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Biodegradability and Nitrogen Use Efficiency of Crotonaldehyde Diurea in Wheat

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Abstract

A field experiment was conducted to test the efficacy of Rhodococcus spp for biodegradability of crotonaldehyde diurea and its N use efficiency in wheat (Triticum aestivum L.)"at different critical growth stages and five treatments of nitrogen. The soil sample was collected and attempted to isolate the Rhodococcus spp. on selective medium. The Rhodococcus spp. showed profuse growth in a medium containing 0.1 % urea and slightly less profuse growth on the medium containing 0.1 % CDU as a sole nitrogen source. The CDU contained ammonical and nitrate nitrogen 19.6 and 13.0 %, respectively. It thus appeared that the soil microorganism Rhodococcus spp. can utilize the CDU probably suggesting that microorganism could have enzymatic activity which can degrade CDU. The activity of nitrate assimilating enzyme was assayed from the leaf tissues at four critical stages showed that the treatment T5 (100 % CDU) recorded highest activities of NR, NiR, and GS, high nitrogen up take, high NUE and higher grain yield. Thus it appeared that Crotonaldehyde Diurea (CDU) is biodegradable by the Rhodococcus spp. present in the soil rhizosphere and applied once at the time of sowing.

Key words: Crotonaldehye Diurea, Nitrogen Use Efficiency, Wheat, *Rhodococcus spp.* Nitrate Assimilating Enzymes

INTRODUCTION

The nitrogen is the major nutrients for the crop plants that can be derived from the soil organic and inorganic commercial fertilizers applied by the farmers. In plant nutrition, soil and plants are two antagonistic systems that compete for the nutrients available in or applied to the soil. This competition is the main reason why only a portion of nutrients is taken up and used by the plant and crops grown, while another portion is immobilized in the soil or lost by denitrification/volatization and leaching. The fertilizer industry has developed special types of fertilizers and fertilizer modification which avoid or at least reduce such losses through the use of foliar fertilizers and slow and controlled nutrient release fertilizers.

A more practical route is the use of nitrogen fertilizer which releases the nutrient according to the plants' requirement. The microbial degradable N-product such as urea–formaldehydes (UFs and other urea-aldehyde composition) are commonly referred to in the trade as slow release fertilizers and coated or encapsulated products as controlled– release fertilizer.

Cyclodiurea was first patented as a slow-release fertilizer in 1959. The Chisso Corporation developed an economic, continuous industrial process to produce CDU from acetaldehyde and urea in 1962(Hauck and Koshino 1971) CDU is produced in Japan using the Chissoasahi process (acetaldehyde+urea) but, in Germany, BASF use crotonaldehyde + urea. The molecule has a cyclic structure and is formed by the acid-catalyzed reaction of urea and acetaldehyde. CDU decomposed by both hydrolysis and microbial processes in the soil, and temperature, soil moisture and biological activity affect the rate of release. The degradation is slower than that of IBDU (Isobutyraldehyde urea), even in acid soils. As with IBDU, the particle

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size of CDU greatly influences the rate of N release and the agronomic performance is similar to that of IBDU. A number of condensation products of urea and different aldehydes are used as slow- release nitrogen fertilizer on professional turf, in green houses, on lawns, for gardening and landscaping and they have been employed to improve the nitrogen status of forest soils or in bioremediation processes. At a molar ratio of urea to formaldehyde of 1.2- 1.5: 1 during synthesis, condensates suitable for use as slow release fertilizers are formed, consisting of a mixture methyleneurea polymers having a short chain length, such as methylenediurea (diureidomethane [MDU],) dimethylenetriurea [DMYU], trimethylenetetraurea [TMTU], etc. These are known to be mineralized by microbial activity and approximately 220,000 tons per year are used in the United State, Western Europe, Israel, and Japan. Recently, it was shown that methyleneureas are degraded via hydrolysis catalyzed by a specific enzyme, methylenediurea deiminase (methylenediureas).

