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FOLIAR APPLICATION OF LBU INFLUENCES THE LEAF NITROGEN LEVEL, VEGETATIVE AND REPRODUCTIVE PERFORMANCE OF 'BLOOD RED' SWEET ORANGE

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Abstract

Winter leaf drop in sweet orange has been considered as the main cause for loosing the tree vigour and yield, which has resultantly almost vanished it from the citrus industry of Punjab-Pakistan. The current study was aimed at determining the optimum time of low-biuret urea (LBU) foliar spray to improve leaf age, fruit set, and yield of 'Blood Red' sweet orange (*Citrus sinensis* L. Osbeck.) through nitrogen (N) enrichment of over wintering leaves. A 2% solution of LBU was sprayed on to 15 years old 'Blood Red' sweet orange trees to the point of run off, during mid October, November and December along with control sprayed with simple water. The changes in the level of total N and NH_4-N in the leaves were studied after 0, 3 and 6 days of LBU spray and then during February (flushing), March (flowering), and April (fruit set). The maximum total leaf N contents were found in December treatment (3.3%) followed by November (3%) and October (2.8%) compared with control (2.4%). Likewise, maximum NH_4-N was observed in December and November sprayed trees (1.7% each). The LBU treatments did not significantly influence total number of shoots per branch, vegetative shoots (%), generative shoots (%), length of shoot and number of leaves per shoot. All the LBU treatments significantly increased the leaf age along with total number of buds per branch and bud drop intensity (%) compared with control, while flower maleness and flower opening tendency were significantly decreased by all treatments compared with control. There was no significant difference among treatments in case of flower drop, fruit set on current shoots and old shoots. In conclusion, winter application of LBU significantly improved the leaf age, tree vigour and production of 'Blood Red' sweet oranges through improved flowering without any improvement in fruit setting.

Introduction

The main problem of sweet orange growers in Punjab, Pakistan, is their shy bearing and low quality fruit and short life span compared with 'Kinnow' mandarin dominating the local citrus industry (Chaudhary, 1992). The monoculture in citrus industry may be a serious threat in the case of a disease outbreak in 'Kinnow' mandarin. Presently, in Pakistan, the average yield of citrus including Kinnow is about 10 t ha^{-1} which is far less than world average yield of 30 t ha^{-1} (Anon., 2008). Annually, 1.70 MMT citrus is produced from a total area of 185 thousand ha in Pakistan (Anon., 2005). Today, Pakistan stands at 13^{th} and 10^{th} position among top citrus producing and exporting countries of the world, respectively (Anon., 2005). Sweet orange may be induced in our citrus industry provided its production and quality is improved. The major reasons ascribed to low productivity are severe leaf shedding during winter, poor fruit set and higher fruit drop. This may be attributed to malnutrition, improper management and insect pest attack.

In sweet oranges, winter leaf drop has been noted as a serious production constraint (Chaudhary, 1992). Leaves developed during spring act as sink till they are 4-6 weeks old (Goldschmidt, 1997) which reveals the prime importance of old leaves. In citrus there is considerable evidence that the presence of old leaves, is crucial to improve the fruit setting, and fruit weight and size is determined by leaf area (Sinclair, 1984). The spring flush of citrus, soon followed by floral development, anthesis and fruit set, requires photosynthates in higher amount for organ growth and to meet the respiratory requirements (Goldschmidt, 1997). The persistence of previous year foliage in citrus undoubtedly plays a critical role to supply photo-assimilates during the emergence of the spring flush prior to full expansion of new leaves (Moss *et al*., 1972). More than 25% defoliation affects flower initiation for the next crop in Olive (Hartmann, 1972; El-Tamzini, 1982), while removal of either old or young leaves increase abscission, in Satsuma (*Citrus unshiu,* cv. Clausellina) mandarin trees (Mehouachi *et al*., 2000). Ruan (1992) reported that the assimilate supply to fruitlets and young leaves of *Citrus unshiu* is mainly affected by the current photosynthesis by the old leaves, instead of from the re-mobilization of stored reserves from the trunks. Younger leaves gradually substitute the older ones to supply assimilates to the developing fruits about day 20 after full bloom. Legaz *et al*., (1995) studied the mobilization of the reserves N in 'Valencia' sweet orange and found that older leaves were the main reserves organs, contributing about 40-50% of the total N exported.

