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Research Article

Seasonal changes in carbohydrate and nitrogen contents of olive trees 'Fishomi' cultivar in several parts under alternate bearing conditions

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Abstract

Some olive cultivars possess high tendency to alternate bearing, which have a minus economic effect on olive industry. The experiment was performed in an olive orchard located in Shiraz region. Monthly monitoring of concentrations of unstructured carbohydrates (glucose, fructose, sucrose, mannitol and starch), protein, nitrogen, potassium and phosphorus in leaves, branches and roots of olive trees ON and OFF was performed from October 2018 to September 2019. The aim of this study was to evaluate carbohydrate and nutrient reserve changes on flower initiation in ON and OFF olive trees. The results showed that there was a significant difference between carbohydrate concentrations (glucose, fructose, sucrose, mannitol and starch), protein, nitrogen, potassium and phosphorus in the leaves, branches and roots between ON and OFF trees during bearing cycle. During autumn and winter, carbohydrate concentrations showed an upsurge trend in different parts in OFF trees. The content of nitrogen ramped up in the leaves in both ON and OFF trees; whereas, it was declined in the branches as well as roots in both trees during autumn and winter. Furthermore, the content of potassium showed a downward trend in the leaves and branches during autumn and winter. In spring and summer, a better percentage of carbohydrates was recorded in olive organs in OFF trees than in ON trees. In July, the concentration of carbohydrates and amount of nitrogen of OFF trees in all samples was higher than that in ON trees. In July, carbohydrate content and mineral content declined in ON trees. The deficiency of carbohydrate and nutrient reserves during the flowering induction affected vegetative growth strength, fruit size, and flowering in the spring of the subsequent year. Simple correlations were shown for 21 measured traits for 'Fishomi' seed olive cultivar in different months, respectively. The correlation coefficient showed that there was the most positive correlation between leaf starch and total leaf carbohydrate. The presence of carbohydrate and nitrogen reserves played a key role in flower induction of olive trees and was expressed as crucial parameters to stimulate alternate bearing.

Keywords: Olive, Nutrient reserve, Carbohydrate reserve, *Olea europaea*, ON-OFF trees

Introduction

Olive (*Olea europaea* L.) is an ever-green tree originated in semi-arid Mediterranean conditions (Gholami and Zahedi, 2019b). It is one of the most economically and socially important tree for the output of olive fruit and oil products in the world. Depending on the course of the vegetative and reproductive processes over the biennial period, olive crops have a high propensity for alternate bearings (Ebrahimzadeh *et al*., 2003; Yanik *et al*., 2013; Annabi, *et al*., 2019). Olive trees face large yield fluctuations and go from a year with a heavy fruit load (ON) to a year with a low fruit load (OFF) (Beya-Marshall and Fichet, 2017). The yield difference in olives may reach 5-30 tons per hectare between ON and OFF years (Lavee, 2007). To increase and maintain fruit yield, researchers should use cultivars and genotypes with high adaptability that their physiological evolution, especially the stage of flowering and fruit formation is compatible with optimal climatic cycles and high physiological and genetic resistance to reduced fruit yield due to have tension (Gholami and Zahedi, 2019a).

The problems caused by alternate bearing of this important industrial crop are of great economic significance (Lavee, 2007). It is found that in the post-flowering period, olive yield is determined by two variables: The alternative bearing habit and the large fruit abscission (Haouari, 2013). Olive flower induction, as a critical stage in flower production, occurs in mid-summer about 7 to 8 weeks after full blooming, which coincides with pit hardening of the nucleus of growing fruits (Connor and Fereres, 2005). Floral transition process in olives may take up to 8 months, low temperature (around 7°C) during fall and winter is required for floral differentiation and development in olives (Connor and Fereres, 2005). Dag *et al.* (2010) found that the primary factor in the olive tree determining the flowering and fruit yield was the amount of shoot growth and the presence of new well-developed mature buds. Flowering in olives is

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controlled by combination of physiological processes and environmental conditions (Ulger *et al*., 2004).

Turktas *et al*. (2013) demonstrated that the nutritional and hormonal situation of the olive tree played a vital impress in its alternation. Carbohydrates reserves and winter cold tolerance play an important role in the formation of buds and the beginning of shoot and root growth (Erel *et al*., 2016; Marino *et al*., 2018). Inhibitory effect of scarce carbohydrate reserves on floral bud formation during and following a high-yield fruit production, as well as due to abiotic and biotic stresses that may unload such reserves was reported (Turktas *et al*., 2013). The dependence of the reproductive period on the reserves of the previous year has been stated to be subject to timing: The number of fruits or fruit growth and production may be decreased before new vegetative growth matures due to the depletion of reserves (Spann *et al*., 2008; Marra *et al*., 2009). It has been revealed that amount of carbohydrates stored in tissues of olive trees in the ON and OFF years is different and there is a relationship between the carbohydrate metabolism and fruit (Barranco *et al*., 2010). Several studies have examined the seasonal dynamics of carbohydrates in different tissues of the olive trees (Bustan *et al*., 2011; Zouari *et al*., 2014; Erel *et al*., 2016; Zouari *et al*., 2020).

Nejad and Niroomand (2007) noted that the concentration of glucose, fructose, and mannitol in olives increased up to 90 days after fruit set but decreased during fruit ripening. A temporary increase in the concentration of these sugars was observed until the onset of fruit color change in ON tree, and during the OFF year, glucose was higher than mannitol (Zouari *et al*., 2014). The effect of carbohydrates and mineral nutrition on formation of flower buds in olive trees was shown by Ulger *et al*. (2004). In addition, the content of carbohydrates and minerals varied significantly in both ON and OFF year in the leaves of olive trees. (Fernandez-Escobar *et al*., 1999; Erel *et al*., 2008). It has been found that during the winter, starch accumulated in the flower buds but was consumed at the same time as the buds open. It is recorded that during winter, starch accumulates in the flower buds and is consumed when the buds sprout. Starch in leaves and buds is high in olive bearing trees until fruit development in OFF trees in autumn (De la Rosa *et al*., 2000; Fabbri and Benelli, 2000). Nutrient status affects other physiological processes in the olive production process directly or indirectly, including flowering and fruit formation (Fabbri and Benelli, 2000). Fernandez-Escobar *et al*. (2000) reported that during OFF year, the nitrogen content of old leaves and stems of rain-fed olive trees showed a stable pattern followed by a decrease from April to October during ON year, resulted in accumulating significant amounts of nitrogen during the OFF year and mobilizing it during the ON year to support growth. Troncoso *et al*. (2008) showed that nitrogen and potassium levels were significantly reduced in leaves at the end of the ON year, the quantity of these substances is high in the OFF year, and returns to optimum values. While, Stateras *et al.* (2017) reported that the amount of nitrogen, phosphorus, potassium, calcium and magnesium showed significant fluctuations during the OFF year in olive leaves (*Olea europaea* var. Minor rotunda). Lavee and Avidan (1994) found that the amount of protein in the leaves and annual branches of ON and OFF trees was different (Kour *et al*., 2018).

In the leaves of OFF trees, the total extractable protein content was significantly less than that of ON trees, while the amount of protein in the bark of ON trees decreased (Kour *et al*., 2018). In this study, we have attempted to explain the involvement of reserves of non-structural carbohydrates and nutrient reserves during flower induction as a causal factor regulating reproductive or vegetative growth and alternate bearing in intensively grown olive trees.

Materials and methods

Plant material and sampling: The experiment was performed in an olive grove located in the Shiraz region. ‘Fishomi’ olive cultivar possesses an alternate bearing habit. This commercial garden is located at 52°25´ E, 29°37´ N; and 1590 m above sea level. This region has a semi-arid Mediterranean stenothermal mesothermal temperate climate, with the maximum temperatures of 28.7 °C in January and minimum temperature of 3.4 °C in July. The average annual precipitation was 330 mm, concentrated between mid-autumn and winter, with a dry period of 6 mo and a sandy-loam soil. The 25-year-old olive trees were propagated by cuttings. The irrigation method in the orchard was drip irrigation system. The trees were selected with opposing bearing patterns (ON-vs. OFF- simultaneously growing trees) and in autumn 2018, were split into two groupings of three heavily fruiting trees (ON) and three non-or lightly fruiting trees (OFF). Leaves, shoots, and roots were sampled monthly from each tree from October 2018 to September 2019. Around the boundary of each tree, four one-year-old shoots were sampled. The younger leaves positioned 5 cm from the twigs at the terminal were discarded, so only adult leaves were located in the survey. At least two holes were dug in the shade area of the tree and small roots with a diameter of 5-10 mm were sampled (Bustan *et al*., 2011).