At variance with urea-formaldehyde the result of the condensation of isobutyraldehyde or crotonaldehyde (or acetaldehyde) with urea yields single oligomers, isobutylidenediurea (IDU) or crotonaldehyde diurea (CDU), the former consisting of two urea moieties linked via an aliphatic branched residue, the latter of one urea moiety linked to a heterocyclic residue. Approximately 100,000 tons of these products are used annually in Western Europe, Japan and United States; In Western Europe, IDU represent the largest group of the slow-and controlled release fertilizers used. Environmental factors such as moisture, temperature, and pH are essential affecting the hydrolysis and decomposition products. Attempt to classify these fertilizers, with regard to the mechanism of their degradation, led to grouping into three patterns. The microbial decomposition pattern (urea formaldehyde), the hydrolytic and microbial decomposition pattern (urea crotonylaldehydes) and abiotic hydrolytic pattern (IDU) has been taken into consideration. In the present study, strains of different bacteria degrading these fertilizer were isolated from soil and the enzymes involved in there degradation were purified and characterized to elucidate the pathways of degradation of these compounds. Currently, there are over 40 species classified under the genus *Rhodococcus* (Euzeby, 2011). *Rhodococci* have many enzymes that allow them to carry out a number of chemical reactions that have been useful in the environmental and industrial biotechnology fields. Strain *R. erythropolis* are capable of carrying out dehydrogenation, hydrolysis, oxidation, desulfurization, hydroxylation, dehalogenation and epoxidation reactions (De Carvalho 2005). Due to this enzyme, some *Rhodococci* are capable of using gaseous hydrocarbons like butane, propane, and acetylene as sole carbon source to grow and isolated from soils contaminated with such compounds (Bell *et al.*, 1998).

Nitrate reduction catalyzed by the enzyme nitrate reductase (NR, EC, 1.6.6.1) is a key step in the overall process of nitrate assimilation and its activity is often correlated with the N-status of the plant (Mishra and Srivastava, 1983). However, in several other systems usually in young seedling, total organic nitrogen content could not be correlated with NR activity in the plants (Kumar *et al.*, 1993; Bharti and Singh, 1993). These characteristics indicated that nitrate reductase plays an important role in the utilization or assimilation of NO_3^- N from soil both before and after flowering (Kothari and Saraf, 1987). Nitrite reductase (NiR, EC 1.7.7.1) catalyzes the reduction of nitrite to ammonium. As nitrite in cytotoxic, its reduction by NiR is very essential. There is a marked specificity for reduced ferrodoxin as the nature of electron donor for the enzyme. Among the artificial substitutes for ferredoxin, methyl viologen is the

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most effective (Losada and Paneque, 1971). Sawhney and Naik (1972) studied the role of light in the synthesis of nitrite reductese in rice seedlings, Experiments with cycloheximide suggested that the fresh protein synthesis in light was necessary for the formation of active enzyme. More activation by light of inactive enzymes or their precursors was not involved. In green seedlings synthesis of nitrite reductase was more sensitive to chloromphenicol than that of nitrate reductase. Glutamine synthetase (EC 6.3.1.2) enzyme plays a central role in nitrogen metabolism. There are multiple regulatory controls at the gene and protein level to modify its activity (Miflin and Habash, 2001). Both the discovery of plastidic and ferrodoxin dependent GOGAT has demonstrated that most of ammonia is assimilated or re-assimilated through GS/GOGAT cycle (Lea and Miflin, 2003). Active functioning of GS/GOGAT cycle is essential not only for prevention or ammonia accumulation but also to maintain adequate level of glutamate in illuminated leaves of wheat (Walker *et al.*, 1984).

In view of the above facts in mind and judicious use of nitrogenous fertilizer by crop plants, wheat one of the important staple food crop has been considered for its nitrogen metabolism from slow release nitrogenous fertilizer formulation manufactured by sugar industry from sugarcane molasses a by product of sugar industry in order to know its biodegradability and nitrogen use efficiency.

Material and methods-The present investigation entitled “Biodegradability of crotonaldehyde diurea and its effect on growth and performance of wheat. [*Teiticum aestivum* (G)]” was carried out at PG farm of Department of Agronomy for field trial and laboratory studies were carried out at Department of Biochemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri-413 722, Dist. Ahmednagar, Maharashtra during 2014-15.The details of the material used and methods adopted while conducting the field experiment and used in the laboratory studies are described under different sub heading in this chapter.

