

## FOLIAR APPLICATION OF LOW-BIURET UREA AND FRUIT CANOPY POSITION IN THE TREE INFLUENCE THE LEAF NITROGEN STATUS AND PHYSICO-CHEMICAL CHARACTERISTICS OF KINNOW MANDARIN (*CITRUS RETICULATA BLANCO*)

AHMAD S. KHAN<sup>1\*</sup>, A.U. MALIK<sup>1</sup>, M.A. PERVEZ<sup>1</sup>, B.A. SALEEM<sup>1</sup>,  
I. A. RAJWANA, T. SHAHEEN<sup>3</sup> AND R. ANWAR<sup>1</sup>

<sup>1</sup>*Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan*

<sup>2</sup>*University College of Agriculture, Bahauddin Zakariya University Multan, Pakistan*

<sup>3</sup>*National Institute for Biotechnology & Genetic Engineering,  
PO Box 577 Jhang Road, Faisalabad, Pakistan*

### Abstract

Experiments were conducted to study i) the influence of supplemental foliar application of low-biuret urea (LBU) on the leaf nitrogen status and fruit quality and ii) the role of fruit canopy positions in the trees on the physico-chemical characteristics of Kinnow mandarin fruit. In first experiment, foliar application of LBU twice (before blooming and after fruit set) significantly brought the leaf N content of the Kinnow trees from deficient to optimum level as compared to single application before blooming or after fruit set and control trees. Trees sprayed with LBU twice also exhibited higher juice and pulp weight compared to other treatments. However, other quality parameters were not significantly affected with LBU application. Results of second experiment revealed that fruit harvested from inside of the tree canopy exhibited higher fruit volume; fruit, peel and pulp weight in contrast to the fruit harvested from other canopy positions. Highest juice percentage was recorded for fruit harvested either from top or outer periphery of the tree canopy. Soluble solids content (SSC), SSC: titratable acid ratio; reducing, non-reducing and total sugars; ascorbic acid contents were higher for fruit harvested from the top of the tree canopy as compared to the fruit harvested from other canopy positions. In conclusion foliar application of LBU can be used effectively to increase the leaf N status of Kinnow trees. Fruit at different canopy positions vary in their physico-chemical characteristics at harvest and should be harvested separately to have uniform quality and get maximum economic return.

### Introduction

Citrus occupy 35% of the total fruit production in Pakistan. It is grown over an area of 192 thousand hectares with a total production of little over 2.4 million tones. Among citrus fruits Kinnow mandarin is the leading citrus cultivar produced in the Punjab and contributes 65% of the total citrus production in the country (Anon., 2006). For consistent bearing and quality citrus production, nitrogen (N) plays an important role in the tree nutrition management in citrus. It is however noticed that the average N level in our trees remains low (Aziz, 1997; Sattar, 1999). Reduced level of N in the trees is one the limiting factor affecting fruit yield and as well as fruit quality.

Usually, the common way to supply N to citrus trees is through soil application. However, its efficacy depends upon many unrelated factors and there is always risk of soil and ground water nitrate pollution (Alberigo, 1999). Urea applied as foliar spray is absorbed rapidly and efficiently by leaves of most fruit crops (Johnson *et al.*, 2001). Studies have shown about 48% to 65% up take and translocation efficiency of foliar