Non-structural carbohydrates determination: Sample preparation was based on the procedure described by Vemmos (1995). 100 mg of dried tissue was put in a centrifuge tube, 5 ml of 40-60˚C petroleum ether was added and centrifuged for 5 min at 3000 rpm. The petroleum ether extract was discarded. 4 ml of 80% ethanol were added to the tube and vortexed. The supernatant was piped to another tube after centrifugation at 3000 rpm (5 min). The remaining plant material was then re-extracted with 4 ml of 80% ethanol, centrifuged and the supernatant was added to the first one, before drying under a nitrogen gas flow. 4 ml of distilled water was surcharged to the dried residue

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in the tube. In the solution, activated charcoal (10 mg) was added, and the mixture centrifuged for 5 min at 3000 rpm. Then filtered before injection through a 0.45 µm membrane filter (10 µl) onto an Alltech 700CH carbohydrate HPLC analysis was performed with HPLC model AZURA (KNAUER, Germany), equipped with a column (300 × 7.9 mm, 5 µm) at 60 °C. Concentrations of sugars were obtained based on dry weight mg /g.

Starch determination: The starch content was calculated by the Mc Cready *et al*. (1950) procedure. After elimination of all soluble content, the solid residue remaining in the centrifuge tube was washed, the measurement was mixed with 5 ml distilled water and 6.5 ml $HClO_4$ 52% and stirred for 5 min. After adding 5 ml of distilled water, the sample tubes were centrifuged twice and placed at 0˚C for 30 min. Then, 10 ml of the anthrone reagent was applied to 2.5 ml of the extract that was collected. Absorbance was read at 630 nm by microplate reader spectrophotometer (Epoch Biotech, USA).

Nitrogen determination: Using Kjeltec system 2300 Analyzer Unit, Foss, Sweden, the amount of nitrogen in samples was determined using the Kjeldahl method (Foss Tecator, Sweden). Approximately 0.2 g of material was hydrolyzed with 15 ml concentrated sulfuric acid (H_2SO_4) Contains 0.5 g of both copper and selenium catalyst tablets in a heat block (Kjeltec system 2300 digestor) at 420˚C for 4 h. After cooling, 20 ml H_2O was added to the hydrolysates before neutralization and titration. The value of total nitrogen was determined with Kjeldahl (Baninasab *et al*., 2007).

Potassium and phosphorus determination: Potassium (k) concentration in samples was calculated with minor modifications by the Nejat and Sadeghi (2016) process. The 1 gr of dried sample powder was powdered at 550˚C for 5 h in the furnace; Then, dissolved in 10 ml of 2 N hydrochloric acid and diluted to 50 ml with distilled water. By a flame photometer the K concentration (PFP7, England), and phosphorus (P) concentration was measured colorimetrically using the blue phosphomolybdate compound at 660 nm (Baninasab *et al*., 2007).

Soluble protein determination: By the Bradford (1976) method, soluble protein content was measured. Samples (500 mg tissue DW) in liquid nitrogen and the resulting powder were mixed with 5 ml of a buffer solution [50 mM Tris-HCl, 0.4 mlL^{-1} β-mercaptoethanol and 2 mM ethylene-diaminetetraacetic acid, pH 7.5]. A Coomassie brilliant blue G-250 solution (0.1 g L^{-1}) was mixed with 4.9 ml of the obtained supernatant. Absorbance was read at 595 nm by microplate reader spectrophotometer (Epoch Biotech, USA) (Lin *et al*., 2013).

The research was carried out as a randomized complete block design with the factorial arrangement and three replications. Variance analysis (ANOVA) was used and mean separation ($P < 0.05$) was applied to the least significant difference (LSD) test. Using the SAS v. 9.01 package (Institute Inc., Cary, NC), using the SPSS software. Correlation coefficient and parsing were performed to the main componentsstatistical analyses.

Results and discussion

Carbohydrate, protein and mineral changes during autumn and winter in different parts of olive ON and OFF trees: The glucose content in OFF trees was higher in the leaves, branches, and roots than in ON trees. During the autumn and winter, the glucose content in all sampling date ramped up (Fig. 1-a; b; c). The fructose content in the leaves, branches, and roots, was higher in OFF trees than ON trees at all dates for sampling. Fructose content in the leaves and roots showed moderate fluctuations during the months of study and reached to the maximum in February and March, but fructose content of the branches decreased in January (Fig. 1-a; b; c)

In all sampling dates, in the leaves, branches, and roots, the sucrose content was higher in OFF trees than in ON trees. The results of the sucrose content in all sampling dates showed that sucrose content decreased from October to December whereas it increased thereafter with some fluctuations during the winter until reaching to the maximum content in February and March (Fig. 2-a; b; c). The content of mannitol in the leaves, branches, and roots, of OFF trees was higher than in ON trees. During the autumn, mannitol content showed a consistent trend, followed by an increasing trend in all samples during the winter, but an upsurge trend was observed for the mannitol concentration until December (Fig. 2-a; b; c).

Vegetative growth and anthesis began in early-April, mid-May, respectively in olive trees, and full bloom occurred about two weeks after in 2019. The physiological proceeding of flowering starts in the preceding summer when environmental factors intervene in this process. Induction of flower buds in olive occurs at the time of endocarp sclerification as early as July or around 7-8 weeks after full bloom, which was in accordance with previous researches (Connor and Fereres, 2005; Fernandez-Escobar *et al*., 1999). The results showed that there were substantial differences in content of glucose, fructose, sucrose, mannitol, starch, and minerals between OFF and ON trees. In perennial fruit trees, reproductive process were mainly controlled by a variety of different factors (Sharma *et al*., 2019). Carbohydrate and nitrogen reserves played the important roles in stimulation of flowering in fruit trees (Erel *et al*., 2016), although Ulger *et al*. (2004) believed that carbohydrate did not influence the flower induction, and solely affected the plant survival. Most fruit trees store carbohydrates to fulfill the metabolic requirements during their growth season and to support annual cycles (Bustan *et al*., 2011). These carbohydrates are a rich source of energy for the use of sprouted buds during the spring and their growth season period (Lombard, 2003). In this reading, changes in the levels of non-structural carbohydrates and mineral

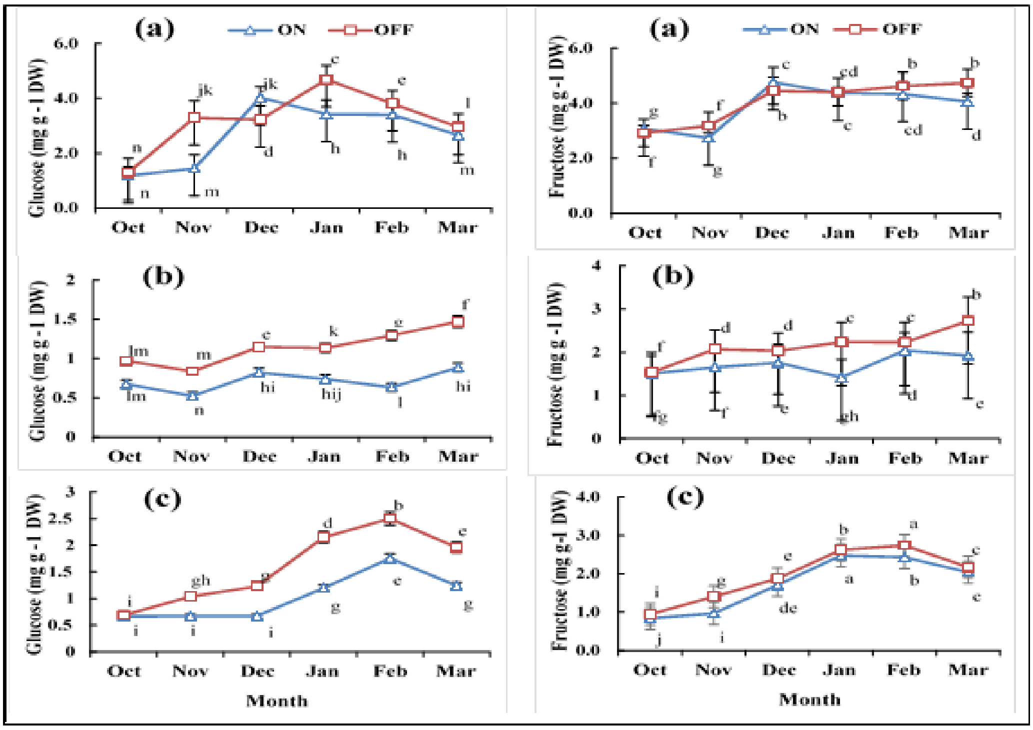

Fig. 1. Changes in glucose and fructose (mg/g dry weight) in autumn and winter (October 2018 to March 2019) in leaves (a), branches (b) and roots (c) of OFF and ON trees of 'Fishomi' cultivar. Mean values with common letters aren't significantly different (P<0.05). (±SE, standard error)

