

# Effect of Different Nitrogen Doses and Calcium Sprays on Bio-Chemical and Antioxidant Characteristics of Peach

Kamal Kumar Pande<sup>1\*</sup> and D.C. Dimri<sup>2</sup>

<sup>1</sup>Krishi Vigyan Kendra (ICAR- VPKAS), Kafligair - 263628, Bageshwar (Uttarakhand)

<sup>2</sup> Department of Horticulture, G.B. Pant University of Agriculture and Technology, Pantnagar- 263145 (Uttarakhand)

## Abstract

An investigation was conducted for two consecutive years in peach with ten treatments comprising three doses of nitrogen (375, 500, 625 g/ tree through neem coated urea) along with three concentrations of calcium chloride (0.5, 1.0, 1.5 %), and a control (500 g N per tree) through neem coated urea with water spray. The experimental findings showed that N @ 375 g per tree along with 1.0 per cent calcium chloride sprays resulted in best bio-chemical attributes and antioxidant activities, while further increase in nitrogen fertilization adversely affected these quality parameters.

**Keywords:** Peach; biochemical; antioxidant; nitrogen doses; calcium spray.

## \*Correspondence

Author: Kamal Kumar Pande

Email: pande4kamal@gmail.com

## Introduction

Peach (*Prunus persica* (L.) Batsch) is an important temperate fruit of excellent appearance and quality. This stone fruit belongs to family Rosaceae and subfamily Prunoideae, which originated in an area near the city Xian, China and recorded to be grown as far back as 2000 BC [1]. Peach is a potential source of bioactive compounds having relevant health implications [2]. The carotenoids and antioxidant molecules viz., procyanidins, anthocyanins, catechins and phenolic acids are present in peach [3, 4]. Moreover, the delicious flavor together with sugars and organic acids contribute to the sensory quality and popularize it as a luscious commodity worldwide. The dietary use of peach can reduce the generation of reactive oxygen species (ROS) and provide protection from several chronic diseases [5]. Peach has laxative properties and is appropriate to prevent constipation and for the cure of duodenum ulcers [6, 7]. In addition to this, chlorogenic and neochlorogenic acids are two specific phenolic acid components of peaches able to kill the breast cancer cells [8].

The phyto-chemical content and consequently the quality of fruits are influenced significantly by various factors such as cultivar, rootstock, horticultural practices and climatic conditions. Among them horticultural practices are of great value under the established orchards which may be amended for better quality attributes. Mineral nutrition of trees is an integral part of horticultural practices which plays a vital role in fruit's bio-chemical properties. It has also been found that plant nutrition influences the antioxidant ability of horticultural produce [9]. It was concluded that management of nutrients, particularly nitrogen and calcium have an important relationship with the quality of fruits [10]. Mineral nutrition in general and nitrogen fertilization especially has been found to influence the antioxidant composition of fruits and vegetables [11, 12]. The beneficial effects of calcium on maintaining fruit quality of temperate fruits was also documented [13]. Some of the researches also reported the beneficial effects of calcium chloride sprays on quality of temperate fruits including bio- chemical attributes [14, 15]. However, the information with context of the effects of different concentrations of calcium chloride on studied parameters is very meager for peach under the agro-climatic conditions of north-western Himalayas.

Thus, different levels of nitrogen fertilization and calcium chloride sprays were identified as the interventions whose effect need to be investigated on bio-chemical attributes and antioxidant activities of peach. Moreover, urea is the major source of nitrogen fertilization in India and from January 2015, Government of India has made it mandatory for the domestic urea manufactures to produce "neem coated urea" up to a minimum of 75 per cent of their total production of subsidized urea from 35 per cent earlier and allowed them to go up to 100 per cent [16]. Although, adequate information has been generated with regard to neem coated urea for the use in field crops viz., maize, rice and wheat [17-19] but such type of work in fruit crops particularly in peaches is very negligible. Therefore, it has become imperative to study the effects of different nitrogen regimes through neem coated urea along with calcium chloride sprays on quality attributes of peach.

## Materials and Methods

The present study was conducted at Krishi Vigyan Kendra (ICAR- VPKAS, Almora) Kafligair- Bageshwar (Uttarakhand) in two consecutive years i.e., 2016 and 2017. The experimental site is situated in the mid Himalayas between 29°45'07" N latitude and 79°44'03" E longitude at an altitude of 1245 meters above the mean sea level which represents the humid sub- temperate climate with average annual rainfall of 1256 mm. The experiment was conducted on 6 -7 year's old peach cv. Red June trees, raised on seedling rootstocks planted in 2010 with planting spacing of 3m x 3m.



Figure 1. The Experimental site



Figure 2. Neem coated urea application at scheduled rates



Figure 3. Calcium chloride spray at scheduled concentrations

The experiment was conducted in randomized block design with three replications and ten treatments (Figures 1-3). The details of treatments are presented in **Table 1**.



