Effect of different nitrogen fertilizers on leaf micronutrients in apple (*Malus domestica* Borkh)

ABSTRACT

Aims: To evaluate the effect of different nitrogen sources on leaf micronutrients of apple in pot culture studies and field studies.
Study design: The experiment was laid out in a Completely Randomized Design factorial in pot studies and in a Randomized Block Design factorial in field studies.
Place and Duration of Study: The pot culture experiment was carried out at the experimental farm of Department of Soil Science and Water Management, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) and field experiment was carried out at Krishi Vigyan Kendra, Rohru, Shimla (H.P.) and Horticultural Research Station, Seobagh, Kullu (H.P.) in 2016 and 2017.
Methodology: Experiments were comprised of fourteen treatments and each treatment was replicated three times, having one plant under each replication. Calculated amount of fertilizers was added in the soil at appropriate time and leaf samples were collected for determination of leaf micronutrients. Copper (Cu), iron (Fe), manganese (Mn), zinc (Zn) contents were determined by using atomic absorption spectrophotometer and boron (B) was estimated by using Azomethine- H method.
Results: In pot culture studies, Cu was 14.96 and 15.05 ppm, Fe was 188.71 and 194.48 ppm, Zn was 31.52 and 32.51 ppm and 64.83 and 65.09 ppm in 2016 and 2017, respectively. The highest B content was recorded under urea alone application (35.64 and 35.48 ppm in 2016 and 2017, respectively). In field studies, Mn was 76.48 and 78.16 ppm at Seobagh and 83.23 and 85.15 ppm at Rohru, Fe was 218.25 and 225.15 ppm at Seobagh and 235.64 and 247.18 ppm at Rohru, Cu was 16.83 and 17.02 ppm at Seobagh and 17.87 and 18.03 ppm at Rohru, Zn was 39.40 and 40.84 ppm at Seobagh and 39.28 and 41.16 ppm at Rohru in 2016 and 2017, respectively. At Seobagh the highest B content in leaves was recorded under urea alone application (37.87 ppm) in 2016 and under the treatment 12:32:16 + calcium nitrate (38.14 ppm) in 2017, while at Rohru highest B content was under urea alone application (33.56 ppm) in 2016 and under calcium nitrate treatment (30.36 ppm) in 2017.
Conclusion: Leaf micronutrients (Fe, Cu, Mn, Zn and B) were significantly affected on application of nitrogen fertilizers and were the highest under urea alone application and were found to have a direct relation with the reduction in pH of the soils.

Keywords: apple, nitrogen sources, fertilizers, leaf, micronutrients

1. INTRODUCTION

Apple (*Malus domestica* Borkh) a native to South East Asia is one of the widely grown and most important fruit crop in temperate region of the world. In India, it was introduced in the middle of the nineteenth century and is a predominant crop of North Western Himalayan region and ranks first in area and production among the temperate fruits. The crop is being grown commercially in the states of Jammu & Kashmir, Himachal Pradesh and Uttarakhand. Its cultivation however, has also been extended to North Eastern States (Arunachal Pradesh and Sikkim) and Southern parts of the country (Nilgiri hills). Apple is the mainstay of Himachal's economy and emerged as a leading cash crop amongst fruit crops. Its cultivation has revolutionized the socio-economic condition of farmers as 88 per cent of total fruit area is under this crop.

Nitrogen is one of the most important nutrients necessary for many functions of plants like shoot growth, fruit and flower bud set and fruit size. Insufficient uptake of nitrogen in apple tree results in weak shoot growth, light green to yellowish-green leaves that causes negative effect on photosynthetic intensity [1-2]. Nitrogen is required for the initial growth of deciduous trees in the spring during cell division [3-4]. Initial growth of fruit trees is supported by remobilization of N reserves and there is a positive relationship between growth and the amount of N reserves for many species and varieties. The efficient use of nitrogenous fertilizers to increase crop yield is an important goal in all agricultural systems [5].

Previous studies showed that soil chemical analysis is the primary factor that determined the nutrient requirement of crops. On the other hand, results from field and orchards suggested that the soil analysis did not give sufficient information about the actual fruit crop requirement for particular nutrients. Due to this reason, in recent time the most trusted method to confirm the disorders of a specific nutrient is foliar diagnosis by analysis of leaves. Also, it is a good method to diagnose the excess or insufficiency of a particular nutrient and helps to correct the deficiencies of nutrients, if necessary [6]. Therefore, to study the effect of different nitrogen sources on leaf micronutrients of apple present study was undertaken.

2. MATERIALS AND METHODS

2.1 Experiment details and locations

The study was carried out at three different locations i.e. Experimental farm of Department of Soil Science and Water Management, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), located at 30° 52' N latitude and 77° 11' E longitude and at an elevation of 1175 m above mean sea level; Krishi Vigyan Kendra, Dr. Y. S. Parmar University of Horticulture and Forestry Rohru (H.P.) which is about 120 km away from Shimla town, located at 31° 13' N latitude and 77° 43' E longitude and at an elevation of 1710 m above mean sea level; and Horticultural Research Station, Dr. Y. S. Parmar University of Horticulture and Forestry, Seobagh, Kullu (H.P.) located at 31° 59' N latitude and 77° 08' E longitude and at an elevation of 1341 m above mean sea level.