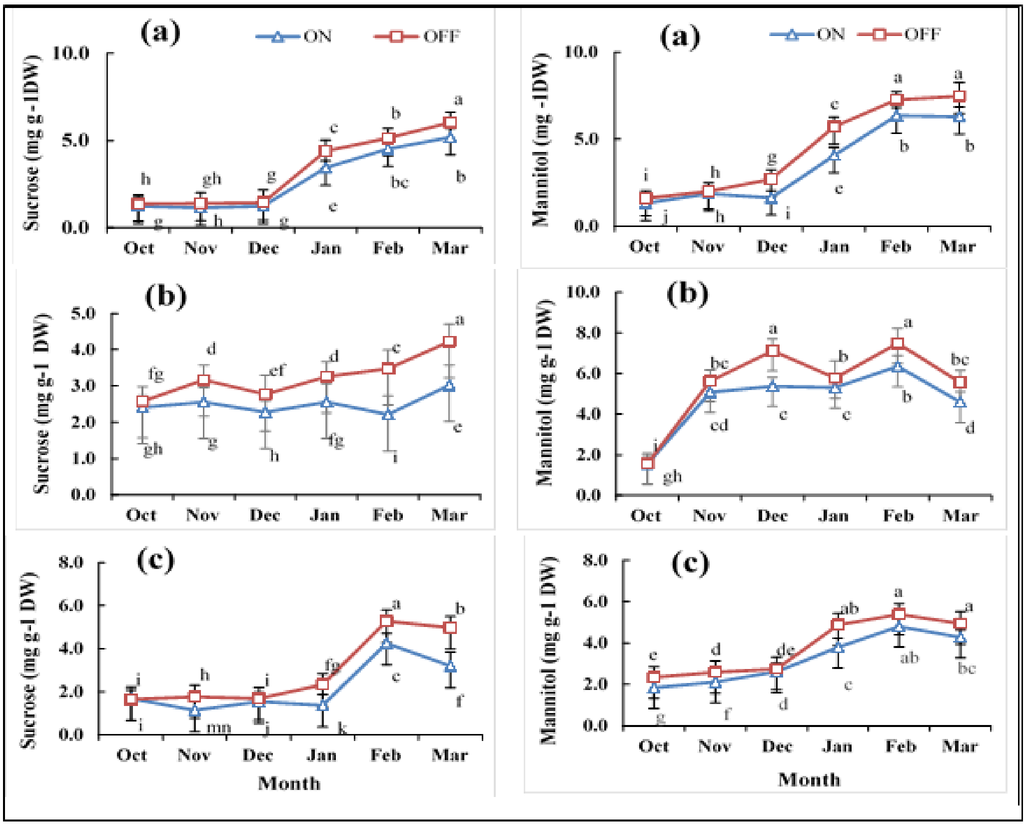

Fig. 2. Changes in sucrose and mannitol (mg/g dry weight) in autumn and winter (October 2018 to March 2019) in leaves (a), branches (b) and roots (c) of OFF and ON trees of 'Fishomi' cultivar. Mean values with common letters aren't significantly different (P<0.05). (±SE, standard error)

nutrients were compared in the leaves, branches and roots of 'Fishomi' olive cultivar between ON and OFF trees in 2018-2019. In autumn and winter (2018), carbohydrate contents and mineral nutrients

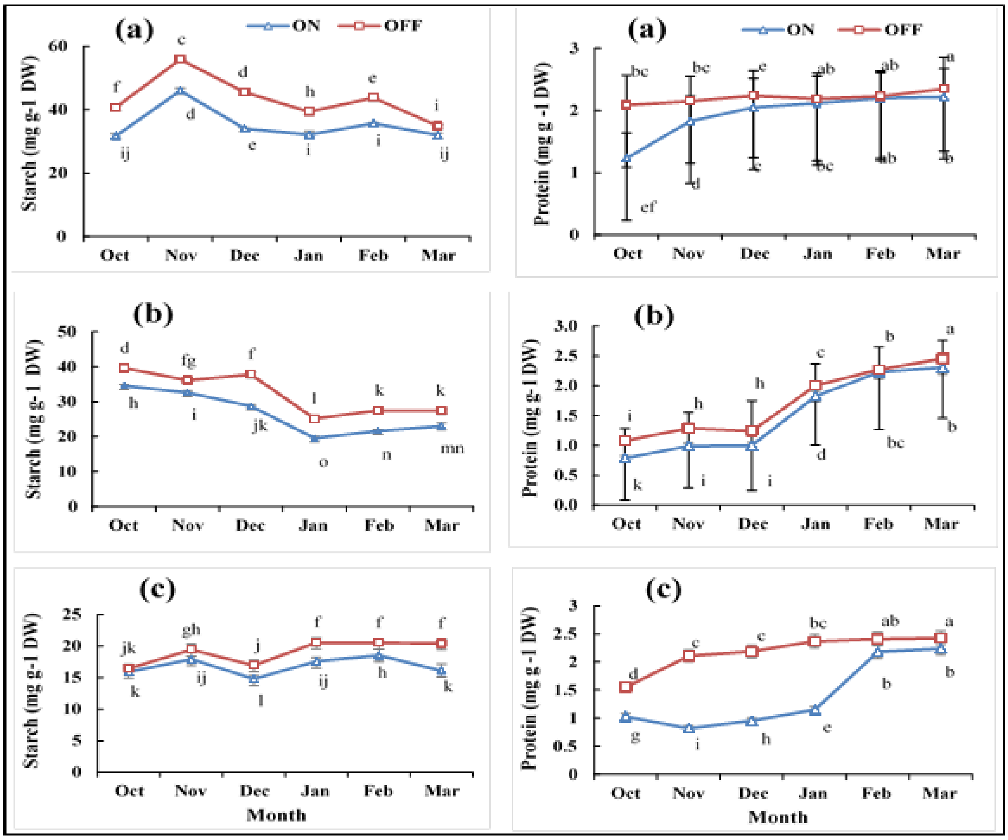

Fig. 3. Changes in starch and protein (mg/g dry weight) in autumn and winter (October 2018 to March 2019) in leaves (a), branches (b) and roots (c) of OFF and ON trees of 'Fishomi' cultivar. Mean values with common letters aren't significantly different (P<0.05). (±SE, standard error)

exhibited some extent fluctuations between different organs analyzed in ON and OFF trees. According to the results, the concentration of glucose, fructose, sucrose, mannitol, starch, protein contents and mineral nutrients were greater in the leaves and shoots than those of in roots. Fernandez-Escobar *et al*. (2000) reported that nitrogen is stored in olive leaves and stems and released during the development of reproductive and vegetative organs. During autumn and winter, the level of glucose, sucrose, fructose, mannitol and starch showed an upsurge trend, resulted in carbohydrates accumulation in many tissues in late January and February, and also, these contents were higher in OFF trees than in ON trees (Fig. 1, 2, 3). Starch reserved were greater in the leaves and branches than in the roots of ON and OFF trees. The starch content was higher in OFF trees than in ON trees in all samples. During autumn, starch content showed an upward trend, then steadily decreased from December and reached to the minimum in March, but in the roots, starch content showed an increasing trend until March (Fig. 3-a; b; c).

