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Enzyme Activity, Agronomic Nitrogen Use Efficiency and Yield of Rainfed Maize (*Zea mays* L.) as Influenced by Natural Nitrification Inhibitors

D. Sravanthi*, G. Pratibha, B. Padmaja and T. Prabhakar Reddy

Central Institute for Dryland Agriculture (CRIDA), Santoshnagar, Hyderabad-500059, Telangana, India
**Corresponding author*

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A B S T R A C T

Nitrogen is a major essential yield limiting nutrient in crop production, it serves as a constituent of many plant cell components, such as amino acids and nucleic acids. Hence it is important to improve the productivity as maize is an exhaustive crop. The low productivity of maize during *kharif* season is attributed to a wide range of constraints including the low nitrogen use efficiency. Nitrogen in the soil is highly dynamic and mobile element and significant loss occurs as a result of NO_3^- leaching, denitrification, runoff, NH_3 volatilization, and gaseous emissions of N_2O and NO to the atmosphere. Among those nitrification is the major loss in drylands. Inefficient use has resulted in serious environmental consequences like ground water pollution and N_2O emissions which is a potent greenhouse gas. It leads to low nitrogen use efficiency, this calls for worldwide attention. The remedy for this is urea coating with natural compounds neem cake, karanj cake and *Vitex negundo* leaf powder (2.5%) by using adjuvants like castor oil and coal tar (1 % v/w) exhibited the lower urease activity and nitrification. Further N availability was increased throughout crop growth periods. So, the ANUE and grain yield were improved.

Introduction

Maize is an exhaustive crop. The low productivity of maize during *kharif* season is attributed to a wide range of constraints including the low nitrogen use efficiency. Urea is the most common nitrogen fertilizer used by the farmers. When applied to soil, urea is hydrolyzed by urease enzyme to form ammonium and is subsequently converted to nitrate and finally nitrate by the nitrifying bacteria, (*Nitrosomanas* and *Nitrobacter* sps.), which can be either leached or denitrified [1] (Kiran and Patra, 2003). Average estimates indicate that recovery of applied urea by *kharif* crops in India is 30-50% because it can be lost through different processes [2] (Patra *et al.*, 2001). Generally in drylands and rainfed situations major loss of nitrogen occurs through nitrification. Efficiency of fertilizer nitrogen particularly under tropical agriculture rarely exceeds 50 per cent and is usually only 30-40 per cent [3] (Sahrawat, 1980). This loss of N either through volatilization, nitrate leaching and denitrification, nitrification. It leads to low nitrogen use efficiency of crops, ground water contamination and serious environmental pollution. To prevent this losses become a major strategy hence the urea coated with

several natural compounds are known for inhibition of urea hydrolysis and nitrification.

Materials and Methods

The experiment was conducted during *Kharif* season of 2014 at Central Research Institute for Dryland Agriculture (CRIDA), Hayathnagar, Hyderabad (Telangana). The Hayathnagar farm is geographically situated at 17^0 18^1 N latitude, 78^0 36^1 E longitude and at an altitude of 542.6 m above the mean sea level in Southern Telangana. According to Trolls classification, the site falls under semi-arid tropics (SAT). Total rainfall of 215 mm was received in 25 rainy days during the crop growth period. The weekly mean minimum and maximum temperatures during the crop period i.e., July to October ranged from 22.1 to 25.2 ^{0}C and 28.3 to 34.9 ^{0}C respectively. The mean relative humidity ranged between 47.2 to 88.6 per cent. The weekly mean evaporation during crop season varied from 3.9 to 6.9 mm day $^{-1}$. The soil was neutral in reaction (pH 7.0), EC (0.16 dS m^{-1}), Bulk density (1.39 g cc^{-1}) with low organic carbon (0.49 %) and available nitrogen (172.1 kg ha^{-1}), high available phosphorus (22.4 kg ha^{-1}) and medium in available potassium (233.0 kg ha^{-1}).

