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# Comparative efficiency of different nitrogen sources on soil microbiological properties in apple

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#### Abstract

The study was conducted at three different locations viz. Solan, pot culture (cv. Starking Delicious), Seobagh, Kullu (cv. Vance Delicious) and Rohru, Shimla (cv. Red Chief) during 2015-16 and 2016-17 with 14 treatments comprising different sources including urea (soil and foliar) and liming, calcium nitrate, calcium cyan amide, 12:32:16, 15:15:15 and their combinations replicated thrice in a RBD under field conditions and CRD under container grown experiment. The different sources were given on N equivalence basis depending upon the age of the plants. In Starching Delicious apple plants grown in containers the highest values of phosphatase were noticed under the application of calcium nitrate (7.93 and 7.83 µmoles of p-nitrophenol (g soil) -1 hr-1 in 2016 and 2017 respectively); dehydrogenase was highest under application of calcium nitrate + urea + liming i.e. 4.28 and 4.32 mg TPF (g soil)<sup>-1</sup> during 2016 and 2017, respectively; and urease enzyme was maximum (0.47 µg (NH<sub>4</sub>)<sup>+</sup> (g soil)<sup>-1</sup> hr<sup>-1</sup>) in urea + liming (In March) in 2016 and under calcium nitrate + urea + liming  $(0.45 \ \mu g \ (NH_4)^+ \ (g \ soil)^{-1} \ hr^{-1})$  in 2017. Total microbial count was highest under calcium nitrate + urea + liming i.e.  $139.05 \times 10^5$  and  $143.05 \times 10^5$  cfu g<sup>-1</sup> soil in 2016 and 2017 respectively. At Seobagh highest total microbial count was recorded under calcium nitrate + urea + liming  $(133.27 \times 10^5 \text{ and } 138.05 \times 10^5 \text{cfu g}^{-1} \text{ soil in 2016 and }$ 2017 respectively) and also at Rohru it was highest under calcium nitrate + urea + liming  $(145.30 \times 10^5)$ and  $138.14 \times 10^5$  cfu g<sup>-1</sup> soil in 2016 and 2017 respectively).

Keywords: Nitrogen sources, fertilizer, microbiological properties, soil enzymes, microbial count

# Introduction

Apple (*Malus domestica* Borkh) a native to South East Asia is one of the most important and widely grown fruit crop in temperate region of the world. It occupies a significant place in horticultural wealth of temperate zone. The farmers have gone for intensive cultivation of apple and as a result have been using the fertilizer resources without any sound knowledge of the requirements as decided by the soil test values. The application of nitrogenous fertilizers is of paramount importance in addition to phosphatic and potassic fertilizers. This is because nitrogen is one of the basic mineral elements required by plants. Nitrogen is often more limiting factor influencing plant growth than any other nutrient. Application of nitrogen to apple every year has a direct effect on growth, yield and fruit quality. Some growers prefer to apply nitrogen in excess to avoid problems related with insufficient availability of N to plants, especially in orchards (Weinbaum *et al.*, 1992<sup>[1]</sup>; Tagliavini *et al.*, 1996<sup>[2]</sup>).

Soil organisms play an important role in nutrient cycling. They can affect the availability of Nutrients through decomposition of organic matter, immobilization and mineralization of nutrients, bioturbation and biological N fixation (Davies *et al.* 2022)<sup>[3]</sup>. Inorganic N fertilizer such as urea, plays a quick role in enhancing the crop growth and also affects the microbial community. According to Allison and Martiny (2008)<sup>[4]</sup> 84% of 38 studies showed a significant change in soil microbial community population with the use of chemical fertilization. It can also results in change in soil enzyme activity. Therefore, to study the effect of various nitrogen sources on soil microbial count and on soil enzymes present study was undertaken.

# **Materials and Methods**

**Experiment 1:** To evaluate the effect of different nitrogen sources on soil microbiological properties and plant growth of apple seedlings (Pot culture studies).

**Location:** The 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,

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Nauni, Solan (H.P), located at  $30^{\circ}$  52' N latitude and  $77^{\circ}$  11' E longitude and at an elevation of 1175 m above mean sea level.

**Experiment details:** 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.

**Quantity of fertilizers:** 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 treatments  $T_7$  to  $T_{12}$  the amount of P and K added through mixed fertilizers (12:32:16 and 15:15:15) was calculated and the remaining amount of P and K was added through SSP and MOP as in the case of other treatments.