Monitoring of starch changes in several textures of olive trees during different months showed that the amount of starch was higher in autumn, reaching a minimum content in mid-winter and increased thereafter in early spring. In the current study, the peak time of dissolved sugar and starch content was not the same, indicating the conversion of starch to soluble sugars during this period. In line with the current results, Lavee (2007), and Fabbri and Benelli (2000) reported that the starch accumulated in the tissues in early autumn (October) and reached to its lowest level in February and March (simultaneous with the olive flower differentiation). Moreover, the initial increase in carbohydrate concentration results from starch accumulation during the winter which was in accordance with our results (De la Rosa *et al*., 2000). Protein content in all sampling time in OFF trees was higher than in ON trees. Protein content showed an increase trend in the leaves and roots from October to March (Fig.3-a, Fig. 3-c). During autumn, the protein content in the branches showed a stable pattern, followed by a rise in January and reached to a peak in March (Fig. 3-b). Protein content exhibited various differences between several olive tissues. Eris *et al*. (2007) stated that monthly changes in protein content in olive leaf and bark tissues increased over the winter whereas sharply dropped in the leaf, branch, and root tissues during the restart of growth in the spring, which was in agreement with the current study. The present study revealed that the amount of nitrogen in the leaves of olive trees ramped up during the autumn and its concentration was higher in OFF trees than in ON trees during the spring and summer, it seemed that the differences in nitrogen content between ON and OFF trees might be due to the consumption of nitrogen by fruits

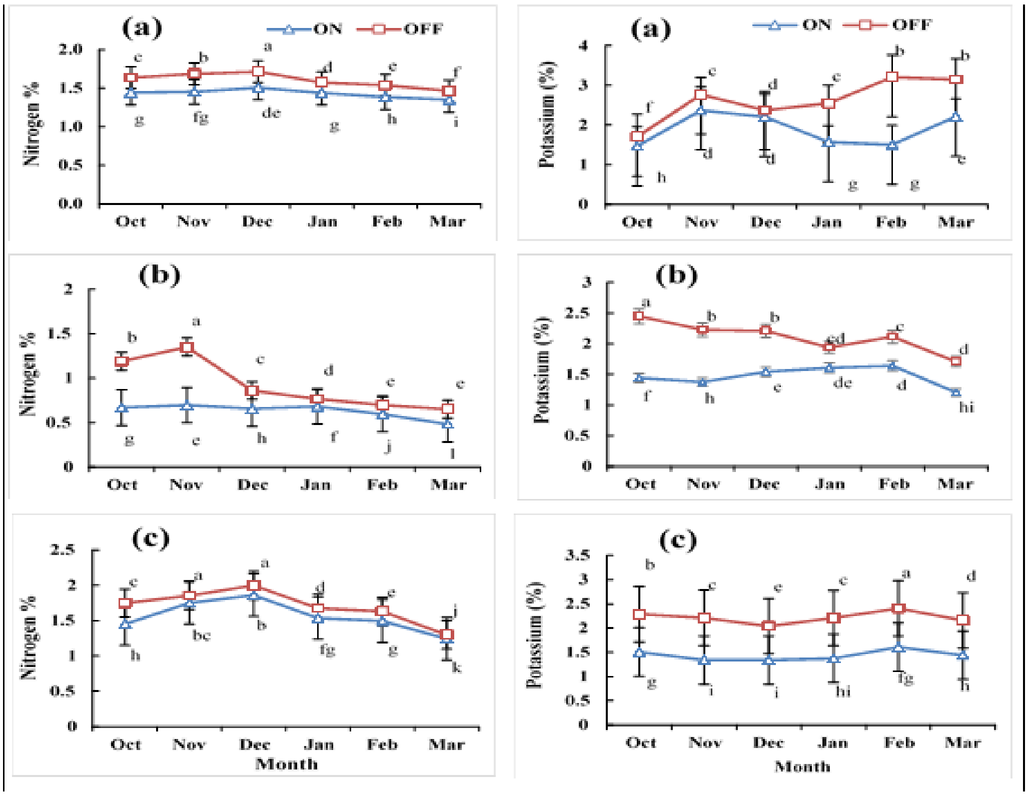

Fig. 4. Changes in nitrogen (%) and potassium (%) in autumn and winter (October 2018 to March 2019) in leaves (a), branch (b), and roots (c) of OFF and ON trees of 'Fishomi' cultivar. Mean values with common letters aren't significantly different (P<0.05). (±SE, standard error)

produced in the ON year, which also can reduce the shoot growth.

The nitrogen content in all samples was higher in OFF trees than in ON trees. Nitrogen content in all samples increased from October to December, followed by a gradual decreased from October to March (Fig. 4-a; b; c). The amount of nitrogen showed a gradual increase during autumn and winter, corresponding to the beginning of the rest period in olive trees. In consistent with our results, Perica (2001) and Stateras *et al.* (2017) stated that the amount of nitrogen was high in winter and then showed a consistent trend from November to February (rest period), and significantly dropped thereafter to reach the minimum level along with the vegetative and reproductive development. Nutrient deficiencies, especially nitrogen, during the olive bud differentiation period (late February) affected vegetative growth strength, fruit size, and flowering in spring. The potassium content of the leaves and roots were higher than those in the shoots. The potassium content in all samples was higher in the OFF trees than in the ON trees. In the leaves, the potassium content increased from October to November, followed by a decrease in December to reached the minimum level in ON trees in February (Fig. 4-a). Potassium content in the branches showed a decreasing trend from October to March (Fig. 4-b), and in the roots showed the consistent trend from October up to March (Fig. 4-c). The minimum amount of potassium was recorded in March. It seemed that low winter temperatures reduced the supply and mobility of potassium resulted in decrease in potassium content (Stateras *et al.*, 2017). Phosphorus level accumulated in various olive tissues during autumn and winter. The phosphorus content in the leaves, branches and roots was higher in the OFF trees than in the ON trees in all sampling time. The phosphorus content increased from October to December in all sampling time, but followed by a downward trend during the winter season (Fig. 5-a; b, c). Phosphorus accumulation was higher in OFF trees than in ON trees. The results of this study was in accordance with the reports of changes in leaf phosphorus concentrations in olive (FernaAndez-Escobar *et al.*, 1999) and citrus (Embleton *et al.*, 1973) trees.

As the new growth was began in the olive trees in spring (2019), the ON trees changed to OFF trees and vice versa. During spring and summer, we determined the changes in carbohydrates and minerals, especially during flower induction process in July.

Carbohydrate, protein and mineral changes during spring and summer in different parts of olive ON and OFF trees: From June to July, substantial differences in glucose content were observed in the ON and OFF trees. The content of glucose in the leaves decreased by 14% in ON trees in July, (Fig. 6-a). Our results exhibited that the decrease in glucose content in the branches in ON trees was about twice