CDU (Crotonaldehyde diurea): The CDU was obtained from Godavary Biorefineries Ltd. Sakarwadi, Dist. Ahmednagar.

Seeds: The seeds of the *Triticum aestivum* genotype Traymbak were obtained from the Department of Agronomy, MPKV, Rahuri.

Soil: A piece of fairly well developed land with uniform fertility was selected for conducting the experiment in complete randomized design as per layout depicted in the table 2 with the following treatments in the month of December, 2015 with *Traymbak* cultivar on post Graduate Research Institute MPKV, Rahuri.

Fertilizer: The initial available nutrient (N, P and K) in the soil before sowing of wheat crop were 161.34:8.67:470.4 kg/ha NPK. As per the available nutrients the soil was fertilized with the nitrogen at the rate of 120 kg ha^{-1} in the form of urea and crotonylidene diurea and P_2O_5 and K_2O was applied at the rate of 60 kg ha^{-1} respectively in the form of single super phosphate and murate of potash respectively.

After care: One irrigation was given immediately after sowing and subsequent irrigations are given as and when required at critical growth stages of crop. The weeding and interculturing operations were carried out two times and experimental plot was kept clean throughout the experimental period. Observations of the experiment were recorded at four different critical growth stages of wheat viz., Crown root initiation(18-21),Tillering (35-42), Flowering (60-62), Milk stage(80-85) days after sowing.

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Experimental details: The seeds were sown at 22.5 cm spacing. Gross plot size was 3.0×2.25 m² and the net plot size was 2.40×1.80 m². There were eight rows in each plot. Urea and crotonylidene as source of nitrogen with other nutrients as per recommended dose were applied before sowing. The crop was studied for growth performance and nitrogen assimilating enzymes (NR, NiR and GS) at critical growth stages. Crop was grown till physiological maturity harvested and the data of grain yield was recorded. After the harvest of crop soil was analyzed for available NPK nutrients(Table 1).

Treatments of field experiment

Treatments	Treatment Details
T_1	Control (50 % N through urea in two split doses at sowing and 30 DAS)
T_2	75 % N (urea) + 25 % N (crotonaldehyde diurea) at sowing
T_3	50 % N (urea) + 50 % N (crotonaldehyde diurea) at sowing
T_4	25 % N (urea) + 75 % N (crotonaldehyde diurea) at sowing
T_5	100 % N (crotonaldehyde diurea) at sowing

Total P and K was given as basal dose at sowing to all treatments and plant was analyzed at different growth stages for enzyme activities and other growth parameters and yield was recorded at harvest.

Growing of bacterial strain of *Rhodococcus sp.*

Rhodococcus spp. a soil bacteria capable of utilizing MDU, IDU or CDU as a nitrogen source were isolated from garden soil and grown on culture medium containing 1 per cent glucose as a carbon source and 0.1 per cent urea and CDU as the sole source of nitrogen in the laboratory.

One gram soil + 9 ml distilled water

↓

Mixed well allowed to settle

↓

1 ml suspension was drawn and transferred into fresh test tube + 9 ml distilled water was added mixed well (1 x 10^{-1} cell dilution)

↓

Above steps repeated with 1 ml suspension with addition of 9 ml distilled water till cell suspension were diluted to 1 x 10^{-9}

↓

Cell suspension diluted to 1 x 10^{-6} added to the growth media containing 0.5 % glucose (C-source), 0.1 % urea or 0.1 % CDU(N source)

↓

Cell were grown on the above media at 28 $\pm$ 2^0C in incubator for 48 hrs

↓

Cells were subcultured in required media 28 $\pm$ 2^0C for 48 hrs

↓

Harvesting of cell suspension

↓

Harvested cell suspension subjected to centrifugation at 10000 Xg 20 min at 4^0C

↓

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Pellet collected
↓
The pellet was washed two time with 50 mM Na_2HPO_4 containing β-mercaptoethanol
↓
One milliliter of lysozyme was added (20 mg/ml)
↓
Incubation 37^0C for 30 min
↓
Centrifugation (30000 Xg 60 min at 15^0C)
↓
Suprnatant of cell suspension
↓
Used as source of CDUase enzyme

Fig. 1. Flow diagram for isolation of *Rhodococcus spp*. from the soil

0.2 ml suspension + 0.8 ml phosphate buffer + 0.1 ml CDU solution
↓
Incubation at. 30^0C for 30 min
↓
Reaction terminated by addition of 2.5 ml sodium phenate +
1 ml sodium nitroprusside + 1 ml sodium hypochlorite
↓
Transferred in hot water bath at 55^0C for 10 min
↓
Blue colour intensity measured at 630 nm on
Spectronic-20

Fig. 2. Flow diagram for assay of CDUase

Isolation of bacterial strain of *Rhodococcus Spp*.

