IMPACT OF THINNING AND FOLIAR-BORON-ZINC SPRAY ON SOME MORPHOLOGICAL AND PHYSIOLOGICAL INDICATORS IN APPLE TREE (*MALUS DOMESTICA* CV. GOLDEN DELICIOUS)-"TARTOUS GOVERNORATE"

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ABSTRACT

The research was conducted in Tartous governorate on apple trees cv. *Golden Delicious* grafted on the rootstock "*Malus domestica* Borkh", during two seasons 2019-2020 to study the impact of manual and chemical thinning using foliar boron /zinc spray separately or in combination on some morphological and physiological indicators. The results showed a significant increase in leaf area and its content of total chlorophyll and carotenoids in the second season (low fruit load) compared to the first season (heavy load) due to a rise in vegetative growth and a reduction in fruiting. During the heavy load season, a balance between vegetative and fruiting growth was noted when the manual and chemical thinning applied with no significant differences was found between the two seasons in leaf area, total chlorophyll and carotenoids content. On the other hand, the application of manual thinning, chemical thinning and foliar spraying with boron and zinc separately or together pointed out an increase in the branches content of carbohydrates and nitrogen and C/N ratio in the low load season compared to the control. Consequently, fruit thinning was more effective to obtain a balance between the seasons in term of leaf area, its pigments content, and C/N ratio in branches, which in turn mitigate the alternate bearing phenomenon.

Key words: total chlorophyll, carotenoids, C/N ratio, leaf area, naphthalene acetic acid.

مجلة العلوم الزراعية العراقية -2023 :54(3):874-883 الاحمد وآخرون

تأثير الخف والرش الورقي بعنصري البورون والزنك في بعض المؤشرات المورفولوجية والفيزيولوجية لشجرة التفاح صنف غولدن ديليشيس في "محافظة طرطوس"

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المستخلص

نُفذ البحث في محافظة طرطوس على أشجار التفاح صنف غولدن ديليشيس المطعمة على الأصل Malus domestica Borkh، خلال موسمي النمو لعامي 2019 و2020 وذلك بهدف دراسة تأثير الخف اليدوي والخف الكيميائي والرش الورقي بالبورون والزنك منفصلين والزنك والبورون معاً في بعض المؤشرات المورفولوجية والفيزيولوجية. أظهرت النتائج زيادة معنوية في مساحة الورقة ومحتواها من الكلوروفيل الكلي ومن الكاروتينات في الموسم الثاني للدراسة " الحمل الخفيف" مقارنة بالموسم الأول "الحمل الغزير" بسبب زيادة النمو الخضري وتراجع النمو الثمري، في حين أدى تطبيق الخف اليدوي والخف الكيميائي في موسم الحمل الغزير إلى توازن بين النمو الخضري والثمري في كل من الموسمين فلم نجد فروقاً معنوية بين الموسمين في مساحة الورقة ومحتواها من الكلوروفيل الكلي ومن الكاروتينات. أدى تطبيق الخف اليدوي والخف الكيميائي والرش الورقي بالبورون والزنك منفصلين والزنك والبورون معاً إلى زيادة في محتوى الفروع من الكربوهيدرات والآزوت ونسبة C/N في موسم الحمل الخفيف مقارنة مع الشاهد. كان خف الثمار هو الأفضل فقد حقق توازناً بين الموسمين المدروسين في مساحة الورقة ومحتواها من الصبغات وفي نسبة C/N في الفروع الأمر الذي يخفف من ظاهرة تبادل الحمل.

الكلمات المفتاحية: لكلوروفيل الكلي، الكاروتينات، نسبة الكربوهيدرات/الآزوت، مساحة الورقة، نفتالين أسيتيك أسيد.

