulk soils, the stimulatory effect of DCD may also be attributed to the increased root exudation due to NH_4^+ (Mahmood *et al*., 2002; 2005). In the present study, such indirect stimulatory effect of DCD on the microbial activity in the root-zone soil was obvious in treatments receiving higher N level. At the lower N application rate, the magnitude of the stimulatory effect in the unplanted soil that was at least as much as that observed in the root-zone soil, suggests that the role of NH_4^+ and DCD-N *per se* was also important besides the indirect stimulatory effects of NH_4^+ through increased root sugar exudation.

In the present study, the lower urea-N application rate (60 mg kg^{-1}) is close to that recommended for cotton crop in this region (~140–160 kg ha^{-1}). Therefore, the observed stimulatory effects of DCD may also be expected under field conditions. Moreover, considering the relatively high concentrations of DCD required for inhibiting nitrification under warm climates, and the role of DCD *per se* in stimulating the microbial activity in soil, the use of DCD under semiarid subtropics may have significant implication in nutrient transformations in soil besides nitrification.

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