\*Corresponding author: ahmad\_khan157@yahoo.com

applied urea to all other organs of the trees including roots (Tagliavini *et al.*, 1998). Foliar application of low-biuret urea (LBU) below 0.5% has produced its value and thus has become quite common to spray on large scale citrus plantation in the world as supplemental supply of nitrogen without any phytotoxic effects (Alberigo, 2002; Jones & Embleton, 1954). Application of N fertilization has been reported to greatly influence the quality of fruits and particularly foliar application of urea sprays is an efficient and cost-effective way to supply N to citrus, which enhanced the fruit size, peel thickness, juice contents and yield in some citrus species (Agabbio *et al.*, 1999; El-Otmani *et al.*, 2002; Rabe, 1994). Singh & Singh (1981) noted that 1% nitrate sprays increased the juice, pulp, total soluble solids (TSS) and acid contents of citrus. Aso & Dantur (1971) reported that high N rate increased the acid content in Valencia Late oranges. Different sources of foliar applied N have been tested on various citrus species at different agro-ecological conditions in the world, but the results are sporadic and inconclusive. Presently, little information is available on the effect of foliar applied LBU on leaf N status and quality of Kinnow mandarin fruit. It was hence tried to upkeep the N status of Kinnow mandarin trees through foliar application of N and to determine its impact on yield and fruit quality.

The quality of citrus fruit mainly depends upon the cultivar, rootstock and cultural practices such as exogenous application of growth regulators and tree nutrition (Davies & Albrigo 1994; Sattar, 1999; Saleem *et al.*, 2008a, Saleem *et al.*, 2008b). However, the position of the fruit in the tree canopy has also great influence on fruit physico-chemical characteristics (Datio & Tominaga, 1981). The yield and quality of fruits have been found to be affected with position in tree canopy and closely correlated with light transmission on the tree (Tustin *et al.*, 1988). Light levels above the critical minimum are required for the production of high quality fruits. In general the outer sunny sides of the tree canopy produce fruits of higher quality than shaded or lower inside canopy positions (Agabbio *et al.*, 1999). Juice percentage, acidity and TSS have been reported to be higher and fruit size and rind percentage lower in the upper sides of the citrus tree (Jawanda *et al.*, 1973; Wallace *et al.*, 1965). Orange fruit harvested from top position of the tree were found to be higher in reducing sugars (Uchida *et al.*, 1985), whilst no impact of fruit position in the canopy on total sugar contents were observed in Satsuma mandarin (Datio & Tominaga, 1981). Izumi *et al.*, (1990) has reported that citrus harvested from the southern top canopy sides have higher concentrations of soluble solids and higher juice contents than from other tree sides. Apparently, no information is available on the effects of fruit canopy position at harvest on the physico-chemical characteristics of Kinnow mandarin.

The present studies were therefore carried out to determine: 1) the influence of supplemental foliar application of LBU on the N status and fruit quality and 2) the effects of fruit canopy positions in the trees at harvest on the physico-chemical characteristics of Kinnow mandarin fruit.

## Materials and Methods

**Plant material:** To elucidate the role of low-biuret urea (LBU) and fruit canopy positions on the physico-chemical characteristics of citrus fruit, studies were conducted at Postgraduate Agricultural Research Station, University of Agriculture, Faisalabad. Twelve years old 32 uniform Kinnow mandarin (*Citrus reticulata* Blanco) trees grafted on rough lemon (*Citrus jambhei* L.) root stock, with 6.5 m row and plant distances,

apparently free from any pest and diseases were selected for the experiments. All the experimental trees received the similar cultural practices except the experimental treatments. The standard soil applications of N, P and K were done uniformly for all the experimental trees @ 990g, 600g and 600g respectively. First half of N and whole of P and K was applied 2 weeks before the anticipated full bloom and second half of N was applied 2 weeks after fruit set.

**Experiment-I:** During first experiment effects of foliar applications of LBU as supplemental sprays on the leaf nutrient status and quality of Kinnow mandarin fruit were investigated. Whole trees were sprayed with an aqueous solution of 0.3% LBU before blooming, after fruit set and before blooming + after fruit set. Trees sprayed with water without LBU were kept as control. To determine the changes in the endogenous levels of N, P and K in the leaves of experimental trees, leaf samples were taken before soil application of N, P and K, and one week after second half of soil N application. To observe the effects of foliar application of LBU samples were taken on 2, 3 and 4 days after foliar sprays both before blooming and after fruit set. Physico-chemical characteristics of fruit including fruit weight, volume, peel thickness; seed weight and numbers; juice and pulp weight; soluble solids content (SSC), titratable acidity (TA); ascorbic acid; reducing, non-reducing and total sugars were determined after fruit harvest.