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INTRODUCTION

The cultivation of apples in Syria is concentrated in the mountain areas from 600 m up to 1800 m above sea level; it is a temperate tree prefers <26 ºC during the growing season (23). The cultivated area in Syria is about 52.231 hectares with a production of ~476.635 tons. 70.05% of the total area relies on rainfed agriculture concentrated mainly in the governorates of Al Suwayda- Homs countryside, Latakia and Tartous while most of irrigated area, is located in Damascus and Homs countryside. The majority of the crop is concentrated in Tartous particularly in Drakeish and Safita, due to the appropriate climatic conditions in terms of altitude above sea level, the amount of precipitation and the number of chill hours. Golden Delicious is the main cultivar in Tartous and its cultivation is about 70-80% compared to the other cultivars (17). Many fruit trees show what is called the Alternate Bearing Phenomenon (Biennial Bearing), in which one year of high fruit load (heavy load) followed by a year of very low load or no production (low fruit load) (15). It occurs as a result of nutrients depletion by fruits, so the trees need an entire year to restore their activity and produce more in the following year, and thus production fluctuates from year to another (29). (22) Showed that the carbohydrates deficiency is the main reason for the alternate bearing in trees while (30) underlined a greater formation of new vegetative branches (not fruitful) in low load year after a heavy production year. On the other hand, the availability of sufficient nutrients during high load season can ensure a production with a good quality next year when the tree sprayed with nutrients solution (18, 27). (6) mentioned that adding fertilizers to Anna apple increased the leaf area which positively reflected on tree nutrition, raised the production and improved the fruits quality. The application of boron fertilization, regardless of the addition method, improved the growth (36), increased flowers pollination and fruit setting, and enhanced the productivity and quality of fruits in many temperate regions (34 , 11) and improved the marketing qualities of fruits by reducing the incidence of physiological disorders (21). Zinc is essential element in the formation of auxins produced in the *stem tips*, promotes cell division, shoots and leaves growth and fruits development. It plays a crucial role in chlorophyll synthesis in leaves for carbohydrates formation by photosynthesis (19). Which many researches showed the significant effect of fertilizers or extracts have zinc on plants (2, 9, 10, 24) Due to the economic importance of apple tree in Syria, Golden Delicious is known to be the most cultivated variety, especially in Tartous governorate, that highly requires fruit thinning as a result of alternative bearing occurrence and characterizes by a low production per area. Besides, the lack of adequate local studies on the best agricultural practices (pruning, fertilization management to increase soil fertility) that would improve the nutritional status of trees and provide favourable growth conditions to enhance a regular production and affect positively on the quality specifications of apple fruits. This research aimed to evaluate the effect of spraying Golden Delicious apples with boron and zinc and applying manual and chemical thinning on some morphological and physiological indicators to improve the nutritional status of the trees, maintain the productivity and reduce the alternative bearing phenomenon.

MATERIAIS AND METHODS

Experiment site and soil analysis

The research was conducted in Beit Yusef, Al-Dreikish in Tartous, Syria (34º55`37``N 36º12`57``E, 890 m asl). It is characterized by a very cold-humid winter, cold-humid spring and mild-humid summer with an annual rainfall average of 1213 mm and with an annual temperature average of 17.5°C (18). The soil is a clay loam, table (1) reports the main characteristics of soil layers in the study area and the normal levels of each element (33).

Plant material and treatments

The experiment was carried out from 2019 to 2020 on 15-year-old apple trees (cv. "Golden delicious"), planted at spacing of 5 × 5 m and trained to a *modified* central *leader*. These trees were originally grafted on the rootstock "*Malus domestica* Borkh", which is characterized by great growth power that it gives to the grafted variety, extending the life

of the tree, forming a strong deep root system, resistance to frost, drought and diseases, adapting to different types of soil and high percentage of seed germination (16). During the experimental period, all the practices of pruning, tillage, weeding and pest control were applied regularly