The level of N fertility has more influence on the growth and yield of citrus than any other single plant nutrient (Thompson *et al*., 2002). Foliar N fertilization offers an opportunity to apply a significant portion of the total tree N needs in a more efficient manner than traditional flood or ground applications (Wright & Pena 2000). Low-biuret urea (LBU) is the best choice for foliar applications on high value horticultural crops. LBU sources have been applied as the foliar component of a typical N application program. Foliar application of LBU during winter significantly increased yield compared with soil applied urea, although this application of LBU to the foliage of 'Washington Navel' orange did not significantly increase total leaf N at the end of three years (Ali & Lovatt 1994; Saleem *et al*., 2008a).

In the nitrification process, NH_4-N is converted into NO_2-N, which is further converted into NO_3-N, since plants can absorb only NH_4-N and NO_3-N (Ishikawa, 1999). Zilkah *et al*., (1996) found 26% increase in N concentration of avocado leaves with three foliar applications of LBU. Urea application significantly increased N levels in 'Clementine' mandarin and the maximum leaf N concentration was recorded two days after each application, which declined thereafter (El-Otmani *et al*., 2004). Nitrogen being the most frequently applied nutrient is reported to be indispensable for enhanced fruit set in citrus (Saleem *et al*., 2005). Low leaf N levels promote excessive flowering, nonetheless the fruit set and yields are poor (Smith 1969). Therefore, maintaining leaf N levels in the optimum range produce a moderate number of flowers with maximum fruit set.

Although significant work on foliar application of LBU to improve production and quality of citrus has been done in the past (Lovatt *et al*., 1988; Lovatt *et al*., 1992; Ali & Lovatt, 1995; El-Otmani *et. al*., 2004) however, optimum time of winter application of low-biuret urea, has never been investigated. Nonetheless, such work is lacking for sweet oranges in Pakistan. Therefore, this study was conducted to reduce over wintering leaf drop to improve vigour of trees, flower production and fruit set of 'Blood Red' sweet orange through leaf N enrichment as a result of LBU application.

Material and Methods

Plant materials: The study was conducted on 15 years old sweet orange (*Citrus sinensis* Osbeck L) cv. Blood Red trees, growing at Experimental Fruit Garden Sq. No. 9, Institute of Horticultural Sciences, University of Agriculture, Faisalabad (Latitude 31°- 26´ N and Longitude 73°- 06´ E), Punjab, Pakistan. The experimental trees were spaced at about 7 m x 7 m, grafted on rough lemon (*Citrus jambheri* Lush.) rootstock, growing under similar agro-climatic conditions and received same cultural practices during the period of investigation (Saleem *et al*., 2004; Saleem *et al*., 2008b).

Before start of the experiment, the trees were evaluated for uniformity of growth, fruit yield potential and possible disease incidence. Twelve uniform trees with no apparent disease incidence were selected for the experiment in the form of a block. To check the fertility status of the soil, samples were collected at different depths from four sites of the experimental orchard and from under the tree separately.

Foliar spray of low-biuret urea: Low-biuret urea (LBU) @ 2% aqueous solution was applied as foliar spray with hand sprayer onto whole trees to run off during the mid of the months of October, November and December 2004. There were three replications with one tree as the treatment unit. Detail of treatments is given as under:
T_0 Control
T_1 Foliar Spray of 2% LBU in October
T_2 Foliar Spray of 2% LBU in November
T_3 Foliar Spray of 2% LBU in December

N estimation: The total N and NH_4-N status of experimental trees, was monitored through leaf analysis on day 0, 3, 6 after spray application and then during February (flushing), March (flowering) and April (fruit set). The total leaf N was determined following method of Chapman & Parker (1961). It involved the digestion of plant material with concentrated H_2SO_4 and digestion mixture comprising K_2SO_4: CuSO4: Fe_2SO_4 (10: 0.5: 1). NH4-Nwas estimated by modifying the protocol of Bhargava & Raghupathi (1993).