#### **Treatments details:**

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

# **RD** (Recommended dose) of fertilizers:

i) One year old apple tree: N:  $P_2O_5$ : K<sub>2</sub>O: 70: 35: 70 g/tree. ii) Ten year or above old apple tree: N:  $P_2O_5$ : K<sub>2</sub>O: 700: 350: 700g /tree.

**Experiment 2:** To study the effect of different nitrogen sources on soil properties, growth and productivity of apple (Field studies).

**Location:** The experiment was carried out at two locations i.e. Krishi Vigyan Kendra, Rohru, Shimla which is about 120 km away from Shimla town, located at  $31^{\circ}$  13' N latitude and 77° 43' E longitude and at an elevation of 1710 m above mean sea level and at Horticultural Research Station, Seobagh, Kullu, located at  $31^{\circ}$  59' N latitude and 77° 08' E longitude and at an elevation of 1341 m above mean sea level

**Experiment details:** The experiment was laid out in a Randomized Block Design factorial and comprised of fourteen treatments and each treatment was replicated three times having one plant under each replication. At Rohru, the experiment was conducted on variety Red Chief and at Seobagh, variety was Vance delicious.

# **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<sub>14</sub>: Calcium Nitrate + Urea + Liming

#### **RD** (Recommended dose) 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.

**Quantity of fertilizers:** 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.

**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.

**Soil sampling and methodologies for analysis:** Representative soil samples from 0-15 cm depth were collected 30 cm away from tree trunk. The soil samples were collected after crop harvest during both the years of study. Collected soil samples were air dried in shade and ground with the help of wooden pestle and mortar. These ground samples were then passed through 2 mm sieve for further analysis.

Determination of total microbial count (bacteria, fungi and actinomycetes count in soil): The serial dilution and plating technique suggested by Rao (1999) <sup>[5]</sup> was employed for isolation and identification of viable bacteria, actinomycetes and fungi count. Media were prepared for desired micro flora. The autoclaved and cooled ( $45^{\circ}$ C) medium was poured into sterile plates and allowed to solidify. One gram of sieved (2 mm) soil was added to 9 ml sterile water blank and shaked for 15-20 minutes. Serial dilutions of  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$  and  $10^{-7}$  were prepared and 0.1 ml of aliquots of various dilutions were added, cooled and solidified medium in petri plates. Pour plate method was employed. The plates were rotated for uniform distribution of bacterial cells and fungal spores in the aliquot under the media and allowed

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to solidify. After the media solidified, the plates were inverted and incubated at  $28^{\circ}$ C for 3-4 days. The appearances of colonies on the surface of medium in the plates were observed. Soil extract media was used for total microbial count.

#### **Determination of Soil enzymes**

**Dehydrogenase:** The dehydrogenase enzyme estimation was carried out by method given by Casida *et al.* (1977) <sup>[6]</sup>. One g of soil was incubated for 12 hours with 1 ml of 3% TTC (Triphenyltetrazolium chloride) and 0.5 ml of 1% glucose. After incubation 10 ml of methanol was added. Then the test tube was shaken and allowed to stand in dark for 24 hours. Supernatant was withdrawn and colour intensity was measured using blue filter at 485 nm wavelength. The amount of formazan formed from standard curve prepared from TPF (Triphenylformazan) was in the range of 0.04 to 0.5 mg 10 ml<sup>-1</sup>. The results were expressed in the terms of mg TPF per hour per gram of soil.

**Phosphatase:** The phosphtase enzyme estimation was carried out by method given by Tabatabai and Bremner (1969) <sup>[7]</sup>. One gram of soil taken in test tube was incubated with 1 ml of 5mM buffered sodium p-nitrophenyl phosphate in acetate buffer (pH 5.2) and 0.3 ml toluene at 37°C for 1 hour. Determination of p-nitrophenol involved the colorimetric analysis of the extract obtained by treating the incubated soil sample with 4 ml water, 10 ml of 0.5 M NaOH and by filtering it through Whatman no. 42 filter paper, The suspension obtained by shaking the mixture for 1 minute and absorbance of yellow color of p-nitrophenol released was determined spectrophotometrically at 420 nm wavelength. The standard curve was prepared by p-nitrophenol (10-100 ppm). The result was expressed as  $\mu$  mole of p-nitrophenol per gram soil per hour.