**Table 1** Details of treatments

Sl. No.	Treatment combinations	Treatments	Particulars of Treatments
1.	N <sub>1</sub> C <sub>1</sub>	T <sub>1</sub>	375g N per tree + 0.5% Calcium chloride as foliar spray
2.	N <sub>1</sub> C <sub>2</sub>	T <sub>2</sub>	375g N per tree + 1.0% Calcium chloride as foliar spray
3.	N <sub>1</sub> C <sub>3</sub>	T <sub>3</sub>	375g N per tree + 1.5% Calcium chloride as foliar spray
4.	N <sub>2</sub> C <sub>1</sub>	T <sub>4</sub>	500g N per tree + 0.5% Calcium chloride as foliar spray
5.	N <sub>2</sub> C <sub>2</sub>	T <sub>5</sub>	500g N per tree + 1.0% Calcium chloride as foliar spray
6.	N <sub>2</sub> C <sub>3</sub>	T <sub>6</sub>	500g N per tree + 1.5% Calcium chloride as foliar spray
7.	N <sub>3</sub> C <sub>1</sub>	T <sub>7</sub>	625g N per tree + 0.5% Calcium chloride as foliar spray
8.	N <sub>3</sub> C <sub>2</sub>	T <sub>8</sub>	625g N per tree + 1.0% Calcium chloride as foliar spray
9.	N <sub>3</sub> C <sub>3</sub>	T <sub>9</sub>	625g N per tree + 1.5% Calcium chloride as foliar spray
10.	N <sub>2</sub> C <sub>0</sub>	T <sub>10</sub>	500g N per tree + Water spray (Control)

Foliar sprays of calcium chloride were given thrice, first at petal fall stage, second at 25 days after I<sup>st</sup> spray and third at 25 days after II<sup>nd</sup> spray. Common doses of FYM (25 kg/tree), P<sub>2</sub>O<sub>5</sub> (250 g/tree) and K<sub>2</sub>O (500 g/tree) were also applied uniformly in each tree. Source of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O were neem coated urea, single super phosphate and muriate of potash, respectively. Whole quantity of FYM, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O were applied in December. Half of the N was applied in mid February about three weeks before flowering and remaining half in last week of March after fruit set.

Fruit of almost same maturity were harvested carefully in the last week of May in both the years. A representative sample of ten fruits per treatment per replication was taken randomly from all directions of the plant and various biochemical attributes and antioxidant activities were estimated by using the methods described underneath;

#### **Total soluble solids (TSS <sup>o</sup>Brix)**

The total soluble solid content of fruits was measured by a digital refractometer (Extech Instrument, MI 722-01).

#### **Titrateable acidity**

The acidity of fruits was estimated by titrating the fruit pulp extract with 0.1N NaOH using phenolphthalein as indicator [20].

#### **Sugars**

Reducing sugar, non reducing sugars and total sugars were determined by the method of Lane and Eynon [20].

#### **Antioxidant metabolites**

For extraction of antioxidant metabolites, 5 g fresh peach fruit sample was homogenized in 25 ml extraction solvent (400 ml acetone + 400 ml methanol+ 200 ml water+ 10 ml acetic acid) with some modifications [21]. The homogenate was transferred into a 50 ml centrifuge tube and incubated at 60<sup>o</sup> C in a water bath for 1 hour. These samples were centrifuged at 5000 at 20<sup>o</sup> C rpm for 5 min. and then filtered with Whatman filter paper No. 1 and diluted to a final volume of 50 ml. The antioxidant activity was estimated through the assays of total polyphenol, DPPH radicals, ABTS radicals and total antioxidant activity. All assays were carried out in triplicate for each sample and their mean were calculated.

#### **Total polyphenol**

Total polyphenol (TP) content was measured spectrophotometrically by the Folin- Ciocalteu method [22]. 100 µl sample extract was mixed with 50µl Folin- Ciocalteu reagent 1N and 2.5 ml sodium carbonate and allowed to stand in dark at room temperature for 30 min. A blank was also prepared in the same manner except using double distilled water instead of sample extract. The absorbance was measured at 725 nm using UV- VIS Double Beam Spectrophotometer- 2201 (Systronics). A standard curve of gallic acid was constructed (20- 100 µl) and results were expressed as mg gallic acid equivalent (GAE) per 100 g fresh weight (FW).

#### **DPPH [2, 2- diphenyl- 2- picryl hydrazyl] radicals**

The DPPH assay was done by quantifying the decrease in absorbance of methanolic DPPH solution at 515 nm in the

presence of peach fruit extract with some modifications [23]. 24 mg DPPH was dissolved in 100 ml methanol to prepare the stock solution. The working solution was prepared by mixing 10 ml of stock solution with 45 ml methanol that gave an absorbance of  $1.17 \pm 0.02$  units at 515 nm. 150  $\mu$ l peach fruit extract was poured in 2850  $\mu$ l working solution of DPPH and allowed to stand for 24 hours in dark. Then the absorbance was recorded at 515 nm using UV-VIS Double Beam Spectrophotometer- 2201 (Systronics). DPPH radical scavenging activity was calculated as percentage of DPPH discoloration by employing the following equation;

$$\text{DPPH radical scavenging (\%)} = [A_{\text{control}} - A_{\text{sample}}] \times 100$$

Where,  $A_{\text{control}}$  = Absorbance of DPPH solution without adding the peach fruit extract,  $A_{\text{sample}}$  = Absorbance of DPPH solution when the peach fruit extract was added

*ABTS [2, 2- azino-bis (3- ethylbenzothiazoline-6- sulphonic acid)] radicals:*

The ABTS assay was done by measuring the decrease in absorbance of methanolic ABTS solution at 745 nm in the presence of peach fruit extract [24]. There were two stock solution; 7.0 mM ABTS solution and 2.3 mM ammonium persulphate solution. The working solution was made by mixing the two stock solutions in equal amount and allowed them to react for 12 hours in dark at room temperature. The solution was diluted by mixing 1 ml working ABTS solution with 3 ml methanol that gave an absorbance of  $0.9 \pm 0.02$  units at 745 nm. Peach fruit extract (150  $\mu$ l) was mixed with 2850  $\mu$ l of diluted working ABTS solution and incubated for 30 min in dark. Then the absorbance was recorded at 745 nm using UV- VIS Double Beam Spectrophotometer- 2201 (Systronics). ABTS radical scavenging activity was calculated as percentage of ABTS discoloration by using the following equation;

$$\text{ABTS radical scavenging (\%)} = [A_{\text{control}} - A_{\text{sample}}] \times 100$$