Nauni falls under the mid hills sub-humid agro-climatic zone of Himachal Pradesh and the average annual rainfall ranges between 800-1300 mm. Rohru (Shimla) falls under the high hills temperate wet agro-climatic zone of Himachal Pradesh and average annual rainfall is 850 mm. Seobagh (Kullu) falls under high hills temperate wet agro-climatic zone of Himachal Pradesh and average annual rainfall is 1100 mm.

At Nauni, effect of different nitrogen sources on leaf micronutrients was evaluated in apple seedlings in a pot culture study. At Rohru and Seobagh, effect of different nitrogen sources on leaf micronutrients was evaluated in apple trees in a field study.

2.1.1 Treatments details

T_1 : Control (No application)
T_2 : Urea
T_3 : Calcium Nitrate
T_4 : Calcium Cyanamide
T_5 : Urea + Liming (In October)
T_6 : Urea + Liming (In March)
T_7 : 12: 32: 16 + Urea
T_8 : 12: 32: 16 + Calcium Nitrate
T_9 : 12: 32: 16 + Calcium Cyanamide
T_{10} : 15:15:15 + Urea
T_{11} : 15: 15: 15 + Calcium Nitrate
T_{12} : 15: 15: 15 + Calcium Cyanamide
T_{13} : 50% Urea (soil) + Foliar N
T_{14} : Calcium Nitrate + Urea + Liming

2.2 Recommended dose (RD) of fertilizers

i) One year old apple tree: N: P_2O_5: K_2O :: 70: 35: 70 g/tree
ii) Ten year or above old apple tree: N: P_2O_5: K_2O :: 700: 350: 700g /tree

At Nauni, the experiment was laid out in a Completely Randomized Design factorial and comprised of fourteen treatments and each treatment was replicated three times, having one plant under each replication, planted in drums having 30 cm radius and 60 cm height. At Rohru and Seobagh, the experiment was laid out in a Randomized Block Design factorial [11] and comprised of fourteen treatments and each treatment was replicated three times having one plant under each replication.

Table 1: Experiment details of Rohru and Seobagh

	Rohru	Seobagh
Variety	Red Chief	Vance delicious
Spacing	3.5m × 3.5m	6m × 6m
Number of treatments	14	14
Number of replications	3	3
Experimental design	RBD Factorial	RBD Factorial

Table 2: Physio-chemical properties of experimental soil before the start of experiment

Soil properties	Nauni	Rohru	Seobagh
pH (1:2)	6.76	5.49	5.60
EC (dSm^{-1})	0.401	0.390	0.367
Organic carbon (%)	1.42	1.60	0.97
Available N ($kg\ ha^{-1}$)	274.16	332.49	285.24
Available P ($kg\ ha^{-1}$)	51.60	76.15	56.72
Available K ($kg\ ha^{-1}$)	387.48	486.15	365.09
Available Ca (cmol (p^+) kg^{-1})	5.71	5.23	4.15
Available Zn ($mg\ kg^{-1}$)	2.30	2.52	2.28
Available Cu ($mg\ kg^{-1}$)	1.24	1.89	1.09
Available Fe ($mg\ kg^{-1}$)	18.94	28.70	17.11
Available Mn ($mg\ kg^{-1}$)	13.11	23.02	16.43

2.3 Quantity of fertilizers:

At Nauni, Fertilizer quantity was calculated using the soil volume calculations and twice the calculated volume of the fertilizer/amendment was added to the respective drums. In treatment T_7 to T_{12} the amount of P and K added through mixed fertilizers (12:32:16 and 15:15:15) was calculated and remaining amount of P and K was added through SSP and MOP as in the case of other treatments.
At Rohru and Seobagh, the quantity of fertilizers under each treatment was calculated to fulfill the recommended dose of nitrogen which was calculated on the basis of per cent nitrogen contained in the respective fertilizer. The recommended dose of phosphorus was applied through single super phosphate and recommended dose of potassium was applied through muriate of potash to all the trees under study. The foliar application of 50 % RD of nitrogen was calculated as 1 % urea spray. In treatment T_7 to T_{12} the amount of P and K added through mixed fertilizers was calculated and remaining amount of P and K was added through SSP and MOP as in the case of other treatments.

2.4 Time and method of fertilizers application

The NPK mixture 15:15:15 and 12:32:16 were applied during the month of December along with P, K fertilizers and FYM. The urea, calcium cyanamide and calcium nitrate were applied in two equal split doses, half dose fifteen days before flowering and remaining half dose one month after flowering. The fertilizers were broadcasted under the spread of tree in entire basin area, 30 cm away from the tree trunk. After broadcasting fertilizers were thoroughly mixed with the soil to reduce the loss of nutrients.