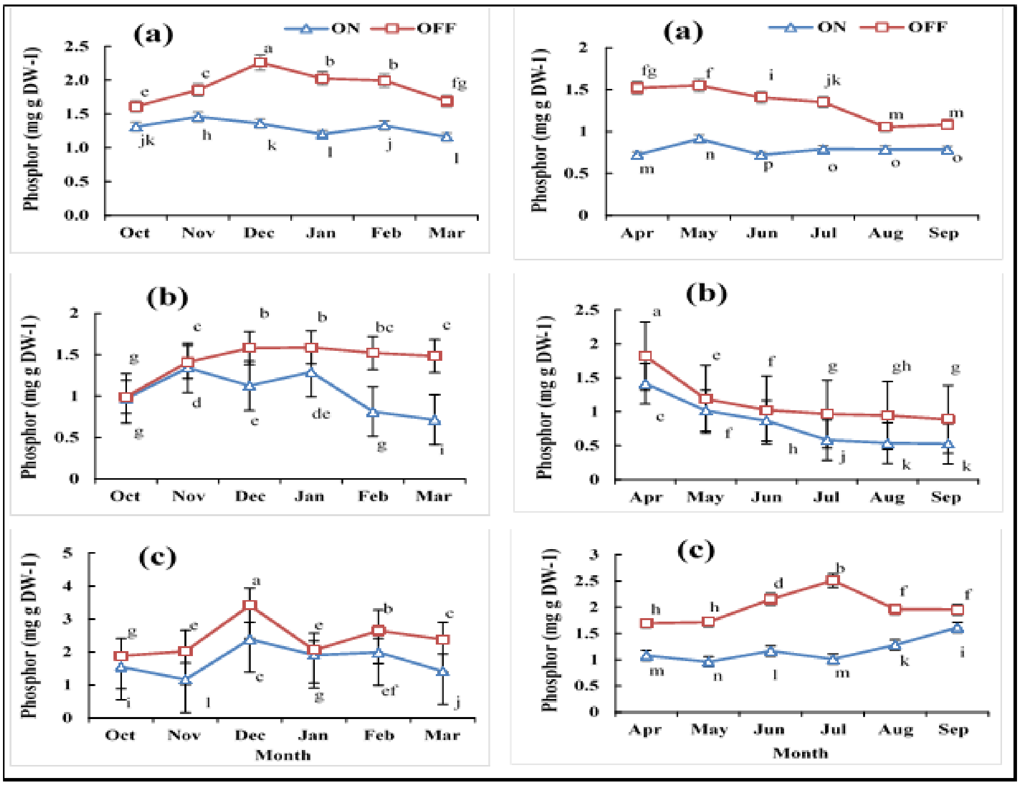

Fig. 5. Changes in phosphorus (mg/g dry weight) in autumn and winter (October 2018 to March 2019) (Figure left), spring and summer (April 2019 to September 2019) (Figure right) in leaves (a), branch (b), and roots (c) of OFF and ON trees of 'Fishomi' cultivar. Mean values with common letters aren't significantly different (P<0.05). (±SE, standard error)

as much as OFF trees during the summer (Fig. 6-b). The results showed that a 49% reduction in glucose content was recorded in July for ON tree trees (Fig. 6-c). Fructose content decreased in the leaves, branches, and roots from April to June, and there were significant variations in the sucrose content between June and July in ON and OFF trees. In the leaves in ON trees, fructose content was reduced by 43% in July (Fig.6-a). In July, fructose content in the branches of ON trees decreased by 33% (Fig. 6-b). Fructose content sucrose content decreased in the leaves, branches, and roots in ON and OFF trees from April to June, There were significant variations in the sucrose content between June and July in ON and OFF trees. In July, the reduction of sucrose content in OFF trees was twice as high as that of in ON trees (Fig. 7-a). In the branches of ON trees, sucrose content reduced by 65% in July (Fig. 7-b). In ON trees, in July a 60% decrease was observed in the sucrose content of the roots (Fig. 7-c). Mannitol content decreased in the leaves, branches, and roots in ON and OFF trees from April to June. Between June and July, significant variations in mannitol content were observed in the ON and OFF trees. Mannitol content in ON trees showed a downward trend in the leaves during the months of analysis and then 62% ramped down in July, (Fig.7-a). Mannitol content decreased in the branches, up to 28% in ON trees (Fig. 7-b). Mannitol content declined in the roots of ON trees, In July, up to a 46% (Fig. 7-c). According to the results of this study, in spring and summer, a higher carbohydrates was observed in OFF trees compared with ON trees. It's been found that along with bud opening and also the expansion of new leaves, the amount of carbohydrate reserves decreased due to the conversion of stored carbohydrates into structural carbohydrates. On the other hands, fruit production is known to inhibit the vegetative growth of olives at the same time (Lavee, 2007). Developing fruit is a strong sink for absorb photoassimilates and deplete the photoassimilates, especially carbohydrates from the bud, resulted in the avoidance of flower induction (Sharma *et al*., 2019). These carbon demands are often met by increased use of stored carbohydrate reserves (Bustan *et al*., 2016). In olive trees, the fruits were developed on well-lignified shoots grown in the previous year, and flower induction will begin at the time of pit hardening in the current year's growing fruits and will last about three months. The existence of fruit on the tree at this time affects unique metabolic pathways and thus the induction of the flower bud is affected by the signals producing by the development of embryos. The reverse rivalry between developing fruits and embryos and differentiation of flower buds was also reported in other fruit trees for the following year's return bloom (Dastkar *et al*., 2020). These reserves also are

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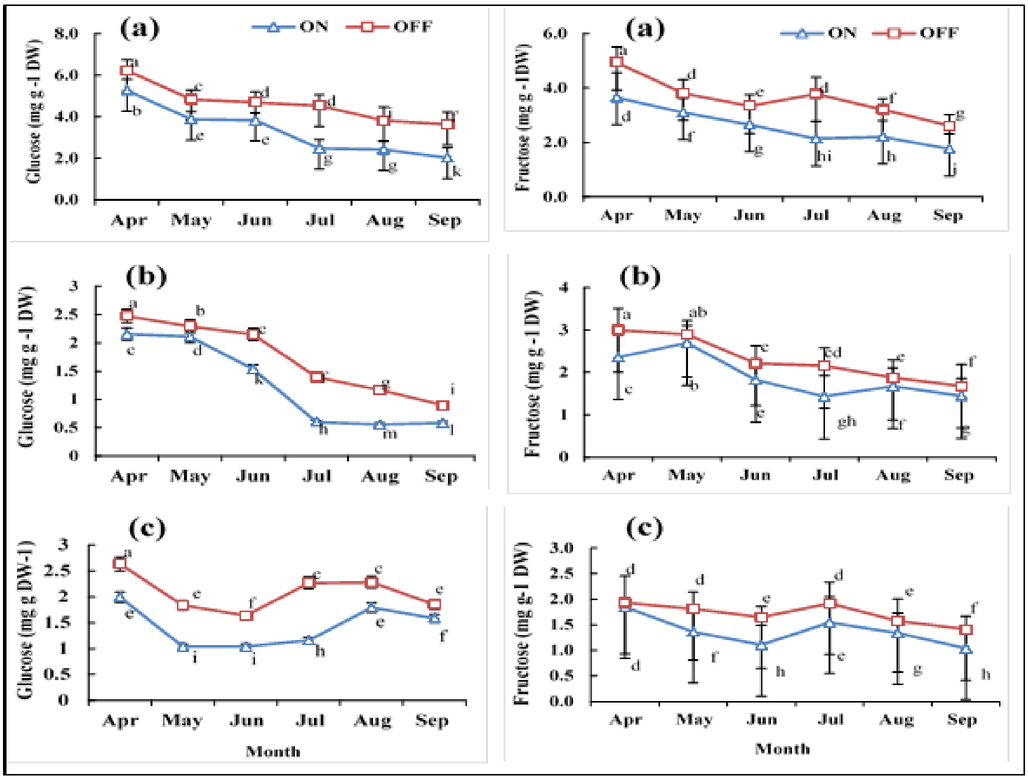

Fig. 6. Changes in glucose and fructose (mg/g dry weight) in spring and summer (April 2019 to September 2019) in leaves (a), branch (b), and roots (c) of OFF and ON trees of 'Fishomi' cultivar. Mean values with common letters aren't significantly different (P<0.05). (±SE, standard error)

