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Effect of Ni on important nitrogen transforming enzymatic activities at different crop growth stages in maize plant and soil

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Abstract
A pot experiment was carried out at Net House of Department of Soil Science and Agricultural Chemistry of Navsari Agricultural University, Navsari in *Rabi* season in the year 2016-17. This experiment was including 16 treatment combinations comprising four N levels (UR_{80}, UR_{120}, AS_{80} and AS_{120}) and four Ni levels (Ni_0, $Ni_{2.5}$, Ni_5 and $Ni_{7.5}$ ppm). The Ni application through $NiCl_2.6H_2O$ was given before fifteen days of sowing. Different parameters like urease enzyme activity in soil, urease enzyme activities in plants along with N as well as Ni concentration were analysed at different time intervals of N split application. The application of urea at the rate of 80 and 120 kg N ha^{-1} enhanced urease enzyme activity in maize leaves as compared to the ammonium sulphate. Whereas application of Ni at 5.0 and 7.5 mg kg^{-1} were equally effective in increasing urease enzyme activity in maize leaves. The Ni application either at 5.0 or 7.5 mg kg^{-1} with urea N level (80 kg ha^{-1}) were equally effective in increasing urease enzyme activity in maize leaves. The levels of N application, urea 80 kg N ha^{-1} and urea 120 kg N ha^{-1} showed significant increase in soil urease enzyme activity after 3rd day and 7th day of first and second N split application while, application of Ni at 5.0 mg kg^{-1} and 7.5 mg kg^{-1} showed higher soil urease enzyme activity but below this level resulted in reduced activity of urease enzyme in the soil. The combined effect of N application at 80 kg ha^{-1} along with Ni application at 7.5 mg kg^{-1} resulted into maximum urease enzyme activity being at par with $UR_{80}Ni_{2.5}$ and $UR_{80}Ni_5$ in soils after 3rd day and 7th day of first and second N split application.

Keywords: Urease activity, ammonium sulphate and urea

Introduction
Essentiality of nickel (Ni) for higher plants was first reported by Dixon *et al.* (1975) [4] as an essential component of urease enzyme in plants followed by the findings of Polacco (1977) [16], who reported that soybean cells had an absolute requirement for Ni, when grown with urea as a sole N source. Afterwards, several researchers reported essentiality of Ni for higher plants grown with urea as an N source (Shimada *et al.*, 1980; Eskew *et al.*, 1983; Walker *et al.*, 1985) [18, 6, 22]. Nickel is involved in activation of urease enzyme, hence most of Ni essentiality studies were focused on legumes due to higher urease activity in seeds of legumes and transportation of absorbed N as ureides compounds within plant body, which requires urease (Holland *et al.*, 1987; Welch, 1981; Bollard, 1983; Walker *et al.*, 1985) [9, 1, 6, 22]. Maize is a multiple purpose crop being used as food, feed and fodder in India. It is pre-dominantly grown in urban and peri-urban areas of South Gujarat for food and fodder purpose. The maize crop requires high rate of nitrogenous fertilizers. Among the nitrogenous fertilizers sources, the farmers utilize urea as N source than ammonium sulphate and other N sources. In fact, urea fertilizer is highest utilized high analysis fertilizer in India. However, urea N requires conversion of nitrogen into inorganic form; it is hydrolyzed by urease enzyme before its utilization by the plant roots. Therefore the significance of N source and Ni supply for maize plant was investigated and special attention was paid to the key enzyme (Urease) of urea conversion.

Materials and Methods
A pot experiment in *rabi* season on maize during 2016-2017 was carried out in the Net House of Soil Science and Agricultural Chemistry Dept., Navsari Agricultural University, Navsari. The soil of the experimental field was clayey in texture, medium in available nitrogen (235.2 kg ha^{-1}) and medium in phosphorus (49 kg ha^{-1}), while fairly rich in available

potassium (281 kg ha^{-1}). The soil was slightly alkaline in reaction (pH-8.29) with normal electrical conductivity (0.28 dS m^{-1}). The treatments were involving two factors viz., four N levels and four Ni levels comprising sixteen treatment combinations were laid out in factorial completely randomized design with three repetitions. Nickel was applied in the form of $NiCl_2.6H_2O$ as per the treatment 2.5, 5 and 7.5 ppm. The Nitrogen fertilizer as basal application *i.e*. 50 kg N ha^{-1} was supplied in the form of urea and ammonium sulphate and remaining dose was applied in two split applications *i.e.* 25 kg N ha^{-1} per split dose while whole quantity of P_2O_5 of recommended dose *i.e.* 50 kg P_2O_5 ha^{-1} was applied in the form of KH_2PO_4 as basal dose. The maize variety GM-3 was used in this present investigation. The crop was grown for 60 days only.

