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Effect of crotonylidene diurea on soil enzyme activity and nutrient availability in a Vertisol

ATUL BHAGAWAN PAWAR[1], M R CHAUHAN[2], K D KALE[3], KAPIL ATMARAM CHOBHE[4], ANIL KUMAR VERMA[5], VIVEK KUMAR TRIVEDI[6], GANPAT LOUHAR[7] and RAVINDRA KUMAR REKWAR[8]

Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra 413 722, India

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ABSTRACT

The release of nitrogen (N) from slow release N fertilizers (SRNF) is affected by soil microbial activity. However, several studies have shown variable results regarding the effect of microbial population size on the release of nutrient from slow release fertilizers. To understand this relationship clearly, changes in activities of two soil enzymes (urease and dehydrogenase), microbial population and available macronutrients *viz* nitrogen (N), phosphorus (P) and potassium (K) were assessed in an incubation study conducted under ambient condition. The six treatments were used for present investigation as control, 100% urea and crotonylidene diurea (CDU) @ 100, 75, 50 and 25% of recommended dose. The results indicated that the addition of N through commercially available urea and CDU as per general recommended dose of nutrients (GRDN) showed an increase in soil enzyme activity, microbial population, available N, P and K. The urease enzyme activity was found to be highest of nutrients in GRDN treatment at 60 days after addition (DAA) (39.55 µg NH_4^+-N/g soil/hr) and in 100% N through CDU (37.45 µg NH_4^+-N/g soil/hr) at 90 DAA. The soil dehydrogenase enzyme activity was significantly higher in GRDN at 7, 14, 21 and 30 DAA (1.32, 1.44, 1.56 and 1.68 µg TPF/g soil/hr, respectively). However, at 60 and 90 DAA the dehydrogenase enzyme activity was significantly higher in 100% N through CDU (1.89 and 1.63 µg TPF/g soil/hr, respectively). The soil available N content increased up to 60 DAA in all the treatments and it was significantly higher in GRDN followed by 100% N through CDU. However, it was significantly higher at all the periods of incubation over 75, 50, 25 and 100% N through CDU.

Key words: Bacterial population, Crotonylidene diurea, Incubation study, Enzyme activity, Nitrogen release, Urea

Nitrogen is an essential and primary nutrient, required by all the crops in large amount. However, nitrogen fertilizer added in soil get leached or washed out. It not only causes economic loss but also gives invitation to soil, water and environmental pollution. The use of slow release fertilizer up to some extent useful and eco-friendly option to overcome these problems. Slow-release fertilizers (SRFs) are condensation products obtained by reacting urea, the most common mineral fertilizer characterized by high nitrogen (N) content and relatively low cost, with several aldehydes. These SRFs release N at slower rates compared to conventional N-fertilizers, such as urea releasing N rapidly by hydrolysis, thus theoretically SRFs facilitate better N uptake and utilization by crop plants. Among SRFs, urea-formaldehyde (UF), isobutylidene diurea (IBDU), and Crotonylidene diurea (CDU) have gained popularity (Trenkel 1997). CDU, a ring-structured compound, is produced by condensation of urea with acetic aldehyde. The microbial activity and hydrolysis drive the degradation of CDU. Thus, soil moisture, temperature, and pH influence the CDU-N release (Trenkel 1997). Studies of enzyme activities in soil are important as they indicate the potential of the soil to support biochemical processes which are essential for the maintenance of soil fertility (Dkhar and Mishra 1983). Among all enzymes in the soil environment, dehydrogenases are one of the most important, and are used as an indicator of overall soil microbial activity (Gu *et al.* 2009; Salazar *et al.* 2011), because of their intracellular nature (Moeskops *et al.* 2010; Zhao *et al.* 2010). Slow-release fertilizers have been largely studied under different temperature and soil

