Journal of Plant Process and Function, Vol. 7, No. 25, Year 2018

Changes of phenolic compounds and non-structural carbohydrates on alternate bearing cycle in 'Kinnow' mandarin trees

Abbas Mirsoleimani[1*], Ali-Reza Shahsavar[2] and, Vahid Rowshan[3]

[1]Department of Plant Production, College of Agriculture and Natural Resources of Darab, Shiraz University, Shiraz, Iran, [2]Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz Iran, [3]Agricultural and Natural Resources Research Center of Fars, Shiraz, Iran

(Received: 11/07/2017-Accepted: 15/04/2018)

Abstract

In this study, we have demonstrated the variations of carbohydrates and phenolic compounds present in the leaves and stems of 'Kinnow' mandarin (*Citrus reticulata* Blanco) trees in alternate bearing cycle and the possible involvement of these compounds to flower bud formation process. The amounts of these compounds were determined in the leaves and stems of "on" and "off" trees monthly from Nov. 2010 until Mar. 2011 coinciding with citrus trees floral bud induction and differentiation in northern hemisphere. The experiments were designed as a randomized complete block with three replications and two trees per each replication. Results showed that the presence of fruits on "on" trees inhibited flower bud formation, decreased total sprouted buds and resulted in the reduction of vegetative growth of these trees. The endogenous levels of soluble sugars and total non-structural carbohydrates in leaves and the starch contents of stems were affected by fruiting state of trees. Variations of total phenol contents in the leaves and stems were related to presence or absence of fruits on trees. As in the last three months of the experiment, the total phenol content of the leaves of non-bearing trees was about 1.2 times higher than the leaves of bearing trees and in the first two months, it was 1.73 and 1.34 times lower respectively. The phenolic contents of the stems were significantly lower in non-bearing trees than in fruit-bearing ones. Among the five phenolic compounds analyzed, the change pattern of changes in the contents of chlorogenic acid, caffeic acid and naringin were different in two types of trees. For example leaves chlorogenic acid content of non-bearing trees was 1.73, 1.6 and 1.72 times higher than the leaves of bearing trees in the last three months of the study respectively. Similar trend was observed for the amounts of all phenolic compounds in the stems of bearing and non-bearing tress.

Keywords: Biennial bearing, Caffeic acid, Chlorogenic acid, *Citrus*, Polyphenols, Soluble sugars, Starch.

Introduction

Alternate bearing (biennial or uneven bearing) is the tendency of fruit trees to produce a heavy crop in one year (on-year), followed by a light crop or no crop in the next year (off-year). This phenomenon is widespread among deciduous fruit trees as well as evergreens (Monselise and Goldschmidt, 1982). Alternate bearing is a major problem in citrus fruit production all over the world especially within the mandarin and their hybrids (Wheaton, 1997). Alternate bearing in citrus cultivars is known to be due to a lack of flowering in the next spring following a heavy crop in previous year, but not to a negative effect of a heavy crop on fruit set (Goldschmidt and Golomb, 1982). Both internal and external factors which might affect alternate bearing have been described by some research (Goldschmidt, 2005; Jonkers, 1979; Monselise and Goldschmidt, 1982; Singh, 1948a; Singh, 1948b). In most cases alternate bearing is the result of poor flower initiation and differentiation. Lack of flower bud formation has been attributed to carbohydrate levels in citrus (Li *et al.*, 2003; Monerri *et al.*, 2011; Stander *et al.*, 2017; Valiente and Albrigo, 2004), in olive (Lavee, 2006; Ulger *et al.*, 2004), and in pistachio (Rosecrance *et al.*, 1998; Vemmos, 1999), and also to growth regulators in citrus (Goldschmidt *et al.*, 1985; Koshita and Takahara, 2004; Koshita *et al.*, 1999; Li *et al.*, 2003; Valiente and Albrigo, 2004) and in olive (Baktir *et al.*, 2004; Lavee, 2006; Ulger *et al.*, 2004). On the other hand in citrus trees the presence of fruits reduces bud sprouting, vegetative growth and shoot biomass (Martínez-Alcántara *et al.*, 2015; Mirsoleimani *et al.*, 2014; Verreynne and Lovatt, 2009).

Phenolic compounds are secondary metabolites, which possess an aromatic ring bearing one or more hydroxyl groups and their structures may range from a simple phenolic molecule to those of complex high-molecular mass polymers. These compounds, which are classified as phytochemicals, occur widely in plant kingdom and play important both physiological and morphological roles in plants. Due to their bioactive properties, phenolic compounds play an important role in plant growth and development (Ignat *et al.*, 2011). The involvement of phenolic acids such as chlorogenic

*Corresponding Author, Email: Soleiman@shirazu.ac.ir

[DOR: 20.1001.1.23222727.1397.7.25.2.9]

acid, cinnamic acid, ferulic acid and caffeic acid in controlling the alternate bearing of olive trees has been reported by Lavee *et al.* (1985). (Lavee *et al.* (1993) hypothesized that the active phenolic compounds on one hand and the metabolic pathway leading to it on the other hand, are somewhat different and apparently specific in the various tissues of different plants. The effects of phenolics in flower bud differentiation have not been explored for other fruit trees such as citrus in detail (Goldschmidt, 2005).

Positive correlations have been shown between carbohydrates accumulation and flowering, which have led researchers to assume that the levels of carbohydrates may be a limiting factor in flower bud formation in citrus (Goldschmidt and Golomb, 1982). This hypothesis is supported by the fact that girdling (Li *et al.*, 2003; Vemmos, 2005) and or reduction in crop load before winter by thinning (Dag *et al.*, 2010; Martínez-Fuentes *et al.*, 2010) or early fruit harvesting (Vemmos, 1999; Yahata *et al.*, 2006) can enhance carbohydrates and starch accumulation leading to flower formation in the next growth season.