Table 1. Characteristics of soil conditions and limits of main elements and organic matter

	pH	Nitrogen (ppm)	Potassium (ppm)	Phosphor (ppm)	Organic matter (100g/g)	Active lime (6-12%)	Calcium Carbonate (%)
0-30 cm	**7.8**	**12.8**	**103.44**	**11.24**	**2.42**	**Tr**	**Tr**
30-50cm	**7.78**	**9.9**	**59.64**	**9.62**	**1.88**	**Tr**	**Tr**
50-70cm	**7.74**	**5.8**	**38.3**	**7.04**	**0.82**	**1.70**	**4.4**
Limits	**6.0-6.5**	**20 <**	**150 <**	**15 <**	**1.29 <**		

to all the trees in each treatment. According to the soil analysis, a fertilization of nitrogen, phosphorous and potassium was done, in fall around each tree, at the rate of (1) kg urea, (1) kg of superphosphate and (1.5) kg of potassium sulphate with (20) kg of organic cow manure. Manual and chemical thinning technique was applied in the heavy bearing season (2018 before the starting of the experiment). The experiment consisted of the following 6 treatments, as independent treatments arranged in a completely random block design with three replicates and each replicate contained two trees.

T_1: Control

T_2: Manual thinning by leaving 2 fruits of each flower bud during the June drop period

T_3: Chemical thinning using NAA (10 mg/L) after 20 days of full bloom (petals fall)

T_4: Spraying of boric acid solution (H_3BO_3) at 1 g/L

T_5: Spraying of zinc sulphate solution ($ZnSO_4.7H_2O$) at 2 g/L

T_6: Spraying of boric acid and zinc sulphate together at 1 g/L of H_3BO_3 and 2 g/L of $ZnSO_4.7H_2O$

T4, T5 and T6 were applied twice: in flower cluster and then in full bloom stage.

Studied parameters

Leaf area (cm^2): The measurement was done using millimeter graph paper method, on 30 fully-extended leaves from each tree collected from the middle third of the branch at the end of growing season.

Content of chlorophyll (mg/g) and carotene pigments (mg/100g):

The content of chlorophyll a and chlorophyll b was estimated according to the method of. (14). modified by (8). Briefly, 100 mg of leaves from each treatment were crushed with 10 cm^3 of 80% acetone using a pestle and mortar until obtaining a uniform solution, then centrifuged at 3,000 rpm for 5 minutes. The supernatant was poured in volumetric flasks and then volume made up to 20 cm^3 using 80% acetone. Lastly, the absorbance was read at 645 and 663nm with the spectrometer, chlorophyll a and chlorophyll b concentrations (mg/g) were calculated based on the equations proposed by (14):

$Chl.a=(12.7(D_{663})-2.69(D_{645}))\times V/(1000\times W)$

$Chl.b=(22.9(D_{645})-4.68(D_{663}))\times V/(1000\times W)$

$Tot.chl=(20.2(D_{645}))+(18.2(D_{633}))\times V(100\times W)$

V=Final volume of the extract (cm^3)

D=Optical absorption in wavelengths 663 and 645 nm

W=Fresh weight (FW, mg)

Tot.chl=Total amount of chlorophyll

The content of carotene pigments was measured with the method described by (26) at a wavelength of 480 nm and calculated following the formula:

$$\text{Carotene content (mg/100g FW)} = \frac{[(\text{Absorption at wavelength (480)} \times V \times (1000)}{100 \times 2500} \times 10$$

Percentage of carbohydrates

One-year-old branches were dried at 70 ºC until constant weight and then ground before further analysis. According to the method (26), 0.2 g of dried material with a perchloric acid was placed in water bath (60 ºC) for 60 minutes (carried out 3 times) before centrifugation at 3000 rpm for 15 minutes (per each). The three supernatants were collected in volumetric flask and distilled water was added to bring the volume to *100 ml*. 1 ml of the diluted solution was mixed with 1 ml of phenolic solution (5%) and (5) ml of concentrated sulfuric acid, then the read was done at 490 nm using spectrophotometry.

Concentration of total nitrogen and C/N ratio: A powdered sample of 0.5 g was digested by sulfuric and perchloric acids and then placed in Micro-Kejldahl equipment to estimate the total nitrogen content. For the

calculation of C/N ratio, the results of carbohydrates analysis divided by the results of nitrogen analysis.

Statistical analysis

Comparisons among treatments were performed by one-way analysis of variance (ANOVA). The *means* were *compared* by *least significant differences (LSD)* method. Statistical analyses were realized using *GenStat* software.