Vegetative and reproductive behaviour: To study the vegetative and reproductive behaviour of trees, 4-5 cm thick branches were tagged, one each of the four sides of the selected trees. Data regarding different parameters such as total number of shoots per twig, vegetative shoots, mix shoots, generative shoots, shoot length, number of leaves per shoot, number of flowers buds per branch, bud drop, hermaphrodite flowers, flower drop, male flowers, fruit set, periodic and total fruit drop were recorded from those tagged branches. The data regarding vegetative growth was collected during the last week of March, while that of number of leaves/shoot, length of shoot was collected during last week of April. Leaf age was calculated in terms of over-wintering leaf retention percentage, during last week of January.

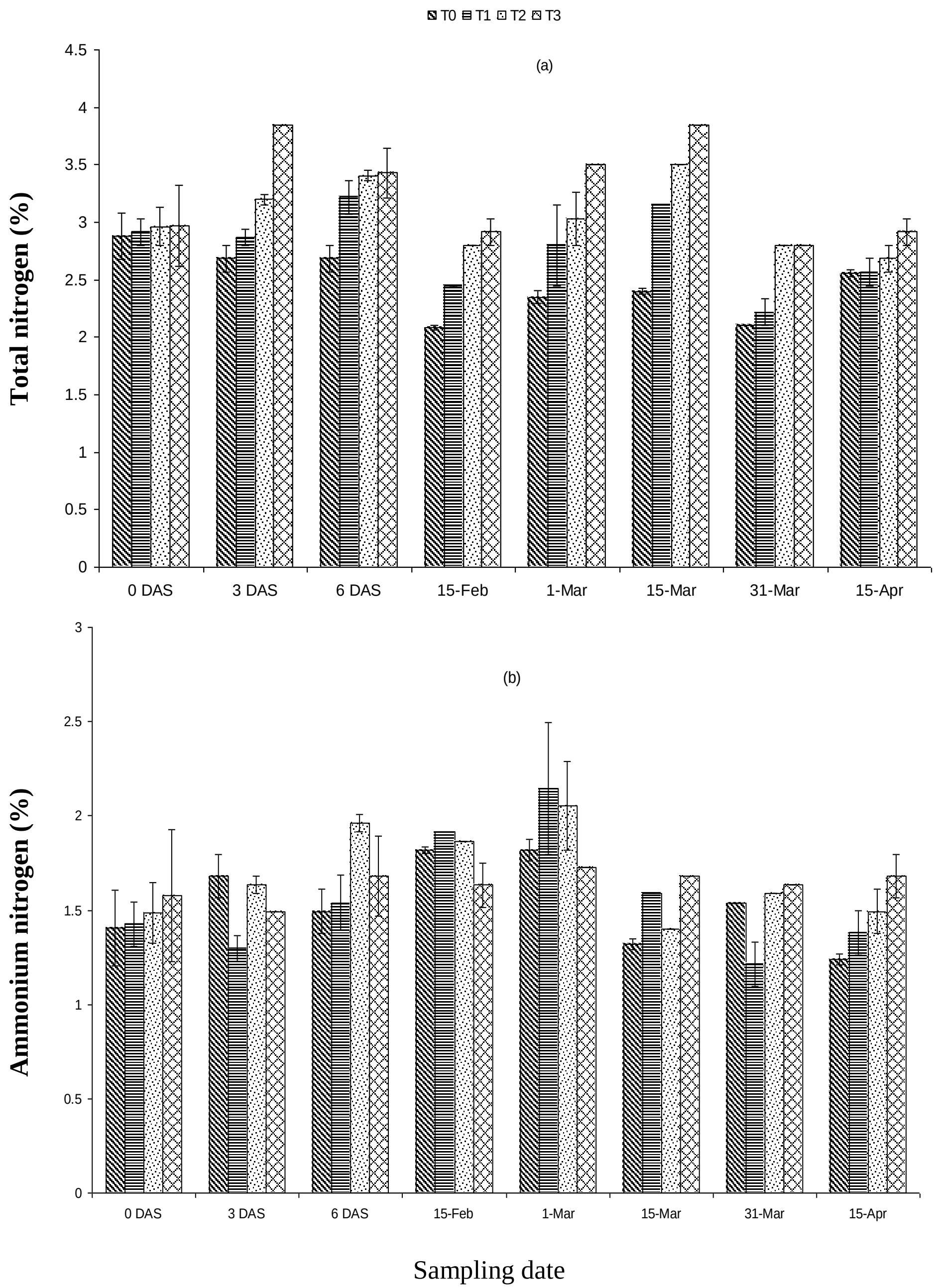
Statistical analysis: The data were subjected to ANOVA using RCBD. A single tree was selected as treatment unit replicated three times. The response of experimental trees to different treatments was evaluated by statistical analysis of data using the computer software MSTAT-C (Freed & Scott, 1994), while DMR test was used to compare the differences among the treatment means at 5% probability level.