[1]Ph D Scholar (e-mail: atulpawar2006@gmail.com), [4]Scientist (e-mail: chobhekapil27@gmail.com), [5]Ph D Scholar (e-mail: anilv0560@gmail.com), [6]Research Associate (e-mail: vivektrivedi002@gmail.com), [7]Ph D Scholar (e-mail: loharganpat95@gmail.com), [8]Ph D Scholar (e-mail: ravindra.rekwar001@gmail.com), Division of Soil Science and Agricultural Chemistry, ICAR-Indian Agricuraltul Research Institute, New Delhi 110 012. [2]Professor (e-mail: mahavirsinghchauhan5@gmail.com), Department of Soil Science and Agricultural Chemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri 413722 (India). [3]Assistant Professor (e-mail: kd_kale@rediffmail.com), Department of Irrigation Water Management, Mahatma Phule Krishi Vidyapeeth, Rahuri 413 722 (India).

moisture regimes (Engelsjord *et al.* 1997; Agehara and Warncke 2005), whereas the effect of size and activity of soil microflora on SRF degradation has received less attention with contrasting results observed (Koivunen and Horwath 2004). To date, the question of whether N release from SRFs proceeds faster in soils with higher enzyme activity is still open. The aim of current study was therefore to better understand such relationships by determining N release from CDU and urea in soil with different enzyme activities and bacterial population. Given that N release from GRDN through Urea and CDU should be mainly driven by enzyme activity, we hypothesized a faster N release from Urea and CDU in soils with higher enzyme activity and bacterial population.

MATERIALS AND METHODS

The soil for conducting present investigation was collected from ICAR-Soil Test Crop Response Correlation Project (STCR), Research Farm, Mahatma Phule Krishi Vidyapeeth, Rahuri during 2016, grouped under Inceptisols order belong to Sawargaon (Pather) soil series which comprises fine montmorillionitic isohyperthemic family of *Vertic Haplustepts*. The soil was medium black with 80cm depth having swell shrink property. The texture of the soil was clayey with slightly alkaline in reaction (pH 8.12) with calcium carbonate content of 7.90%. The soil was low in available nitrogen (188.16 kg/ha), medium in available phosphorus (21.43 kg/ha) and very high in potassium (548.80 kg/ha), and micronutrients, viz. Fe, Mn, Zn, Cu was 6.27, 12.78, 0.96, 2.92 mg/kg, respectively. The initial urease, dehydrogenase activity and microbial population was 19.60 µg NH_4^+-N/g soil/hr, 0.97 µg TPF/g soil/hr, 17.25 cfu × 10^6/g soil, respectively. The six treatments were used for present investigation as control, 100% urea and CDU @ 100, 75, 50 and 25% of recommended dose and replicated four times. Plastic cylindrical bowls of 11 cm × 24 cm (height × diameter) size were used for incubation study. Pots were filled with 2 kg air dry, 2 mm sieved soil and saturated with water at field capacity and maintained throughout the incubation study. Nitrogen was applied as per treatment through urea and CDU while recommended P and K were added through single super phosphate (SSP) and muriate of potash (MOP) in solution form. Equal amount of organic manures was added in all the pots. The periodical nutrient release, i.e. NH_4^+-N, NO_3^--N and soil enzymes activities, viz. urease and dehydrogenase were assessed by conducting an incubation study in the laboratory under ambient condition. Soil samples were drawn periodically from each treatment at 0, 7, 14, 21, 30, 60, and 90 days and collected samples were analyzed for NH_4^+-N, NO_3-N, urease (Tabatabai and Bremmer 1972) and dehydrogenase (Casida *et al.* 1964) determination. The microbial population was determined by the method described by Halvorsun and Zeiglar (1993). The data were analyzed statistically as per standard procedures of completely randomized design (CRD).

RESULTS AND DISCUSSION

Effect on urease, dehydrogenase and microbial population

The soil urease enzyme activity as influenced by the nitrogen application through CDU and GRDN are reported in Table 1. An increment in soil urease enzyme activity irrespective of treatments was recorded with increased period of incubation. The highest urease enzyme activity (39.55 µg NH_4^+-N/g soil/hr) was observed at 60 DAA in GRDN and at 90 DAA in 100% N through CDU (37.45 µg NH_4^+-N/g soil/hr). The treatment GRDN recorded significantly higher values of soil urease enzyme activity at all the periods of

Table 1 Effect of CDU on periodical soil urease enzyme activity under incubation study