**Experiment-II:** In another experiment effects of fruit canopy positions on the physico-chemical characteristics of Kinnow mandarin fruit were investigated. Fruits were harvested from the following four canopy positions, i) from in-side the tree canopy, ii) from middle outer periphery of the tree canopy, iii) from top of the tree canopy and iv) from lower side of the tree canopy (Fig. 1). All the physico-chemical characteristics of the fruit were studied as explained in the experiment-I.

**Leaf N, P and K:** Healthy uniform 50 mature (8-10 weeks old) leaves, free from any visual symptoms of nutrient deficiency or toxicity, and insects or disease attack were taken from non-fruiting terminals at shoulder height, uniformly from all around the tree. Leaf N, P and K were determined as reported earlier by Chapman & Pratt (1978).

**Average yield per tree:** At harvest, average yield including total number and weight of fruit per experimental trees were recorded. Total number of under sized fruits (less than 8 cm diameter) was also counted per treatment and percentage of unmarketable fruit was calculated dividing the number of under size fruit to total fruit and then multiplied by 100.

**Physical characteristics of fruit:** To determine fruit weight (g), volume (ml), peel thickness (mm), peel weight (g), pulp weight (g), juice weight (g), seed number and seed weight (g) 10 fruits were taken as sample unit and were replicated four times.

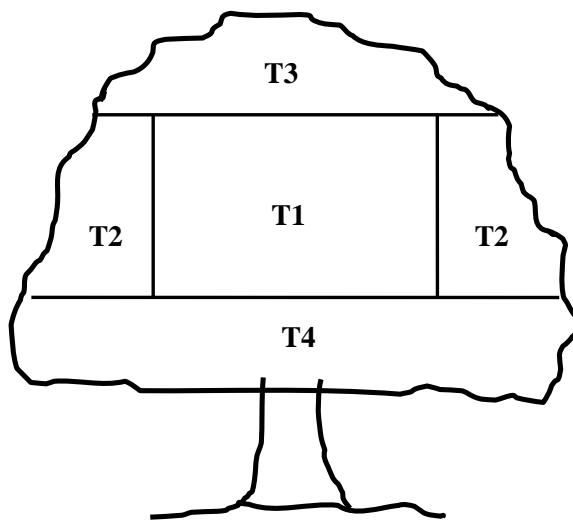


Fig. 1. Canopy positions of Kinnow mandarin fruit at the time of harvest. T1 = inside of the tree canopy, T2 = outer periphery of the tree canopy, T3 = top of the tree canopy, T4 = lower side of the tree canopy.

**SSC, TA and TSS:TA ratio:** The SSC of the juice was determined by using the automatic temperature compensating type ATC-20E hand refractometer (32-10 Honcho, Itabashi-ku, Tokyo 173-0001, Japan) and was expressed as °Brix (%). The TA of the fruit juice was determined by titrating to pH 8.2 with 0.1 N NaOH, using phenolphthalein as an indicator, and was expressed as % citric acid. The SSC:TA ratio was calculated by dividing SSC by the corresponding TA value. For each parameter 10 fruits were taken as treatment unit and were replicated four times.

**Sugars:** Reducing, non-reducing and total sugars from the juice were determined by using the method of Hortwitz (1960).

**Ascorbic acid:** To determine the ascorbic acid contents in the fruit juice, 10 ml of juice was diluted with 0.4% oxalic acid solution and 5 ml of filtered aliquot was titrated against 2, 6-dichlorophenol indophenol dye to light pink colour end point. Ascorbic acid contents were determined as mg 100 ml<sup>-1</sup> FW as reported by Ruck (1969).