However, in some cases, a positive relationship between carbohydrate levels and flower bud formation could not be demonstrated (García-Luis *et al.*, 1995; Goldschmidt and Golomb, 1982; Ulger *et al.*, 2004).

The objectives of our investigation were to determine: 1) the relation between carbohydrates, soluble sugars and starch levels in the leaves and stems and alternate bearing in 'Kinnow' mandarin and 2) changes in phenolic compounds in relation to biennial bearing pattern of these trees 3) relation between these changes and flower bud formation stages in trees.

Materials and Methods

Plant materials and sampling: This study was conducted on 10-year old 'Kinnow' mandarin (*Citrus reticulata* Blanco) trees (without any annual pruning) grafted on 'Mexican' lime (*Citrus aurantifolia*) rootstock grown in a commercial orchard in the city of Darab, Fars Province, Iran. Trees were irrigated by micro-sprinkler and standard commercial cultural practices were performed during the experiment. Twelve uniform size trees (six for on- and six for off-year) were selected in September, 2010. The experiments were designed as a randomized complete block with three replications and two trees per replication. Samples for chemical analysis were taken from "on" and "off" trees in approximately monthly intervals from Nov. 2010 till Mar. 2011. Each time, fifteen current season growth branches were collected for each replication. At first, fully expanded leaves were taken from the tip end of these shoots for chemical analysis and then the rest of the leaves were removed to collect the stems. Leaves and stems were washed with water and kept in liquid nitrogen and brought to the laboratory. Mature fruits were harvested on Feb 5, 2011.

Flower bud development: Ten shoots (15-20 cm long) from each replication were sampled at one month intervals. In order to study the flower bud development, the shoots were defoliated and sprayed with 100 mg/l benzyladenine and then placed in a vase contain distillated water in a growth chamber kept at 28˚C for three weeks (Yahata *et al.*, 2006).

Carbohydrates analysis: Tissues were dried in a forced air oven at 65˚C for 48 hrs. and then ground into fine powder. Dry tissue samples (100mg each) were used for assaying the soluble sugars and starch content of leaves and stems. Soluble sugars were extracted in hot 80% ethanol and assayed using phenol-sulforic acid method using glucose as standard (Masuko *et al.*, 2005). The remaining materials left in the centrifuge tubes after the removal of soluble sugars were washed and extracted with percholoric acid/water (5%) mixture for starch determination. The starch content in extracts was determined colorimetrically using the anthrone method (Saini, 2001). The solution absorbance was determined at 630 nm in a digital spectrophotometer (WPA Biowave II) and the starch content was calculated by multiplying the glucose content by 0.9. The amounts of total non-structural carbohydrates (TNC) were calculated by adding the amount of starch and soluble sugars together (Li *et al.*, 2003).

Phenolic compounds analysis: Extraction, separation and quantification of phenolics were performed according to Misan *et al.* (2011) method with some modifications.

Sample preparation: Plant extracts were prepared by macerating 200 mg frozen samples (leaf or stem) with a solution of methanol/acetic acid mixture (85:15) for 24 hrs. at 4˚C and subsequently extracted in an ultrasonic bath at room temperature for 15 mins. The resulting suspension was then centrifuged at 10000 rpm for 20 mins at 0˚C. To remove compounds such as chlorophylls and lipids, the supernatant was extracted with 1 ml n-hexane and centrifuged at 10000 rpm for 10 mins. After removing the supernatant, the resulting solution was used for the analysis of both total phenolic contents and their components.

Determination of total phenolics: Total phenolics were determined spectrophotometerically using Folin-Ciocalteu,s reagent and results were expressed as gallic acid equivalents. Quantification of total phenolics were performed by using a microplate reader (Bio Tek ELx808) at 750 nm and gallic acid calibration curve.

HPLC analysis of phenolic compounds: The HPLC system employed consisted of a high performance liquid chromatograph (Agilent 1200 series) equipped with a UV-Vis multiwavelength detector at 280 and 330 nm. Data were evaluated using a Chemstation Software (Agilent Technologies) data processing system. The separation of components was achieved by an Agilent, XDB-C18, 5 μm, 4.6×150 mm column, at a flow-rate of 1 ml min^{-1}. Solvent gradient was performed by varying the proportion of solvent A (methanol) to solvent B (2% acetic acid in water) to separation of vanillic acid, catechin and naringin in 280 nm and chlorogenic acid and caffeic acid in 330 nm.

[DOR: 20.1001.1.23222727.1397.7.25.2.9]

The total running time and post-running time were 30 and 10 mins, respectively. The column temperature was 30ºC. The volumes of samples and standards injected were 20 µL which was done automatically using autosampler (Misan *et al*., 2011).

Statistical analysis: Statistical analysis was performed using the SAS software for Windows V9 (SAS Institute Inc. Cary, NC, USA). Differences among the mean values were detected by Least Significant Differences (LSD) test at %5 level.

Results

Flower bud development: Table 1 shows that the percentage of bud-break in "on" trees were very low and all those buds broken were vegetative. On the other hand, generative shoots appeared only in the last two sampling dates and were only in non-fruiting trees.