Results and Discussion

Leaf N content: The application of LBU improved the leaf N content of 'Blood Red' sweet orange trees. After three days of LBU spray, a significant increase in N contents was observed in November and December treatments, while October and control treatment had non significant change in N contents. Nonetheless after six days, December treated trees showed significant decrease in leaf N contents. However, N contents in these trees were still higher than before foliar spray, while October and November treated trees exhibited a significant increase in the N contents compared with control (Fig. 1a). All the treated trees had significantly higher N compared with control. After the danger of frost was over i.e., 15 February (by the time of fertilizer application), there was overall significant decrease in leaf N contents in all experimental trees. The total N contents in all the treated trees (2.45%, 2.80% and 2.92% for October, November and December treated trees respectively) were significantly higher compared with control (2.08 %) trees. On 15 March, all the treatments had significant differences among each other with maximum total N (3.85 %) in December treated trees compared with control. On 31 March there was decrease in N contents with equal status in all treatments while, on 15 April again the N status started to increase with non significant differences among treatments (Fig. 1a).

Among nutrients, N has the greatest influence on growth and development of a plant, affecting the production of branches, leaves and fruits. During winter season nutrient uptake by roots is not much efficient, foliar nutrition through leaves can supply the N required for cell/tissue renewal and activity (Ali & Lovatt, 1995). There was about 30% increase in leaf N in December treatment, while 7.7% increase in November treated trees ranging between 3.19 -3.85%, which supports the assumption that citrus leaf N can be increased by the foliar application of urea (Zilkah *et al*., 1987). The increase in N on 1st and 15 March was due to soil application of fertilizer/spring doze. The increment in N after foliar application was maintained up to six days when it started declining (Fig. 1) indicating that winter application of LBU can provisionally elevate the citrus leaf N status to improve growth, vigour and production of sweet oranges, since earlier studies suggest that total growth and fruitfulness were found greatly dependent on N levels in 'Valencia' oranges (Smith *et al*., 1953).

Three days after the LBU spray, all the treatments had significantly lower NH_4-N compared with control, except trees sprayed in November. The NH_4-N contents were decreased in October and December treatments, whilst, the control and November treated trees had higher NH_4-N contents compared with the previous analysis. After six days of LBU application, the significant increase in NH_4-N contents was observed in November treatment (1.9%) compared with the control. Remaining treatments including control had increasing but non significant trend compared with previous reading as well as among the treatments. On 15 February, all the treatments had significant difference among each other with maximum NH4-N in October (1.91%) application, while December treatment had minimum concentration of NH4-N (1.6%) in the tree leaves, however, the change in concentration was non significant compared with previous observation except control having significant difference from its previous reading (1.49%). On 1st March, there was again increase in NH_4-N but the difference was non significant compared with previous reading as well as among treatments except December treatment. On 15 March, all the treatments increased the leaf NH_4-N concentration compared with control with maximum value (1.7%) being in December application and minimum (1.4%) in control trees. On 31