2.5 Leaf analysis

2.2.1 Collection and preparation of leaf foliage samples

Leaf samples from each experimental tree were collected from the middle of the current season's growth around the periphery of the trees, as recommended by [7], during the second fortnight of July in both the years. The leaf samples were washed first under tap water followed by 0.1N HCl and finally with double distilled water. The samples were spread on filter paper for air drying and were subsequently put in paper bags, which were kept in hot air oven at 60 ± 5°C for 72 hours for drying. The dried samples were crushed, ground and stored in butter paper bags for further analysis.

2.2.2 Digestion of leaf samples and methods for determination of micronutrients

For estimation of Cu, Fe, Mn, Zn contents well ground samples of known weight of leaf were digested in diacid mixture prepared by mixing concentrated HNO_3 and $HClO_4$ in the ratio of 4:1 observing all relevant precautions as laid down by [8] and were determined by using atomic absorption spectrophotometer [9]. For estimation of Boron the leaf samples were dry ashed. 0.5 g of dried sample was placed in a silica crucible and ashed at 550° C in a muffle furnace. The grey white ash was dissolved in 10 ml of 6 N HCl and heated to 80° C on a hot plate to evaporate to dryness. The residue was dissolved in double distilled water and transferred to 50 ml volumetric flask and volume was made up the mark with double distilled water. The solution was then filtered through Whatman No. 42 filter paper. After digestion Boron was estimated by using Azomethine- H method [10].

2.6 Statistical analysis

The data generated from these investigations were appropriately computed, tabulated and analyzed by applying CRD Factorial and RBD Factorial designs. The level of significance was tested at 5% for different variables [11]. The data recorded was statistically analyzed by using MS-Excel and OPSTAT.

3 RESULTS AND DISCUSSION

3.1 Effect of different nitrogen sources on leaf micronutrients in apple seedlings (pot culture study) at Nauni

3.1.1 Boron

The data on presented in table 3 on the effect of nitrogen fertilizers on leaf boron content did not show any definite trend. During 2016, leaf boron content was non-significant among the treatments with maximum (35.64 ppm) being under urea application. During 2017, maximum boron content of 35.48 ppm was noticed under urea followed by calcium nitrate alone application (35.01 ppm) and minimum leaf boron content of 26.16 ppm was recorded under control. Pooled data analysis revealed that the maximum boron was recorded under trees receiving urea (35.56 ppm) followed by 50% urea (soil) + foliar N (33.98 ppm) and minimum under urea + liming (In March) (27.50 %). The interaction between treatment and year (Y×T) as well as effect of years was non-significant.

Table 3. Effect of different nitrogen sources on leaf boron and Copper of container grown plants

Treatment	Boron (ppm)			Copper (ppm)		
	2016	2017	Pooled	2016	2017	Pooled
Control (No application)	29.84	26.16	28.00	8.05	7.98	8.02
Urea	35.64	35.48	35.56	14.96	15.05	15.00
Calcium Nitrate	32.76	35.01	33.88	13.35	13.42	13.39
Calcium Cyanamide	31.57	34.04	32.81	9.78	9.86	9.82
Urea + Liming (In October)	29.09	27.80	28.44	11.87	11.91	11.89
Urea + Liming (In March)	28.57	26.43	27.50	11.42	11.51	11.46
12: 32: 16 + Urea	33.79	31.57	32.68	14.05	14.11	14.08

12: 32: 16 + Calcium Nitrate	32.24	33.15	32.70	12.85	12.89	12.87
12: 32: 16 + Calcium Cyanamide	30.35	34.51	32.43	9.92	9.97	9.95
15:15:15 + Urea	33.45	32.08	32.77	13.85	13.92	13.89
15: 15: 15 + Calcium Nitrate	30.72	34.15	32.44	12.62	12.66	12.64
15: 15: 15 + Calcium Cyanamide	31.59	32.43	32.01	10.23	10.27	10.25
50% Urea (soil) + Foliar N	34.88	33.08	33.98	10.74	10.82	10.78
Calcium nitrate + urea +Liming	29.57	31.75	30.66	11.09	11.16	11.13
Mean	31.72	31.97		11.77	11.82	
$CD_{0.05}$	NS	4.86		0.50	0.53	
Year (Y):			NS			NS
Treatment(T):			4.00			0.37
Y × T:			NS			NS

3.1.2 Copper

Data depicted in table 3 clearly indicated that leaf copper content was significantly influenced by different nitrogen fertilizers. The highest values of leaf copper were noticed under application of urea i.e. 14.96 and 15.05 ppm during 2016 and 2017, respectively. The lowest values of copper were recorded under control (no fertilizer application) i.e. 8.05 and 7.98 ppm during 2016 and 2017, respectively. Pooled data revealed that the highest leaf copper content was recorded under urea alone application (15.00 ppm) and lowest under control (8.02 ppm). The data on the effect of years as well as interaction (Y×T) was, however, non significant. [12] and [13] also reported that copper contents of the leaves increased with the application of nitrogen fertilizers.