Changes in starch content: Leaves starch contents in "on" trees were constant in the first three months of experiment and increased significantly during the last one and reached the maximum value (1.33 mg/g DW). In "off" trees, leaf starch contents decreased significantly during the three first month and remarkably increased in the last one. These data indicated that leaf starch concentrations in 'Kinnow' mandarin trees were affected by sampling date (Fig. 1). Although leaves starch concentrations in "on" trees were lower than in "off" trees but the differences between them were not significant except for the first sampling date. Thus presence or absence of fruits on shoots did not have effect on leaf starch concentrations.

Stems starch contents in "off" trees increased gradually from beginning to the end of the study. In "on" trees stems starch concentrations were stable during the first three months of experiment but increased significantly during the last one. Stem starch concentrations in "off" trees were higher than in "on" trees all over the experiment except for the first sampling date. This indicated that starch contents in stem tissue were influenced by tree fruiting state (Fig. 1).

Changes in total soluble sugars: Leaf total soluble sugars (TSS) in "off" trees increased significantly ($P < 0.001$) from minimum value (3.98 mg/g DW) at the beginning of the season and reached the highest value (8.92 mg/g DW) in Feb. and decreased significantly during the last month. Leaf TSS contents in fruiting trees had an initial significant increase during the first month but remained steady until the end of the experiment (Fig. 2). Thus sampling time affected the variation of leaves TSS concentrations in both types of trees. Differences between leaf TSS contents in "on" and "off" trees were noticeable in the last three months of the experiment. Thus seasonal changes of leaf TSS contents were influenced by presence of fruits on shoots.

Presented data in figure 2 also shows that stem TSS concentration in "off" trees increased gradually during Nov. and Dec. and decreased significantly during Jan. and Feb. In "on" trees stem TSS contents increased significantly during first month and reduced significantly during the last month of the experiment but were constant during the two rest periods. These results indicated that the time of sample preparation affected significantly on TSS contents of stems. Differences between stem TSS concentrations were significant for sample prepared in Nov. and Feb. and were similar in "on" and "off" trees in remained sampling dates (Fig. 2).

Changes in total non-structural carbohydrates (TNC): Leaf total non-structural carbohydrate (TNC) contents in "on" trees after an initial significant ($P < 0.01$) increase during the first month was stable till the end of the experiment. TNC contents in the leaves of non-fruiting trees had significant increasing trend in the first two months but decreased significantly during the last month of the study. Thus leaf TNC concentrations in "on" and in "off" trees were affected by sampling time (Fig. 3). Fruiting state effect on leaf TNC contents of trees because the differences between leaves TNC contents in "on" and in "off" trees were noticeable in the last three sampling dates. Figure 3 also shows that stem TNC contents in both types of trees influenced by sampling time. Both trees had increasing trend during the first two months and had significant decreasing rate over the last two months of the study. Except for the first sampling date, mean values of TNC contents of stems in "on" and in "off" trees were not different (Fig. 3).

Changes in total phenolic compounds: Pattern of seasonal variations in leaf phenolic contents in "on" trees was constant throughout the experiment and did not affect by time of sampling. Leaf phenolic contents in "off" trees increased during the first three months and from minimum value (46.71 mg/g FW) in the early of experiment reached maximum value (92.98 mg/g FW) in Feb. and then decreased. This demonstrates that sampling date affected on seasonal variation of leaves phenolic compounds in "off" trees (Fig. 4). Total phenolic contents in leaves were significantly different between "on" and "off" trees over the study and these show strong effect of crop load on leaf phenolic compounds.

Similar to leaves, seasonal variations in stems phenolic contents in "on" trees were very small and non-significant. Whereas the rate of stem phenolic compounds accumulation in "off" trees increased during the first two months of experimentation period and it was steady during two last ones. These indicate that the time of sample preparation affected the amount of stem phenolic compounds in "off" trees (Fig. 4). Stem phenolic concentrations were significantly different between "on" and "off" trees throughout the season except for samples of Jan. These differences are the responses to presence of fruits on shoots.

Changes in chlorogenic acid: Time had significant effect in chlorogenic acid (CHA) contents of leaves in both "on" and "off" trees. In "on" trees leaf CHA decreased significantly during the first two months of

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Table 1: Flower bud development in excised shoots

Year	Date of sampling	Tree cropping state	Percentage of bud break (%)	
			Vegetative	Generative
2010	Oct.	ON	1.6	0
		OFF	6.45	0
	Nov.	ON	2.77	0
		OFF	9.64	0
	Dec.	ON	1.58	0
		OFF	16.74	0
2011	Jan.	ON	3.82	0
		OFF	13.65	0
	Feb.	ON	3.81	0
		OFF	21.30	1.54
	Mar.	ON	12.04	0
		OFF	20.85	3.03

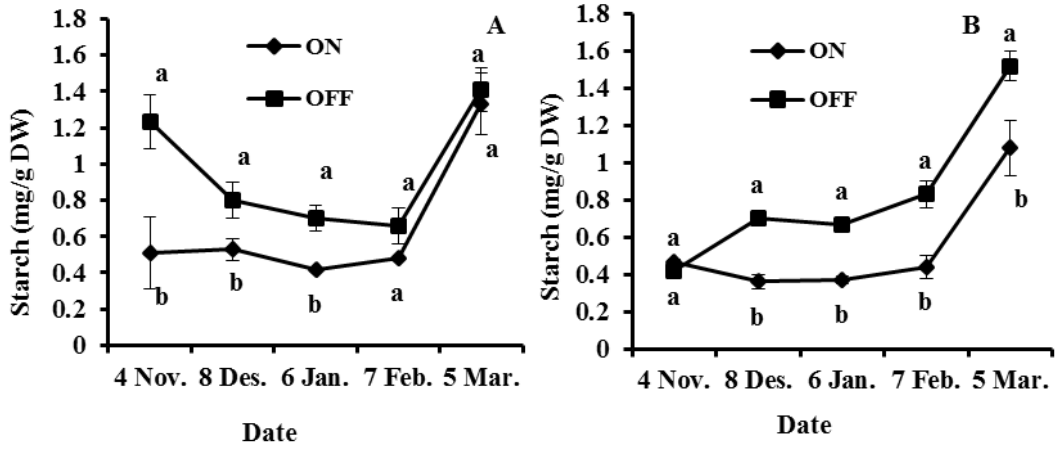

Figure 1: Monthly changes in starch contents of leaves (A) and stems (B) of "on" and "off" 'Kinnow' mandarin trees. Different letters indicate significant differences (P<0.05). Bars indicate ± SE.