Where,  $A_{\text{control}}$  = Absorbance of ABTS solution without adding the peach fruit extract,  $A_{\text{sample}}$  = Absorbance of ABTS solution when the peach fruit extract was added

*Total antioxidant activity*

The total antioxidant activity of the methanolic extract of all the fruit samples were determined spectrophotometrically using the Phosphomolybdenum method [25]. Peach fruit extract (0.3 ml) was mixed with 2.7 ml of reagent solution (0.6 M sulfuric acid, 28 mM tri- sodium phosphate and 4 mM ammonium hepta molybdate). This mixture was capped and incubated for 2 hours at 70°C in waterbath. It was then cooled to room temperature and the absorbance was recorded at 695 nm using UV- VIS Double Beam Spectrophotometer- 2201(Systronics). For taking blank reading, instead of peach fruit extract, 0.3 ml double distilled water was used. The total antioxidant activity was expressed as equivalent of trolox ( $\mu$ g Trolox/ mg fresh weight).

## Results and Discussion

The results of bio-chemical attributes of fruits *viz.*, total soluble solids (TSS), titratable acidity, reducing sugar, non-reducing sugars and total sugars are presented in **Table 2** and the findings pertaining to antioxidant activities are given in **Table 3 & Figure 4**.



Figure 4. (a) TSS Recording with Digital Refractometer



(b) Sample preparation and lab work

**Table 2** Response of different nitrogen regimes through neem coated urea and calcium sprays on bio-chemical attributes of peach

Treatments	TSS <sup>0</sup> B		Titratable acidity (%)		Reducing sugar (%)		Non-reducing sugar (%)		Total sugar (%)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
T <sub>1</sub>	11.07 <sup>b*</sup>	10.93 <sup>b</sup>	0.98 <sup>ef</sup>	0.99 <sup>f</sup>	1.857 <sup>b</sup>	1.857 <sup>a</sup>	5.880 <sup>b*</sup>	5.837 <sup>b*</sup>	7.737 <sup>b*</sup>	7.643 <sup>b*</sup>
T <sub>2</sub>	11.27 <sup>a</sup>	11.10 <sup>a</sup>	0.95 <sup>g</sup>	0.97 <sup>f</sup>	1.890 <sup>a</sup>	1.883 <sup>a</sup>	5.990 <sup>a</sup>	5.923 <sup>a</sup>	7.877 <sup>a</sup>	7.760 <sup>a</sup>
T <sub>3</sub>	11.17 <sup>ab</sup>	11.00 <sup>ab</sup>	0.96 <sup>fg</sup>	0.97 <sup>f</sup>	1.873 <sup>ab</sup>	1.867 <sup>a</sup>	5.933 <sup>a</sup>	5.873 <sup>a</sup>	7.807 <sup>ab</sup>	7.690 <sup>ab</sup>
T <sub>4</sub>	10.53 <sup>e</sup>	10.43 <sup>e</sup>	0.99 <sup>e</sup>	1.03 <sup>d</sup>	1.780 <sup>de</sup>	1.760 <sup>bc</sup>	5.637 <sup>d</sup>	5.603 <sup>de</sup>	7.410 <sup>d</sup>	7.340 <sup>e</sup>
T <sub>5</sub>	10.90 <sup>c</sup>	10.80 <sup>c</sup>	0.99 <sup>e</sup>	1.00 <sup>e</sup>	1.830 <sup>c</sup>	1.787 <sup>b</sup>	5.793 <sup>c</sup>	5.763 <sup>c</sup>	7.620 <sup>c</sup>	7.550 <sup>c</sup>
T <sub>6</sub>	10.70 <sup>d</sup>	10.60 <sup>d</sup>	1.02 <sup>d</sup>	1.06 <sup>bc</sup>	1.797 <sup>d</sup>	1.800 <sup>b</sup>	5.687 <sup>d</sup>	5.657 <sup>d</sup>	7.480 <sup>d</sup>	7.410 <sup>d</sup>
T <sub>7</sub>	10.37 <sup>f</sup>	10.30 <sup>f</sup>	1.06 <sup>ab</sup>	1.08 <sup>ab</sup>	1.737 <sup>f</sup>	1.727 <sup>c</sup>	5.510 <sup>f</sup>	5.497 <sup>f</sup>	7.247 <sup>f</sup>	7.200 <sup>f</sup>
T <sub>8</sub>	10.60 <sup>d</sup>	10.50 <sup>d</sup>	1.03 <sup>cd</sup>	1.04 <sup>cd</sup>	1.770 <sup>e</sup>	1.773 <sup>bc</sup>	5.597 <sup>e</sup>	5.590 <sup>e</sup>	7.363 <sup>e</sup>	7.317 <sup>e</sup>
T <sub>9</sub>	10.53 <sup>e</sup>	10.47 <sup>e</sup>	1.05 <sup>bc</sup>	1.05 <sup>cd</sup>	1.767 <sup>e</sup>	1.757 <sup>bc</sup>	5.600 <sup>e</sup>	5.570 <sup>e</sup>	7.363 <sup>e</sup>	7.293 <sup>e</sup>
T <sub>10</sub>	10.33 <sup>f</sup>	10.23 <sup>f</sup>	1.08 <sup>a</sup>	1.09 <sup>a</sup>	1.730 <sup>f</sup>	1.740 <sup>c</sup>	5.493 <sup>f</sup>	5.460 <sup>f</sup>	7.223 <sup>f</sup>	7.153 <sup>f</sup>
CD (0.05)	0.13	0.11	0.02	0.02	0.025	0.044	0.068	0.061	0.089	0.079
SE (m) ±	0.04	0.04	0.01	0.01	0.008	0.015	0.023	0.020	0.030	0.027