Treatment	Urease enzyme (µg NH_4^{+}-N/g soil/hr)						
	7 DAA	14 DAA	21 DAA	30 DAA	60 DAA	90 DAA	Cumulative Urease
GRDN (200 :40:200 g N:P_2O_5: K_2O + 10 kg FYM plant^{-1})	31.1	33.6	35.3	38.1	39.5	35.3	213 (35.5)
100 % N–CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	27.3	29.4	31.5	33.9	35.7	37.4	195 (32.5)
75 % N- CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	25.5	27.3	28.3	29.7	31.8	33.9	176 (29.4)
50 % N-CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	23.4	25.2	25.9	27.6	29.7	31.5	163 (27.2)
25 % N- CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	22.4	23.8	24.5	25.5	26.9	28.3	151 (25.2)
Absolute control	19.9	21.3	21.3	21.3	20.6	20.6	125 (20.8)
Initial value				19.9			
SE $\pm$	0.16	0.17	0.10	0.14	0.13	0.13	-
CD at (P=0.05)	0.49	0.52	0.30	0.42	0.39	0.39	-

Figures in parentheses are mean of periodical analysis. CDU: Crotonylidene diurea, DAA: days after addition, GRDN: general recommended dose of nutrients.

incubation except at 90 DAA. Slow release products have been developed to delay urease enzyme activity because of delay in bacterial oxidation of NH_4^+ by depressing the activities of nitrifier microorganisms in soil, whereas urease inhibitors are 3 compounds that delay the hydrolysis of urea also reported by Kissel *et al.* (2008).

The soil dehydrogenase activity was significantly higher in GRDN at 7, 14, 21 and 30 DAA (1.32, 1.44, 1.56 and 1.68 µg TPF/g soil/hr, respectively) with mean value of 1.48 µg TPF/g soil/hr. However, at 60 and 90 DAA, dehydrogenase enzyme activity was significantly higher in 100% N through CDU (1.89 and 1.63 µg TPF/g soil/hr in that order) with mean value of 1.56 µg TPF/g soil/hr (Table 2), meaning that microbial metabolic activity had increased due to incorporation of compounds capable of activating the soil's autochthonous biomass (Pascual 1998). The reduced levels of CDU for providing the nitrogen reduced the dehydrogenase enzyme activity throughout the incubation period. Thus, the dehydrogenase enzyme activity in soil was enhanced by treatment GRDN but at later stage, addition of 100% N through CDU enhanced the dehydrogenase enzyme activity at 60 and 90 DAA. It was increased with an advanced period of incubation. Similar relationship between microbial biomass, total N and soil dehydrogenase activity were reported by Goyal *et al.* (1992) and Perucci (1992). The bacterial population (Fig 1) at 7 DAA was significantly higher in GRDN (25.50 cfu × 10^6/g soil) and statistically at par with 100% N through CDU at 90 DAA (27.0 and 26.50 cfu × 10^6/g soil).

Soil ammonical nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N)

The ammonical nitrogen content was increased up to 60 DAA in all the treatments and it was significantly higher in treatment GRDN followed by 100% N through CDU. However, it was significantly higher at all the periods of incubation over 75, 50 and 25% N through CDU. At 60 DAA significantly highest (65.10 mg/kg soil) ammonical nitrogen was observed in treatment GRDN followed by 100% N through CDU (62.30 mg/kg soil). All the treatments recorded highest ammonical nitrogen at 60 days of incubation, whereas the values for ammonical nitrogen

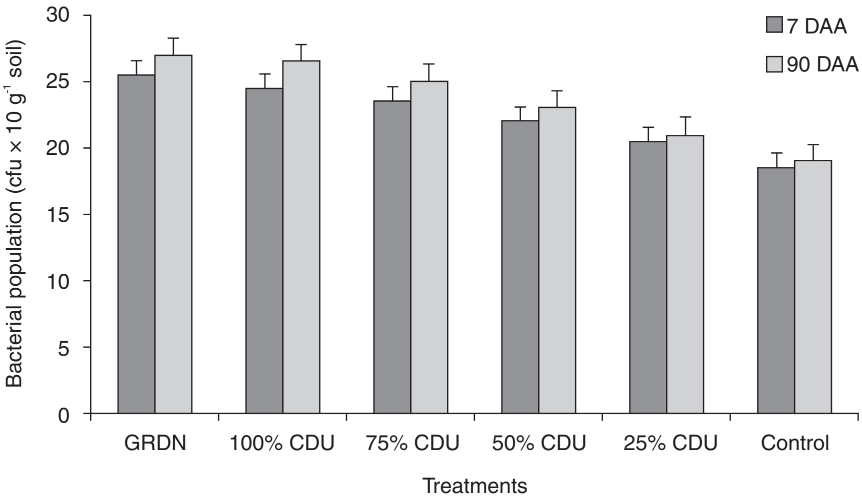

Fig 1 Effect of crotonylidene diurea (CDU) on periodical bacterial population under incubation study. Bar represents the CD value at P<0.05.