The bacterial strain *Rhodococcus* was grown on the media containing 0.1 % urea and 0.1 % CDU as nitrogen source separately (Plate.1). It was observed that the slightly less profuse growth of bacteria on culture media containing CDU as compared to the growth on media containing urea as a source of nitrogen indicating easy degradability of the CDU by the bacteria.

Serial dilution and pour plate method

Serial dilution and pour plate method was followed for isolation of *Rhodococcus spp*. As per method given by (Pikoyaskya, 1948).One gram soil sample was weighed and added to 9 ml sterile water in a test tube. Shaken thoroughly for 1 minute. This has given soil dilution of 10^{-1}.Then 1 ml of suspension of 10^{-1} dilution was transferred to next 9 ml sterile water blank and mixed thoroughly by shaking for one minute. Thus 10^{-2} dilution of original soil sample was prepared. The above steps were repeated and dilution of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and $10^{-8}$of the soil sample were prepared in test tubes containing sterile water. The 10^{-6} to 10^{-8} dilutions

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were used for isolation. The 1 ml aliquot of the desired dilution was transferred to sterile Petri plate and molten agar medium (45^0C) was poured. The soil dilution with molten medium was mixed by gentle rotating the plates for uniform mixing and allowed the medium in the plates to solidify. The petri plates were placed in BOD incubator at 30 ± 2^0C and observations regarding growth were recorded. Rich medium (RM) consisting of 500ml distilled water, 5g glucose, 4 g nutrient Broth, 0.25 g yeast extract and 7.5 g agar was used growth of *Rodoccocus spp*.

Procedure

After mixing all ingredients, 0.1 per cent urea and CDU as the sole source of nitrogen in the laboratory was autoclaved for 20 minutes at 121°C. The agar was left out when preparing RM broth. When preparing RM agar plates, the media was cooled to 55°C in a water bath and then poured in sterile petri dishes that were allowed at cool to room temperature to solidify and were then stored at 4°C for later use. The colonies were transferred to rich medium (RM) plate in order to observe their colour. *Rhodococcus* is known for having a colorful appearance on nutrient media that varies from strain to strain.

Crotonaldehyde diurease-The CDUase was assayed as per method described by Jahns *et al.* (2003) and Zawada and Sutdiff**,** (1981).

Ammonical: Ammonical nitrogen was determined by the direct distillation of ammonia with MgO. Ammonical nitrogen 19.6 per cent was available in CDU.

Nitrate nitrogen -Nitrate nitrogen was determined by the direct distillation Devarda alloy method. Nitrate nitrogen 13.0 per cent was available in CDU.

Nitrate reductase (NR) activity-The *in vivo* method as described by Hageman and Huckleshy (1971) was used for nitrate reductase activity from fresh leaf materials.

Nitrite reductase (NiR)- The method as described by Munjal, (1986) was for assaying nitrite reductase from fresh leaf material.

Glutamine synthetase activity- This enzyme was assayed by measuring the formation of γ glutamyl hydroxamate which is reacted with ferric chloride and the brown colour in the acid medium was measured at 540 nm. The method as described by Mohanty and Fletcher (1980).

Soluble proteins- The soluble protein in the leaves were determined by the colorimetric method of Lowry's *et al.* (1951).

Available Nitrogen - The alkaline permanganate method (Subbiah and Asija, 1956) was used for determination of soil mineralizable nitrogen.

Available Phosphorus -The method as ascorbic acid reduced molybdophosphatic blue colour sulphuric acid system (Watanabe and Olsen, 1965).

Available Potassium -Available which is free to exchange with cation of salt solution added to the soil plus water soluble K by Knudsen and Peterson (1982).