Fi.g. 1. Influence of winter application of low-biuret urea on periodic changes in (a) total leaf nitrogen and (b) ammonium nitrogen in 'Blood Red' sweet orange ± s.e.
T_0 = Control, T_1 = October application of LBU, T_2 = November application of LBU,
T_3 = Dec application of LBU, DAS = Days after spray

March, all the treatments had significant differences among each other with respect to NH_4-N concentration, maximum (1.6%) being in December, while minimum concentration (1.2%) was noted in October treatment. The change in concentration of leaf NH_4-N was significant compared with last readings (1st March). On 15 April, the concentration of NH_4-N in treated trees were significantly higher than control, but the difference between the treatments was non significant.

There was maximum increase (44.3%) in NH_4-N concentration on 1st March, in October treated trees followed by 38.5% increase in November treated trees and 32.4% in December treatment and all the trees resumed the status during mid April as before the foliar spray. The decrease in concentration of NH_4-N after March may be due to its involvement in flowering (Lovatt *et al.*, 1988). However, in November treatment there was significant increase in NH_4-N after six days of spray and there was increasing trend in ammonium concentration up to beginning of March (Fig. 1). Our results are in accordance with previous reports that NH_4-N may be increased by spray of LBU (Lovatt *et al.*, 1988;1992). Besides increment in NH_4-N by winter stress (Southwick & Davenport, 1987), tissue N status may also be temporarily increased by foliar spray of LBU (Lovatt *et al.*, 1988;1992) with maximum increase by November spray.

Vegetative growth: There was no difference in number of shoots per twig in response to 2% spray of LBU during winter compared with control, however, number of mixed shoots was significantly decreased compared with control (Table 1). Maximum mixed shoots (85.6%) were recorded in control trees, while minimum mixed shoots (75.4%) were observed in December treated trees. There was no effect of treatments on emergence of vegetative shoots and generative shoots, number of leaves per shoot and length of shoot. All the treatments significantly improved the leaf age compared with control with maximum leaf age (60.1%) in December treated trees and minimum leaves (12.2%) were observed in control trees (Table 1).

In subtropical regions 2-5 vegetative growth flushes usually occur annually with main vegetative growth flush in spring after a long rest period due to low temperature. There was no effect of treatments on shoot intensity and vigour because there was no deficiency of N in experimental trees as the leaf N concentration remained in optimum to excess levels during whole the period of experiment. Increased concentrations of leaf N produced healthy leaves and retained them over longer period. According to previous reports as total growth and fruitfulness were found greatly dependent on N levels in 'Valencia' oranges (Smith *et al.*, 1953) and also raising the NH_4^+ status of trees by applying LBU did not increase number of vegetative shoots in 'Washington Navel' sweet orange (Lovatt *et al.*, 1988). However, leaf age was significantly enhanced with foliar application of N (Davies & Albrigo, 1994), while low N caused more shedding of citrus (Smith 1969), and avocado leaves (Zilkah *et al.*, 1996). An inverse correlation between N contents and leaf senescence has been established in avocado trees by Zilkah *et al.*, 1996 as the trees treated with low-biuret urea foliar spray @ 2% had a significantly lower degree of leaf senescence than control trees in avocado.

Reproductive growth: All the three LBU treatments significantly increased the bud drop compared with control, with maximum bud drop (39.7%) in trees sprayed with LBU during December. Foliar treatments significantly reduced flower opening compared with control as maximum opened flowers were recorded in control trees. However, flower drop was not significantly affected by any treatment compared with control. Incidence of male flowers was significantly decreased by all the treatments compared with control with maximum male flowers (10.41%) in control trees and minimum (1.42%) in December treated trees. Similar fruit set, occurrence of fruit on old shoots as well as current shoots were observed in all treatments compared with control.