**Urease:** 10 g of dry and sieved soil was incubated for 15 min with 15 ml of toluene. 10 ml of urea solution and 20 ml of citrate buffer were added, mixed and incubated for 3 hours at 37° C. Then diluted to 100 ml with water, mixed and filtered. Pipette out 1 ml of filtrate, added 9 ml of water, 4 ml phenate solution and 3 ml of sodium hypochlorite solution. Mixed and allowed standing for 20 minutes until the maximum colour was obtained. Diluted to 50 ml with water, mixed well, and read the transmittance or absorbance at 630 nm against the blank. The standard curve was prepared from ammonium sulphate solution (10 µg N ml<sup>-1</sup>). Results were expressed as µg NH<sub>4</sub><sup>+</sup> per g soil per hr to get Urease number. Urease number was multiplied by 0.32 to obtain urease units (Kandeler and Gerber, 1988) <sup>[8]</sup>.

# Results and Discussion Experiment 1

Phosphatase enzyme: Application of different nitrogen sources exerted a significant influence on phosphatase enzyme (Table 1). The highest values of phosphatase were noticed under application of calcium nitrate i.e. 7.93 and 7.85 µmoles of p-nitrophenol (g soil) <sup>-1</sup> hr<sup>-1</sup> during 2016 and 2017, respectively. The lowest values of phosphatase were recorded under control (no fertilizer application) i.e. 4.21 and 4.08 µmoles of p-nitrophenol (g soil) <sup>-1</sup> hr<sup>-1</sup> during 2016 and 2017, respectively. Pooled data analysis reveals that the highest phosphatase content was recorded under calcium nitrate application (7.89 µmoles of p-nitrophenol (g soil) <sup>-1</sup> hr<sup>-1</sup>) and lowest under control (4.14 µmoles of p-nitrophenol (g soil)<sup>-1</sup> hr<sup>-1</sup>). The data on interaction (Y×T) as well as on years reveals a non-significant effect. The present findings are in agreement with Ling et al. (2017)<sup>[9]</sup>, who reported the increase in the activity of phosphatase enzyme on application of nitrogen fertilizers.

	Phosphatase			Dehydrogenase			Urease		
Treatment	(µmoles of p-nitrophenol (g soil) <sup>-1</sup> hr <sup>-1</sup> )			(mg TPF (g soil) <sup>-1</sup> )			(µg (NH4) <sup>+</sup> (g soil) <sup>-1</sup> hr <sup>-1</sup> )		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
Control (No application)	4.21	4.08	4.14	2.21	2.14	2.18	0.14	0.13	0.14
Urea	6.46	6.38	6.42	2.75	2.81	2.78	0.43	0.40	0.42
Calcium Nitrate	7.93	7.85	7.89	4.07	4.13	4.10	0.37	0.32	0.34
Calcium Cyanamide	5.72	5.78	5.75	2.53	2.62	2.58	0.21	0.25	0.23
Urea + Liming (In October)	5.26	5.21	5.24	3.59	3.45	3.52	0.40	0.37	0.38
Urea + Liming (In March)	6.89	6.81	6.85	3.92	3.86	3.89	0.47	0.42	0.44
12: 32: 16 + Urea	4.97	5.06	5.02	2.83	2.87	2.85	0.34	0.39	0.36
12: 32: 16 + Calcium Nitrate	7.72	7.78	7.75	3.84	3.75	3.79	0.38	0.42	0.40
12: 32: 16 + Calcium Cyanamide	4.78	4.86	4.82	3.04	3.15	3.10	0.21	0.26	0.24
15:15:15 + Urea	6.03	6.08	6.06	3.52	3.58	3.55	0.30	0.35	0.32
15: 15: 15 + Calcium Nitrate	7.21	7.15	7.18	3.71	3.74	3.73	0.37	0.41	0.39
15: 15: 15 + Calcium Cyanamide	5.55	5.58	5.57	3.38	3.46	3.42	0.26	0.21	0.24
50% Urea (soil) + Foliar N	5.13	5.18	5.15	3.31	3.35	3.33	0.28	0.27	0.27
Calcium nitrate + urea +Liming	7.37	7.31	7.34	4.28	4.32	4.30	0.42	0.45	0.43
Mean	6.09	6.08		3.36	3.37		0.33	0.33	
CD <sub>0.05</sub>	0.29	0.31		0.12	0.16		0.05	0.06	
Year (Y)			NS			NS			NS
Treatment(T)			0.21			0.10			0.04
$Y \times T$ :			NS			NS			0.06