**Table 3** Response of different nitrogen regimes through neem coated urea and calcium sprays antioxidant activities of peach

Treatments	Total polyphenol (mg GAE/ 100 g)		DPPH radical scavenging activity (%)		ABTS radical scavenging activity (%)		Total antioxidant activity	
	2016	2017	2016	2017	2016	2017	2016	2017
T <sub>1</sub>	122.747 <sup>a</sup>	125.427 <sup>a</sup>	69.707 <sup>a</sup>	71.160 <sup>a</sup>	85.193 <sup>a</sup>	86.293 <sup>a*</sup>	1.413 <sup>a*</sup>	1.430 <sup>a*</sup>
T <sub>2</sub>	122.943 <sup>a</sup>	126.137 <sup>a</sup>	70.327 <sup>a</sup>	71.920 <sup>a</sup>	85.850 <sup>a</sup>	86.973 <sup>a</sup>	1.417 <sup>a</sup>	1.427 <sup>a</sup>
T <sub>3</sub>	123.843 <sup>a</sup>	127.877 <sup>a</sup>	70.953 <sup>a</sup>	71.863 <sup>a</sup>	86.023 <sup>a</sup>	87.137 <sup>a</sup>	1.418 <sup>a</sup>	1.423 <sup>a</sup>
T <sub>4</sub>	107.143 <sup>b</sup>	109.283 <sup>b</sup>	59.703 <sup>b</sup>	61.343 <sup>b</sup>	73.740 <sup>b</sup>	75.257 <sup>b</sup>	1.207 <sup>b</sup>	1.223 <sup>b</sup>
T <sub>5</sub>	106.580 <sup>b</sup>	110.097 <sup>b</sup>	59.263 <sup>b</sup>	60.103 <sup>b</sup>	73.393 <sup>b</sup>	74.863 <sup>b</sup>	1.203 <sup>b</sup>	1.227 <sup>b</sup>
T <sub>6</sub>	105.650 <sup>b</sup>	108.477 <sup>b</sup>	60.370 <sup>b</sup>	61.203 <sup>b</sup>	74.103 <sup>b</sup>	75.513 <sup>b</sup>	1.213 <sup>b</sup>	1.220 <sup>b</sup>
T <sub>7</sub>	83.610 <sup>c</sup>	85.267 <sup>c</sup>	41.330 <sup>c</sup>	43.017 <sup>c</sup>	47.330 <sup>c</sup>	49.207 <sup>c</sup>	1.027 <sup>c</sup>	1.033 <sup>c</sup>
T <sub>8</sub>	84.163 <sup>c</sup>	85.943 <sup>c</sup>	42.443 <sup>c</sup>	43.777 <sup>c</sup>	48.100 <sup>c</sup>	50.037 <sup>c</sup>	1.020 <sup>c</sup>	1.030 <sup>c</sup>
T <sub>9</sub>	83.893 <sup>c</sup>	85.380 <sup>c</sup>	41.817 <sup>c</sup>	43.380 <sup>c</sup>	47.923 <sup>c</sup>	49.970 <sup>c</sup>	1.023 <sup>c</sup>	1.033 <sup>c</sup>
T <sub>10</sub>	107.030 <sup>b</sup>	110.260 <sup>b</sup>	60.283 <sup>b</sup>	61.983 <sup>b</sup>	73.910 <sup>b</sup>	75.227 <sup>b</sup>	1.213 <sup>b</sup>	1.227 <sup>b</sup>
CD (0.05)	4.742	4.752	1.570	1.488	1.341	1.000	0.013	0.016
SE (m) ±	1.584	1.587	0.524	0.497	0.448	0.334	0.004	0.005

**Total soluble solids (TSS <sup>0</sup>Brix)**

The first as well as second year of the study (Table 2) showed that the highest TSS was recorded under T<sub>2</sub> (11.27 <sup>0</sup>Brix in 2016 and 11.10 <sup>0</sup>Brix) that was statistically *at par* to T<sub>3</sub> (11.17 <sup>0</sup>Brix in 2016 and 11.00 <sup>0</sup>Brix in 2017) and followed by T<sub>1</sub>, T<sub>5</sub> and T<sub>6</sub>, while the minimum TSS was found under control T<sub>10</sub> (10.33 <sup>0</sup>Brix and 10.23 <sup>0</sup>Brix in first and second year, respectively). The possible reason of high TSS with lower doses of nitrogen fertilization could be the adequate scope of nutrients to the plant, which hydrolyze starch into sugars and thus increases the TSS of fruits. The influence of more vegetative growth under medium and high nitrogen application (data not shown) acting as competing sinks with maturing fruits supposed to be another important explanation for less TSS. Therefore, it could be assumed that better nitrogen utilization efficiency throughout the growing season with the use of neem coated urea led to the fulfillment of nitrogen requirement of peach with lowest applied nitrogen regime (375 g per tree) and further increment in nitrogen levels significantly reduced the fruit TSS due to impaired plant metabolism and translocation of metabolites. The adequate dose of nitrogen stimulates the functioning of various enzymes in the physiological processes which might have increased the TSS content of fruits [26]. Decrease in fruit TSS with increase in nitrogen fertilization above a certain level was also observed in peach and apricot [27, 28]. Calcium may function directly in several aspects of photosynthesis. It appears to modulate activity of the phosphatase enzymes in the carbon reduction cycle and also regulate chloroplast NAD<sup>+</sup> kinase activity through a calmodulin-like protein [29]. Thus, adequate supply of calcium leads to proper carbohydrate synthesis and their further translocation, utilization and conversion into sugars take place in maturing fruits. Positive influence of calcium chloride sprays on fruit TSS was also reported in peach [15] and apple [30].