3.1.3 Iron

A data presented in table 4 revealed that leaf iron contents were significantly influenced by different nitrogen fertilizers. In 2016, highest leaf iron content was recorded under urea (188.71 ppm) which was at par with calcium nitrate (183.32 ppm), 12:32:16 + urea (185.46 ppm) and 15:15:15 + urea (184.58 ppm) and lowest iron content (143.65 ppm) was recorded under control. During 2017, highest leaf iron content was under urea application (194.48 ppm) which was at par with calcium nitrate (189.35 ppm) and 15:15:15 + urea (192.70 ppm) and lowest (138.16 ppm) under control. Pooled data analysis reveals that the highest leaf iron content was recorded under urea alone application (191.60 ppm) which was at par with calcium nitrate (186.34 ppm) and 15:15:15 + urea (188.64 ppm) and lowest (140.91 ppm) under control. The data on years and the interaction between year and treatment (T×Y) revealed significant effect. Increase in leaf iron content might be due to soil acidification properties of nitrogen fertilizers that increase the availability of iron in soil, hence the uptake by the plant. The results are in agreement with those of [12] and [13] who reported that iron contents of the leaves increased on application of nitrogen fertilizers.

3.1.4 Zinc

Data depicted in table 4 clearly indicate that leaf zinc content was significantly influenced by different nitrogen fertilizers and its behavior was similar to other micronutrients. In 2016, highest leaf zinc content was recorded under urea (31.52 ppm) which was statistically at par with 12:32:16 + urea (29.80 ppm) and the lowest (17.34 ppm) was under control. During 2017, highest zinc content was recorded under urea application (32.51 ppm) which was at par with 12:32:16 + urea (29.05 ppm), whereas, the lowest zinc content (17.12 ppm) was recorded under control. Pooled data analysis revealed that the highest leaf zinc content was recorded under urea alone application (32.01 ppm) and the lowest under control (17.23 ppm). The data on years and interaction between year and treatment (Y×T) reveals non significant effect. Nitrogen and zinc are the examples of nutrients showing relative high mobility and when taken up by the leaves they can be distributed throughout the entire plant [14]. Zinc contents of the leaves increased with the use of nitrogen fertilizers [12].

3.1.5 Manganese

A perusal of the data presented in table 4 revealed that nitrogen fertilizers have significant effect on leaf manganese content. In 2016, the highest leaf manganese content was recorded under urea with mean value of 64.83 ppm which was statistically at par with 12:32:16 + urea (62.58 ppm) and 15:15:15 + urea (61.42 ppm). The lowest manganese content (45.32 ppm) was recorded under control. During 2017, the highest manganese content was recorded under urea application with mean value of 65.09 ppm which was at par with 12:32:16 + urea (62.06 ppm) and 15:15:15 + urea (62.16 ppm). The lowest manganese content (44.64 ppm) was recorded under control. Pooled data analysis revealed that the effect of different nitrogen sources on leaf manganese content was significant. The highest leaf manganese content was recorded under urea alone application (64.96 ppm). The values between the year and the interaction between year and treatment (Y×T) were non-significant. It might be due to soil acidification properties of nitrogen fertilizers mainly urea. Similar findings were also recorded by [13].

Table 4: Effect of different nitrogen sources on leaf iron, zinc and manganese of container grown plants

Treatment	Iron (ppm)			Zinc (ppm)			Manganese (ppm)		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
Control (No application)	143.65	138.16	140.91	17.34	17.12	17.23	45.32	44.64	44.98
Urea	188.71	194.48	191.60	31.52	32.51	32.01	64.83	65.09	64.96
Calcium Nitrate	183.32	189.35	186.34	27.76	28.16	27.96	59.91	60.24	60.08
Calcium Cyanamide	157.04	161.27	159.15	22.82	22.94	22.88	53.47	53.91	53.69
Urea + Liming (In October)	174.34	182.28	178.31	20.86	21.16	21.01	48.29	49.40	48.84
Urea + Liming (In March)	170.40	178.19	174.29	21.58	21.24	21.41	50.32	50.56	50.44
12: 32: 16 + Urea	185.46	180.18	182.82	29.80	29.05	29.42	62.58	62.06	62.32
12: 32: 16 + Calcium Nitrate	179.17	186.27	182.72	26.24	27.16	26.70	59.32	60.31	59.82
12: 32: 16 + Calcium Cyanamide	160.83	154.76	157.79	23.67	24.34	24.01	54.32	54.82	54.57
15:15:15 + Urea	184.58	192.70	188.64	27.05	27.90	27.48	61.42	62.16	61.79
15: 15: 15 + Calcium Nitrate	177.06	172.06	174.56	26.81	27.42	27.12	56.64	57.28	56.96
15: 15: 15 + Calcium Cyanamide	162.52	158.84	160.68	22.04	23.27	22.65	52.78	53.82	53.30
50% Urea (soil) + Foliar N	163.61	170.13	166.87	25.49	26.43	25.96	54.88	55.47	55.17
Calcium nitrate + urea +Liming	167.36	169.14	168.25	22.87	23.57	23.22	52.05	52.81	52.43