Table 1: Methods used for analysis of soil and plant samples

Determination	Method	Reference
Soil Analysis		
Soil Reaction (pH)	Potentiometry (1:2.5) Soil: water suspension	Jackson (1973)
Electrical conductivity (EC)	Conductometry (1:2.5) Soil: water suspension	Jackson (1973)
Organic carbon(OC)	Walkley and Black wet oxidation method	Jackson (1973)
Available N	Alkaline KMnO4 (0.32%) method	Subbiah & Asija (1956)
Inorganic N forms(NH_4^+& NO_3^--N) content	2 M KCl extracted distillation with and without Devarda's alloy	Bremner (1965)
Micronutrients (Fe, Cu, Mn, Zn) & Ni	Atomic absorption spectrophotometer 0.005M DTPA extract (pH 7.3)	Lindsay and Norvell (1978)
Urease enzyme activity	Citrate buffer (pH 6.7) Extracted	Hofman (1965)
Plant Analysis		
Nitrogen	Kjeldahl's digestion Method	Jackson
Micronutrients (Fe, Cu, Mn, Zn) & Ni	Di-acid digestion method, Atomic absorption Spectrophotometer	Jackson (1973)
Urease enzyme Activity	20% Glycerol extracted	Hofman (1965)

Results and Discussion

Urease activity in plants

After first N split application

The urease activity in plants after third day and seventh day of first split application of Nitrogen was analyzed and is presented in table 2 and 3. Application of N as well as Ni significantly influenced urease activity in plants. The significantly highest urease activity (7.279 mg NH_3/g. F.Wt/hr) and (8.284 mg NH_3/g. F.Wt/hr) was noticed on 3rd day and 7th day after first N split application at urea 80 kg N ha^{-1}, respectively compared to other treatments. The application of Ni at 7.5 mg kg^{-1} stimulated plant urease activity after 3rd day and 7th day after first N split application showed highest activity (7.348 mg NH_3/g. F.Wt/hr) and (8.605 mg NH_3/g. F.Wt/hr), respectively. Among different combinations, Ni application at 7.5 mg kg^{-1} and urea application at 80 kg N ha^{-1} showed significantly highest urease activity after 3rd day (8.320 mg NH_3/g F.Wt/hr) and 7th day (10.473 mg NH_3/g F.Wt/hr) after first N split application.

For urea hydrolysis, a urease apoenzyme needs to be activated and this process requires participation of several accessory proteins that incorporate nickel into urease forming catalytic site. The primary role of urease is to allow organism to use external or internally generated urea as a nitrogen source (Mobley and Hausinger, 1989; Mobley *et al.*, 1995) [15, 14]. This compound derives from arginine and possibly from degradation of purines and ureides (Polacco and Holland, 1993) [17]. The nitrogen present in urea is unavailable to the plant unless hydrolyzed by urease. The product of urease activity-ammonia is incorporated into organic compounds mainly by glutamine synthetase. The urease enzyme plays an important role in hydrolysis of internally produced urea in order to tone down the toxicity effects arose from accumulation of urea in plant tissues.

Table 2: Effect of levels and sources of N and levels of Ni on urease enzyme activity after 3rd day of first N split application in maize plant

Treatments	Ni_0	$Ni_{2.5}$	$Ni_{5.0}$	$Ni_{7.5}$	Mean
UR_{80}	6.183	7.210	7.403	8.320	7.279
UR_{120}	6.257	6.997	7.487	8.197	7.234
AS_{80}	4.703	6.067	6.950	6.827	6.137
AS_{120}	4.723	5.957	6.833	6.050	5.891
Mean	5.466	6.557	7.168	7.348	
	Nitrogen (N)		Nickel (Ni)		Interaction
S. Em. ±	0.24		0.24		0.47
CD @ 5 %	0.680		0.680		1.360
CV %	11.88				

Table 3: Effect of levels and sources of N and levels of Ni on urease enzyme activity after 7th day of first N split application in maize plant