RESULTS AND DISCUSSION

Leaf area: A significant increase in the leaf area was indicated among all the treatments in the first study season (Figure 1). Manual and chemical thinning reported 28.46 and 28.38 cm^2 respectively in comparison to 25.80 cm^2 in the control. The thinning of fruit led to more vegetative growth in accordance with (37), because the heavy load declines vegetative growth and plant increases of nutrients flow to fruits formation. Also, bigger leaf areas were measured in foliar spray treatments with boron/zinc separately or together (27.67, 27.78, 28.13 cm^2). In second season, although leaf area significantly increased regardless of the treatment compared to control (30.75 cm^2), no considerable differences were noted between manual and chemical thinning and the foliar spray application. These results are agreed with (6) which showed that foliar spray with boron on the apple trees (Anna) increased the leaf area and with (28) who found that spraying urea, boric acid and zinc sulphate on olive trees (*Nabali*) was effective in optimizing the leaf area. Comparing the two seasons reveals a remarkable increasing in the leaf area in boron and zinc treatments individually (33.04, 33.16 cm^2) or combined (33.82 cm^2) in the second year compared to the first year (27.78, 27.67, 28.13 cm^2, respectively). Leaf area in control increased from 25.80 cm^2, in the first season, to 30.75 cm^2 in the second season as result of light fruits load in the second season. No noticeable differences were reported in the manual and chemical thinning between the two seasons- due to the balance in the annual load; resulting of the thinning in high load year -first season. Similarly, (4) explained that the flow of nutrition and the metabolism shift to fruiting or vegetative growth as two reverse phenomena which the prevalence of fruiting affects the balance of metabolic flow causing less vegetative development and leading to minimum new growth that are important for fruiting in the next year, and then the occurrence of alternative bearing.

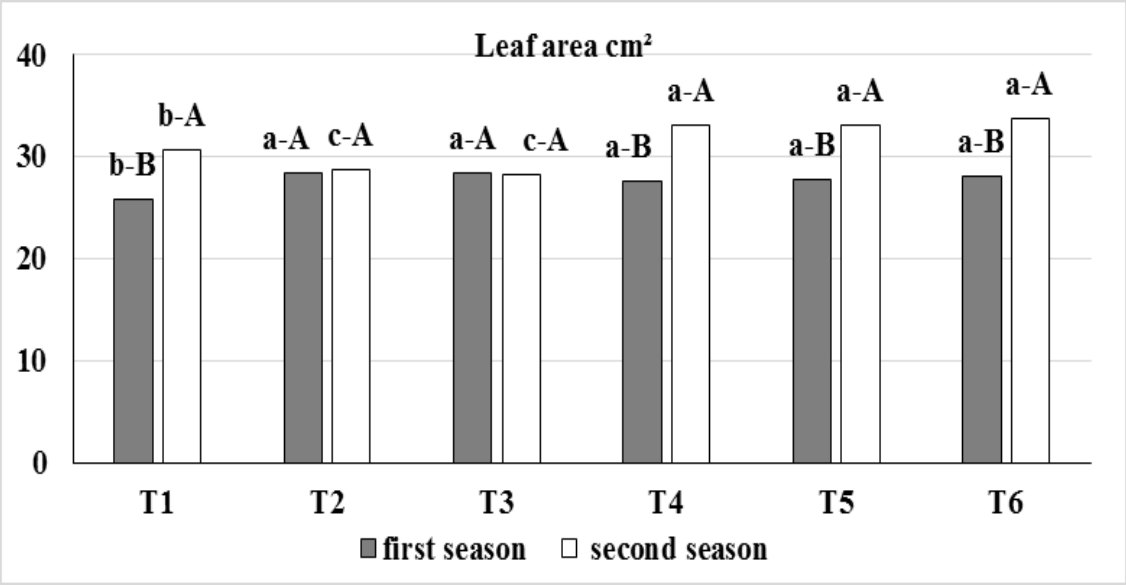

Figure 1. Effect of different treatments on leaf area (cm^2) in the two study seasons