The experiment was arranged in a randomized block design with three replicates in plot sizes of 6.3m x 5m. Treatment level of N @ 100 kg ha^{-1} in form of urea coated with neem seed cake (*Azadirachta indica*), karanj cake (*Pongamia* sps), *Vitex negundo* leaf powder @ 2.5% by using adjuvant like castor oil and coal tar (1%) applied in splits as basal and knee high stage. The Phosphorus and potassium were applied at the rates of 60 kg P_2O_5 and 40 kg K_2O per hectare applied as basal. Maize seed variety, DHM-117 was selected. Data involving plant height, dry matter, nitrogen use efficiency, factor productivity grain and stover yields benefit cost ratio were collected. To estimate the activity of enzymes collected the soil samples at 2 DATD (45 DAS), 18 DATD (61 DAS) and 23 DATD (67 DAS) in the plots treated with uncoated urea and NNIs. Data collected on dehydrogenase and urease enzyme activity, agronomic nitrogen use efficiency and grain yield.

Urease enzyme activity (µg NH_4^+ g^{-1} soil hr^{-1})

Urease activity in the soil is good indicator of soil to hydrolyze urea. Urease (urease amino hydrolase) is a unique enzyme which catalyses the hydrolysis of urea to ammonia (NH_3) which is subsequently transformed to ammonium (NH_4^+), nitrite and nitrate (NO_3^-) ions. Urease activity was assayed by quantifying the rate of release of NH_4^+ from the hydrolysis of urea as described by [4] Tabatabai and Bremner (1972) but with some modifications as suggested by [5] Sankara Rao (1989). Five grams of soil sample was taken in 25 mm × 150 mm capacity screw capped tubes. Nine ml of distilled water was added. The contents were gently mixed followed by addition of one ml of 0.2 M urea. The contents of fuse were swirled and incubated at 37 ± 0.5^{0}C for 2 hrs in BOD incubator. The reaction is terminated by addition of 15 ml of KCl- Ag_2SO_4 solution. The contents were agitated on a mechanical shaker for 1 hr to release all the NH_4^+ formed and the suspension was allowed to settle. Control samples were run simultaneously in the same way except for addition of 1 ml of 0.2 M urea solution after termination reaction.

Dehydrogenase enzyme activity (µg TPF g^{-1} soil hr^{-1})

Dehydrogenase enzyme activity was determined by as release of 2, 3, 5- triphenyl formazan (TPF) from the triphenyltetrazolium chloride (TTC) demonstrated by [6] Casida *et al.*, (1964). One gram of soil sample was taken in screw capped tubes. 0.2 ml of 3% triphenyltetrazolium chloride (TTC) and 1%

of 0.5 ml glucose solution were added, after addition the contents were thoroughly shaken and kept for incubation in BOD for 24 hours. After 24 hours the bottles were removed and 10 ml methanol (AR grade) was added and kept for 6 hours without any disturbance from outside. The supernatants reading at 540 nm in spectrophotometer was taken.

Results and Discussion

The enzyme, urease influences the hydrolysis of urea and this enzyme is produced by number of bacteria, fungi and actinomycetes hence the urease activity was estimated at different time intervals after fertilizer application and presented in Table 1 and Figure 1. Urease activity estimated at 2 DATD (45 DAS), 18 DATD (61 DAS) and 23 DATD (67 DAS) indicated that it was activity significantly influenced by uncoated urea and NNIs.

The data on urease activity after basal dose was not presented whereas the urease activity after top dressing only was presented and it was significant among different treatments at different days. The urease activity estimated after basal dose of fertilizer application was very low since the soil moisture content was low at this stage due to dry spell of 14 days at emergence stage (2-15 DAS). The urea hydrolysis takes place at soil moisture content ranging from near air dry to water logging in the soils [7] (Fertilizer Association of India, 1977). But urea hydrolysis is slow in the dry soils (near wilting point) as compared to field capacity. If the soil is air dry, 80% applied urea is not hydrolyzed even after 14 days, whereas under continuously moist conditions the urea is hydrolyzed completely within 7 days [8] (Low and Piper, 1961). In general, the enzyme activity was higher at 2, 18 days after fertilizer application and decreased at later stages. The higher enzyme activity in control (no nitrogen) at 2 and 18 DATD was due to the fact that soil organic matter content is associated with the urease activity. The higher enzyme activity immediately after fertilizer application might be due to the availability of more urea for hydrolysis. At 2 DATD and 18 DATD, urea and adjuvants coated urea recorded significantly higher urease activity than the control and NNI coated urea. However the urease activity in adjuvants coated urea and uncoated urea were on par with each other.