Results and discussion

Isolation of bacterial strain of *Rhodococcus Spp* and assay of CDUs activity

The bacterial strain *Rhodococcus* was grown on the media containing 0.1 % urea and 0.1 % CDU as nitrogen source separately (Fig-1). It was observed that the slightly less profuse growth of bacteria on culture media containing CDU as compared to the growth on media containing urea as a source of nitrogen indicating easy degradability of the CDU by the bacteria. An assay of CDUase from the cell free extract of *Rhodococcus spp.* grown on 0.1 per cent CDU revealed an increased activity of CDUase (245.31 µmols of ammonia released mg^{-1} protein min^{-1}) from crude enzyme extract of *Rhodococcus spp*. The crotonaldehyde diurea, CDU contained

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19.6 percent ammonical and 13.0 percent nitrate nitrogen. Condensation products of urea and different aldehydes such as formaldehyde, isobutyraldehyde, crotonaldehyde etc are used in large amounts as resins, binders, and insulating materials for industrial applications, as well as controlled-release nitrogen fertilizer for greens, lawns, or bioremediation processes. The biodegradability of these condensates and the enzymatic mechanism of their degradation were studied in microorganisms isolated from the soil, which were able to use these compounds as the sole source of nitrogen for growth. Different pure cultures of both gram positive and gram negative bacteria completely degraded methylenediurea, dimethylenetriurea, isobutylidenediurea and crotonylidenediurea to urea, ammonia, and the corresponding aldehydes and carbon dioxide (Hauck and Koshino 1971).

***In vivo* nitrate reductase–**

The in *vivo* nitrate reductase activity in wheat ranged from 1.22 to 2.62 (stage I), 1.17 to 3.05 (stage II), 1.5 to 2.53 (stage III) and 1.10 to 1.66 (stage IV) with mean values of a 1.83, 2.04, 1.91 and 1.31(µ moles of NO_2^- formed g^{-1} fr.wt. hr^{-1}) respectively. Among the different levels of nitrogen through CDU to the wheat crop the highest activity of nitrate reductase was found at all growth stages with 100 per cent nitrogen through CDU fertilizer (Table 2). The maximum *in vivo* nitrate reductase activity of 3.056 (µ moles of NO_2^- formed g^{-1} fr.wt.hr^{-1}) was observed at stage II (Tillering stage of wheat) with 100 per cent CDU as source of nitrogen ,which was declined during later growth stage of crop. Its activity was higher in treatment five than other treatments .This indicated that most of nitrogen applied through either urea or CDU has been assimilated by the crop till stage-II and least chances of nitrogen loss through leaching or by other means were observed.

***In vitro* nitrite reductase**

The nitrite reductase activity in wheat at different growth stages and treatments, was estimated and depicted in Table 2. It was observed that nitrite reductase activity was in the range of 101.88 to 121.80 (stage-I), 114.6 to 136.02 (stage-II), 114.54 to 150.48 (stage-III) and 112.70 to 136.50 µ moles of NO_2^- disappeared g^{-1} fr.wt.hr^{-1} (stage-IV) with mean values of a 122.10,124.47, 132.97 and 124.28 respectively. In all the critical growth stages nitrite reductase activity was highest when 100% N was applied in the form of CDU that is 150.48 µ moles of NO_2^- reduced g^{-1} fresh wt.hr^{-1} at (stage-III) was found to be decreases to 136.5 µ moles of NO_2^- disappeared g^{-1} fr. wt.hr^1 (stage-IV). Among all these critical growth stages the nitrite reductase activity was highest at flowering (stage-III).

Jones and Sheard (1977) reported that 0.5 to 1.0 per cent (v/v) n-propanol concentration enhanced nitrite production by 10 to 40 per cent in 6-day-old wheat leaves. It has been reported earlier that the levels of NiR were remarkably higher than NR levels both in leaves and roots. This observation appeared to correlate that NO_3^- reduction was the rate-limiting step in the nitrate reduction pathway (Barro *et al.,* 1991). Salunkhe (2002) observed that 4 per cent n-propanol was optimum in the infiltration medium of *in vivo* nitrate activity assays in the wheat leaves. Similar results have been reported in by Hageman and Hucklesby (1971), Munjal (1986) in winged bean. The level of nitrate reductase activity of oat leaves decreased rapidly as the age of plants increased.(Schrader *et al.*,1974). The ammonium nitrate at high concentration leads to decrease in nitrite reductase activity (Red *et al.*, 2013). When nitrogen supply is higher than the plant demand, the decreases in nitrate assimilation might be due to the decreases of nitrogen up take as a result of the negative feedback regulation by accumulated nitrate (Sivasankar *et al.*,