**Statistical analysis:** The data in both experiments were subjected to analysis of variance (ANOVA) using MSTAT-C statistical software (Freed, 1994) using randomized complete block design. The effects of various treatments were accessed within the ANOVA for various parameters and least significant differences (LSD) were calculated by using the F test ( $p \leq 0.05$ ). All assumptions of the analysis were checked to ensure the validity of the statistical analysis. There were four treatments replicated four times and two trees were taken as a treatment unit.

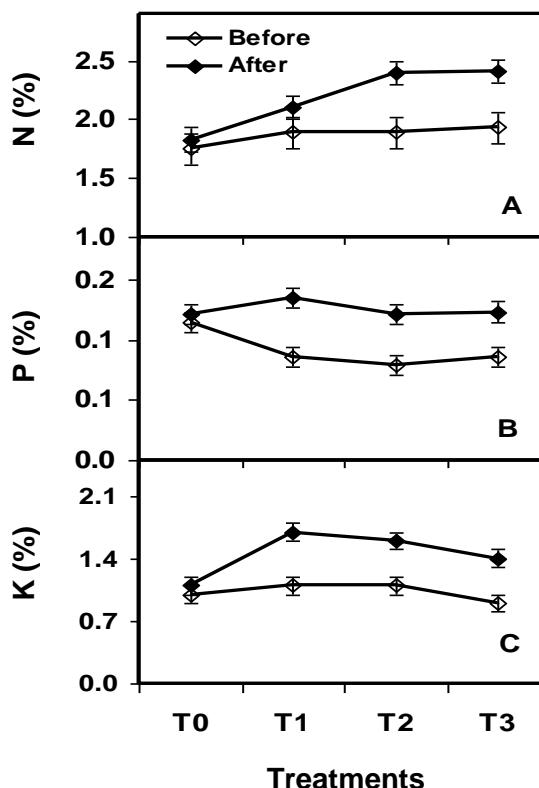


Fig. 2. Effects of foliar application of Low-biuret urea (LBU) on the leaf N, P and K status of Kinnow mandarin trees before and after fertilizer application. Vertical bars represent  $\pm$  SE of means. T0 = control, T1 = LBU application before blooming, T2 = LBU application before blooming + after fruit set, T3 = LBU application after fruit set, n = 4 replicates.

## Results and Discussion

**Leaf N, P and K:** The levels of N, P and K before fertilizer application in the Kinnow mandarin leaves were between 1.4-1.7%, 0.11-0.16% and 0.9-1.1% respectively (Fig. 2) as reported earlier by Chapman (1960). Soil N, P, K and supplemental foliar LBU spray brought a significant ( $p \leq 0.05$ ) increase in leaf N, P and K levels of LBU sprayed Kinnow trees as compared to control trees (Fig. 2). After foliar LBU application the levels of both P and K were at the optimum range from 0.17 to 0.19% and 1.1 to 1.7% respectively (Fig. 2B & 2C), whilst N was from deficient to optimum range 1.83 to 2.4% (Fig. 2A). Trees sprayed with LBU twice (T3) showed highest increase in N level (2.4%) about 0.8-fold higher than unsprayed trees. After fruit set, all the LBU treated trees exhibited high N levels compared to untreated trees. The N level of untreated trees remained continuously