NNI had registered 20.2 to 50.2 % and 17.4 to 42.1 % lower urease activity over uncoated urea at 2 and 18 days after fertilization, respectively. The urease activity inhibition recorded with NNI was in the order of VCTU (42.1, 50.2%), VCU (40.8, 36.6%), KCTU (29.4, 25.2 %) NCTU (24.3, 30.6%) KCU (23, 20.2 %), NCU (17.4, 42.9 %) over uncoated urea at 2, 18 DATD respectively. At 23 DATD, the urease activity of uncoated urea and NNI coated urea was on par with each other and was superior to control. NCU recorded 57.7 % inhibition over uncoated urea. At all the stages control recorded lower activity since it has no nitrogen. Among the NNI, VCU and VCTU recorded lower urease activity up to 18 DATD but increased at 23 DATD. This indicates that NNI inhibited urease activity only up to 18 days after fertilization and later the influence of NNI on urease activity was not observed. The retardation in urease activity in NNI coated urea is due to inhibition of growth of the microbes secreting urease enzyme similar retardation in urease activity with neem cake was reported by [9] Purkayastha (1997), [10] Mohanty *et al.,* (2008) and [11] Patra *et al.,* (2006), [12] Dharani *et al.,* (2009) and [13] Saha *et al.,* (2013) also reported higher urease activity with *Pongamia* application.

The dehydrogenase enzyme activity (DHA) was significantly influenced with application of NNI coated fertilizers. The enzyme activity

was estimated at 2, 18 and 23 DATD, it indicates the microbial growth of the particular soil. DHA was progressively increased up to 18 DATD afterwards it was decreased (Table 1 and Figure 2).

Table.1 Influence of urea and different NNI coated urea on enzyme activity at different days after top dressing in rainfed maize

Treatments	Urease (µ g NH_4^+-N kg soil ha^{-1})			Dehydrogenase (µ g TPF kg $soil^{-1}$)		
	2 DATD	18 DATD	23 DATD	2 DATD	18 DATD	23 DATD
Control	73.6	73.5	59.5	14.4	25.4	23.6
U	98.0	105.1	87.5	15.0	28.8	21.6
CU	91.3	94.4	83.0	13.1	28.8	22.3
CTU	92.5	93.9	84.5	14.3	31.3	20.8
NCU	76.0	73.5	55.8	12.5	28.5	25.3
KCU	65.0	87.5	77.0	10.9	32.0	31.6
VCU	66.5	77.0	84.0	16.2	23.4	22.6
NCTU	77.0	80.5	73.8	12.3	26.5	24.5
KCTU	77.0	84.0	80.0	10.7	31.7	18.3
VCTU	67.9	70.0	84.0	11.9	28.2	22.0
SEm ±	3.64	4.73	4.73	0.82	1.84	1.66
CD (P=0.05)	10.9	14.1	14.1	2.47	5.51	4.97
CV (%)	8.0	9.7	9.7	10.9	11.2	12.3

Control – 0 Kg N ha^{-1};U –Uncoated urea (100 Kg N ha^{-1}); CU-Castor oil coated urea; CTU- Coal tar coated urea ; NCU- Neem cake coated urea with castor oil adjuvant; KUC- Karanj cake coated urea with castor adjuvant ; VCU - *Vitex negundo* leaf powder coated urea with castor oil adjuvant ; NCTU- Neem cake coated urea with coal tar adjuvant ; KCTU- Karanj cake coated urea with coal tar adjuvant ; VCTU- *Vitex negundo* leaf powder coated urea with coal tar adjuvant.