***Small letters indicate the differences between treatments within the same season ($LSD_{0.05}$= 0.24, first season- 0.17, second season) while the capital letters compare the treatment during the two seasons where values of $LSD_{0.05}$= T_1= 1.24, T_2=0.68, T_3=0.71, T_4=1.71, T_5= 1.32, T_6= 2.46.**

Content of chlorophyll

Figure (2) shows an increase in the total chlorophyll content of leaves in the first season in each treatment compared to the control (1.28 mg/g). The values of manual and chemical thinning (1.53 and 1.59 mg/g), and the combined foliar spray of boron and zinc (1.81 mg/g) differed significantly while the

single foliar spray using boron or zinc (1.44, 1.48 mg/g) proved to be non-significance. On the contrary, in the second season the single zinc treatment (1.78 mg/g) and the boron/zinc together (2.22 mg/g) reported a significant increase compared to the control (1.53 mg/g) with no significant differences in the manual (1.55 mg/g) and chemical (1.58 mg/g) thinning and the foliar boron spray (1.70 mg/g) alone. These results agree with the findings of (5) who explained the important and critical role of nutrients in chlorophyll *synthesis* in leaves where the zinc participates in the chlorophyll production through its direct effect on the formation processes of amino acids, carbohydrates and energy compounds. Further, higher chlorophyll content in two grape cultivars (Halwani and Kamali) were marked when vineyards sprayed with nutritional solution consisting of Fe, Mn, B, Cu and Zn (12) or with 200 mg/l of zinc (7). Also, (3) found that spraying citrus with zinc considerably increased the total chlorophyll in the leaves. The comparison between the two seasons revealed a significant increase in the content of chlorophyll pigment in the second season in the control, the foliar spray by boron/zinc separately and together. This increase attributed to more vegetative growth and bigger leaf area resulting from the light bearing, which provides a higher chlorophyll biosynthesis and greater number of new shoots (usually are non-fruiting *shoots)* after a heavy load year (29). The data of two seasons exhibited no significant differences in both manual and chemical thinning treatments due to a balance between vegetative growth and fruit production in the second season (light bearing season).

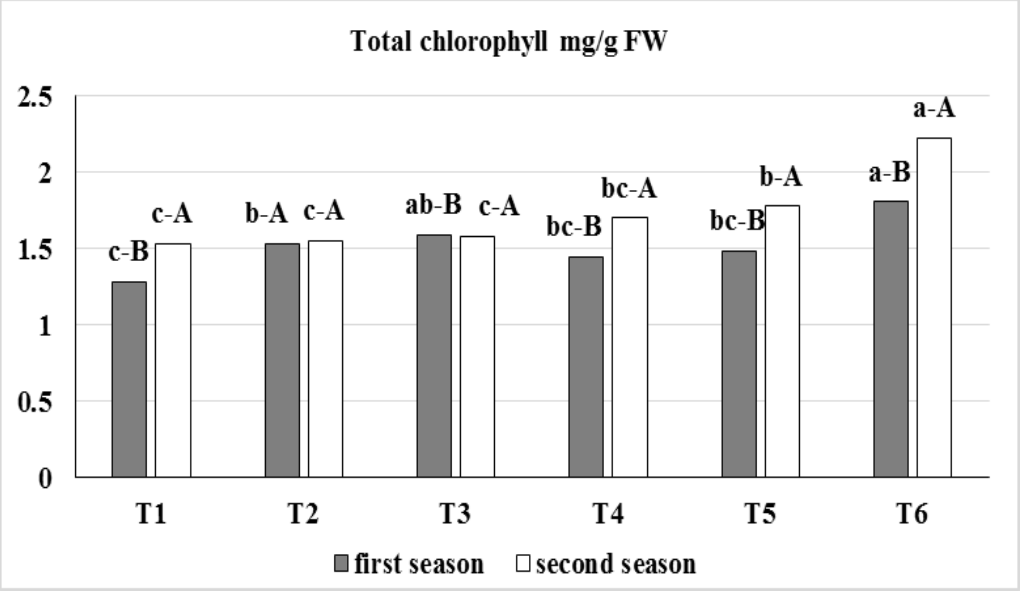

Figure 2. Effect of different treatments on the total chlorophyll content (mg/g FW)