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1997). The rate of nitrate up take relies on the activity of nitrate transport system in the plasma membrane of root cells. External factor such as nitrate concentration as well as internal factors such as nitrogen metabolites all regulate the rate of nitrate uptake (Orsel *et al.*, 2002).Fentam *et al.* (1983) reported ammonia assimilating enzyme activities in nitrate and ammonia grown barley seedlings and the results showed the highest enzyme activities in ammonia nutrition.

Glutamine Synthetase

It was observed that glutamine synthetase activity recorded in the range of 0.55-0.68 (stage-I), 0.62-0.75 (stage-II), 0.60-0.81 (stage-III) and 0.59-0.68 µ mole γ – glutamyl hydroxamate mg^{-1} protein min^{-1} at (stage-IV) with mean values of 0.63, 0.70, 0.71 and 0.64 respectively(Table 2). In all critical growth stages, glutamine synthetase activity was highest when 100% N was applied in the form of CDU (0.81 µ mole γ–glutamyl hydroxamate mg^{-1} protein min^{-1}) at flowering which was found to be decreased to 0.68 µmole γ–glutamyl hydroxamate mg^{-1} protein min^{-1} at Milk stage. Higher activity of GS recorded at flowering stage. In wheat, GS activity is one of the best physiological marker to depict the whole plant N status irrespective of developmental stage or N fertilizer level. Further, a strong correlation has been found between GS activity and the amount of N remobilized from shoots to grain yield by using five cultivars depicting contrasting N use efficiency (Fontaine *et al.*, 2009). It has been reported that GS activity in the wheat crop remained at higher level up to 14 days after flowering which was decreased dramatically until 28 days after flowering (Kichey *et al.*2005). In wheat leaves higher GS and GDH activities in ammonia nutrition than nitrate nutrition was recorded (Orachani *et al.*, 2011). It has been also demonstrated that level of GS activity in the chloroplasts was much higher than that of GOGAT (Suzuki and Gadal, 1982).

Soluble protein

The soluble protein content ranged from 3.33-5.00 (stage I), 3.78-5.10 (stage II), 3.75-5.23 (stage III) and 3.35-4.45 (stage IV) with mean values of a 4.20, 4.35, 4.63 and 4.00 mg g^{-1} respectively. Among different levels of nitrogen through CDU to the wheat crop the highest content of soluble protein was found at all growth stages with 100 per cent nitrogen through CDU fertilizer (Table 2). The highest protein content of 5.23 mg g^{-1} was observed at flowering with 100 per cent CDU as source of nitrogen, which was declined during later growth stage of crop. Its protein content was higher in treatment five than other treatments. This indicated that most of nitrogen applied through either urea or CDU has been assimilated by the crop till flowering. The decrease in the activity of these enzymes may limit to a large extent the protein yield in cereals (Kwinta and Cal, 2005).

N, P, K uptake and Nitrogen use efficiency and grain yield of wheat

It is observed that the nutrient uptake of N in all the treatment was in the range of 98-115.96 kg/ha, the P uptake was in the range of 17.36-23.73 kg/ha and the K uptake was in the range of 90.66-116 kg/ha (Table 3). It was observed that nitrogen uptake (115.96 kg/ha) was highest in T_5 treatment that is when 100 % nitrogen applied in the form of CDU, followed by the treatment T_4 (107.63 kg/ha) and T_1 (103.1 kg/ha), while the treatment recorded T_2 lowest N uptake of 98 kg/ha as compared to the other treatments. The uptake of P and K were almost similar in all treatments, with slight higher in treatment T_3. It is also observed that the NUE in all the treatment was in the range of 81.66-96.63 per cent, the P use efficiency was in the range of 28.93-39.55 per cent and the K use efficiency was in the range of 151.1-193.33 per cent. It was found that NUE was highest in T_5 treatment (96.63 %) followed by T_4 (89.72 %) and

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Lowest in T_2 (81.66 %). The P and K use efficiency was almost similar in all treatments even through little higher was found in T_3.