The increased leaf age reflected on as more bud drop in urea treated trees with lesser flower opening. Number of flowers produced is directly related to the tissue N status because during flower development N is translocated from leaves to the flowers (Smith & Reuther 1954). In contradiction to this study, Jeelani (1994) reported that N application before flowering reduced flower drop. Retention of green older leaves for longer time reduced the male flowers in treated trees compared with control. Weak sweet orange trees produce more male flowers, while foliar N application improved the leaf NH_4-N status and health and vigour of trees, therefore male flower production was reduced. NH_4-N may directly affect flowering via ammonia regulation and bud polyamines level (Lovatt *et al*., 1988). The foliar application of LBU to citrus trees subjected to less low temperature stress (reduced from 8 weeks to 4 weeks) increased the NH_4-N contents of leaves and doubled the intensity of flowering (Lovatt *et al*., 1988). The extension in leaf age improved complete flower formation which led to better yields of treated trees without any significant effect on flower drop and fruit set on different kinds of shoots. As the N status was in optimum range even in control, the addition of N through foliar spray could not have substantial impact on flower drop and fruit set. The results regarding fruit set confirm the findings of Smith (1969), who reported that the higher N resulted in poor fruit set as all the trees had leaf N in excess (Fig. 1).

Fruit drop: One week after fruit set (10 April), all the treatments significantly reduced the fruit drop compared with control (Fig. 2). Maximum fruit drop (68.1%) was observed in control trees, while minimum fruit drop (33.17%) was noted in trees sprayed with LBU in October (Fig. 3). On 17 April (2 weeks after fruit set) all the treatments had significantly different intensity of fruit drop with minimum fruit drop (9.43%) in control and maximum fruit drop (38.9%) in October treatment. On 1st May (4 weeks after fruit set) all the treatments had statistically similar intensity of fruit drop (19.42–23.11%). The treatments had no effect on intensity of fruit drop on 30 May as well as on final fruit count (18 October). The total fruit drop at final fruit count was significantly decreased by all LBU application compared with control (Fig. 3). Maximum fruit drop (99.22%) was recorded in control trees, nonetheless minimum fruit drop (94.50%) in November treatment, which was statistically similar to other treatments.

Fruit drop is a natural phenomenon, meant for load management and it avoids the excessive drain of food reserves from tree. Climate, nutritional status and health of tree are the main factors responsible for the extent of fruit drop. Three waves of fruit drop (Racsko *et al*., 2006) have been reported in fruit crops including citrus (Davies & Albrigo, 1994) i.e., just after fruit set, June drop and preharvest drop. The results pertaining to periodic fruit drop indicated that most of the fruit drop (50-70%) occurred just one week after the fruit set (three weeks after full bloom). The LBU treatments significantly retained more fruit compared with control just after fruit set, which was temporary. In crux, there was no difference among treatments including control. The range of initial fruit drop was 29-43 % (Sattar, 1999) in Kinnow and 50–70% in ‘Blood Red’ sweet orange. October treatment proved to be the best in reducing initial fruit drop (33%) followed by November (51%) and December (55%) treatments. Our results partially confirm the findings of Singh & Singh (1973) in Kaghzi lime.