### ***Titratable acidity***

In 2016, the highest titratable acidity was estimated under control i.e., T<sub>10</sub> (1.08%) that was statistically *at par* to T<sub>7</sub> (1.06%) and followed by T<sub>9</sub>, T<sub>8</sub> and T<sub>6</sub> (Table 2). The lowest titratable acidity in the same year was recorded under T<sub>2</sub> (0.95%) that was statistically *at par* to T<sub>3</sub> (0.96%). During second year of the study, the maximum acidity at harvest was again observed with T<sub>10</sub> (1.09%) that was having non significantly differences with T<sub>7</sub> (1.08%) and these were followed by T<sub>6</sub>, T<sub>9</sub>, T<sub>8</sub> and T<sub>4</sub>. In this year (2017), the minimum fruit acidity (0.97%) was measured in T<sub>2</sub> as well as in T<sub>3</sub> and these two treatments were statistically *at par* to T<sub>1</sub> (0.99%). Present findings in respect to the minimum titratable acidity under lowest nitrogen doses that increased with further increment in nitrogen levels are also in agreement with the previous reports in guava [31] and Kinnow mandarin [25]. However, some of the workers documented non significant effect of nitrogen fertilization on fruit acidity [32, 33]. The fruit acidity depends on nitrogen and potassium nutrition, though the mechanisms involved are complex and the results in the field experiments are often contradictory [34]. Nevertheless, the citation that increases in fruit acidity with higher doses of nitrogen was due to increased synthesis and translocation of organic acids in fruits [35], supports present findings. Moreover, similar results with respect to calcium chloride sprays were also documented in peach [15] and apple [29].

### ***Sugars***

#### ***Reducing sugar***

The data presented in Table 2 reveals that in 2016, the maximum reducing sugar was estimated in T<sub>2</sub> (1.890%) that was statistically *at par* to T<sub>3</sub> (1.873%) and followed by T<sub>1</sub> (1.857%), T<sub>5</sub> (1.830%) and T<sub>6</sub> (1.797%), whereas, minimum reducing sugar was observed under T<sub>10</sub> (1.730%) that differed non significantly to T<sub>7</sub> (1.737%). T<sub>4</sub> was statistically *at par* to T<sub>6</sub>, T<sub>8</sub> and T<sub>9</sub>. In the second year (2017) also treatment T<sub>2</sub> exhibited the maximum reducing sugar (1.883%) that was statistically *at par* to T<sub>1</sub> and T<sub>3</sub> which had 1.857% and 1.867% reducing sugar, respectively, while, in the same year, the minimum reducing sugar was estimated under T<sub>10</sub> (1.740%) which had non significant difference with T<sub>9</sub> (1.757%), T<sub>8</sub> (1.773%), T<sub>7</sub> (1.727) and T<sub>4</sub> (1.760%).

#### ***Non-reducing sugar***

In first year of the study, the values of non- reducing sugar ranged from 5.493% to 5.990%. The maximum value recorded under T<sub>2</sub> was statistically *at par* to T<sub>3</sub> (5.933%) followed by T<sub>1</sub>, T<sub>5</sub> and T<sub>6</sub>, whereas the minimum non reducing sugar was estimated under T<sub>10</sub> which had non significant difference with T<sub>7</sub>. During second year of the study also the maximum value of non- reducing sugar (5.923%) was recorded with T<sub>2</sub>. It was found to be statistically *at par* with T<sub>3</sub> (5.873%) and followed by T<sub>1</sub>, T<sub>5</sub> and T<sub>6</sub>, whereas the minimum non- reducing sugar was found under T<sub>10</sub> (5.460%).

#### ***Total sugar***

The results of first year experiment showed that maximum total sugar content was found under T<sub>2</sub> (7.877%) followed by T<sub>3</sub> (7.807%) and T<sub>1</sub> (7.737%). Treatment T<sub>3</sub> was statistically *at par* to T<sub>1</sub> and T<sub>2</sub>. In the same year, the minimum total sugar content was estimated under T<sub>10</sub> (7.223%) that varied non significantly with T<sub>7</sub> (7.247%). In second year of the experiment i.e., 2017 the maximum total sugar content was estimated under T<sub>2</sub> (7.760%) that was statistically *at par* to T<sub>3</sub> (7.690%). Treatment T<sub>3</sub> differed non significantly with T<sub>1</sub> also. In 2017, the minimum total sugar content was recorded with control i.e., T<sub>10</sub> (7.153%) that was statistically *at par* with T<sub>7</sub> (7.200%).