***Small letters indicate the differences between treatments within the same season ($LSD_{0.05}$= 1.73, first season-2.18, second season) while the capital letters compare the treatment during the two seasons where values of $LSD_{0.05}$= T_1= 0.36, T_2=0.29, T_3=0.27, T_4=0.25, T_5= 0.218, T_6= 0.14.**

Content of carotene pigments

The presence of carotene pigment in leaves was studied (Figure 3). In the first season, there was no significant difference in the leaves of the manual (2.66 mg/100g) and chemical (3.05 mg/100g) thinning, foliar zinc spray (2.53 mg/100 g) and foliar zinc/boron spray (2.85 mg/100g) compared to the control (2.49 mg/100 g). A similar result was obtained also in the second season where all treatments showed non- significance in comparison to the control (2.97 mg/100g). Considering the two year of study, a significant increase in carotene content was observed in the second season compared to the first one, the control (2.49 vs 2.97 mg/100g), the single foliar spray with boron (2.74 vs 2.32 mg/100g) or zinc (3.02 vs 2.53 mg/100g) and the combination (3.47 vs 2.85 mg/100g) while the significance was absent in the manual (2.70 vs 2.66 mg/100g) and chemical (3.04 vs 3.05 mg/100g) thinning. Carotenoids may have more than one obligatory role in higher plants, but a basic function is the protection of chlorophyll from photooxidation (13)

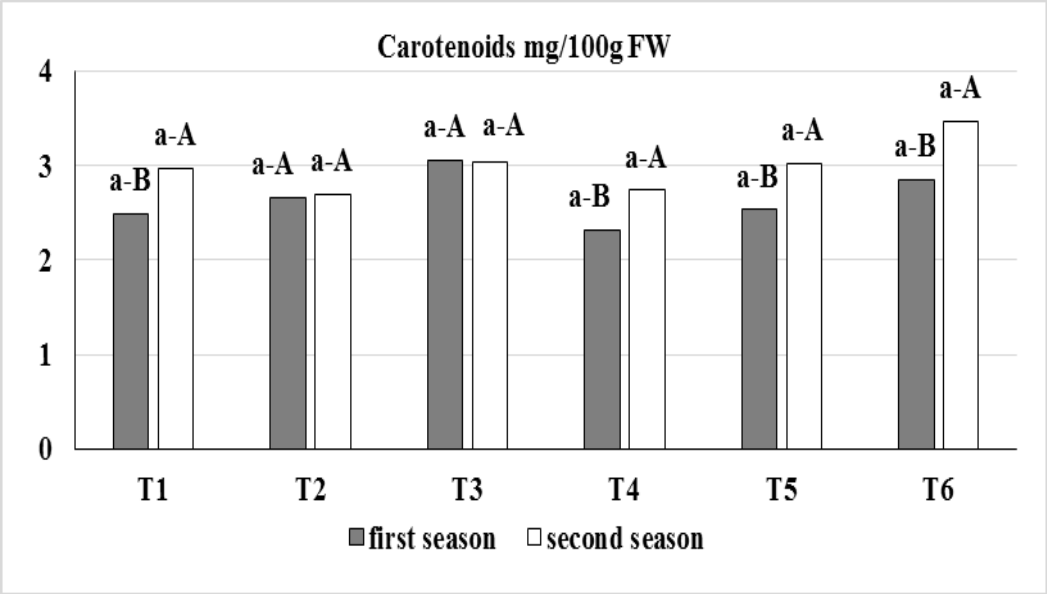

Figure 3. Effect of various treatments on the content of carotene pigments (mg/100g FW)

***Small letters indicate the differences between treatments within the same season ($LSD_{0.05}$= 0.90, first season- 0.35, second season) while the capital letters compare the treatment during the two seasons Where values of $LSD_{0.05}$= T_1= 1.24, T_2=1.22, T_3=0.27, T_4=1.83, T_5= 0.31, T_6= 0.52.**