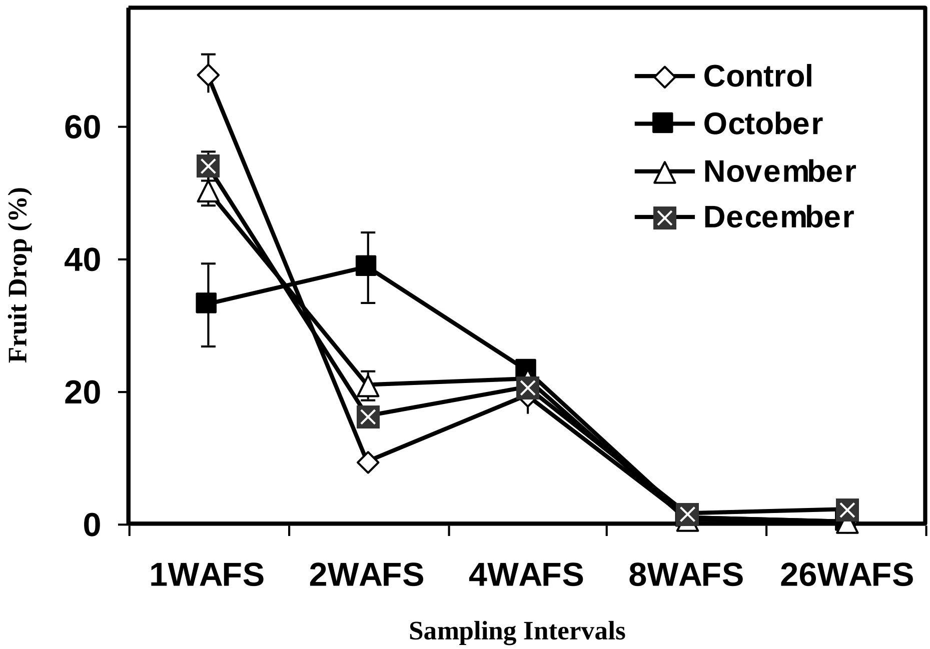


Fig. 2. Periodic fruit drop (%) of 'Blood Red' sweet orange in responseto winter application of low-biuret urea (± s.e.) WAFS = Weeks after fruit set

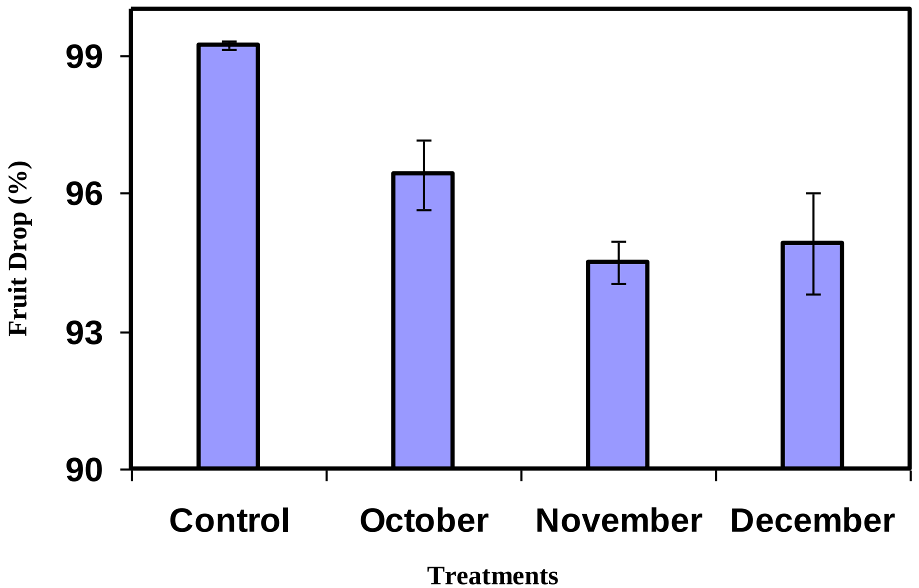


Fig. 3. Effect of exogenous application of low-biuret urea, on total fruit drop percentage, of 'Blood Red' sweet orange (± s.e.).

Fruit yield: The results regarding production are presented in Table 3 which shows that the LBU treatments significantly increased the total yield per tree compared with control. Maximum yield (689.70 fruit per tree) was observed in December treated trees. October and November treatments produced statistically similar number of fruit per tree, while minimum yield (340 fruit per tree) was recorded in control trees.