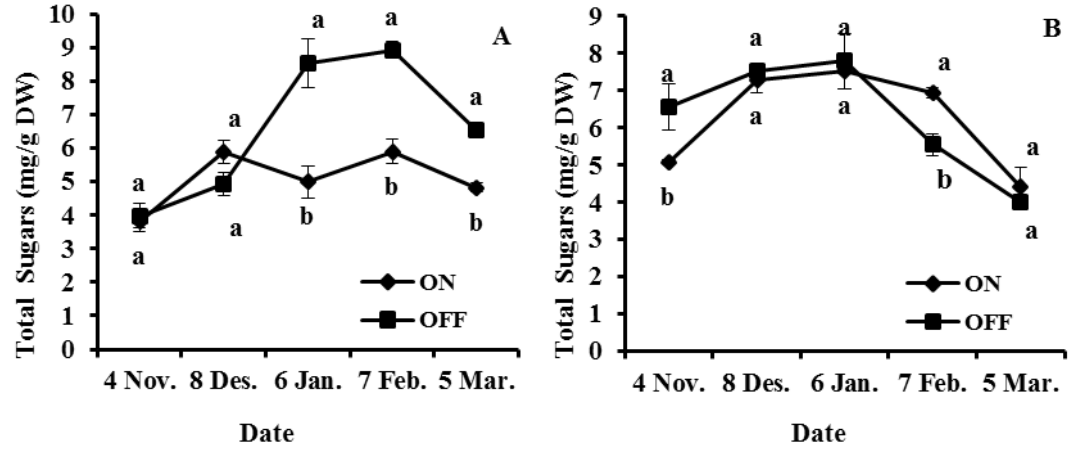

Figure 2: Monthly changes in total sugar contents of leaves (A) and stems (B) of "on" and "off" 'Kinnow' mandarin trees. Different letters indicate significant differences (P<0.05). Bars indicate ± SE.

experiment and from 0.96 mg/g FW in Nov. reached to the lowest value (0.49 mg/g FW) in Jan. and was stable after that till the end of study. In "off" trees leaf CHA contents increased significantly during the second and fourth month and were constant during remaining times (Fig. 5). Figure 5 also shows that seasonal changes in CHA contents of leaves were affected by fruiting state.

CHA concentrations in stems of "off" trees were constant all over the study but in "on" trees time of sampling had significant effect. The differences between CHA amounts in stems of two types of trees were noticeable in Nov. Jan. and Mar. These indicated that fruiting state had significant effect on stem CHA contents (Fig. 5).

Changes in caffeic acid: Leaf caffeic acid (CAA) contents in "on" trees were constant during the first

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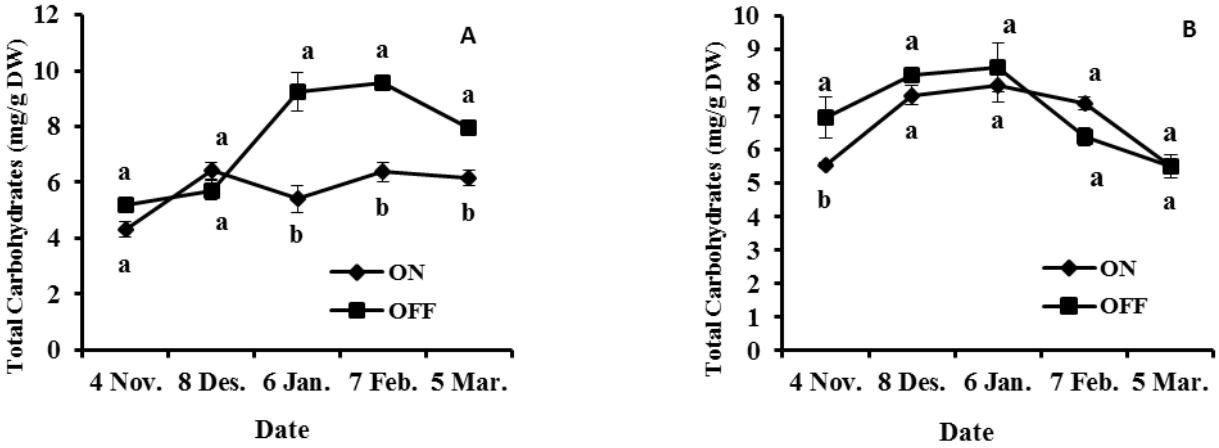

Figure 3: Monthly changes in total carbohydrate contents of leaves (A) and stems (B) of "on" and "off" 'Kinnow' mandarin trees. Different letters indicate significant differences (P<0.05). Bars indicate ± SE.