Table 2 Effect of CDU on periodical soil dehydrogenase activity under incubation study.

Treatment	Dehydrogenase enzyme activity (µg TPF/g soil/hr)						
	7 DAA	14 DAA	21 DAA	30 DAA	60 DAA	90 DAA	Cumulative DHA
GRDN (200 :40:200 g N:P_2O_5: K_2O + 10 kg FYM plant^{-1})	1.32	1.44	1.56	1.68	1.51	1.35	8.86 (1.48)
100 % N–CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	1.28	1.40	1.51	1.62	1.89	1.63	9.33 (1.56)
75 % N-CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	1.24	1.36	1.46	1.56	1.77	1.55	8.94 (1.49)
50 % N-CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	1.21	1.32	1.41	1.52	1.68	1.47	8.61 (1.44)
25 % N- CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	1.17	1.29	1.36	1.48	1.61	1.46	8.37 (1.39)
Absolute control	1.03	1.03	1.12	1.12	1.10	1.06	6.46 (0.98)
Initial value				0.97			
SE±	0.00	0.01	0.00	0.00	0.01	0.00	-
CD at (P=0.05)	0.01	0.02	0.01	0.01	0.03	0.01	-

Figures in parentheses are mean of periodical analysis. CDU: Crotonylidene diurea, DAA: days after addition, GRDN: general recommended dose of nutrients.

Table 3 Effect of CDU on periodical soil ammonical nitrogen (NH_4^+-N) under incubation study

Treatment	Ammonical nitrogen (NH_4^+–N) (mg/kg soil)						
	7 DAA	14 DAA	21 DAA	30 DAA	60 DAA	90 DAA	Cumulative NH_4^+–N
GRDN (200 :40:200 g N:P_2O_5: K_2O + 10 kg FYM plant^{-1})	58.1	60.2	63.7	64.4	65.1	60.9	372.4 (62.0)
100 % N–CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	55.3	55.3	58.8	60.9	62.3	58.1	350.7 (58.4)
75 % N- CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	52.5	53.9	55.3	56.7	57.5	55.3	331.2 (55.2)
50 % N-CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	50.4	52.5	53.9	55.3	56.0	53.8	321.9 (53.7)
25 % N- CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	49.7	50.4	51.8	53.2	53.9	51.8	310.8 (51.8)
Absolute control	46.9	47.6	48.3	49.0	48.3	46.9	287.0 (47.8)
Initial value					44.7		
SE±	0.29	0.39	0.33	0.39	0.24	0.26	-
CD (P=0.05)	0.85	1.15	0.98	1.15	0.70	0.78	-

Figures in parentheses are mean of periodical analysis. CDU: Crotonylidene diurea, DAA: days after addition, GRDN: general recommended dose of nutrients.

declined at 90 DAA than 60 DAA (Table 3). The decrease in ammonical nitrogen in CDU application with an advanced period of incubation might be associated with the decrease in substrate concentration to forms NH_4^+-N in soil.

The application of nitrogen as per GRDN through urea and through CDU significantly varied the periodical nitrate nitrogen content in soil (Table 4). It increased with an advanced incubation period and the highest content of nitrate nitrogen was observed at 30 DAA in all the treatment. The highest nitrate nitrogen content was observed in GRDN at 30 DAA (18.90 mg/kg soil) followed by 100% N through CDU (16.80 mg/kg soil). The decreased levels of nitrogen through CDU decreased the nitrate nitrogen content at all the periods of incubation. The application of urea fertilizer release more nitrate nitrogen (NO_3^--N) throughout the periods of 60 days of incubation in treatment GRDN. Similar findings have also been reported by Sonar and Kamire (1979) and Bhuyia *et al.* (1974).