In terms of total fruit weight, production was significantly higher in all LBU treatments compared with control. Maximum fruit production (78.7 kg per tree) was recorded in October treatment statistically similar to November and December treatment, while minimum fruit production (66.6 kg per tree) was attained from control trees.

Adequate supply of N during the critical stages of fruit initiation and development is important to support optimal yields of good quality citrus (Davies & Albrigo, 1994; Alva *et al*., 2006). The low N in tree during flowering and fruit setting may lead to lower yield regardless of the N levels during the remaining fruiting cycle (Jones & Embleton, 1954). Fruit production of 'Blood Red' sweet orange was improved by foliar application of the LBU, possibly owing to reduced initial fruit drop resulting in more fruit per tree reaching to mature stage (Fig. 2). Fruit drop in control trees was 99.01%, while that of treated trees were 96.12%, 94.48%, and 94.51% for October, November and December treated trees, respectively and accordingly was final fruit yield. Moreover the increased number of fruit my be attributed to production of more number of flowering buds (474.50, 660.25, 815.83 and 740.58 buds per branch for control, October, November and December treatments respectively, Table 2). The N application improved the general health and vigour of trees which also improved the final yield of trees. Our results confirmed the findings of Franciosi (1985) and Lovatt *et al*., (1992) and Sattar (1999), who reported an increase in yield through foliar application of N on citrus. But the results do not support the finding of Pedrera & Hernandez (1984) who stated that foliar application of N was very weakly correlated with yield in citrus cultivars.

Conclusion

In crux, winter application of 2% LBU extended the leaf age, which finally led to better yield of 'Blood Red' sweet orange. The extended leaf age improved general health and vigour of trees which ultimately produced more complete flowers, whilst discouraging male flowers caused more fruit on treated trees. Hence exogenous application of LBU in winter may be recommended to enhance the production of sweet ornages.

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Table 1. Effect of winter application of low-biuret urea on vegetative characters of 'Blood Red' sweet orange.

Treatments	Total No. of shoots/branch	Mixed shoots (%)	Vegetative shoots (%)	Generative shoots (%)	Length of shoot (cm)	No. of leaves/shoot	Leaf age (%)
Control	29.55	85.59a	5.83	8.58	1.75	3.13	12.22c
October	34.97	77.25bc	10.02	12.73	2.93	3.03	51.57ab
November	36.37	81.61ab	10.37	8.02	2.22	3.14	60.09a
December	29.97	75.38c	9.64	14.95	2.59	3.48	35.45b
	NS	*	**NS**	**NS**	**NS**	**NS**	*

NS = Non Significant. * = Figures sharing the same letters in the same column differ significantly at $P \leq 0.05$

Table 2. Effect of winter application of low-biuret urea on reproductive characters of 'Blood Red' sweet orange.

Treatments	Number of buds/ branch	Bud drop (%)	Male flowers (%)	Opened flowers (%)	Flower drop (%)	Fruit on old shoots (%)	Fruit on current shoots (%)	Fruit set (%)
Control	474.50	18.94b	10.41a	81.06a	79.91	6.32	93.68	15.46
October	660.25	26.29ab	1.60b	73.71ab	75.19	4.47	95.53	18.41
November	815.83	38.28a	1.94b	61.75b	76.61	7.19	92.81	14.22
December	740.58	39.77a	1.42b	60.23b	77.69	8.07	91.93	14.11
	*	*	*	*	**N.S.**	**N.S.**	**N.S.**	**N.S**

N.S = Non Significant, * = Figures sharing the same letters in the same column differ significantly at $P \leq 0.05$

Table 3. Effect of winter application of low-biuret urea on yield of 'Blood Red' sweet orange fruit.

Treatments	Fruit yield		
	No/tree	**kg /tree**	**t/h**
Control	340.70c	66.61b	16.46b
October	589.00b	78.70a	19.45a
November	689.70a	74.70a	18.46a
December	543.30b	72.64ab	17.95ab
	*	*	*

* = Figures sharing the same letters in the same column differ significantly at $P \leq 0.05$

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