Mean	171.29	173.41		24.70	25.16		55.44	55.90	
$CD_{0.05}$	7.79	8.99		3.18	3.59		3.44	3.50	
Year (Y):			NS			NS			NS
Treatment(T):			5.80			2.34			2.39
Y × T:			8.20			NS			NS

3.2 Effect of different nitrogen sources on leaf micronutrients in apple plants (Field study) at Rohru and Seobagh

3.2.1 Boron

The data on effect of nitrogen fertilizers on leaf boron content did not reveal definite trend and is presented in table 5. At Seobagh, during 2016, the difference in the leaf boron content were non-significant with maximum (37.87 ppm) being under urea application.

During 2017, maximum boron content of 38.14 was noticed under 12:32:16 + calcium nitrate followed by 37.42 ppm in trees receiving 15:15:15 + calcium cyanamide and minimum leaf boron content (27.86 ppm) was recorded under urea + liming (In March). Pooled data analysis revealed that the effect of different nitrogen sources on boron content varied with a maximum in trees receiving 12:32:16 + calcium nitrate (36.71 ppm) followed by calcium nitrate alone (36.49 ppm) and minimum under control (29.64 %). The effects of year and the data on interaction between year and treatment (Y×T) were found to be non-significant.

Table 5. Effect of different nitrogen sources on leaf boron (ppm) in apple

Site / Treatment	Seobagh			Rohru		
	2016	**2017**	**Pooled**	**2016**	**2017**	**Pooled**
Control (No application)	32.39	30.28	31.34	24.45	22.51	23.48
Urea	37.87	34.14	36.01	33.56	27.43	30.49
Calcium Nitrate	35.84	37.15	36.49	28.58	30.36	29.47
Calcium Cyanamide	33.78	35.48	34.63	26.24	29.41	27.83
Urea + Liming (In October)	31.87	28.14	30.00	23.81	20.31	22.06
Urea + Liming (In March)	31.43	27.86	29.64	23.07	19.91	21.49
12: 32: 16 + Urea	36.46	33.05	34.76	29.61	26.58	28.09
12: 32: 16 + Calcium Nitrate	35.28	38.14	36.71	27.51	29.75	28.63
12: 32: 16 + Calcium Cyanamide	32.91	35.31	34.11	24.92	27.36	26.14
15:15:15 + Urea	36.37	33.05	34.71	28.23	23.14	25.69
15: 15: 15 + Calcium Nitrate	33.44	36.47	34.95	25.57	27.54	26.56
15: 15: 15 + Calcium Cyanamide	34.61	37.42	36.02	26.83	28.06	27.45

50% Urea (soil) + Foliar N	36.79	34.03	35.41	29.11	25.17	27.14
Calcium nitrate + urea +Liming	32.14	35.43	33.79	24.17	28.47	26.32
Mean	34.37	34.00		26.83	26.14	
$CD_{0.05}$	NS	5.66		NS	5.45	
Year (Y):			NS			NS
Treatment(T):			3.48			4.00
Y × T:			NS			NS

At Rohru, during 2016, boron content was non-significant with maximum leaf boron content of 33.56 ppm being recorded under urea alone treatment. During 2017, maximum boron content was noticed in trees receiving calcium nitrate (30.36 ppm) followed by trees receiving 12:32:16 + calcium nitrate (29.75 ppm) and minimum leaf boron content of 19.91 was recorded in trees receiving urea + liming (In March). Pooled data analysis revealed that the effect of different nitrogen sources on boron content was significant. Maximum boron was noticed in urea application (30.49 ppm) followed by calcium nitrate (29.47 ppm) and minimum under urea + liming (In March). The interaction between treatment and year (Y×T) and year (Y) presented in table 4 revealed a non-significant effect.

Table 6. Effect of different nitrogen sources on leaf copper (ppm) in apple

Site / Treatment	Seobagh			Rohru		
	2016	**2017**	**Pooled**	**2016**	**2017**	**Pooled**
Control (No application)	10.35	10.19	10.27	12.09	11.86	11.98
Urea	16.83	17.02	16.93	17.87	18.03	17.95
Calcium Nitrate	14.93	15.03	14.98	15.85	15.97	15.91
Calcium Cyanamide	11.23	11.26	11.25	12.94	13.05	13.00
Urea + Liming (In October)	13.95	14.09	14.02	14.88	14.97	14.92
Urea + Liming (In March)	13.10	13.24	13.17	14.79	14.86	14.83
12: 32: 16 + Urea	15.74	15.86	15.80	16.72	16.80	16.76
12: 32: 16 + Calcium Nitrate	14.32	14.41	14.37	15.43	15.46	15.45
12: 32: 16 + Calcium Cyanamide	11.40	11.49	11.44	13.29	13.38	13.33
15:15:15 + Urea	15.21	15.34	15.27	16.34	16.45	16.40
15: 15: 15 + Calcium Nitrate	14.00	14.24	14.12	15.14	15.21	15.17
15: 15: 15 + Calcium Cyanamide	11.45	11.51	11.48	13.74	13.81	13.78
50% Urea (soil) + Foliar N	12.52	12.56	12.54	13.89	13.96	13.93
Calcium nitrate + urea +Liming	12.70	12.84	12.77	14.32	14.45	14.39