Table 1: Effect of different nitrogen sources on soil phosphatase, dehydrogenase and urease activities under container grown plants

**Dehydrogenase enzyme:** Application of different nitrogen sources exerted a significant influence on dehydrogenase enzyme (Table 1). The highest values of dehydrogenase were noticed under application of calcium nitrate + urea + liming i.e. 4.28 and 4.32 mg TPF (g soil) <sup>-1</sup> during 2016 and 2017, respectively. The lowest values of dehydrogenase were recorded under control (no fertilizer application) i.e. 2.21 and 2.14 mg TPF (g soil) <sup>-1</sup> during 2016 and 2017, respectively.

Pooled data analysis also revealed that the effect of different nitrogen sources on dehydrogenase was significant. The highest dehydrogenase content was recorded under calcium nitrate + urea + liming (4.30 mg TPF (g soil) <sup>-1</sup>) and the lowest under control (2.18 mg TPF (g soil) <sup>-1</sup>). The data on interaction between year and treatment (Y×T) and year (Y) presented in table 1 reveals a non-significant effect. Grigaliuniene *et al.* (2003) <sup>[10]</sup> suggested that fertilizer application with mineral fertilizer or organic manure and their combination increased the biological activity in the soil. The annual application of NPK fertilizer significantly increased the activity of enzymes in the soil. Mandal *et al.* (2007) <sup>[11]</sup> and Guo *et al.* (2008) <sup>[12]</sup> also reported the increase in activity of dehydrogenase on the application of nitrogen fertilizers.

Urease enzyme: Application of different nitrogen sources exerted a significant influence on the urease enzyme (Table 1). During 2016, highest urease enzyme (0.47  $\mu$ g (NH<sub>4</sub>)<sup>+</sup> (g soil) <sup>-1</sup> hr<sup>-1</sup>) was recorded under urea + liming (In March) which was statistically at par with urea alone application (0.43  $\mu g (NH_4)^+ (g \text{ soil})^{-1} \text{ hr}^{-1}$  and calcium nitrate + urea + liming  $(0.42 \ \mu g \ (NH_4)^+ \ (g \ soil)^{-1} \ hr^{-1})$ . The lowest urease (0.14  $\mu g$  $(NH_4)^+$  (g soil) <sup>-1</sup> hr<sup>-1</sup>) was recorded under control. In 2017, the highest urease was recorded under calcium nitrate + urea + liming  $(0.45 \ \mu g \ (NH_4)^+ \ (g \ soil)^{-1} \ hr^{-1})$  which was statistically at par with urea alone application (0.40  $\mu$ g (NH<sub>4</sub>)<sup>+</sup> (g soil)<sup>-1</sup> hr<sup>-1</sup>), 12:32:16 + calcium nitrate (0.42  $\mu$ g (NH<sub>4</sub>)<sup>+</sup> (g soil)<sup>-1</sup> hr<sup>-1</sup> <sup>1</sup>) and  $15:15:15 + \text{calcium nitrate } (0.41 \,\mu\text{g (NH}_4)^+ \,(\text{g soil})^{-1} \,\text{hr}^{-1}$ <sup>1</sup>). The lowest urease content of 0.13  $\mu$ g (NH<sub>4</sub>)<sup>+</sup> (g soil) <sup>-1</sup> hr<sup>-1</sup> was recorded under control. Pooled data analysis revealed that the effect of different nitrogen sources on urease was significant. The highest urease content of 0.44  $\mu$ g (NH<sub>4</sub>)<sup>+</sup> (g soil)<sup>-1</sup> hr<sup>-1</sup> was recorded under urea + liming (In March) which was statistically at par with urea alone application (0.42  $\mu$ g  $(NH_4)^+$  (g soil) <sup>-1</sup> hr<sup>-1</sup>), 12:32:16 + calcium nitrate (0.40 µg (NH<sub>4</sub>)<sup>+</sup> (g soil) <sup>-1</sup> hr<sup>-1</sup>) and calcium nitrate + urea + liming  $(0.43 \ \mu g \ (NH_4)^+ \ (g \ soil)^{-1} \ hr^{-1})$  and lowest  $(0.14 \ \mu g \ (NH_4)^+ \ (g \ soil)^{-1} \ hr^{-1})$ soil) <sup>-1</sup> hr<sup>-1</sup>) was recorded under control. The data on interaction between year and treatment (Y×T) presented reveals a significant effect, with the maximum urease activity of 0.47  $\mu$ g (NH<sub>4</sub>)<sup>+</sup> (g soil) <sup>-1</sup> hr<sup>-1</sup> in urea + liming (In March) treatment during 2016 and minimum under control in 2017. The years have a non significant impact on urease activity. The results are in line with the findings of Vetanovetz and Peterson (1992)<sup>[13]</sup> who studied the effect of nitrogen sources (ammonium sulphate and urea) on urease activity and found that the activity of urease was higher under application of urea as compared to ammonium sulphate. Guo et al. (2008) [12] and Jingjing et al. (2015)<sup>[14]</sup> also found that the activity of urease increases on application of nitrogen fertilizers.