Nutrient use efficiency could be improved considerably through the use of foliar sprays because of any immobilization or leaching from soil (Amberger, 1996). Another possible route of improving nutrient use efficiency is the use of slow release fertilizer, particularly N fertilizers, which release the nutrients according to the plant's requirements (Tenkel, 2010). In season N applied with point injection or top dressing can maintain or increase NUE compared with N applied through broadcasting in wheat (Sowers *et al.*, 1994).The present results revealed that nitrogen can be improved by using CDU to avoid the N losses.

The total grain yield ranged from 1508.64-2424.683 kg ha^{-1} (Table 3). It is observed that the grain yield was highest (2424.65 kg ha^{-1}) in the treatment T_5, indicated the use of nitrogen in the form of CDU resulted significantly higher grain yield than the use of nitrogen in the form of urea.

Conclusion -Among all these treatments, the treatment T_5 (100 % CDU) recorded highest activities of nitrogen assimilating enzymes such as NR, NiR, and GS, high nitrogen up take, high NUE and higher grain yield, followed by the treatment T_4 (25 % N through urea + 75% N through CDU) which is comparable to the treatment T_1 (100 % N through urea).

Thus it appeared that the condensation product of urea and crotonaldehyde, CDU is biodegradable by the *Rhodococcus spp*. present in the soil rhizosphere. Unlike urea which is given in two split doses, the CDU can be applied once at the time of sowing.

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Table 1 Soil N, P and K status

Treatments	Before sowing (kg/ha)		
	N	P	K
	161.34	8.67	470.4
	After harvest of crop (kg/ha)		
T_1	144.3	8.2	347.25
T_2	122.35	8.6	436.85
T_3	128.55	7.7	414.45
T_4	150.55	9.2	481.65
T_5	163.1	10.25	459.25
Range	122.3-163.1	7.7-10.25	336-481
Mean	141.77	8.79	427.89
S.E.±	0.028	0.028	0.028
C.D. at 5%	0.090	0.090	0.090

Table 2 Effect of crotonaldehyde diurea on *in vivo* nitrate, nitrite reductase glutamine synthetase activity and soluble protein of wheat leaves

Treatments	*In vivo* nitrate reductase activity (µ moles of NO_2^- formed g^{-1}fr. wt. hr^{-1})			
	Stages: Days after sowing			
	Stage-1	Stage-2	Stage-3	Stage-4
T_1	1.50	1.44	1.58	1.25
T_2	1.22	1.17	1.50	1.10
T_3	1.65	1.94	1.89	1.19
T_4	2.13	2.58	2.04	1.31
T_5	2.62	3.05	2.53	1.66
Range	1.22-2.62	1.17-3.05	1.50-2.53	1.10-1.66
Mean	1.830	2.043	1.913	1.307
S.E. ±	0.090	0.106	0.131	0.045
C.D. at 5 %	0.283	0.334	0.414	0.143
Treatments	**Nitrite reductase activity (µ moles of NO_2^- disappeared g^{-1} fr. wt.hr^{-1})**			
T_1	118.44	129.18	140.64	132.48
T_2	101.88	114.60	114.54	112.70
T_3	104.64	116.52	121.80	117.18
T_4	113.76	126.06	137.40	122.04
T_5	121.80	136.02	150.48	136.50
Range	101.88-121.8	114.6-136.02	114.54-150.48	112.70-136.5
Mean	112.100	124.470	132.970	124.180
S.E. ±	0.823	2.262	2.004	2.561
C.D. at 5 %	2.593	7.130	6.316	8.070