Mean	13.41	13.51		14.81	14.88	
$CD_{0.05}$	0.48	0.61		0.77	0.81	
Year (Y):			NS			NS
Treatment(T):			0.37			0.53
Y × T:			NS			NS

3.2.2 Copper

Data depicted in table 6 clearly indicated that leaf copper content was significantly influenced by different nitrogen fertilizers. At Seobagh and Rohru, the highest values of leaf copper were noticed under application of urea i.e. 16.83, 17.02, 17.87 and 18.03 ppm during 2016 and 2017, respectively. The lowest values of copper were recorded under control (no fertilizer application) i.e. 10.35, 10.19, 12.09 and 11.86 ppm during 2016 and 2017, respectively. The pooled data analysis also revealed that the highest leaf copper content was recorded under urea alone application (16.93 and 17.95 ppm for Seobagh and Rohru, respectively). The data on interaction between year and treatment (Y×T) and year (Y) presented in table 6 revealed a non-significant effect.

Saini and Singh [12-13] also reported that leaf copper contents of the leaves increased on application of nitrogen fertilizers, which may be ascribed to better root growth as well as improved physiological activity in the plants as well as its increased availability in the soil.

3.2.3 Iron

A perusal of the data presented in table 7 revealed that leaf iron content was significantly influenced by different nitrogen fertilizers. The highest values of leaf iron were noticed under application of urea at both the locations and in both the years. At Seobagh, 218.25 and 225.15 ppm during 2016 and 2017, respectively and at Rohru, 235.64 and 247.18 ppm during 2016 and 2017, respectively. The lowest values of copper were recorded under control (no fertilizer application) i.e. 170.15, 162.42 ppm (at Seobagh) and 182.15, 171.64 ppm (at Rohru) during 2016 and 2017, respectively. The pooled data analysis also revealed that the highest leaf iron content was recorded under urea alone application (221.70 and 241.41 ppm for Seobagh and Rohru, respectively). The interaction between year and treatment (Y×T) and year (Y) presented in table 7 revealed a non-significant effect.

Increase in leaf iron content might be due to soil acidification properties of nitrogen fertilizers that increase the availability of iron in soil, hence the uptake by the plant. The results are in agreement with that of Saini and Singh [12-13] who reported that iron content of the leaves increased with application of nitrogen fertilizers.

Table 7. Effect of different nitrogen sources on leaf iron (ppm) in apple

Site / Treatment	Seobagh			Rohru		
	2016	**2017**	**Pooled**	**2016**	**2017**	**Pooled**
Control (No application)	170.15	162.42	166.29	182.15	171.64	176.90
Urea	218.25	225.15	221.70	235.64	247.18	241.41
Calcium Nitrate	200.34	209.48	204.91	217.38	224.35	220.86
Calcium Cyanamide	176.15	184.54	180.34	190.08	198.47	194.28
Urea + Liming (In October)	194.05	203.43	198.74	207.18	219.24	213.21
Urea + Liming (In March)	189.06	195.06	192.06	207.59	217.80	212.69
12: 32: 16 + Urea	208.19	216.35	212.27	226.35	235.45	230.90

12: 32: 16 + Calcium Nitrate	199.21	211.42	205.32	213.65	219.74	216.69
12: 32: 16 + Calcium Cyanamide	178.38	185.42	181.90	194.54	202.06	198.30
15:15:15 + Urea	203.08	211.26	207.17	221.35	228.47	224.91
15: 15: 15 + Calcium Nitrate	197.46	208.58	203.02	211.40	215.61	213.50
15: 15: 15 + Calcium Cyanamide	181.06	192.45	186.76	196.74	201.09	198.92
50% Urea (soil) + Foliar N	184.71	187.06	185.88	201.87	207.19	204.53
Calcium nitrate + urea +Liming	185.15	189.41	187.28	204.38	209.41	206.90
Mean	191.80	198.72		207.88	214.12	
$CD_{0.05}$ **Year (Y):** **Treatment(T):** **Y × T:**	8.79	8.90	 2.32 6.13 NS	9.00	8.41	 2.28 6.03 NS

<u>3.2.4 Manganese</u>

A perusal of the data presented in table 8 revealed that nitrogen fertilizers had significant effect on leaf manganese content. At Seobagh in 2016 and 2017, the highest leaf manganese content was recorded under urea alone treated trees (76.48 and 78.16 ppm, respectively) which was statistically at par with 12:32:16 + urea (74.23 and 75.41 ppm, respectively) and 15:15:15 + urea (73.56 and 75.84 ppm, respectively). The lowest manganese content (51.08 and 51.89 ppm, respectively) was recorded under urea + liming (In October). The pooled data revealed that the highest leaf manganese content was recorded under urea alone application (77.32 ppm) which was statistically at par with 12:32:16 + urea (74.82 ppm) and 15:15:15 + urea (74.70 ppm). The interaction between year and treatment (Y×T) was non-significant.