in the deficient range throughout the sampling dates (Fig. 2A). Trees treated with LBU twice (T2) at one week after second-half of soil N application showed 1.7 folds increase in leaf N level as compared to the beginning of the experiment (Fig. 2A). LBU application to Kinnow mandarin trees before blooming brought a significant and rapid increase in leaf N level within 2 days after LBU application as compared to unsprayed trees (Fig. 3). Trees sprayed with LBU before blooming (T1 & T2) exhibited 1.5 and 1.9 folds increase in their leaf N as compared to their N levels before soil N, P and K application. Foliar application of LBU before blooming brought the leaf N status of T1 and T2 from deficient (1.7 to 1.4%) to optimum (2.5 to 2.6%) range after two days of LBU application respectively. Later on, after three and four days of LBU application, N levels of T1 and T2 reduced but remained higher than unsprayed trees. A similar trend in changes in leaf N status of Kinnow mandarin trees was also observed after fruit set and second LBU application, where LBU spray significantly ( $p \leq 0.05$ ) increased the leaf N level of Kinnow mandarin trees after two days of treatment. Trees sprayed twice with LBU (T3) exhibited higher leaf N content throughout the sampling dates. N levels in these trees on day-4 after LBU application remained in the optimum range. The N level of trees sprayed with LBU once after fruit set (T4), was significantly lower than trees sprayed with LBU twice (T3) from 2 to 4 days after LBU application. Treatment with two sprays of LBU increased the N content to optimum range as compared to control trees where N content were still in the deficient range, this might be due to enhanced utilization of N during the critical period of flowering and fruit setting in Kinnow mandarin trees. A combination of foliar application of LBU and soil N application is considered most favorable for improving N uptake efficiency and to minimize potential leaching of nitrate to the ground water along with optimum fruit production and leaf N status for citrus (Alva *et al.*, 2003).

**Fruit yield:** At harvest LBU applications significantly increased the fruit yield compared to control. Trees sprayed with LBU twice (T2) exhibited highest increase in the fruit yield both in terms of average fruit number and average fruit weight per tree (Fig. 4). Percentage of marketable fruit in T2 was three folds more as compared to untreated control fruit. Supplemental foliar application of LBU resulted in 2.3 folds reduction in the mean unmarketable fruit per tree in contrast to unsprayed trees (Fig. 4). This increase in the final fruit yield and reduction in the percentage of unmarketable fruit per tree could be due to increased N status of these trees with supplemental LBU application at the most critical stage (fruit set) of fruit growth and development (Fig. 2 & 3). Similarly foliar LBU application to 30 year old 'Washington Navel' oranges (*Citrus sinensis* Osbeck) has been reported to increase the yield in terms of total fruit weight and fruit number per tree for 3 consecutive years (Lovatt, 1999). LBU application twice at intervals of 10-14 days about 6-8 weeks before anthesis markedly increased the yield of Navel and Shamouti sweet oranges (Rabe & Walt, 1993).

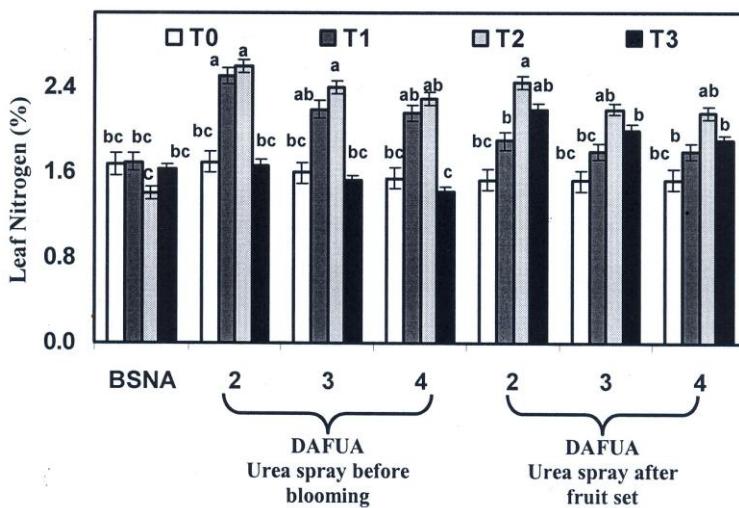


Fig. 3. Changes in N status of Kinnnow mandarin leaves after foliar LBU application. BSNA = before soil nitrogen application. DAFUA = days after foliar urea application. Means followed by different letter are significantly different at  $p \leq 0.05$ . LBU = low-biuret urea, T0 = control, T1 = LBU application before blooming, T2 = LBU application before blooming + after fruit set, T3 = LBU application after fruit set. Vertical bars represent  $\pm$  SE of means,  $n = 4$  replicates.