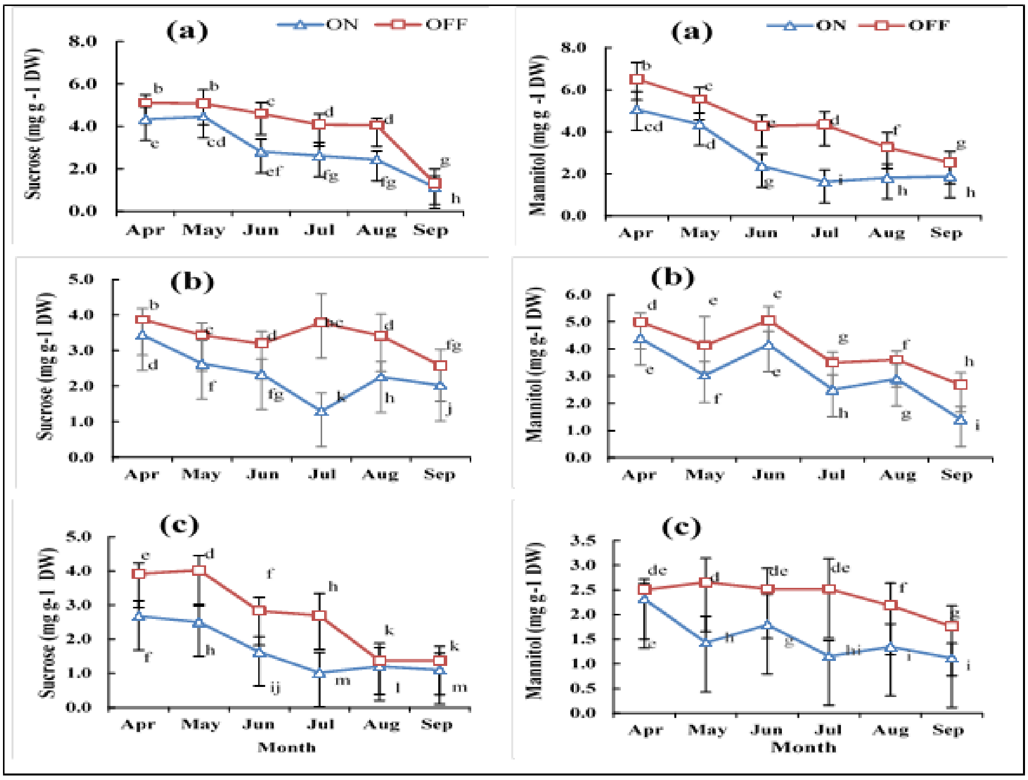

Fig. 7. Changes in sucrose and mannitol (mg/g dry weight) in spring and summer (April 2019 to September 2019) in leaves (a), branch (b), and roots (c) of OFF and ON trees of 'Fishomi' cultivar. Mean values with common letters aren't significantly different (P<0.05). (±SE, standard error)

used in the formation and growth of new organs, and because of respiration and increased plant development, some of these reserves were lost (Sivaci, 2006; De la Rosa *et al.*, 2000). Therefore, reducing the number of nodes and leaf area declines the capacity of flower induction within the next season (Kour *et al*., 2018). Kour *et al*. (2018) showed that the prevention of bud formation in olive trees occurs as the carbohydrate reserves are reduced, which usually occurs after the production of high-yielding fruit resulted in a reduction of the reserves of trees and minimal crops in the following year in the roots dropped by, 19% in July (Fig. 6-c).

There were major differences in the content of the starch in the leaves, branches, and roots in ON and OFF trees. Starch content showed a declining trend in the leaves from May to September and in July in ON trees and reduced by 19% (Fig. 8-a). Starch content in the branches reached a maximum level in June and July, Whereas, in ON trees a 18% dropping was observed in the starch content in July (Fig. 8-b). Starch content increased in the roots from April to June. In OFF trees. In July, solely a 11% increase was observed in starch content compared to the ON trees (Fig. 8-c). Starch is considered as an energy source in early spring and its rate tends to decrease in the early stages of olive bud flowering (De la Rosa *et al*., 2000). Theory of nutritional concept clarified how the developing fruit provides photo-assimilates with a strong sink and also indicated the depletion mechanism of photo-assimilates, especially carbohydrates from the bud, leading to the prevention of flower induction (Sharma *et al*., 2019). There were significant differences in protein content of the leaves, branches, and roots in ON and OFF trees. Protein content decreased in the leaves, and roots in ON and OFF trees but in the branches, the lowest content of protein was observed in May, then significantly increased and reached to the peak in June and then declined with some fluctuations in July. A downward trend was observed in protein content of the leaves from May to September in ON trees. Protein content was ramped down by 27% in July (Fig. 8-a). The lowest content of protein in ON trees was recorded in the branches in May, then significantly increased and reached to the peak in June and then ramped down with some fluctuations in July (Fig. 8-b). Protein content gradually decreased in the roots of ON trees from April to September, and in July, a 22% reduction was obtained in protein content (Fig. 8-c). Present study revealed that protein levels in olive decreased during spring and summer. The protein content of the leaves, branches as well as roots in OFF trees was more than in ON trees, which is accordance with the results of Lavee and Avidan (1994) who stated that the protein content of OFF trees was more than in ON trees. Since different and specific proteins are formed during the ON and OFF years, the differences in protein content seems to be rational. Protein probably influenced the physiology of olive production, because growing inflorescences are strong sinks for water-soluble proteins (Lavee and Avidan, 1994; Kour *et al*., 2018).

There were significant variations in the content of nitrogen in the leaves, branches, and roots in ON and OFF trees. The results showed that the nitrogen content of the leaves exhibited an upsurge trend from April to May, followed by a decreasing trend in June. In July, the nitrogen content was 15% higher in OFF trees than that of ON trees (Fig. 9-a). Nitrogen content increased in the branches of ON trees from April to June and rapidly dropped in July, reaching to the minimum level in September. A 45% reduction of nitrogen content was recorded in July (Fig. 9-b). Nitrogen content decreased in the roots of ON trees from April to May, followed by an increasing trend until June, and remained constant from July to September. In July, a 21% decrease was observed in the nitrogen content (Fig. 9-c). Since both flower bud and vegetative growth required nitrogen in the spring, the decrease in the amount of nitrogen was inevitable and nitrogen deficiency resulted in reduced growth, fewer leaves, and less flowering and yield and defective flower formation (Garcia *et al*., 1997). FernaAndez-Escobar *et al*. (1999) reported that the nitrogen concentration in young olive leaves began to decrease in June to reach the minimum content in August. They also indicated that the highest nitrogen accumulation was recorded in the OFF year. Also, a high nitrogen accumulation in OFF year has been reported in olive leaves (Ulger *et al*., 2004), which is in line with the results of this study. According to the current results, the lowest amount of nitrogen was observed in ON trees in May. Lower levels of nitrogen in July and August are often due to the coincidence with the hardening period of the fruit endocarp and floral induction. Deficiency in nitrogen has been established in olive trees as a limiting factor for flowering and fruit formation (Freeman *et al*., 2005; Erel *et al*., 2008).

In protein biosynthesis, nitrogen is an essential factor. In reality, developing inflorescences have been shown to be a strong sink for nitrogen and water-soluble proteins, whereas availability of which is crucial to flowering processes (Erel *et al*., 2008; Noori *et al*., 2015). Furthermore, fruit set, yield, and shoot growth are negatively affected by N deficiency (Freeman *et al*., 2005). It was stated that high levels of carbohydrates alone can't promote flowering provided that there's sufficient nitrogen in the leaves (Ulger *et al*., 2004). There were significant variations in the content of potassium of leaves, branches, and roots in ON and OFF trees. Potassium content in the leaves of ON trees showed a downward trend from April to August followed by an increasing trend in September. In July, a 55% decrease was recorded in potassium content (Fig. 9-a). The lowest potassium content of the branches in ON trees was recorded in May and July, respectively, with a 33% reduction in July (Fig. 9-b).