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Treatments	**Glutamine synthetase activity (μ mole γ – glutamyl hydroxamate mg^{-1} protein min^{-1})**			
T_1	0.65	0.74	0.74	0.67
T_2	0.55	0.62	0.60	0.59
T_3	0.60	0.67	0.66	0.62
T_4	0.64	0.69	0.74	0.65
T_5	0.68	0.75	0.81	0.68
Range	0.55-0.68	0.62-0.75	0.60-0.81	0.59-0.68
Mean	0.630	0.700	0.710	0.640
S.E. ±	0.007	0.014	0.015	0.008
C.D. at 5 %	0.024	0.046	0.049	0.027
Treatments	**Soluble protein (mg g^{-1})**			
T_1	4.449	4.391	4.871	4.170
T_2	3.350	3.782	3.758	3.537
T_3	3.995	3.843	4.351	3.815
T_4	4.230	4.632	4.949	4.047
T_5	5.008	5.104	**5.239**	4.456
Range	3.33-5.00	3.78-5.10	**3.75-5.23**	3.35-4.45
Mean	4.20	4.35	4.63	4.005
S.E. ±	0.109	0.090	0.096	0.045
C.D. at 5 %	0.344	0.284	0.304	0.143

Table 3 N, P K uptake and use efficiency of wheat

Treatments	**N, P and K uptake (Kg/ha)**		
	After harvest of crop		
	N	**P**	**K**
T_1	103.10	19.13	93.00
T_2	98.00	18.96	102.13
T_3	99.53	23.73	116.00
T_4	107.63	22.06	90.66
T_5	115.96	17.36	91.73
Range	98-115.96	17.36-23.73	90.66-116.00
Mean	104.840	20.250	98.700
S.E. ±	1.560	0.240	0.686
C.D. at 5 %	4.940	0.770	2.160
Treatments	**(N, P and K) use efficiency (%)**		
	N	**P**	**K**
T_1	85.90	31.88	155.06
T_2	81.66	31.60	170.20
T_3	82.94	**39.55**	**193.33**
T_4	89.72	36.76	151.10
T_5	**96.63**	28.93	152.88

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Range	**81.66-96.63**	28.93-39.55	151.1-193.33
Mean	87.370	33.744	164.514
S.E. ±	0.021	0.011	0.061
C.D. at 5 %	0.066	0.036	0.192
	Grain yield (kg ha^{-1}) of wheat		
T_1	2187.65		
T_2	1508.64		
T_3	1891.35		
T_4	2150.613		
T_5	**2424.683**		
Range	1508.64-2424.683		
Mean	2032.588		
S.E. ±	56.04503		
C.D. at 5 %	176.6015		

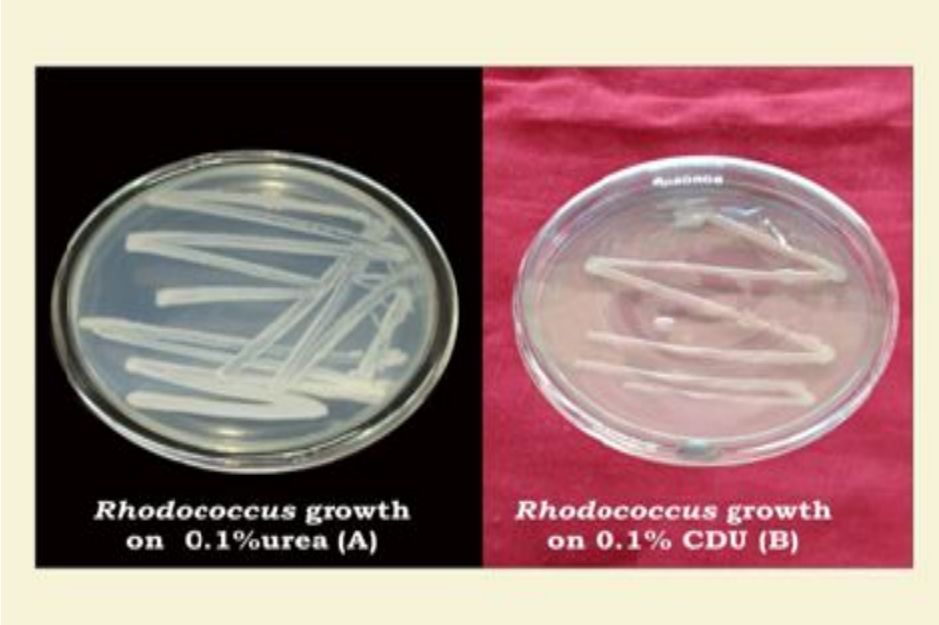

Fig-1 Growth of Rhodococcuss bacteria on A) 0.1% Urea B) 0.1% CDU