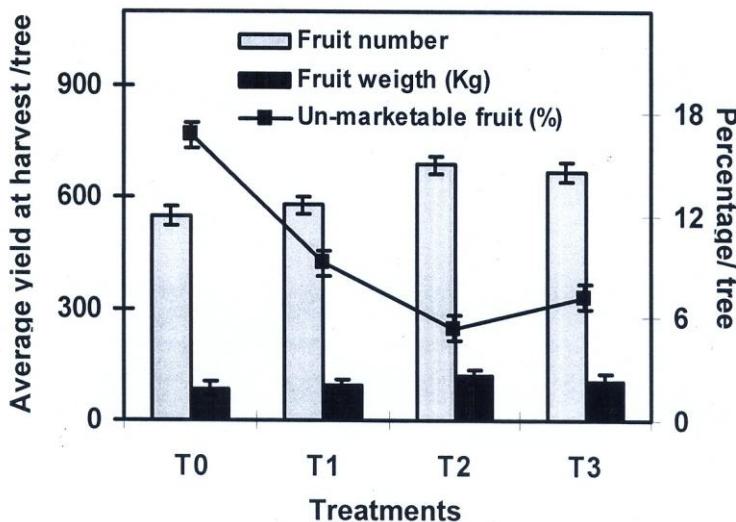


Fig. 4. Effects of foliar application of low-biuret urea (LBU) on the average fruit number, average fruit weight and percentage of un-marketable Kinnnow mandarin fruit per tree. T0 = control, T1 = LBU application before blooming, T2 = LBU application before blooming + after fruit set, T3 = LBU application after fruit set. Vertical bars represent  $\pm$  SE of means,  $n = 4$  replicates.

### Fruit quality

**Average fruit weight, volume and rind thickness:** Foliar LBU applications did not affect the fruit quality parameters significantly. However, trees sprayed with LBU after fruit set (T3) produced slightly larger and heavier fruits with thicker peel as compared with the other treatments (Table 1). Fruit canopy positions on the tree significantly affected the fruit weight and fruit volume. Fruits picked from the inside of the tree canopy were heavier (20%) and larger in volume (22.3%) in contrast to fruit harvested from top of the tree canopy respectively (Table 2). Previous study of Jawanda *et al.*, (1973) confirms that fruit from the inner part of the canopy were heavier than from other sides of tree canopy. Fruit peel thickness of fruit was not affected significantly with the LBU application as well as fruit canopy positions on the tree at harvest (Tables 1 & 2). Higher fruit weight and peel thickness of fruits from internal canopies than from external canopies were determined in Red Blush grape fruit, Valencia orange and Lisbon lemon (Fallahi *et al.*, 1989).

**Number and weight of seed:** The LBU spray application significantly reduced the average seed weight (31.3%) as compared to control trees (Table 1). However, no effects of LBU application were observed on the average number of seeds per fruit. Similarly, fruit canopy position at harvest did not significantly affect the seed number as well as the seed weight of Kinnow mandarin fruit (Table 2).

**Peel, juice and pulp weight:** Supplemental application of LBU to Kinnow mandarin trees did not affect the peel weight. However, the fruit sprayed with LBU twice (T2) showed 16% more juice weight and 20% pulp weight as compared to untreated fruit (T0) respectively (Table 1). Similar results were obtained by foliar N application in Navel orange, Shamouti orange and Dancy Tangerine fruits (Azzony *et al.*, 1970; Dasberg *et al.*, 1983; Singh & Singh, 1981). The effects of fruit canopy position in the tree on peel, juice and pulp weight of Kinnow mandarin fruit were highly significant. Fruit from inner sides of the tree canopy exhibited 33% more peel weight than fruit from top of the tree (Table 2). Fruit harvested from the top of tree have higher juice weight % and lowest pulp weight % per fruit as compared to fruit harvested from other canopy position. Results are in agreement with the findings of Wallace *et al.*, (1965) and Jawanda *et al.*, (1973) who observed the higher amount of juice weight in the fruit harvested from the top outer position of the tree. Grapefruit harvested from the sunlit canopy positions were heavier with more juice content than fruit from shaded positions (Syvertsen & Albrigo, 1980). However, higher amount of total fruit per fruit, peel weight per fruit have been reported in Red Blush grape fruit, Valencia orange and Lisbon lemon harvested from internal canopies in contrast from external canopies (Fallahi *et al.*, 1989).