**Total microbial count:** The data depicted in table 2 clearly indicate that total microbial count was significantly influenced by different nitrogen fertilizers. During 2016, the highest total microbial count was recorded under calcium nitrate + urea + liming  $(139.05 \times 10^5$ cfu g<sup>-1</sup> soil) which was statistically at par with calcium nitrate  $(134.35 \times 10^5$ cfu g<sup>-1</sup> soil), and urea + liming (In March)  $(134.03 \times 10^5$ cfu g<sup>-1</sup> soil), whereas, the lowest total microbial count (92.03 ×10<sup>5</sup>cfu g<sup>-1</sup> soil) was recorded under control. During 2017, the highest total microbial count was recorded under calcium nitrate + urea + liming  $(143.05 \times 10^5$ cfu g<sup>-1</sup> soil) and the lowest (87.08 × 10<sup>5</sup>cfu g<sup>-1</sup> soil) was recorded under control. The pooled data

also shows that highest total microbial count was recorded under Cal has + urea + liming (141.05 × 10<sup>5</sup>cfu g<sup>-1</sup> soil) which was at par with urea + liming (In March) (137.09 × 10<sup>5</sup>cfu g<sup>-1</sup> soil). The data on interaction between treatment and year (Y×T) presented in table 2 revealed non-significant effect. Liming helps to maintain the pH of the soil that leads to higher microbial count. Mineral fertilization increases the biological productivity as well as the microbial activity in soil (Barabaszl *et al.* 2002) <sup>[15]</sup>.

Treatment	Total microbial count (×10 <sup>5</sup> cfu/g soil)				
	2016	2017	Pooled		
Control (No application)	92.03	87.08	89.56		
Urea	103.64	109.36	106.50		
Calcium Nitrate	134.35	127.06	130.70		
Calcium Cyanamide	99.65	106.18	102.92		
Urea+ Liming (In October)	125.16	134.65	129.90		
Urea + Liming (In March)	134.03	140.14	137.09		
12: 32: 16 + Urea	105.06	109.86	107.46		
12: 32: 16 + Calcium Nitrate	131.84	136.54	134.19		
12: 32: 16 + Calcium Cyanamide	106.38	112.32	109.35		
15:15:15 + Urea	120.54	128.05	124.30		
15: 15: 15 + Calcium Nitrate	129.61	133.41	131.51		
15: 15: 15 + Calcium Cyanamide	117.80	109.74	113.77		
50% Urea (soil) + Foliar N	110.36	112.43	111.40		
Calcium nitrate + urea +Liming	139.05	143.05	141.05		
Mean	117.82	120.71			
CD0.05	5.74	6.19			
Year (Y)			1.56		
Treatment (T)			4.12		
$Y \times T$ :			NS		

**Table 2:** Effect of different nitrogen sources on total microbial count $(\times 10^5 cfu/g \text{ soil})$  in soil of container grown apples

