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Ni and N sources (Urea and ammonium sulphate) affecting growth, yield and quality in maize plant (*Zea mays*)

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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 growth, yield and protein content were analysed at different time intervals of N split application. The urea N application (120 kg ha^{-1}) registered the highest plant height as compared with ammonium sulphate. The Ni application at 5 mg kg^{-1} caused significant and maximum improvement in plant height as well as dry matter yield. In case of protein with increasing levels and sources of N application, protein content was also increased. Ni application at 2.5 mg kg^{-1} has significant effect on protein content over control. Due to the interaction effect highest protein content was recorded with the treatment $UR_{120}Ni_{2.5}$.

Keywords: Urea, ammonium sulphate and protein

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) [14], 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) [16, 5, 19].

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, 20, 1].

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

Plant growth

The data presented in table 2-4 on effect of levels and sources of N and Ni application on plant height of maize at 15, 30, 45 and 60 DAS indicated that plant height of maize was significantly affected by levels and sources of N and Ni levels.
The data pertaining to plant height (14.2 cm) presented in table 2 at 15 DAS was recorded significantly higher due to urea application at 120 kg N ha^{-1} over the application of ammonium sulphate at 80 kg N ha^{-1} (12.4 cm). The highest plant height at 15 DAS due to Ni application was recorded under Ni application at 2.5 and 5 mg kg^{-1} (14.7cm) over the application of Ni 0 mg kg^{-1} (10.5 cm). The interaction effect was found significant and the plant height (16.6 cm) at 15 DAS recorded significantly highest with combined effect of Ni at 5 mg kg^{-1} and urea application at 120 kg N ha^{-1}
Consequently, the data on plant height at 30 DAS of maize are presented in Table 3. The similar results were obtained. The significantly highest plant height at 30 DAS was recorded due to Urea application at 120 kg N ha^{-1} (37.5 cm) over ammonium sulphate. The highest plant height at 30 DAS due to Ni application was recorded under Ni application at 5 mg kg^{-1} (35.9 cm).
The Table 3 showed that the interaction effect was found significant on plant height at 30 DAS. The plant height (42 cm) was found significantly higher with combined effect of Ni at 5 mg kg^{-1} and urea application at 120 kg N ha^{-1}. As it is well established fact that N fertilization in crops boosts up the growth due to its role in protein synthesis and vital plant metabolic activities, the graded application of N has shown positive influence on plant height, a measure of crop growth. As Ni is closely associated with N-urea assimilation, growth promotion in plants fertilized with Ni can also be attributed to enhanced N-urea assimilation. The stimulating effect of a moderate Ni supply in urea-grown plants is well documented in wide range of plants (Shimada and Matsuo, 1985; Krogmeier *et al.*, 1991; Gerendas and Sattelmacher, 1997) [17, 11, 6].

Table 2: Effect of levels and sources of N and levels of Ni on plant height (cm) of maize at 15 DAS

Treatments	Ni_0	$Ni_{2.5}$	$Ni_{5.0}$	$Ni_{7.5}$	Mean
UR_{80}	10.8	15.4	15.4	12.9	13.6
UR_{120}	11.2	15.3	16.6	14.0	14.2
AS_{80}	11.2	13.2	12.5	12.5	12.4
AS_{120}	8.8	15.2	14.6	12.2	12.7
Mean	10.5	14.7	14.7	12.8	
	Nitrogen (N)		**Nickel (Ni)**		**Interaction**
S. Em. ±	0.205		0.205		0.41
CD @ 5 %	0.6		0.6		1.2
CV %	9.37				

Table 3: Effect of levels and sources of N and levels of Ni on plant height (cm) of maize at 30 DAS

Treatments	Ni_0	$Ni_{2.5}$	$Ni_{5.0}$	$Ni_{7.5}$	Mean
UR_{80}	32.5	32.2	34.0	28.8	31.9
UR_{120}	31.9	41.9	42.0	34.6	37.5
AS_{80}	27.5	33.5	35.6	36.0	33.2
AS_{120}	25.6	33.0	32.0	31.1	30.4
Mean	29.4	35.2	35.9	32.6	
	Nitrogen (N)		**Nickel (Ni)**		**Interaction**
S. Em. ±	0.61		0.61		1.22
CD @ 5 %	1.8		1.8		3.5
CV %	6.30				

Yield

Application of N sources as well as Ni significantly influenced the fresh weight as well as dry weight of maize. The fresh weight (126.4 g $plant^{-1}$) and dry weight (28.11 g $plant^{-1}$) was significantly influenced by the treatment and found highest with the application of urea at 120 kg N ha^{-1}. Whereas with the increasing levels of Ni application (Ni 5 mg kg^{-1}), increment in fresh weight (125.4 g $plant^{-1}$) as well as the dry weight (26.34 g $plant^{-1}$) of the maize plant was observed.
The fresh weight (154.3 g $plant^{-1}$) of plant was found significantly higher with combined effect of Ni at 5 mg kg^{-1} and urea application at 120 kg N ha^{-1}. Similar results were found for dry weight of plant (Table 6) and found significantly highest with combined effect of Ni at 5 mg kg^{-1} and urea application at 120 kg N ha^{-1} (34.13 g $plant^{-1}$). As reviewed by Mishra and Kar (1974) [13] and Welch (1981) [20], an optimum dose of Ni in any crop would enhance grain or economic yield by virtue of its role in N metabolism as well its significant function in several metabolic activities like iron acquisition and phytoalexin synthesis.