**SSC, TA, SSC:TA ratio and ascorbic acid:** Foliar application of LBU did not significantly affect the SSC, TA, SSC:TA ratio and ascorbic acid level of Kinnow mandarin fruit at harvest. However, SSC, SSC:TA ratio was higher and TA was lower in T2 as compared to other treatments. Fruit harvested from top of the tree were significantly ( $p \leq 0.05$ ) higher in SSC (12.4%) and SSC:TA ratio (15.4) compared to fruit harvested from other positions on the tree (Table 4). Fruit harvested from the outer





periphery also exhibited higher SSC:TA ratio compared to fruit harvested from inside and lower sides of the tree canopies. The higher amount of SSC:TA ratio in the fruit harvested from top of tree canopy could be due to increased exposure of the fruit to the sun light. In previous studies similar results had been observed in citrus fruit harvested from outer position on the tree compared to inside of the tree (Jawanda *et al.*, 1973; Wallace *et al.*, 1965). Freshly harvested Torocco orange fruit from the external southern side of the tree had higher SSC and lower acid levels with improved taste in comparison to fruit from northern and interior parts of the canopy (Agabbio *et al.*, 1999). Fruit harvested from top of the tree canopy and outer periphery of the tree canopy showed 11% and 5.6% higher ascorbic acid level compared to the fruit harvested from the inside of the tree. The present study clearly shows the fundamental relationship between fruit orientation on the tree canopy and the light levels within the tree. Higher SSC and lower acidity were observed in the outside canopy positions of Ruby grapefruit than the lower positions (Syvertsen & Albrigo, 1980). Lower Brix: acid ratio was observed in the lychee fruit from lower canopy position than from fruit harvested at higher canopy positions (Tyas *et al.*, 1998). Similarly, Lewallen & Marini (2003) reported that peach fruit from the canopy exterior generally were larger, had more surface darker and redder than fruit harvested from interior positions.

**Reducing, non-reducing and total sugars:** The reducing, non-reducing and total sugar content of Kinnow fruit from trees sprayed with LBU did not show any significant differences. Previously Baldry *et al.*, (1982) have reported that high N rates do not raise the sugar contents in citrus fruits. Observations from different tree canopies revealed that fruits which were harvested from top of the trees had significantly ( $p \leq 0.05$ ) higher reducing (1.3 folds), non-reducing (1.4 folds) and total sugars (1.1 folds) than fruit harvested from inside of the tree canopy (Table 4). The results are in conformity with Jawanda *et al.*, (1973) and Uchida *et al.*, (1985) who reported that reducing and non-reducing sugar contents increased in fruits harvested from the exposed sides of the fruit as compared to inner sides. However, in contrast Datio & Tominaga (1981) observed that position of fruit in the canopy did not significantly affect the total sugar content in Satsuma mandarin fruit juice.

## Conclusions

In conclusion, application of LBU as supplemental foliar spray, twice, before bloom and after fruit set, can be used effectively to bring the leaf N level of N deficient Kinnow mandarin trees from deficient to optimum level. Fruit produced at different positions of the tree canopy vary in the physico-chemical characteristics at harvest and should be harvested separately to maintain uniform quality and fetching highest economic returns.

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