Table 8. Effect of different nitrogen sources on leaf manganese (ppm) in apple

Site / Treatment	**Seobagh**			**Rohru**		
	2016	**2017**	**Pooled**	**2016**	**2017**	**Pooled**
Control (No application)	58.23	57.15	57.69	62.32	61.76	62.04
Urea	76.48	78.16	77.32	83.23	85.15	84.19
Calcium Nitrate	70.96	72.81	71.89	77.92	78.38	78.15
Calcium Cyanamide	65.56	66.84	66.20	70.46	72.06	71.26
Urea + Liming (In October)	51.08	51.89	51.49	65.56	66.42	65.99
Urea + Liming (In March)	61.87	63.48	62.68	66.87	66.94	66.90
12: 32: 16 + Urea	74.23	75.41	74.82	81.26	81.86	81.56
12: 32: 16 + Calcium Nitrate	69.20	70.65	69.93	77.92	79.28	78.60
12: 32: 16 + Calcium Cyanamide	67.10	69.48	68.29	73.05	74.64	73.85
15:15:15 + Urea	73.56	75.84	74.70	78.79	80.16	79.48

15: 15: 15 + Calcium Nitrate	68.05	68.94	68.50	77.04	78.64	77.84
15: 15: 15 + Calcium Cyanamide	64.23	66.10	65.17	68.34	70.61	69.47
50% Urea (soil) + Foliar N	67.72	68.79	68.26	77.10	77.92	77.51
Calcium nitrate + urea +Liming	62.34	63.48	62.91	68.82	70.38	69.60
Mean	66.47	67.79		73.48	74.59	
$CD_{0.05}$	4.08	3.92		4.07	4.29	
Year (Y):			1.06			1.08
Treatment(T):			2.80			2.85
Y × T:			NS			NS

Also at Rohru, the highest leaf manganese content was recorded in trees treated with only urea (83.23 and 85.15 ppm, respectively) which was statistically at par with 12:32:16 + urea (81.26 and 81.86 ppm, respectively). The lowest manganese content (62.32 and 61.76 ppm, respectively) was recorded under control. The pooled data shows that the effect of different nitrogen sources on leaf manganese content was significant with the highest leaf manganese content of 84.19 ppm being in trees receiving urea alone which was statistically at par with 12:32:16 + urea (81.56 ppm). The interaction between treatment and year (Y×T) was, however, non-significant. It might be due to soil acidification properties of nitrogen fertilizers mainly urea which decrease the pH of the soil and consequently the dissolution/availability of manganese in the soil solution manifolds. As a result of this increased availability the nutrient content/uptake in plants also increases. Similar findings were also recorded by [13].

3.2.5 Zinc

The data depicted in table 9 clearly indicated that leaf zinc content was significantly influenced by different nitrogen fertilizers and behavior was similar to other micronutrients. At Seobagh in 2016 and 2017, the highest leaf zinc content was recorded under urea alone treated trees (39.40 and 40.84 ppm, respectively) which was statistically at par with 12:32:16 + urea (37.82 and 39.54 ppm, respectively) and 15:15:15 + urea (37.38 and 37.85 ppm, respectively). The lowest zinc content (21.51 and 21.05 ppm, respectively) was recorded under control. The pooled data revealed that the highest leaf zinc content was recorded under urea alone application (40.12 ppm) which was statistically at par with 12:32:16 + urea (38.68 ppm). The interaction between year and treatment (Y×T) was non-significant.

Table 9. Effect of different nitrogen sources on leaf zinc (ppm) in apple

Site / Treatment	**Seobagh**			**Rohru**		
	2016	**2017**	**Pooled**	**2016**	**2017**	**Pooled**
Control (No application)	21.51	21.05	21.28	22.46	21.86	22.16
Urea	39.40	40.84	40.12	39.28	41.16	40.22
Calcium Nitrate	35.64	37.16	36.40	34.85	35.15	35.00
Calcium Cyanamide	25.87	26.80	26.33	26.90	27.18	27.04
Urea + Liming (In October)	24.42	24.91	24.67	25.37	26.34	25.85
Urea + Liming (In March)	24.81	25.56	25.19	25.21	27.51	26.36
12: 32: 16 + Urea	37.82	39.54	38.68	37.53	39.54	38.54
12: 32: 16 + Calcium Nitrate	33.51	34.06	33.78	33.46	34.06	33.76