Table 4: Effect of levels and sources of N and levels of Ni application on fresh weight (g plant^{-1}) of Maize

Treatments	Ni $_0$	Ni $_{2.5}$	Ni $_{5.0}$	Ni $_{7.5}$	Mean
UR $_{80}$	89.3	133.0	136.7	139.3	124.6
UR $_{120}$	92.0	136.0	154.3	123.3	126.4
AS $_{80}$	91.0	88.7	104.6	111.0	98.8
AS $_{120}$	95.0	105.7	106.0	100.0	101.7
Mean	91.8	115.8	125.4	118.4	
	Nitrogen (N)		**Nickel (Ni)**		**Interaction**
S. Em. $\pm$	4.35		4.35		8.70
CD @ 5 %	12.5		12.5		25.0
CV %	13.35				

Table 5: Effect of levels and sources of N and levels of Ni application on dry weight (g plant^{-1}) of Maize

Treatments	Ni $_0$	Ni $_{2.5}$	Ni $_{5.0}$	Ni $_{7.5}$	Mean
UR $_{80}$	18.85	25.54	31.88	27.33	25.90
UR $_{120}$	20.95	30.01	34.13	27.37	28.11
AS $_{80}$	21.21	22.96	18.70	22.68	21.39
AS $_{120}$	22.70	23.30	20.65	21.24	21.97
Mean	20.93	25.45	26.34	24.66	
	Nitrogen (N)		**Nickel (Ni)**		**Interaction**
S. Em. $\pm$	1.196		1.196		2.391
CD @ 5 %	3.44		3.44		6.89
CV %	17.01				

Protein content

Effect of levels and sources of Nitrogen (N) and levels of Nickel (Ni) on protein content in maize plant

After first N split application

The data pertaining to protein content of maize plants after 3rd day and 7th day of first N split application in table 6 and 7 revealed that levels and sources of N application significantly influenced protein content. It was recorded that with the increasing levels and sources of N application, protein content was also increased. It was found highest with the treatment UR $_{120}$ (1.088 mg/ g F. Wt.) and (1.007 mg/ g F. Wt.) which was at par with UR $_{80}$ (1.040 and 0.953 mg/ g F. Wt.).

It was also revealed (table 6 and 7) that Ni application significantly enhanced the protein content in maize plants after 3rd and 7th day of first N split application. The protein content after 3rd and 7th day of first N split application was found significantly highest with the treatment Ni $_{2.5}$ (1.034 mg/ g F. Wt.) and (0.950 mg/ g F. Wt.) respectively, which was at par with Ni $_{7.5}$ (0.936 mg/ g F. Wt.) after 3rd day of first split and Ni $_{5.0}$ and Ni $_{7.5}$ (0.875 and 0.842 mg/ g F. Wt.) after 7thday of first split over the lowest value (0.878 mg/ g F. Wt.) and (0.726 mg/ g F. Wt.), respectively in control.

Table 6: Effect of levels and sources of N and levels of Ni on protein content 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 $_{80}$	0.953	1.243	0.947	1.017	1.040
UR $_{120}$	1.023	1.287	1.117	0.927	1.088
AS $_{80}$	0.760	0.773	0.800	0.863	0.799
AS $_{120}$	0.777	0.833	0.870	0.937	0.854
Mean	0.878	1.034	0.933	0.936	
	Nitrogen (N)		**Nickel (Ni)**		**Interaction**
S. Em. $\pm$	0.03		0.03		0.07
CD @ 5 %	0.10		0.10		0.20
CV %	12.47				

The interaction effect was found significant. From table 6 it is revealed that protein content after 3rd day of first N split application was found significantly higher with treatment UR$_{120}$ Ni $_{2.5}$ (1.287 mg/ g F. Wt.) which was at par with UR $_{120}$ Ni $_{5.0}$ (1.117 mg/ g F. Wt).

Table 7: Effect of levels and sources of N and levels of Ni on protein content 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 $_{80}$	0.800	1.163	1.013	0.833	0.953
UR $_{120}$	0.917	1.200	1.017	0.897	1.008
AS $_{80}$	0.553	0.723	0.707	0.817	0.700
AS $_{120}$	0.633	0.717	0.763	0.820	0.733
Mean	0.726	0.950	0.875	0.842	
	Nitrogen (N)		**Nickel (Ni)**		**Interaction**
S. Em. $\pm$	0.03		0.03		0.06
CD @ 5 %	0.08		0.08		0.17
CV %	11.96				

Similar results were also obtained after 7thday of first N split application, significantly highest value was observed with the treatment UR $_{120}$ Ni $_{2.5}$ (1.200 mg/ g F. Wt.) which was at par with UR $_{80}$ Ni $_{2.5}$ (1.163 mg/ g F. Wt).