Accumulation of sugars in fruits is a complex phenomenon that involves carbohydrate synthesis, translocation, dilution and conversion into different sugars. All these processes require adequate supply of nutrients that not only lead to sufficient synthesis of assimilates but their balanced partitioning also, consequently it results into better source to sink ratio. It provides the ample amount of substrates for sugar production which was manifested with lowest nitrogen regime in our present findings. The main enzymatic reactions responsible for synthesis of different sugars have been identified in some fruits [36, 37] but little is known about their relative importance and regulation [38]. The lower concentration of sugars with higher doses of nitrogen fertilization might also be due to reaching the nitrogen at toxic levels for enzymatic activities of sugar synthesizing enzymes [25]. The decrease in reducing, non- reducing and total sugars with increase in nitrogen fertilization in Kinnow mandarin was also reported [25]. Fruit nitrogen was also found to be negatively correlated with sugars in apple [39] that further endorse present results regarding the decrease in sugar content with increase in nitrogen fertilization that would have possibly increased the fruit nitrogen also. The observations are also in agreement with the previous findings in peach which showed decrease in total sugar content with increase in nitrogen fertilization above a certain level [32].

Positive influence of calcium chloride sprays on sugar content might be due to the regulatory role of calcium on respiration [15] that would reduce the catabolic degradation of sugars. The present results showing the positive effect of calcium chloride sprays on sugar content (reducing, non-reducing and total sugar) were also reported in apple [14, 29], aonla [40] and litchi [41].

### ***Antioxidant metabolites***

All the antioxidant assays *viz.*, total polyphenols, DPPH radicals, ABTS radicals and total antioxidant activity affected adversely with increase in nitrogen fertilization levels, while sprays of calcium chloride could not impart any significant influence on them under same nitrogen regime (Table 3).

In first as well as second year of the experiment the highest total polyphenols were estimated under T<sub>3</sub> (123.843 mg GAE/100 g FW in 2016 and 127.877 mg GAE/ 100 g FW in 2017) which remained statistically *at par* with T<sub>1</sub> and T<sub>2</sub>. The minimum total polyphenols were recorded under T<sub>7</sub> (83.610 mg GAE/100 g FW in 2016 and 85.267 mg GAE/100 g FW in 2017), which varied non significantly with T<sub>8</sub> and T<sub>9</sub>. Likewise, significantly higher DPPH radicals scavenging activity, ABTS radical scavenging activity and total antioxidant activity was recorded for the treatments under lowest nitrogen regime, while minimum values for all these parameters were estimated for highest nitrogen regime group. Irrespective of calcium chloride sprays the non significant differences for the studied antioxidant assays were observed under same nitrogen regime.

The present study showed adverse effect of increased nitrogen fertilization levels on all the estimations of antioxidant metabolites. This observation might be due to reduced phenyl alanine ammonia-lyase (PAL) activity that was correlated with low C/N ratio, photosynthetic rates and total non structural carbohydrate (TNC) [42]. It was also reported that antioxidant enzymes showed decreased specific activities with nitrogen fertilization in coffee [43]. The present results regarding response of nitrogen on antioxidant metabolites also elucidate the findings in tomato [44]. As far as the response of calcium chloride on antioxidant activities is concerned, negative effect in blueberry [45], while positive effect in cornelian cherry [46] has been reported. However, here the findings did not follow any of them. It might be due to the vast influence of different nitrogen regimes that possibly could not allow calcium chloride treatments to impart their effects.

### **Conclusion**

The array of description presented above elucidates that the applied treatments *viz.*, different nitrogen doses and calcium chloride sprays had significantly influenced the various bio-chemical parameters of peach fruits. The response of different nitrogen fertilization levels on antioxidant activities of peach fruits was also found significant, however calcium chloride sprays could not affect the antioxidant activities of peach fruits significantly, under same nitrogen regime. Therefore, it could be concluded that the lowest applied nitrogen dose (375 g N per tree) through neem coated urea along with 1.0 per cent calcium chloride sprays resulted in best bio-chemical attributes and antioxidant activities, while further increment in nitrogen fertilization adversely affected these quality parameters.

### **Acknowledgement**

The presented results are the part of Ph. D thesis research (GBPUA&T, Pantnagar) of the first author. The experimental and analytical facilities extended by the Director (ICAR- VPKAS), Almora are duly acknowledged.

### **References**

- [1] Kumar, J., Thakur, M.K., Verma K.K., Singh, H., Singh, D., Thakur, D., Jawandha, S.K. and Banyal, S.K. 2015. Peach. In: Temperate Fruit Crop Breeding: Domestication to Cultivar Development, Part-II. Ghosh, S.N., Verma, N.K. and Thakur, A. (eds.) Jaya Publishing House, New Delhi. 435- 475.
- [2] Camejo, D., Marti, M.C. and Roman, P. 2010. Antioxidant system and protein pattern in peach fruits at two maturation stages. *J. Agric. Food Chem.*, 58: 11140- 11147.
- [3] Tomas-Barberan, F.A., Gil, M.I. and Cremin, P. 2001. HPLC- DAD-ESIMS analysis of phenolic compounds in nectarines, peaches and plums. *J. Agric. Food Chem.*, 49: 4748- 4760.
- [4] Loizzo, M.R., Pacetti, D. and Lucci, P. 2015. *Prunus persica* var. *Platycarpa* (Tabacchiera peach): bioactive compounds and antioxidant activity of pulp, peel and seed ethanolic extracts. *Plant Foods Hum. Nutr.*, 70: 331- 337.
- [5] Tsantili, E., Shin, Y., Nock, J.F. and Watkins, C.B. 2010. Antioxidant concentrations during chilling injury development in peaches. *Postharvest Biol. Technol.*, 57: 27- 34.