Table.2 Influence of NNI coated urea on yield grain yield and ANUE of rainfed maize

Treatment	Grain Yield (Kg ha^{-1})	ANUE (kg yield kg N $applied^{-1}$)
Control	1304	0.0
U	2119	8.2
CU	2176	8.7
CTU	2006	7.0
NCU	2446	11.4
KCU	1985	6.8
VCU	3233	19.3
NCTU	2995	16.9
KCTU	2687	13.8
VCTU	3218	19.2
SEm ±	166.05	0.67
CD (P=0.05)	497.18	2.02
CV (%)	11.9	10.5

Higher DHA at 2 DATD, was registered with VCU and this was on par with coal tar adjuvant coated urea, uncoated urea and control. These treatments were superior over the other NNI coated urea. At 18 DATD, all the treatments were recorded higher activity, except *Vitex* coated with castor oil (VCTU) and control. Whereas, dehydrogenase enzyme activity at 23 DATD was significantly higher with KCU which was superior over the other NNI (KCTU, NCU, NCTU, VCU and VCTU) coated urea, coal tar and castor oil adjuvant coated urea, uncoated urea and control. At this stage only, the NNIs recorded higher microbial growth compared to adjuvant coated urea, uncoated urea and control. The recommended dose had increased this soil dehydrogenase enzyme activity [14] (Gopal *et al.*, 2007).

All the NNI coated urea except KCU recorded significantly higher yields over adjuvants coated urea and uncoated urea. Among the NNI coated urea, the increase in grain yield was in the order of VCU (34.5 %) > VCTU (34.2%) > NCTU (29.2 %) > KCTU (21.1%) > NCU (13.4%) compared to uncoated urea. Among all the NNI treatments VCU, VCTU, NCTU were superior over KCTU, NCU, KCU (Table 2). This indicates that the NNI has no significant influence on partitioning of the dry matter.

The highest agronomic nitrogen use efficiency was registered with *Vitex* leaf powder coated urea with castor oil (VCU 19.3 kg yield kg N applied^{-1}) was on par with *Vitex* leaf powder coated urea with coal tar adjuvant (VCTU 19.2 kg yield kg N applied^{-1}).

These two treatments were superior over the other NNIs like NCTU (16.9 kg yield kg N applied^{-1}), KCTU (13.8 kg yield kg N applied^{-1}), NCU (11.4 kg yield kg N^{-1}) and KCU (6.8 kg yield kg N applied^{-1}) which was again superior over the castor oil and coal tar adjuvants coated urea (8.7, 7.0 kg yield kg N applied^{-1} castor oil and coal tar coated urea respectively), uncoated urea (8.2 kg yield kg N applied^{-1}).

The encapsulation or coating of urea with natural nitrification inhibitors controls water entry and rate of dissolution, thus decreases the nitrification. Furthermore the compounds like tetranotriterpenoids in neem [15] (Vyas *et al.*, 1993), furano flavonoids in karanjin and alkaloids like nishindine and flavonoids flavones luteolin-7-glucoside, casticin, iridoid glycosides [16] (Tendon, 2005) in *Vitex* are reported to be responsible for reducing the activity of nitrifying bacteria which in turn helps in inhibition of nitrification.

NNI inhibited urease activity only up to 18 days after top dressing and later the influence of NNI on urease activity was not observed. The retardation in urease activity in NNI coated urea is due to inhibition of growth of the microbes secreting urease enzyme. Dehydrogenase enzyme activity was increased due to the addition of natural nitrification inhibitors since they will encourage the microbe growth in the soil. Agronomic nitrogen use efficiency was also improved due to slow availability of nitrogen from urea. Ultimately grain yield was increased.

Future line of work

The urea coated with natural nitrification and urease inhibitors slows down the process of nitrification and urea hydrolysis release of ammoniacal nitrogen and further nitrate nitrogen. So that availability of ammoniacal and nitrate nitrogen to the crop throughout the growing period. Otherwise there may be loss of nitrogen from urea leads to low nitrogen use efficiency and N_2O release into atmosphere it will cause greenhouse effect and contaminates the atmosphere.

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