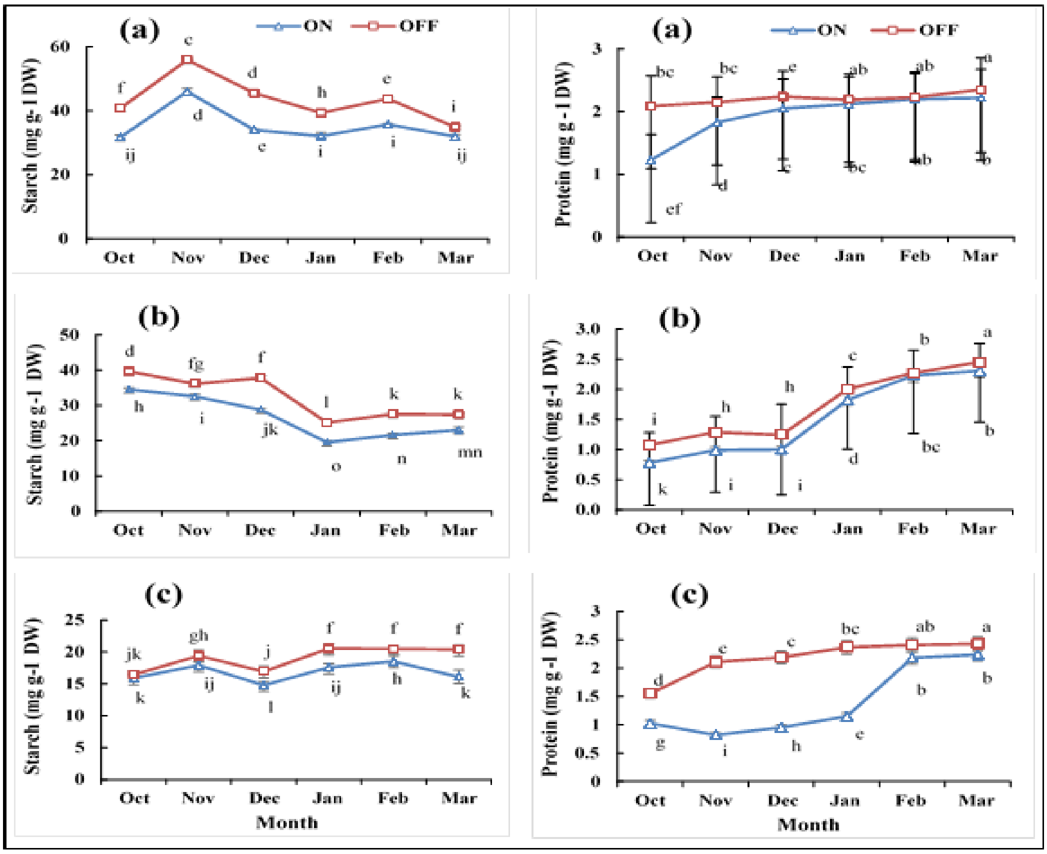

Fig. 8. Changes in starch and protein (mg/g dry weight) in spring and summer (April 2019 to September 2019) in leaves (a), branch (b), and roots (c) of OFF and ON trees of 'Fishomi' cultivar. Mean values with common letters aren't significantly different (P<0.05). (±SE, standard error)

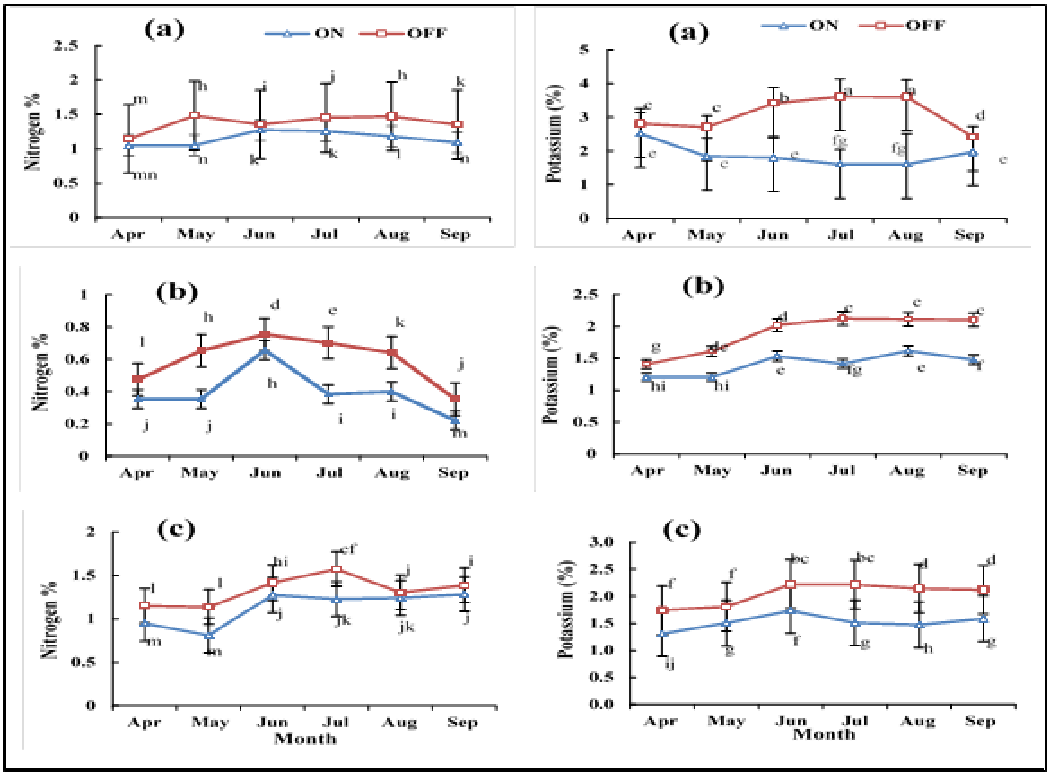

Fig. 9. Changes in nitrogen (%) and potassium (%) in spring and summer (April 2019 to September 2019) in leaves (a), branches (b) and roots (c) of OFF and ON trees of 'Fishomi' cultivar. Mean values with common letters aren't significantly different (P<0.05). (±SE, standard error)

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Our results indicated that potassium content in the roots of OFF trees was 47% higher than in ON trees in July (Fig. 9-c). In line with the results of this study potassium levels in leaves rapidly decreased until July and remained at low levels. High accumulation of potassium in olive leaves after the OFF year followed by a decrease trend in ON year indicated a high demand of reproductive structures to potassium (FernandezEscobar *et al*., 1999; Bouhafa *et al*., 2018). Potassium is additionally essential for photosynthesis, carbohydrate, protein synthesis, and enzyme activation (Bouhafa *et al*., 2018). Potassium is recognized to play a key role in flowering compared with the other macro elements (Fabbri and Benelli, 2000), since it promotes the production of amino acids that induces the formation of IAA oxidase, leading to the induction of flowering. Potassium can also facilitate the formation of pyruvate kinase and thus affects the level of certain amino acids involved in flowering induction. (Fabbri and Benelli, 2000; Troncoso *et al*., 2008).

There were significant variations in the content of phosphorus in leaves, branches, and roots in ON and OFF trees. Phosphorus content ramped down in leaves and branches from April to September, however, a 70% and 66% increase was recorded, respectively in phosphorus content in OFF trees than those of in ON trees in July (Fig. 5-a, Fig. 5-b). Phosphorus content gradually increased in roots of OFF trees from April up to July, then sharply dropped and reached to the consistent trend in September. Phosphorus content was observed about twice as much as that of in ON trees in July (Fig. 5-c). Bouhafa *et al*. (2018) stated that the phosphorus content of olive leaves exhibited a moderate upsurge trend from April to May, followed by a decrease trend in June and July and increased thereafter until peaking in September and then gradually decreased to remain constant, which is accordance with the results of this study. A high phosphorus absorption in leaves during flowering period and fruit enlargement bringing about the highest requirement of olive trees to phosphorus during these periods. Erel *et al*. (2016) reported that reduced flowering and fruit formation in low phosphorus conditions, resulted from the importance of phosphorus for successful fertility. Qualitative characteristics of flowers including complete flowers, inflorescences, pistil biomass, pistil viability, and flower and fruit formation were positively related to the nutritional status of phosphorus (Erel *et al*., 2013). Erel *et al*. (2016) stated that the positive impact of phosphorus is linked to the availability of carbohydrates and transport dynamics on flower formation and fruit set. Signaling and substituting specific sugars or phosphorylated sugars were reported to have a beneficial effect on the role of phosphorus and carbohydrates in reproductive process. It was found that inhibition of flower induction was likely associated with the compounds transferred from the growing fruit and seeds into the olive buds, and competition for nutrients as well. Competition between vegetative and reproductive organs decreased the growth of new shoots in olive trees and the formation of flowers during the ON year (competition with developing fruits during the summer) resulted in alternate bearing phenomena (Connor and Fereres, 2005).