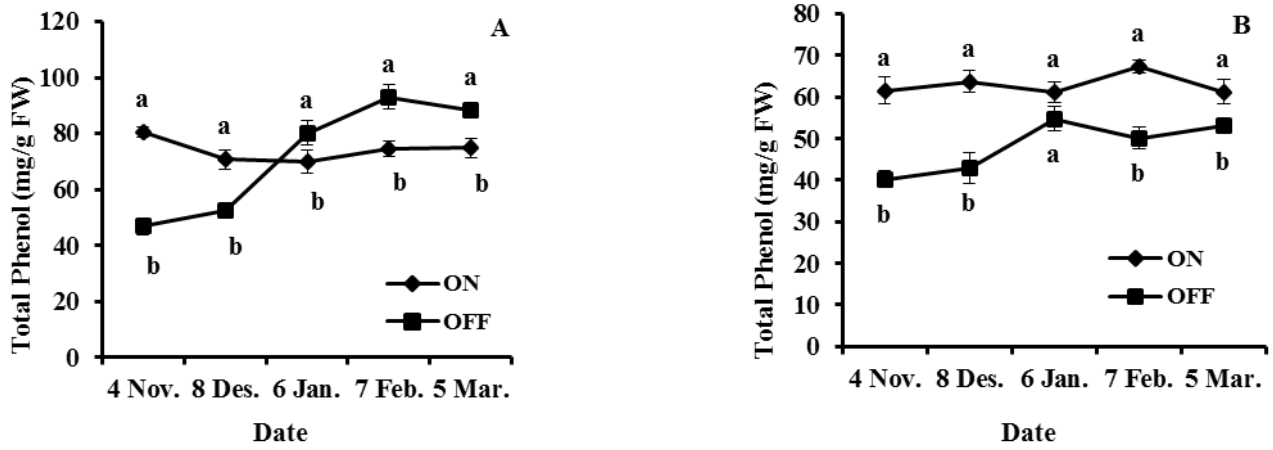

Figure 4: Monthly changes in total phenol contents of leaves (A) and stems (B) of "on" and "off" 'Kinnow' mandarin trees. Different letters indicate significant differences P<0.05). Bars indicate ± SE.

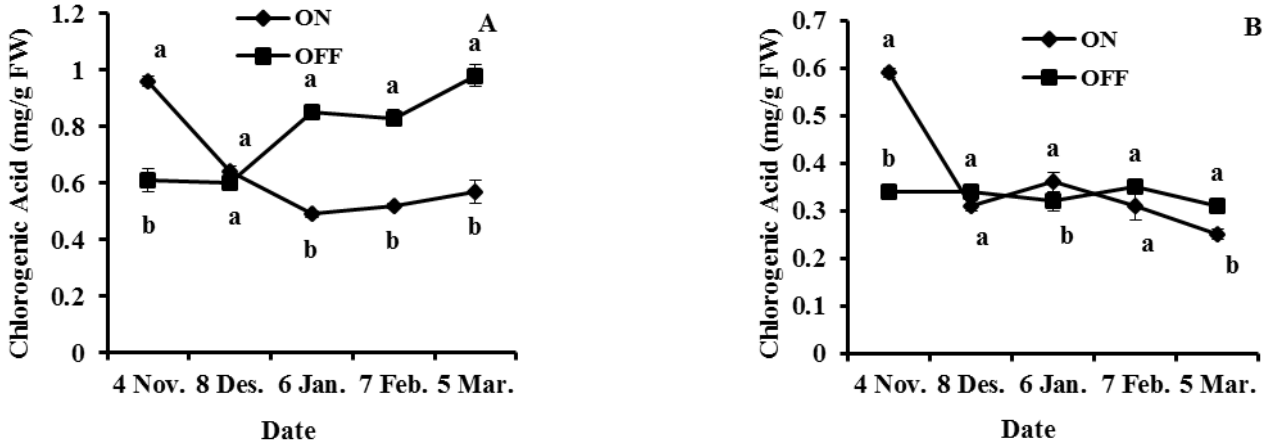

Figure 5: Monthly changes in chlorogenic acid contents of leaves (A) and stems (B) of "on" and "off" 'Kinnow' mandarin trees. Different letters indicate significant differences (P<0.05). Bars indicate ± SE.

three months and decreased significantly during the last month but whole of this pattern did not affect by time of sampling (Fig. 6). In contrast, time had significant effect on seasonal variations of CAA in the leaf of "off" trees. It increased overall the first two months and from 1.17 mg/g FW in the beginning of the study reached to maximum value (4.02 mg/g FW) but it declined and increased again in the last two months respectively. Leaves CAA contents in "off" trees were significantly lower than in "on" trees in Nov. whereas these were higher significantly in Jan. and Mar.

Caffeic acid contents in the stems of "off" trees were constant during the first month. It increased fluctuations but the pattern of these variations were not uniform