Table 4 Effect of CDU on periodical soil nitrate nitrogen (NO_3^--N) under incubation study

Treatment	Nitrate nitrogen (NO_3^-–N) (mg/kg soil)						
	7 DAA	14 DAA	21 DAA	30 DAA	60 DAA	90 DAA	Cumulative NO_3^-–N
GRDN (200:40:200 g N:P_2O_5: K_2O + 10 kg FYM plant^{-1})	16.1	16.8	18.2	18.9	17.5	16.1	103.6 (17.26)
100 % N–CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	14.7	15.4	16.1	16.8	15.4	14.7	93.1 (15.51)
75 % N- CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	13.3	13.3	14.0	15.4	13.3	12.6	81.9 (13.65)
50 % N-CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	11.9	13.3	13.3	14.0	12.6	11.2	76.3 (12.71)
25 % N- CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	10.5	11.2	11.9	13.3	11.9	10.5	69.3 (11.55)
Absolute control	10.3	11.2	11.4	12.6	12.2	10.1	67.8 (11.30)
Initial value				10.6			
SE±	0.29	0.44	0.23	0.31	0.33	0.20	-
CD (P=0.05)	0.85	1.30	0.69	0.92	0.98	0.60	-

Figures in parentheses are mean of periodical analysis. CDU: Crotonylidene diurea, DAA: days after addition, GRDN: general recommended dose of nutrients.

Table 5 Effect of CDU on periodical soil available phosphorus and potassium

Treatment	Av. phosphorus (mg/kg soil)			Av. potassium (mg/kg soil)		
	7 DAA	90 DAA	Cumulative av. P	7 DAA	90 DAA	Cumulative av. K
GRDN (200:40:200 g N:P_2O_5: K_2O + 10 kg FYM plant^{-1})	11.0	11.6	22.6 (11.3)	267.5	272.5	540.0 (270)
100 % N–CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	10.9	11.3	22.3 (11.1)	267.5	271.2	538.7 (269)
75 % N- CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	10.5	11.1	21.7 (10.8)	260.0	265.3	525.3 (264)
50 % N-CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	10.4	10.7	21.2 (10.6)	260.0	261.2	521.2 (263)
25 % N- CDU+ (40:200 g P_2O_5: K_2O + 10 kg FYM plant^{-1})	10.3	10.5	20.9 (10.4)	257.5	260.0	517.5 (260)
Absolute control	9.6	9.7	19.4 (9.7)	227.5	230.0	457.5 (258)
Initial value		9.4			225.0	
SE $\pm$	0.10	0.03	0.52	0.83	0.55	0.69
CD (P=0.05)	0.30	0.09	1.54	2.48	1.64	2.06

Figures in parentheses are mean of periodical analysis. CDU: Crotonylidene diurea, DAA: days after addition, GRDN: general recommended dose of nutrients.

Soil available phosphorus and potassium

The soil available phosphorus (P) and potassium (K) contents in soil was significantly increased over the initial value at 7 and 90 DAA (Table 5). The treatment GRDN and 100% N through CDU was found statistically at par with each other for soil available phosphorus at 7 DAA (11.05 and 10.93 mg/kg soil, respectively) and significantly higher in GRDN at 90 DAA (11.62 mg/kg soil). Whereas, soil available potassium content was found statistically at par with each other in treatment GRDN and 100% N through CDU at 7 DAA (267.5 and 267.5 mg/kg soil, respectively) and at 90 DAA (270.0 and 269.4 mg/kg soil, respectively). Soil available phosphorus and potassium was increased at 90 DAA compared to 7 DAA. This might be because of increased microbial population resulting organically bound phosphorus and potassium being mineralized and become available. Similar results were also recorded by Lal *et al.* (2000).

Conclusion

From the study it may be concluded that soil urease and dehydrogenase enzyme activity and bacterial population enhanced with use of 100% N through CDU due to increased content of organic carbon and nutrients. The results of study indicated that addition of CDU and Urea generally had positive effects on the studied soil enzyme activities. The periodical ammonical and nitrate nitrogen, available phosphorus and potash in an incubation study were significantly higher in treatment GRDN and numerically followed by 100% N through CDU.

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