Percentage of carbohydrates and total nitrogen and C/N ratio:

The first season (heavy bearing): Table (3) shows no significant differences in the content of carbohydrates and nitrogen in the branch because the analysis was conducted before the beginning of experiment (end of 2018- the year of light crop load) where all the trees were similar in term of low fruits bearing. In the following year, the percentage of carbohydrates and total nitrogen and C/N ratio increased according to table (3). During the light crop year, leaves absorb light and carbon dioxide with the presence of water and chlorophyll to produce carbohydrates as plant food and reserve the exceed amount as a starch in the late summer. High accumulation of sugar and nitrogen leads to developing a lot of floral buds and then flowers considering that buds' differentiation is held after a short period of the current year flowering and a long time before the flowering next year (35).

The second season (light bearing): Table (3) presents a significant increase in branches content of carbohydrate and nitrogen in the manual and chemical thinning in comparison with control. The percentage of carbohydrate in manual and chemical thinning treatments was 7.11, 7.01 % respectively, and in control 5.59% while the nitrogen concentration reported 0.83 % in the manual and chemical thinning treatments with 0.82% in the control. Also, the ratio of C/N significantly increased in both manual (8.59%) and chemical (8.47%) thinning when compared to the control (6.99%). Applying the manual and chemical thinning on fruit setting (heavy crop load) leads to provide sufficient nutrition and induces the formation of flowering buds next year instead of its depletion for the overload fruit growth in the current year. Actually, thinning increases the leaf area and supplies the essential nutrients for fruit growth with a possibility to accumulate a greater amount for buds formation next year, obtaining a nutritional balance between fruits setting in the first year and flowering in the following year. Since C/N ratio is the content of carbohydrates in branches divided by nitrogen amount so any increase in carbohydrates content will produce a higher carbohydrate and nitrogen ratio (1). These outcomes correspond to (32) who corelated between the carbohydrates content in organs and the regular cropping, a good and regular bearing is achievable when an adequate percentage of carbohydrates, in any part of the tree, is available during the proper time. That means no competition between the vegetative growth and fruit setting and supplies roots with their requirements of carbohydrates reaching a balance between production and consumption. On other hand, (31, 39) mentioned that

Table 3. Effect of different treatments on the percentage of total carbohydrates, total nitrogen and C/N ratio in the branches during the two seasons (2018, 2019) and (2020, experiment end)

	2018			2019			2020		
Treatment	Carbohydrates	Nitrogen	C/N	Carbohydrates	Nitrogen	C/N	Carbohydrates	Nitrogen	C/N
T_1	9.99a	0.91a	10.9a	5.69d	0.82b	6.99d	10.16a	0.90a	11.2a
T_2	10.07a	0.91a	11.05a	7.11a	0.83a	8.59a	7.34c	0.83c	8.88c
T_3	10.06a	0.91a	11.09a	7.01a	0.83a	8.46a	7.36c	0.83c	8.92c
T_4	9.76a	0.91a	10.72a	6.46b	0.82a	7.843b	8.25b	0.87b	9.48b
T_5	10.04a	0.91a	11.06a	6.12c	0.83a	7.42c	8.3b	0.88b	9.43b
T_6	10.07a	0.91a	11.13a	6.5b	0.82a	7.90b	8.07b	0.86b	9.38b
$LSD_{0.05}$	0.56ns	0.03ns	0.51ns	0.30	0.01	0.36	0.42	0.04	0.32
C.V%	3.22	2.59	4.23	5.28	1.14	1.88	5.27	3.01	1.78