12: 32: 16 + Calcium Cyanamide	29.21	31.05	30.13	29.18	29.86	29.52
15:15:15 + Urea	37.38	37.85	37.62	36.14	37.11	36.63
15: 15: 15 + Calcium Nitrate	31.97	32.56	32.27	31.69	33.51	32.60
15: 15: 15 + Calcium Cyanamide	27.69	29.18	28.44	28.57	30.65	29.61
50% Urea (soil) + Foliar N	30.61	31.26	30.94	29.90	30.28	30.09
Calcium nitrate + urea +Liming	25.02	27.41	26.21	26.31	27.43	26.87
Mean	30.35	31.37		30.49	31.55	
$CD_{0.05}$	4.05	3.30		3.93	3.66	
Year (Y):			0.94			1.02
Treatment(T):			2.49			2.71
Y × T:			NS			NS

Also at Rohru, the highest leaf zinc content was recorded in trees treated with only urea (39.28 and 41.16 ppm, respectively) which was statistically at par with 12:32:16 + urea (37.53 and 39.54 ppm, respectively). The lowest zinc content (22.46 and 21.86 ppm, respectively) was recorded under control. The pooled data shows that the effect of different nitrogen sources on leaf zinc content was significant with the highest leaf zinc content of 40.22 ppm being in trees receiving urea alone which was statistically at par with 12:32:16 + urea (38.54 ppm). The interaction between treatment and year (Y×T) was, however, non-significant. Nitrogen and zinc are the examples of nutrients showing relatively high mobility and when taken up by the leaves they can be distributed throughout the entire plant [14]. Leaf zinc contents of the leaves have also been shown to increase with the increase in the use of nitrogen fertilizers [12].

4 CONCLUSION

Leaf micronutrients (Fe, Cu, Mn, Zn and B) were significantly affected on application of nitrogen fertilizers and were the highest under urea alone application. It was found to have a direct relation with the reduction in pH of the soils as the availability of micronutrients increases at low pH values. It indicates that application of urea may help recover the micronutrient deficiencies that are prevalent in apple.

REFERENCES

1. Cmelik Z, Tojnko S and Unuk T. Fruit quality of 'Fuji' apple as affected by crop load and rates of nitrogen. *Acta Horticulture.* 2006; 721:147–152.

2. Fallahi E, Fallahi B, Neilsen GH, Neilsen D, Peryea FJ. Effects of mineral nutrition on fruit quality and nutritional disorders in apples. *Acta Horticulture.* 2010; 868: 49–60.

3. Ernani PR and Dias. Soil nitrogen application in the spring did not increase apple yield. *Ciencia Rural.* 1999; 29: 645-649.

4. Bi G, Scagel CF, Cheng L, Dong S and Fuchigami H. Spring growth of almond nursery trees depends upon nitrogen from both plant reserves and spring fertilizer application. *Journal of Horticultural Science and Biotechnology.* 2003; 78: 853-858.

5. Dong S, Chang L, Scagel CF and Fuchigami LH. Timining of urea application affects leaf and root N uptake in young Fuji/M9 apple trees. *Journal of Horticultural Science and Biotechnology.* 2005; 80: 116-120.

6. Milosevic T and Milosevic N. Apple fruit quality, yield and leaf macronutrients content as affected by fertilizer treatment. *Journal of Soil Science and Plant Nutrition.* 2015; 15: 76-83.

7. Chapman HD. Suggested foliar sampling and handling techniques for determining the nutrient status of some field, horticultural and plantation crops. *Indian Journal of Horticulture.* 1964; 21: 97-119.

8. Piper CS. *Soil and Plant Analysis*. Hans Publishers, Bombay, India. 368 p.; 1966.

9. Sarma VAK, Krishna P and Budhihal SL. *Soil resource mapping of different states in India- A laboratory manual.* National Bureau of Soil survey and Land Use Planning, Nagpur. 49p.; 1987.

10. Sarkar D, Sheikh AA, Kaushik B and Mandal B. Boron estimation in soil, plant, and water samples using spectrophotometric methods. *Communications in Soil Science and Plant analysis.* 2014; 45: 1538-1550.

11.Gomez KA and Gomez AA. *Statistical Procedure for Agricultural Research.* 2nd Ed. John Wiley and Sons, Inc., New York, USA. 680p; 1984.

12. Saini P. *Comparative studies on the effects of nitrogenous fertilizers on growth, yield and quality in plum cv. Santa Rosa*. Ph.D. Thesis, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan, India; 2011.

13. Singh D, Bhatia HS, Kumar P, Kumari N and Deepika. Nitrogen source fertilization for nutrient profiling of Royal Delicious apples in temperate ecosystem. *International Journal of Farm Sciences.* 2018; 8(2): 169-175.

14. Mengel K. Alternative or complementary role of foliar supply in mineral nutrition. *Acta Horticulturae.* 2002; 594: 33–47.