- [6] Chang, S., Tan, C., Frankel, E.N. and Barrett, D.M. 2000. Low- density lipoprotein antioxidant activity of phenolic compounds and polyphenol oxidase activity in selected clingstone peach cultivars. *J. Agric. Food Chem.* 48: 147- 151.
- [7] Gil, M.I., Tomas- Barberan, F.A., Hess-Pierce, B. and Kader, A.A. 2002. Antioxidant capacities, phenolic compounds, carotenoids and vitamin C contents of nectarine, peach and plum cultivars from California. *J. Agric. Food Chem.*, 50: 4976- 4982.
- [8] Naratto, G., Porter, W., Byrne, D. and Cisneros-Zevallos, L. 2009. Identifying peach and plum polyphenols with chemopreventive potential against estrogen –independent breast cancer cells. *J. Agric. Food Chem.* 57: 5219- 5226.
- [9] Lopez, A., Hellin, P., Davo, M., Herrera, E., Fenoll, J. and Flores, P. 2014. Total antioxidant power of lettuce as affected by nitrogen fertilization. *Acta Hort.*, 1040: 167- 171.
- [10] Prasad, M. and Spiers, T.M. 1991. The effect of nutrition on the storage quality of kiwifruit: A review. *Acta Hort.*, 297: 579- 585.
- [11] Benard, C., Gautier, H., Bourgaud, F., Grasselly, D., Navez, B., Caris- Veyrat, C., Weiss, W. and Genard, M. 2009. Effects of low nitrogen supply on tomato (*Solanum lycopersicum*) fruit yield and quality with special emphasis on sugars, acids, ascorbate, carotenoids and phenolic compounds. *J. Agric. Food Chem.*, 57: 4112- 4123.
- [12] Maggio, A., De Pascale, S., Paradiso, R. and Barbieri, G. 2013. Quality and nutritional value of vegetables from organic and conventional farming. *Sci. Hort.*, 164: 532- 533.
- [13] Bakshi, P., Masoodi, F.A., Chauhan, G.S. and Shah, T.A. 2005. Role of calcium in postharvest life of temperate fruits. *J. Food Sci, Technol.* 42 (1): 1-8.
- [14] Ganal, N.A. 2005. Effect of pre- harvest calcium and boron application on the quality and yield of apple (*Malus domestica* Borkh) cv. Red Delicious under temperate conditions of Kashmir. Thesis Abst. Sher-e-Kashmir University of Agriculture and Technology, Srinagar.
- [15] Wahab, M., Ullah, Z., Sajid, M., Usman, M., Sohail, K., Nayab, S. and Ullah, M. 2016. Effect of calcium sources as foliar applications on fruit quality of peach cultivars. *Progressive Hort.*, 48 (2): 167-172.
- [16] <http://thehindubusinessline.com/economy/agri-business/neemcoated-urea-mandatory-for-75-of-domestic-production/article7049075.ece>
- [17] Chauhan, R.P.S. 1999. Efficiency of coated-urea materials in rice (*Oryza sativa*) - wheat (*Triticuma estivum*) system under partially reclaimed sodic soil. *Indian J. Agri. Sci.*, 69 (5): 311-16.
- [18] Jat, M.L. and Pal, S.S. 2002. Relative efficiency of Pusa neem micro-emulsion-coated and prilled urea in rice (*Oryza sativa*)-wheat (*Triticum aestivum*) cropping system on Ustobrepts of Indo-Gangetic Plain. *Indian J. Agri. Sci.*, 72 (9): 548-50.
- [19] Sanjaykumar, V. V. , Vageesh, T. S. Abhiram and G. J. and Shilpashree Y. P. 2015. Effect of compost enriched with NPK fertilizers and neem oil coated urea on productivity and nutrient use efficiency in maize. *Karnataka J. Agric. Sci.*, 28(2): 185-188.
- [20] Ranganna, S. 1986. Handbook of Analysis and Quality Control for Fruit and Vegetable Products. Tata McGraw Hill publication, New Delhi.
- [21] Rababah, T.M., Ereifej, K.I. and Howard, L. 2005. Effect of ascorbic acid and dehydration on concentration of total phenolics, antioxidant capacity, anthocyanins and color in fruits. *J. Agric. Food Chem.*, 53: 4444- 4447.
- [22] Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, 16: 144-158.
- [23] Brand- Williams, W., Cuvelier, M.E. and Berset, C. 1995. Use of free radical method to evaluate antioxidant activity. *LWT Food Sci. Technol.*, 28: 25-30.
- [24] Arnao, M.B., Cano, A. and Acosta, M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.*, 73: 239-244.
- [25] Prieto, P., Pineda, M. and Aguilar, M. 1999. Spectrophotometric quantization of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal. Biochem.*, 269: 337-341.
- [26] Garhwal, P.C., Yadav, P.K., Sharma, B.D., Singh, R.S. and Ramniw, A.S. 2014. Effect of organic manure and nitrogen on growth, yield and quality of kinnow mandarin in sandy soils of hot arid region. *African Journal of Agricultural Research*, 9 (34): 2638- 2647.
- [27] Jia, H.J., Mizuguchi, K., Hirango, K. and Okamoto, G. 2006. Effect of fertilizer application level on pectin composition of Hakuho peach (*Prunus persica* Batsch) during maturation. *Hort Science.* 41 (7): 1571- 1575.
- [28] Asma, B.M., Colak, S., Akca, Y. and Genc, C. 2007. Effect of fertilizer rate on the growth, yield and fruit characteristics of dried apricots (cv. Hacıhaliloglu). *Asian Journal of Plant Science*, 6 (2): 294-297.