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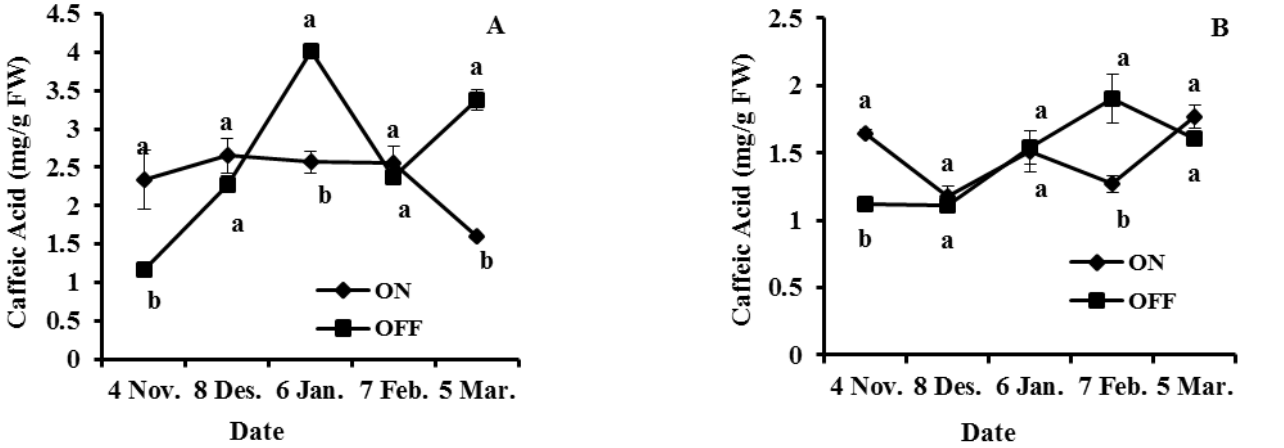

Figure 6: Monthly changes in caffeic acid contents of leaves (A) and stems (B) of "on" and "off" 'Kinnow' mandarin trees. Different letters indicate significant differences (P<0.05). Bars indicate ± SE.

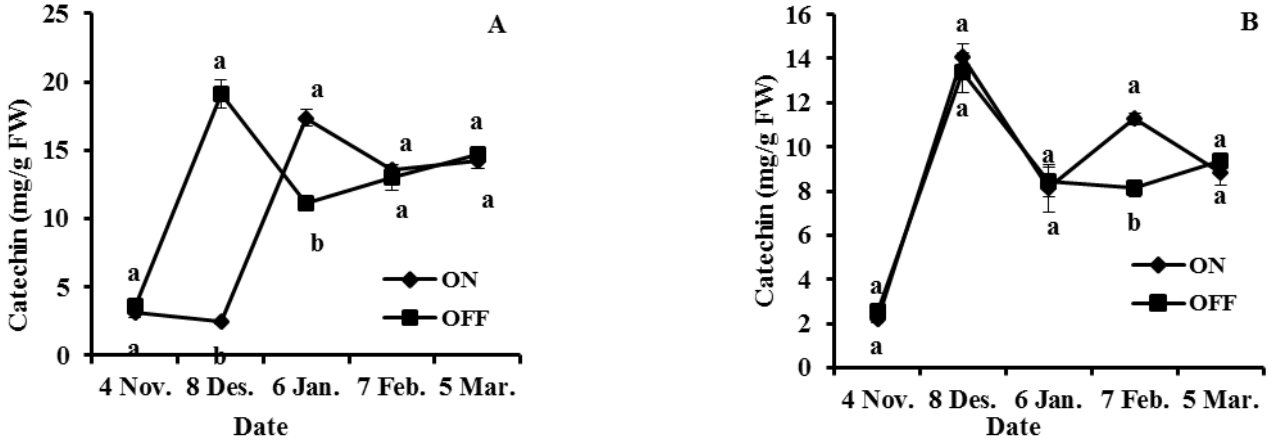

Figure 7: Monthly changes in catechin contents of leaves (A) and stems (B) of "on" and "off" 'Kinnow' mandarin trees. Different letters indicate significant differences (P<0.05). Bars indicate ± SE.

(Fig. 6). Variations in pattern of stem CAA contents in "on" and "off" trees were approximately similar thus it seemed that fruiting state did not affect stem CAA contents of trees.

Changes in catechin: Leaf catechin (CAT) contents in "off" trees increased significantly during the first month and decreased significantly after that during the second month. It was stable over the last two months of study (Fig. 7). Catechin contents of leaves in "on" trees were stable in the first month but increased significantly during the second month and decreased significantly during the third month and constant over the last ones. Our data indicate that time of sample preparation affected on CAT contents of leaves in 'Kinnow' mandarin trees. The differences between leaf CAT contents in "on" and in "off" trees were significant only in samples of Dec. and Jan. These show that fruiting state had effect on variations of leaf CAT contents only at a part of the experiment.

Stem CAT contents in "on" and "off" trees were minimum in the beginning of experiment and increased significantly during the first month and reached to highest value. It decreased significantly after that during the second month and remained constant during the last two months of season (Fig. 7). Comparison of stem CTA contents between "on" and "off" trees showed that the trend of stem CTA content did not affect by fruiting state of trees. However, time of sampling affected the pattern of variation in stem CTA contents of two types of trees.

Changes in vanillic acid: Leaf vanillic acid contents in "on" and "off" trees increased during the first two months and decreased after that till the end of the experiment but these fluctuations were not significant. Therefore, the time of sample preparation did not affect the seasonal changes of vanillic acid contents in leaves of "on" and "off" trees (Fig. 8). Although leaves vanillic acid contents in "on" trees was higher than in "off" trees in the first three sampling dates, the differences between the means at any times of sampling were not significant. Thus, presence or absence of fruits had no effect on leaf vanillic acid contents of trees. Data presented in figure 8 show that neither sampling time nor fruiting state affected on seasonal variation of stems vanillic acid contents in 'Kinnow' mandarin trees.