chemical thinning of apple fruits is a very important management practice to achieve profitable fruit production while the chemical thinning reduces the labour costs required by manual thinning and increases the yield next season. (25) indicates that the manual and chemical thinning improves the fruit size, induces the differentiation of flower buds and reduces the phenomenon of alternative bearing. Table (3) indicates a significant increase in the branches content of carbohydrates and nitrogen, in the light bearing season when foliar spraying with boron (6.46 %) or zinc (6.12%) separately or together (6.50%) compared with the control (5.69%). No significant differences were reported among the tree treatments in term of nitrogen content while C/N ratio significantly increased when foliar spray applied with boron (7.84%) or zinc (7.15%) and together (7.90) versus 6.99% in control treatment. The content of the branches of carbohydrates and nitrogen (%) and the ratio of carbohydrates/ nitrogen were analysed at the end of the second season (2020). Figure (4) presents a percentage of C/N in the control (11.23%) increased significantly compared to the control (6.99%) in the light bearing season, predicting a heavy bearing in the year 2020, while a balance in the ratio was noticed between the manual (8.56%) and chemical (8.46%) thinning treatments in 2019 versus 8.88% and 8.92% in 2020. Also, the foliar spray with boron or zinc separately and combined controlled the incidence of alternate bearing reporting a significant decline (9.48, 9.443 and 9.38%, respectively) compared to the control (11.23%). (29), showed that increasing the amount of production leads to consume large quantities of nutrients, which affects the flowering in the following season, and thus increases the severity of alternative fruit bearing. Thus, reducing the crop load in the first season (heavy load season) helps to provide nutrients, improve production in the second year and contributes to alleviating the phenomenon of alternative fruit bearing.

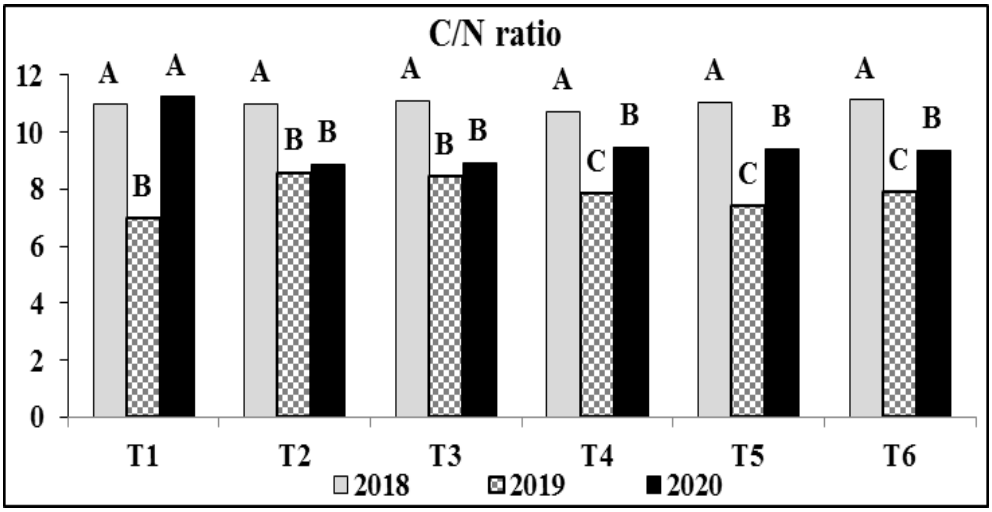

Figure 4. Effect of different treatments on C/N ratio in both study seasons (2018, 2019) and at the end of the experiment (2020).

*Capital letters compare the treatment during the seasons where values of $LSD_{0.05}$= T_1=0.32, T_2=0.32, T_3=0.49, T_4=0.26, T_5= 0.33, T_6= 0.41.

Conclusions

The application of manual thinning, chemical thinning using NAA, and foliar spraying with boron and zinc separately or together led to increase leaf area and its content of total chlorophyll and carotene in each season while an increase in the branches content of carbohydrates and nitrogen and C/N ratio was reported in the low load season. Therefore, both manual and chemical thinning treatments were more effective to control the alternative bearing phenomenon. Further research is required to evaluate the effect of manual and chemical thinning on apple fruit quality parameters (colour, peel, hardness, percentage of sugars and acids, calcium and potassium levels, which are very important for storage and cases of *physiological disorders*) and study its the effect of on other cultivars to reduce the negative impact of alternative bearing.

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