- [29] Brand J.J. and Becker D.W. 1984. Evidence for direct roles of calcium in photosynthesis. *J Bioenerg Biomembr.*, 16(4):239-49.
- [30] Khalifa, R. K.M., Hafez, O.M. and Khair, A.E. 2009. Influencing of foliar spraying with boron and calcium on productivity, fruit quality, nutritional status and controlling of blossom end rot disease of Anna apple trees. *World J. Agric. Sci.*, 5 (2): 237-249.
- [31] Sharma, A., Wali, V.K., Bakshi, P. and Jastora, A. 2013. Effect of organic and inorganic fertilizers on quality and shelf life of guava (*Psidium guajava* L.) cv. Sardar. *The Bioscan*, 8 (4): 1247-1250.
- [32] Rai, M., Dey, P., Gangopadhyay, K.K., das, B., Nath, V., Reddy, N.N. and Singh, H.P. 2002. Influence of nitrogen, phosphorus and potassium on growth parameters, leaf nutrient composition and yield of litchi (*Litchi chinensis*). *Indian J. Agric. Sci.*, 72 (5): 267- 270.
- [33] Sharma, D.P. and Chauhan, J.S. 2004. Response of pruning intensities and fertilizer treatments on yield, fruit quality and photosynthetic efficiency of peach. *Acta Hort.* 662: 237- 241.
- [34] Habib, R. 2000. Modeling fruit acidity in peach trees effects of nitrogen and potassium nutrition. *Acta Hort.*, 512: 141- 148.
- [35] Prasad, R.N. and Mali, P.C. 2000. Effect of different levels of nitrogen on quality characters of pomegranate fruit cv. Jalore Seedless, Haryana *J. Hort. Sci.*, 29 (3-4): 186-187.
- [36] Moriguchi, T., Abe, K., Sanada, T. and Yamaki, S. 1992. Levels and role of sucrose synthase, sucrose phosphate synthase and sucrose accumulation in fruit of Asian pear. *J. Am. Soc. Hortic. Sci.*, 117: 274- 278.
- [37] Moriguchi, T., Sanada, T. and Yamaki, S. 1990. Seasonal fluctuations of some enzymes relating to sucrose and sorbitol metabolism in peach fruit. *J. Am. Soc. Hortic. Sci.*, 115: 278- 281.
- [38] Genard, M., Lescourret, F., Gomez, I. and Habib, R. 2003. Changes in fruit sugar concentrations in response to assimilate supply and dilution: a modeling approach applied to peach fruit (*Prunus persica*). *Tree Physiology*, 23: 373-385.
- [39] Marcelle, R.D. 1995. Mineral nutrition and fruit quality. *Acta Hort.*, 383: 219-226.
- [40] Kumar, A., Tripathi, V.K., Dubey, V., Katiyar, N.K. and Tiwari, P. 2017a. Influence of foliar application of calcium, zinc and boron on fruit drop, yield and quality attributes of aonla (*Emblica officinalis*) cv. NA-7. *Res. on Crops*, 18 (1): 91-97.
- [41] Kumar, D., Mohammed, S., Kathwal, R. and Chander, S. 2017b. Effect of different nutrient sprays on growth, fruit yield and quality of litchi (*Litchi chinensis* Sonn.) cv. Early large red. *Res. on Crops*, 18 (1): 61-65.
- [42] Ibrahim, M.H.; Jaafar, H.Z.E. 2011. The relationship of nitrogen and C/N ratio with secondary metabolites levels and antioxidant activities in three varieties of malaysian kaci fatimah (*Labisia pumila* Blume). *Molecules*, 16: 5514-5526.
- [43] Reis, A.R., Favarin, J.L., Gratao, P.L., Capaldi, F.R. and Azevedo, R.A. 2015. Antioxidant metabolism in coffee (*Coffea Arabica* L.) plants in response to nitrogen. *Theoretical and Experimental Plant Physiology*, 27 (3-4): 203-213.
- [44] Ochoa- Velasco, C.E., Valadez- Blanco, R., Salas- Coronado, R., Sustaita- Rivera, F., Hernandez- Carlos, B., Garcia- Ortega, S. and Santos- Sanchez, N. F. 2016. Effect of nitrogen fertilization and *Bacillus licheniformis* biofertilizer addition on the antioxidants compounds and antioxidant activity of greenhouse cultivated tomato fruits (*Solanum lycopersicum* L. var. Sheva). *Scientia Horticulturae*, 201: 338- 345.
- [45] Ochmian, I. 2012. The impact of foliar application of calcium fertilizers on the quality of highbush blueberry fruits belonging to the 'Duke' cultivar. *Not Bot Horti Agrobo*, 40(2): 163-169.
- [46] Aghdam, M.S., Dokhanieh, A.Y., Hassanpour, H. and Fard, J.R. 2013. Enhancement of antioxidant capacity of cornelian cherry (*Cornus mas*) fruit by postharvest calcium treatment. *Scientia Horticulturae*, 161: 160-164.

© 2024, by the Authors. The articles published from this journal are distributed to the public under CC-BY-NC-ND (<https://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>). Therefore, upon proper citation of the original work, all the articles can be used without any restriction or can be distributed in any medium in any form.

#### Publication History

Received	15.04.2024
Revised	13.06.2024
Accepted	15.06.2024
Online	30.06.2024