Changes in naringin content: Leaf naringin contents in "on" trees was 3.72 mg/g FW in the

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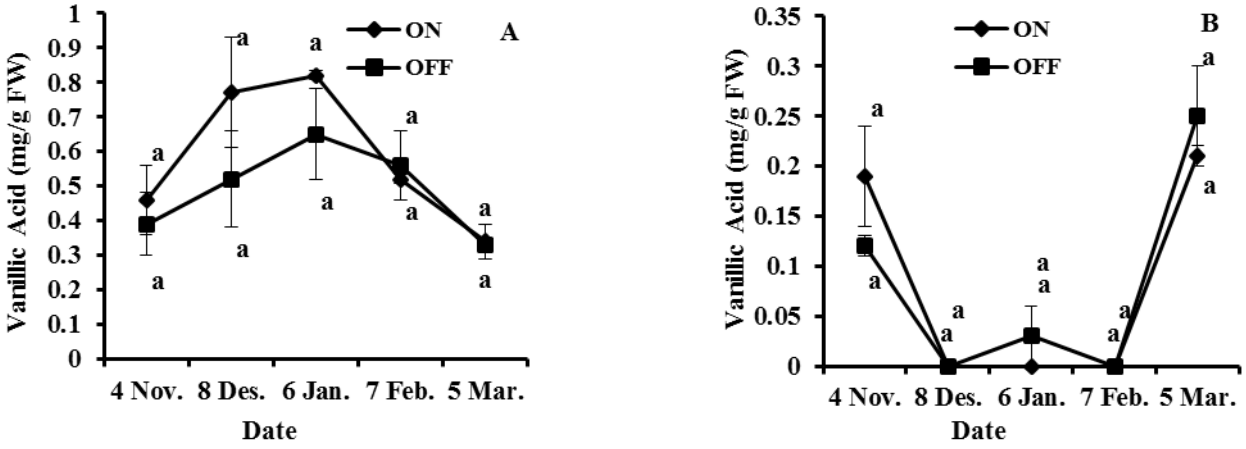

Figure 8: Monthly changes in vanillic acid contents of leaves (A) and stems (B) of "on" and "off" 'Kinnow' mandarin trees. Different letters indicate significant differences (P<0.05). Bars indicate ± SE.

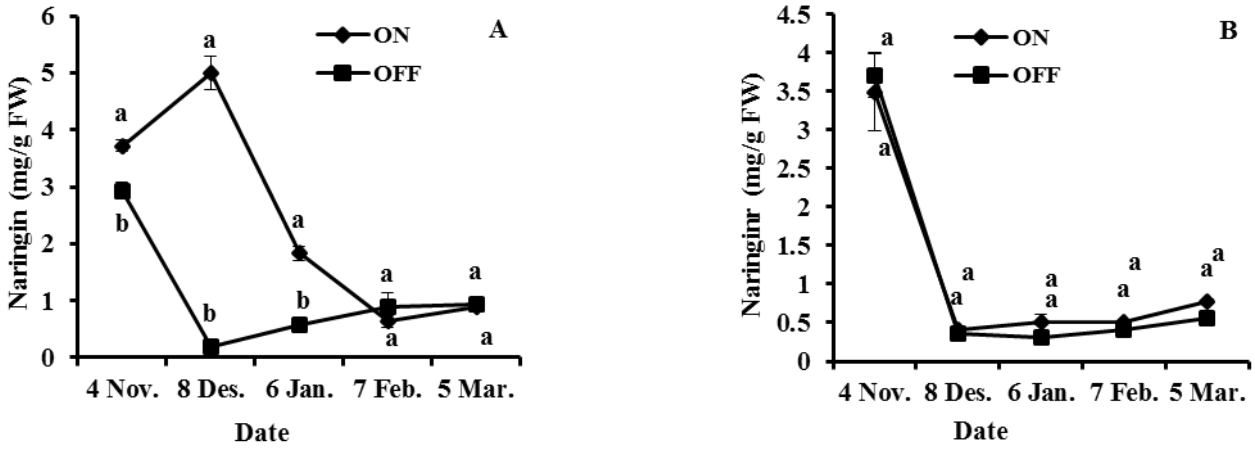

Figure 9: Monthly changes in naringin contents of leaves (A) and stems (B) of "on" and "off" 'Kinnow' mandarin trees. Different letters indicate significant differences (P<0.05). Bars indicate ± SE.

beginning of the experiment and increased significantly during the first month and reached to 5.0 mg/g FW (maximum value) in the second sampling time. Then, it decreased significantly during the second and third months and reached to the lowest value (0.63 mg/g FW) and was constant over the last month of study (Fig. 9). In "off" trees Leaf naringin contents were highest in Nov. and decreased significantly to the lowest value (0.2 mg/g FW) in Dec. It increased gradually after that till the end of the experiment. These data indicate that leaf naringin contents in both "on" and "off" trees were affected by time of sampling. Except for the last two sampling dates, leaf naringin contents in "on" trees were significantly higher than in "off" trees. These showed that tree fruiting status had noticeable effect on leaf naringin contents.

Figure 9 also indicate that the pattern of variation in stem naringin contents in both types of trees were similar. It was the highest in the beginning of season and decreased to the lowest value during the first month but remained constant after that until the end of study. The differences between stem naringin contents in "on" and "off" trees were not significant all over the experiment thus stem naringin concentrations did not affect by fruiting state.