Treatments	Ni_0	$Ni_{2.5}$	$Ni_{5.0}$	$Ni_{7.5}$	Mean
UR_{80}	6.615	7.382	8.663	10.473	8.284
UR_{120}	5.937	6.657	6.787	10.370	7.438
AS_{80}	5.343	5.587	5.933	6.910	5.943
AS_{120}	5.887	5.950	7.007	6.667	6.378
Mean	5.946	6.394	7.098	8.605	
	Nitrogen (N)		Nickel (Ni)		Interaction
S. Em. ±	0.20		0.20		0.40
CD @ 5 %	0.580		0.580		1.160
CV %	9.91				

After second N split application

The maximum significant increase in urease activity (7.970 mg NH_3/g. F.Wt/hr) and (9.508 mg NH_3/g. F.Wt/hr) was noticed on 3rd day and 7th day after second N split application at urea 80 kg N ha^{-1} respectively among all the other treatments (table 4 and 5). Moreover, application of Ni at 7.5 mg kg^{-1} stimulated plant urease activity after 3rd day and 7th day after second N split application showing significantly highest activity (7.766 mg NH_3/g. F.Wt/hr) and (8.635 mg NH_3/g. F.Wt/hr) respectively. Among different combinations affecting urease enzyme activity in maize, Ni application at 7.5 mg kg^{-1} with urea application at 80 kg N ha^{-1} showed

highest urease enzyme activity after 3rd day (10.247 mg NH_3/g. F.Wt/hr) and 7th day (9.950mg NH_3/g. F.Wt/hr) after second N split application.

Table 4: Effect of levels and sources of N and levels of Ni on urease enzyme activity after 3rd day of second N split application in maize plant

Treatments	Ni 0	Ni 2.5	Ni 5.0	Ni 7.5	Mean
UR 80	7.412	8.206	6.017	10.247	7.970
UR 120	7.997	9.200	9.257	6.043	8.124
AS 80	5.953	5.797	7.707	7.543	6.750
AS 120	5.905	6.550	7.916	7.231	6.901
Mean	6.817	7.438	7.724	7.766	
	Nitrogen (N)		Nickel (Ni)		Interaction
S. Em. ±	0.22		0.22		0.44
CD @ 5 %	0.63		0.63		1.27
CV %	10.24				

Table 5: Effect of levels and sources of N and levels of Ni on urease enzyme activity after 7th day of second N split application in maize plant

Treatments	Ni 0	Ni 2.5	Ni 5.0	Ni 7.5	Mean
UR 80	9.000	9.540	9.543	9.950	9.508
UR 120	8.553	9.403	9.167	7.640	8.691
AS 80	6.423	6.560	6.480	8.333	6.949
AS 120	6.723	6.457	6.493	8.620	7.073
Mean	7.675	7.990	7.921	8.635	
	Nitrogen (N)		Nickel (Ni)		Interaction
S. Em. ±	0.15		0.15		0.30
CD @ 5 %	0.430		0.430		0.870
CV %	6.54				

Urease activity in soil

After first split application

An appraisal of data in table 6 and 7 revealed that there was significant effect of levels and sources of N, but it was more pronounced in urea as compared with ammonium sulphate. The significantly highest urease activity was noticed on 3rd day (4.633 mg NH_3/g. F.Wt/hr) and 7th day (6.003 mg NH_3/g. F.Wt/hr) after first N split application at urea 80 kg N ha^{-1}. Ni application at 7.5 mg kg^{-1} also showed significantly highest urease activity (4.864 mg NH_3/g. F.Wt/hr) and (5.709 mg NH_3/g. F.Wt/hr). Ni application at 7.5 mg kg^{-1} with urea application at 80 kg N ha^{-1}, ($UR_{80}Ni_{7.5}$) showed significantly highest value (5.443 mg NH_3/g. F.Wt/hr) after 3rd day and (7.117 mg NH_3/g. F.Wt/hr) after 7th day of second N split application.