#### **Experiment 2**

Total microbial count: The data depicted in table 3 clearly indicates that total microbial count was significantly influenced by different nitrogen fertilizers. At Seobagh, during 2016, the highest total microbial count was recorded under calcium nitrate + urea + liming  $(133.27 \times 10^5 \text{cfu g}^{-1})$ soil) which was statistically at par with calcium nitrate  $(129.08 \times 10^{5}$  cfu g<sup>-1</sup> soil), whereas, the lowest total microbial count (88.16 × 10<sup>5</sup> cfu g<sup>-1</sup> soil) was recorded under control. During 2017 also the highest total microbial count was recorded under calcium nitrate + urea + liming (138.05  $\times$ 10<sup>5</sup>cfu g<sup>-1</sup> soil) which was statistically at par with urea + liming (In March) (135.65  $\times$  10<sup>5</sup>cfu g<sup>-1</sup>) and 12:32:16 + calcium nitrate (135.42  $\times$  10<sup>5</sup> cfu g<sup>-1</sup> soil). The lowest total microbial count (83.16  $\times$  10<sup>5</sup> cfu g<sup>-1</sup> soil) was recorded under control. The pooled data also shows that highest total microbial count was recorded under calcium nitrate + urea + liming (135.66  $\times$  10<sup>5</sup> cfu g<sup>-1</sup> soil). The interaction between treatment and year (Y×T) presented in table 3 revealed nonsignificant effect.

At Rohru, during 2016, the highest total microbial count was recorded under calcium nitrate + urea + liming  $(145.30 \times 10^5 \text{ cfu g}^{-1} \text{ soil})$  and the lowest  $(97.28 \times 10^5 \text{ cfu g}^{-1} \text{ soil})$  was under control. During 2017 also the highest total microbial count was recorded under calcium nitrate + urea + liming  $(138.14 \times 10^5 \text{ cfu g}^{-1} \text{ soil})$  which was statistically at par with urea + liming (In March)  $(133.54 \times 10^5 \text{ cfu g}^{-1} \text{ soil})$ , urea + liming (In October)  $(135.28 \times 10^5 \text{ cfu g}^{-1} \text{ soil})$ , 15:15:15 + calcium nitrate (136.10  $\times 10^5 \text{ cfu g}^{-1} \text{ soil}$ ) whereas, the lowest total

microbial count  $(94.14 \times 10^5 \text{ cfu g}^{-1} \text{ soil})$  was recorded under control. The pooled data also shows that highest total microbial count was recorded under calcium nitrate + urea + liming  $(141.72 \times 10^5 \text{ cfu g}^{-1} \text{ soil})$  which was at par with 12:32:16 + calcium nitrate  $(138.61 \times 10^5 \text{ cfu g}^{-1} \text{ soil})$ . The

interaction between treatment and year (Y×T) was nonsignificant effect. Mineral fertilization increases the biological productivity as well as the microbial activity in soil (Barabaszl *et al.* 2002) <sup>[15]</sup>. Also, liming helps to maintain the pH of the soil that leads to higher microbial count.

**Table 3:** Effect of different nitrogen sources on total microbial count ( $\times 10^5$ cfu/g soil) in apple orchard soils

Site	Seobagh			Rohru			
Treatment	2016	2017	Pooled	2016	2017	Pooled	
Control (No application)	88.16	83.16	85.66	97.28	94.14	95.71	
Urea	97.43	105.51	101.47	110.31	117.16	113.74	
Calcium Nitrate	129.08	122.95	126.02	137.50	129.05	133.28	
Calcium Cyanamide	95.28	98.68	96.98	106.26	115.13	110.70	
Urea + Liming (In October)	123.64	129.36	126.50	126.35	135.28	130.82	
Urea + Liming (In March)	128.34	135.65	132.00	139.17	133.54	136.36	
12: 32: 16 + Urea	103.15	112.35	107.75	114.38	118.08	116.23	
12: 32: 16 + Calcium Nitrate	126.52	135.42	130.97	134.86	142.36	138.61	
12: 32: 16 + Calcium Cyanamide	109.54	106.18	107.86	117.57	123.58	120.57	
15:15:15 + Urea	117.06	128.05	122.56	123.18	118.34	120.76	
15: 15: 15 + Calcium Nitrate	125.32	128.47	126.90	130.05	136.10	133.07	
15: 15: 15 + Calcium Cyanamide	114.75	107.37	111.06	121.21	126.24	123.73	
50% Urea (soil) + Foliar N	110.88	116.14	113.51	118.32	121.03	119.68	
Calcium nitrate + urea +Liming	133.27	138.05	135.66	145.30	138.14	141.72	
Mean	114.46	117.67		122.98	124.87		
CD <sub>0.05</sub>	4.87	4.54		4.26	4.80		
Year (Y)			1.21			1.19	
Treatment (T)			3.19			3.18	
$Y \times T$ :			NS			NS	

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