Analysis of correlation coefficients of different traits with performance helps to decide on the relative importance of these traits and their value as selection criteria (Yu *et al*., 2015). Simple correlations were shown for 21 measured traits for Quebec seed olive cultivar in different months, respectively. The correlation coefficient table 1 showed that there was the most positive correlation between leaf starch and total leaf carbohydrate (1000**). Skin + root starch has a positive correlation with skin + root carbohydrates (1000**) and skin + root protein (0.528**). Root starch had a positive correlation with root carbohydrates (1000 **). According to the results of the correlation coefficient table in different months, leaf nitrogen has a positive correlation with the characteristics of skin + root niacin (505 **), root nitrogen (0.439 **) and leaf C / N (0.88 **) and has a negative correlation with root phosphorus (-411 **) and skin and root potassium (-547 **). Skin + root nitrogen C / N skin + root (-460**) has a negative correlation with root C / N (-0.624*) and then with root protein (-0.442) and skin + root potassium (-464*) had a negative correlation. Root nitrogen has a negative correlation (-0.539 *) with leaf C / N and (-790 **) C / N root. The correlation between traits in different months shows that leaf C / N has a positive correlation with skin / root C / N (0.589 **) and skin + root potassium (0.623 **). C / N skin + root has a positive correlation (0.542 **) with root potassium. The ratio of leaf starch (-548 **) and skin + root starch (-513 **) has a negative correlation with skin + root protein. And bark + root protein and root protein have a positive correlation with leaf protein (0.694 **) and (547 **). Simple correlation for leaf phosphorus shows a positive correlation (547 **) with root phosphorus. Measured root phosphorus had a positive correlation (420 **) with skin potassium + root. Based on the findings of this study, the demand for the reproductive department early in the season for carbohydrates in off c.v. Fishomi olive trees in contrast, the fruit trees are low and therefore the accumulation of this. The combinations continued until mid-June and in flower induction time (late season) is a remarkable part of sugar reserves to perform the glazing process is used. The highest difference in the starch content of fruit-bearing and non-fruit trees is related to mid-June. In the trees off in autumn and winter carbohydrates and starches accumulated in the leaves, branches and roots, while ON trees have lower carbohydrate levels during this period so most of these reserves are recycled for next year's crop. Abundant flowering after one year off causes rapid consumption of carbohydrate stores that

Table 1. Phenotypic correlation coefficients of different traits in c.v. 'Fishomi' olive trees

	x1	x2	x3	x4	x5	x6	x7	x8	x9	x10	x11	x12	9*/x13	x14	x15	x16	x17	x18	x19	x20	x21
x1	1																				
x2	0.231	1																			
x3	0.29	0.227	1																		
x4	0.081	0.139	0.223	1																	
x5	.435*	0.094	0.307	.505*	1																
x6	-0.092	-0.039	0.155	.439*	-0.111	1															
x7	0.082	0.001	-0.246	-.88**	-.460*	-.539**	1														
x8	-0.191	-0.066	-0.091	-.74**	-.624**	0.012	.589**	1													
x9	0.273	0.175	0.068	-0.342	0.196	-.790**	.509*	-0.062	1												
x10	1.000**	0.231	0.29	0.081	.435*	-0.092	0.082	-0.191	0.273	1											
x11	0.232	1.000**	0.229	0.138	0.094	-0.039	0.002	-0.063	0.175	0.232	1										
x12	0.29	0.227	1.000**	0.223	0.307	0.155	-0.246	-0.091	0.068	0.29	0.229	1									
x13	-0.365	-0.117	-0.278	0.252	-0.162	-0.025	-0.101	-0.399	-0.133	-0.365	-0.121	-0.278	1								
x14	-.548**	-.508*	-0.202	0.279	-0.122	0.183	-0.352	-0.147	-.454*	-.548**	-.513*	-0.202	.694**	1							
x15	-0.361	0.01	0.032	0.368	-0.065	0.242	-0.384	-0.335	-0.284	-0.361	0.005	0.032	.547**	.569**	1						
x16	-0.032	0.205	0.147	-0.235	-0.127	-0.155	0.327	0.202	0.073	-0.032	0.204	0.147	-0.044	-0.253	-0.145	1					
x17	-0.142	-0.358	-0.2	0.173	0.181	0.14	-0.36	-0.283	-0.174	-0.142	-0.358	-0.2	-0.076	0.218	0.126	-.603**	1				
x18	-0.331	-0.153	0.098	-.411*	-.426*	0.191	0.254	.542**	-0.215	-0.331	-0.154	0.098	-0.144	0.037	0.138	.580**	-0.283	1			
x19	0.126	-0.083	0.001	0.043	.450*	-0.397	-0.088	-0.145	.477*	0.126	-0.082	0.001	-0.253	-0.123	-0.216	-.445*	0.338	-.414*	1		
x20	-0.093	-0.051	-0.179	-.547**	-.464*	0.071	.623**	0.401	0.176	-0.093	-0.052	-0.179	0.009	-0.184	-0.095	0.265	-0.203	.420*	-0.332	1	
x21	0.177	-.407*	-0.27	0.133	-0.115	0.155	-0.148	-0.136	-0.234	0.177	-.411*	-0.27	0.129	0.254	0.155	-0.151	.510*	-0.097	-0.05	-0.042	1

*** and **: Significant (α= 5%), highly significant (α= 1%), respectively**

x1: leaf carbohydrate x2: carbohydrate skin+root x3: Root carbohydrate x4: N leaf (%) x5: N skin+root(%) x6: N root (%) x7: C/N leaf (%) x8: C/N skin+root (%) x9: C/N root (%) x10: Leaf starch x11: starch skin+root x12: Root starch x13: Leaf protein x14: protein skin+rootx15: Root protein x16: P Leaf mg/g dw x17:p skin+root mg/g dw x18: P root mg/g dw x19: K Leaf mg/g dw x20: k skin+root mg/g dw x21: K root mg/g dw

run out at full bloom (Martinez- Fuentes *et al*., 2015). Continuing the growing season, the demand for carbohydrates in trees is high, which may lead to a decrease in carbohydrate reserves in different parts and cause a rapid decline in reserves. Carbohydrates show that this decrease in reserves in deciduous trees is more severe, which is well consistent with the findings of Monerri *et al*. (2011).

The obvious difference at the end of the season in terms of level carbohydrate reserves are found between off and on trees can be caused by the continuation of the upward trend of storage.

Decomposition into principal components: The principal component analysis method has many applications in expressing the changes of traits and by using it, the patterns of change can be shown in a multidimensional way. Principal component analysis shows the changes in more than one dimension (number of components) and principal components allows further interpretation of the relationship between trait changes (Jolliffe and Cadima, 2016). Decomposition into principal components was performed using different traits measured in Quebec seed olives. The sum of the eigenvalues is equal to the total variance of the data, and the eigenvalues for a principal component show the share of variance of that component in the total variance. A comparison of the values of the first seven vectors confirms the existence of changes. Among the special vectors, the traits that have the largest share in the value of the absolute value will have the largest share in the variation. Analysis into principal components showed that 7 components had a specific value of more than one and the maximum variability was explained by the first component (19.88) and a total of 81.15% of the changes by the first 7 components. In the first component, which explained the most changes, the traits of skin + root carbohydrates (0.290), skin + root starch (0.291), and skin + root protein (0.408) were the most important in the change. In the second component, leaf nitrogen (0.371), bark + root nitrogen (0.363), bark / root C / N (0.352) and leaf phosphorus (0.338) were the most important factor in the change. In the third component, root protein (0.305) and leaf phosphorus (0.327) traits were the most important factor in the change. In the fourth component, skin + root carbohydrates (0.315), root carbohydrates (0.392) and root starch (0.392) were the most important factor in the changes. In the fifth component, root carbohydrates (0.353), root nitrogen (0.429) and root starch (0.353) had the largest share in justifying the

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changes. In the sixth component, leaf carbohydrates (0.347), leaf starch (0.452), root starch (404) and root potassium (306) were the most important factor in changing the traits and this importance in the seventh component related to skin nitrogen (0.359), root protein (0.327) and leaf phosphorus (374). In general, by selecting the first 7 components, about 81.15% of the changes are justified and only about 19% of the changes are not justified. Commented. Since the two components accounted for 38.36% of the total changes. What emerges from the total is that factor 1 has been a more important factor than other factors, and the traits in this factor play a major role in the initial diversity of performance.

Conclusions

In ON year, carbohydrate and nitrogen reserves were reduced and showed their impact in the following season's crop. Accumulation of nutrient elements during the OFF year is a critical factor for further usage in the reproductive stage in the following year. Carbohydrate reserves serve as an important compound in flowering process and there's a close relationship between flower and fruit formation with the supply of sources such as carbohydrates and mineral nutrients. In general, during the summer that flower induction occurred in olive trees, the application of some proper nutrition and fertilizers and appropriate horticultural practices could increase the soluble sugar and starch content in leaves and branches, resulting in enhancing the flower number in each inflorescence and to some extent alleviated alternate bearing phenomenon in olive trees.

Author contributions

MZ carried out the experiment and performed the data analyses. MR was the project supervisor, designed the research, and provided all the technical support during the laboratory work. SE helped in the design of the experiment and wrote the manuscript.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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