Table 6: Effect of levels and sources of N and levels of Ni on urease enzyme activity3rd day of first N split application in soil

Treatments	Ni 0	Ni 2.5	Ni 5.0	Ni 7.5	Mean
UR 80	4.207	4.453	4.427	5.443	4.633
UR 120	3.133	4.727	4.493	4.963	4.329
AS 80	2.583	3.573	4.490	4.513	3.990
AS 120	3.377	3.290	4.640	4.537	3.961
Mean	3.325	4.011	4.513	4.864	
	Nitrogen (N)		Nickel (Ni)		Interaction
S. Em. ±	0.13		0.13		0.25
CD @ 5 %	0.370		0.370		0.730
CV %	10.43				

Table 7: Effect of levels and sources of N and levels of Ni on urease enzyme activity 7th day of first N split application in soil

Treatments	Ni 0	Ni 2.5	Ni 5.0	Ni 7.5	Mean
UR 80	5.390	5.957	5.547	7.117	6.003
UR 120	5.107	5.690	5.633	6.053	5.621
AS 80	3.963	4.153	5.360	5.550	4.757
AS 120	4.333	4.467	5.870	4.117	4.697
Mean	4.698	5.067	5.603	5.709	
	Nitrogen (N)		Nickel (Ni)		Interaction
S. Em. ±	0.13		0.13		0.27
CD @ 5 %	0.390		0.390		0.780
CV %	8.86				

After second split application

Application of N as well as Ni affected urease activity in the soil. With urea application at 80 kg N ha^{-1} urease activity was found significantly highest after 3rd day (5.871 mg NH_3/g. F.Wt/hr) and after 7th day (6.091 mg NH_3/g. F.Wt/hr) of second N split application. Ni application at 7.5 mg kg^{-1} exerted significant effect and urease activity was found maximum with this treatment among the other treatment. The combined application of urea 80 kg N ha^{-1} and Ni at 7.5 mg kg^{-1} also influenced urease activity significantly after 3rd day and 7th day of second split N application (table 8 and 9). As increase in activity of any enzyme is expected with increase in substrate concentration, the results mentioned above also suggest that urease enzyme activity was enhanced with increase in N application through urea which is substrate for the same. Further, there are several reports which indicate that rate of hydrolysis of urea due to soil urease enzyme activity increases with increase in urea concentration until the amount of urea added is sufficient to saturate the enzyme with substrate (Douglas and Bremner, 1971; Tabatabai and Bremner, 1972; Dalal, 1975) [5, 21, 3]. However, at very high urea concentrations the hydrolysis rate decreases which could be attributed to either uncompetitive substrate inhibition of the enzyme or denaturation of enzyme at very high concentrations of urea.

Table 8: Effect of levels and sources of N and levels of Ni on urease enzyme activity 3rd day of second N split application in soil

Treatments	Ni 0	Ni 2.5	Ni 5.0	Ni 7.5	Mean
UR 80	5.260	6.030	5.867	6.330	5.871
UR 120	5.110	4.683	5.287	5.933	5.253
AS 80	4.433	4.360	5.527	5.487	4.952
AS 120	3.433	5.160	5.847	4.963	4.851
Mean	4.559	5.058	5.632	5.678	
	Nitrogen (N)		Nickel (Ni)		Interaction
S. Em. ±	0.18		0.18		0.35
CD @ 5 %	0.510		0.510		1.020
CV %	11.33				

Table 9: Effect of levels and sources of N and levels of Ni on urease enzyme activity 7th day of second N split application in soil

Treatments	Ni 0	Ni 2.5	Ni 5.0	Ni 7.5	Mean
UR 80	5.047	6.190	6.350	6.777	6.091
UR 120	5.637	5.417	5.587	6.700	5.835
AS 80	3.317	5.333	6.093	5.830	5.143
AS 120	4.780	4.873	5.740	5.980	5.343
Mean	4.695	5.453	5.943	6.321	
	Nitrogen (N)		Nickel (Ni)		Interaction
S. Em. ±	0.16		0.16		0.33
CD @ 5 %	0.470		0.470		0.940
CV %	9.67				

Conclusion

The overall findings suggest the practical significance of Ni application on N transformation, metabolism and utilization in maize. The maize responded to 5.0 to 7.5 mg kg^{-1} levels with advances in crop growth and keeping above results in consideration, Ni application at 5 mg kg^{-1} with urea as source of N at the rate of 120 kg ha^{-1} was found to enhance growth and yield of maize plant. Therefore, the results are also suggestive for judicious use of Ni with N application (through urea) to increase N efficiency and increase crop production under maize base cropping system. However, detailed research is necessary to investigate the harmful effect of Ni on soil-plant-human/animal health due to its entry in food chain, if continuously applied over the years.

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