After second N split application

A glance of protein content of maize plants after 3rdday and 7th day of second N split application is presented in table 8 and 9. Protein content was increased due to application of N levels as well as Ni levels. It was found highest with application of urea 120 kg N ha^{-1} (1.125 mg/ g F. Wt.) and (1.101 mg/ g F. Wt.) being at par with UR $_{80}$ (1.097 mg/ g F. Wt.) after 3rd day and UR $_{80}$ (1.061 mg/ g F. Wt.). However, AS $_{80}$ showed lowest value (0.849 mg/ g F. Wt.) and (0.799 mg/ g F. Wt.) after 3rd and 7th day of second N split application, respectively.

Table 8: Effect of levels and sources of N and levels of Ni on protein content 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 $_{80}$	1.037	1.167	1.127	1.057	1.097
UR $_{120}$	1.123	1.220	1.213	0.943	1.125
AS $_{80}$	0.747	0.880	0.873	0.897	0.849
AS $_{120}$	0.750	0.870	0.880	0.960	0.865
Mean	0.914	1.034	1.023	0.964	
	Nitrogen (N)		**Nickel (Ni)**		**Interaction**
S. Em. $\pm$	0.02		0.02		0.04
CD @ 5 %	0.05		0.05		0.10
CV %	6.32				

The protein content after 3rd and 7th day of second N split application was found significantly highest with application of Ni 2.5 mg kg^{-1} (1.034 mg/ g F. Wt.) and (1.00 mg/ g F. Wt.) respectively being at par with Ni $_{5.0}$ (1.023 mg/ g F. Wt.) and (0.99 mg/ g F. Wt.) over the lowest value (0.914 mg/ g F. Wt.) and (0.871 mg/ g F. Wt.) with control (Ni$_0$), respectively.

The interaction effect was found significant. From table 8 it is revealed that protein content after 3rdday of second N split application was found significantly highest with the treatment UR $_{120}$ Ni $_{2.5}$ (1.220 mg/ g F. Wt.) which was at par with UR $_{80}$ Ni $_{2.5,}$ UR $_{80}$ Ni $_{5.0,}$ UR $_{120}$ Ni$_{.0}$ and UR $_{120}$ Ni$_{.5.0}$.

The table 9 revealed interaction was significant after 7thday of second N split application and protein content was significantly highest with UR $_{120}$ $_{Ni}$ $_{2.5}$ (1.293 mg/ g F. Wt.) over the lowest value (0.720 mg/ g F. Wt.) with AS $_{80}$ Ni $_{0.}$

Table 9: Effect of levels and sources of N and levels of Ni on protein content at 7th day of second N split application in soil

Treatments	Ni_0	$Ni_{2.5}$	$Ni_{5.0}$	$Ni_{7.5}$	Mean
UR_{80}	0.993	1.113	1.107	1.030	1.061
UR_{120}	1.027	1.293	1.133	0.950	1.101
AS_{80}	0.720	0.827	0.850	0.800	0.799
AS_{120}	0.743	0.767	0.870	0.893	0.818
Mean	0.871	1.000	0.990	0.918	
	Nitrogen (N)		**Nickel (Ni)**		**Interaction**
S. Em. $\pm$	0.02		0.02		0.05
CD @ 5 %	0.07		0.07		0.14
CV %	8.84				

After harvest protein content

The data in table 10 showed significant effect on protein content and significantly highest value was observed with application of urea 120 kg N ha^{-1} (1.001 mg/ g F. Wt.) over the lowest value with application of ammonium sulphate 80 kg N ha^{-1}(0.736 mg/ g F. Wt.). In urea-grown plants the consequences of Ni deprivation are usually considerable (Polacco, 1977a; Gerendas and Sattelmacher, 1997a, b, 1999) [14, 7]. As the plants N economy depends heavily on the hydrolysis of urea, which is impaired due to absence of Ni. Amino acid accumulations in plant tissues in response to nutrient deficiencies have been observed previously have been attributed to a depression of protein synthesis. This may be either a direct effect of the deficiency on protein biosynthetic processes or an indirect effect resulting from a nutrient deficiency-induced shortage of metabolites, essential for protein synthesis. Under Ni deficiency conditions, we observed an increase in the shoot concentrations of 10 of the 11 amino acids determined. This could be the result of a Ni deficiency-induced disruption of protein synthesis (Brown *et al.* 1990) [3].

Table 10: Effect of levels and sources of N and levels of Ni on protein content in maize plant after harvest of the crop

Treatments	Ni_0	$Ni_{2.5}$	$Ni_{5.0}$	$Ni_{7.5}$	Mean
UR_{80}	0.833	1.043	0.950	0.933	0.940
UR_{120}	0.953	1.080	1.020	0.950	1.001
AS_{80}	0.590	0.753	0.757	0.843	0.736
AS_{120}	0.610	0.757	0.790	0.873	0.758
Mean	0.747	0.908	0.879	0.900	
	Nitrogen (N)		**Nickel (Ni)**		**Interaction**
S. Em. $\pm$	0.01		0.01		0.03
CD @ 5 %	0.04		0.04		0.08
CV %	5.78				

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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