Discussion

The data presented in table 1 show that the presence of fruits on "on" trees inhibited flower bud formation, decreased total sprouted buds and resulted in the reduction of vegetative growth of these trees. The effects of crop load on the inhibition of citrus flowering, vegetative growth and bud break supported by our results have been also reported by several researchers. Verreynne and Lovatt (2009) reported that for the 'Pixie' mandarin in California, the alternate bearing cycles appeared to be a crop load-dependent inhibitory effect of fruit on bud break. Fruit load had a significant effect on development of flowers per shoot for both 'Valencia' and 'Hamlin' sweet orange trees (Valiente and Albrigo, 2004). The presence of fruits, on branches of "on" trees has an inhibitory effect on the sprouting of new shoots and the basipetal appearance of lateral buds in citrus trees (Garcia-Luis *et al.*, 1986; Koshita *et al.*, 1999; Martínez-Fuentes *et al.*, 2010). The presence of fruits on shoots may reduce the sensitivity of buds to inductive conditions (Valiente and Albrigo, 2004) or may be related to competition for carbohydrates and

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inorganic elements (Goldschmidt, 2005; Mirsoleimani *et al.*, 2014) or the upsetting of the hormonal balance of the tree (Goldschmidt, 2005; Monselise and Goldschmidt, 1982).In agreement to the negative effect of fruits on bud break, we can refer to the noticeable difference (%9) between bud sprouting on the stems of fruit-bearing trees before and after of fruit harvesting in the early of February (Table 1).

Our results suggest that tree fruiting status is reflected in the soluble sugars and TNC contents of leaves. Leaf carbohydrate levels of non-bearing trees increased over the Nov., Dec. and Jan., coinciding with the seasonal decrease in temperature and flower bud initiation. While the pattern of variations in leaf carbohydrate levels was constant in "on" trees all over the season. These indicate that fruits were the most powerful sink for soluble sugars and TNC and thus prevented the accumulation of reserves in 'Kinnow' mandarin leaves. It seems that carbohydrate changes in leaves (but not in stems) between Dec. and Feb. (coinciding with floral bud formation in citrus) can relate to flower bud formation process. It is possible that significant reduction in leaf soluble sugars and TNC contents in "off" trees during the last month of experiment related to bud sprouting that was very high in these trees (Fig. 2).

Our results also indicate that pattern of variation in leaf starch contents did not related to alternate bearing cycle and flower bud formation in 'Kinnow' mandarin trees. However it seems that biennial bearing had effect on stem starch contents of these trees.

It is generally accepted that the alternate bearing cycles affect by the heavily crop load and this is related to reduction of flower bud formation in citrus trees (Goldschmidt and Golomb, 1982). On the other hand it is also accepted that the flower bud formation process has been related to carbohydrate levels in fruit trees and especially in citrus (LI *et al.*, 2003; Valiente and Albrigo, 2004). This role of carbohydrate levels and fruit load on the inhibition of floral bud formation has been supported by our results (Table 1). However we could not find clear evidences for regulatory role of starch in this process. It is believed that, threshold levels of reserve carbohydrates should be required for flower bud differentiation in fruit trees (Garcia-Luis *et al.*, 1995; Goldschmidt, 2005).

There are many reports indicating that, phenolic acids and many other phenols affect growth, morphogenesis and metabolic activity in both in vivo and in vitro systems. These effects of phenolic compounds are mainly due to enhancement or reduction in auxins effects through their involvement in regulating of IAA oxidase activity (Lavee and Avidan, 1981; Lavee and Avidan, 1982). Studies on the involvement of endogenous hormones in flower bud formation stages also have suggested that IAA effect on the stages of floral bud formation especially on bud development (Baktir *et al.*, 2004; Koshita and Takahara, 2004; Koshita *et al.*, 1999).

In agreement with these reports, our results indicated that the presence of fruit on trees affected the total phenolic compounds, chlorogenic acid, naringin and caffeic acid contents of 'Kinnow' mandarin leaves. Total phenolics and chlorogenic acid increase in the leaves of "off" trees from the beginning of the experiment (especially from second sampling date) till the end of season and leaf caffeic acid contents increased significantly over the first two months of experiment. This period coinciding with the seasonal decrease in temperature, increased the total carbohydrates and soluble sugars in these leaf and floral bud development process in citrus trees. But in the leaves of fruit-bearing trees, the pattern of changes in chlorogenic acid was decreasing and the trend of total phenolics and caffeic acid was constant throughout the season. Our results also showed that changes in leaf naringin contents were opposite in non-bearing and fruit-bearing trees in the first three sampling dates. This indicated that presence or absence of fruit can affect the leaves naringin contents but this trend was opposite of the total phenolic compounds, chlorogenic acid and caffeic acid contents of leaves. Our study also showed that tree cropping status was not affected on vanillic acid and catechin contents in leaves of 'Kinnow' mandarin trees. On the other hand, stem total phenolics, chlorogenic acid, caffeic acid and naringin concentrations in 'Kinnow' mandarin trees were affected by alternate bearing habit of trees. In agreement with these results, Lavee *et al.*, (1985) have shown that chlorogenic acid content of olive leaves fluctuate in correspondence with alternate bearing cycle. Different trend in the changes of phenolic compounds in response to physiological processes have also been observed in some other studies. For example Malik et al., (2015) have observed that in the bud tissue of some citrus species, when the vegetative buds begin to sprout chlorogenic acid and naringenin concentration increased while in reproductive buds hesperidin and apigenin-7-glocoside content increased, as well.

Conclusion

It is concluded that the levels of TNC and soluble sugars in leaves and starch content of stems are influenced by alternate bearing of 'Kinnow' mandarin trees, but the patterns of their changes are not the same. Total phenolic compounds, chlorogenic acid, caffeic acid and naringin contents affect by alternate bearing cycle and can relate to flower bud formation process in 'Kinnow' mandarin trees. We suggest that phenolic compounds (except with vanillic acid and catechin) as plant secondary metabolite should be considered as a signal for or a result of flower bud formation and alternate bearing process. Furthermore, the details of this involvement and the stage of this interference are not clear and need to further researches.

[DOR: 20.1001.1.23222727.1397.7.25.2.9]

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