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Original Article

Using chitosan nanoparticles and N-acetyl thiazolidine 4-carboxylic acid for olive trees efficiency raising, improving fruits properties and oil quality

Uso de nanopartículas de quitosana e ácido N-acetil tiazolidina 4-carboxílico para aumentar a eficiência das oliveiras, melhorando as propriedades dos frutos e a qualidade do óleo

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Abstract

Recently exposure of olive trees to many stresses particularly oil varieties led to decline in the olive yield. The target of the study is to improve vegetative growth and increase olive fruits quality as well as the fruit oil % and oil quality by applying chitosan nanoparticles (CHNPs) and N-acetyl thiazolidine 4-carboxylic acid (N-ATCA) under the conditions of Egypt. The experiment was carried out in the seasons of 2021 and 2022 on Arbosana olive trees 8 years old and 4×6 m apart the trees sprayed three times on 15th Sept., 1st Oct. and 15th Oct. with (CHNPs at 500, 1000 and 1500 ppm), (N-ATCA at 50, 100 and 150 ppm) and a combination between them and evaluate the vegetative growth of trees, fruit physiochemical characteristics, and oil properties during both study seasons. The application of CHNPs and N-ATCA and a combination of them led to increasing leaf area, total chlorophyll and proline content also increment fruit weight, flesh weight, oil color and oil % moreover improving the quality of produced oil. The improvement in growth, fruit quality, oil % and oil quality, were associated with increasing concentrations of CHNPs, N-ATCA and a combination of them especially (CHNPs at 1500 ppm + N-ATCA at 100 ppm and CHNPs at 1500 ppm + N-ATCA at 150 ppm). Spraying (CHNPs at 1500 ppm + N-ATCA at 150 ppm) is recommended to improve the tree growth, fruit quality, oil % and quality of Arbosana olive.

Keywords: Arbosana, chitosan nanoparticles, N-ATCA, proline, olive oil, peroxide value.

Resumo

Recentemente, a exposição das oliveiras a muitos estresses, particularmente as variedades de azeite, levou ao declínio no rendimento da azeitona. O objetivo do estudo é melhorar o crescimento vegetativo e aumentar a qualidade dos frutos de oliveira, bem como a % de óleo do fruto e a qualidade do óleo, aplicando nanopartículas de quitosana (CHNPs) e ácido N-acetil tiazolidina 4-carboxílico (N-ATCA) nas condições do Egito. O experimento foi realizado nas temporadas de 2021 e 2022 em oliveiras Arbosana de 8 anos e 4×6 m de distância das árvores pulverizadas três vezes em 15 de setembro, 1º de outubro e 15 de outubro com (CHNPs a 500, 1000 e 1500 ppm), (N-ATCA a 50, 100 e 150 ppm) e uma combinação entre eles e avaliar o crescimento vegetativo das árvores, características físico-químicas dos frutos e propriedades do óleo durante as duas épocas de estudo. A aplicação de CHNPs e N-ATCA e uma combinação deles levou ao aumento da área foliar, teor de clorofila total e prolina, além de incrementar o peso do fruto, peso da polpa, cor do óleo e % de óleo, e melhorou a qualidade do óleo produzido. A melhora no crescimento vegetativo, qualidade da fruta, % de óleo e qualidade do óleo foram associados com concentrações crescentes de CHNPs e N-ATCA e uma combinação deles em especial (CHNPs a 1500 ppm + N-ATCA a 100 ppm e CHNPs a 1500 ppm + N-ATCA a 150 ppm). A pulverização (CHNPs a 1500 ppm + N-ATCA a 150 ppm) é recomendada para melhorar o crescimento das árvores, qualidade dos frutos, % de óleo e qualidade da azeitona Arbosana.

Palavras-chave: Arbosana, nanopartículas de quitosana, N-ATCA, prolina, azeite, valor de peróxido.

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1. Introduction

The olive tree is one of the most important plants for the Mediterranean agricultural economy (***Olea europaea*** L.), which is grown in approximately 95% of the world's olive groves. Most olive fruits are used to produce oil, with the remaining 10% becoming table olives, all over the world (Skodra et al., 2021; Diarte et al., 2019). Since it has a high nutritional content and advantageous effects on human health, olive oil is the main food in the Medi-terranean (El Qarnifa et al., 2019). Olives are one of the most significant oil crops in the world and their oil is the healthiest of all oils due to their high bioactive composition, antioxidant activity, sensory attributes, health benefits, and protection against numerous malignancies (Yubero-Serrano et al., 2019; Lozano-Castellón et al., 2020). Olive oil contains a significant amount of triglycerides, glycerol esters, and unsaturated fatty acids (98-99%) as well as an unsaponifiable fraction (2%), which is made up of phenols, sterols, fat-soluble vitamins, and carotenoids (Salas et al., 2013; Preedy and Watson, 2020; Miho et al., 2021). Many olive oil components, such as tyrosol, oleocanthal, apigenin, luteolin, luteolin-7-glucoside and rutin, are known to be effective antioxidants in treating various cancers, including breast, colon and prostate cancer (Hashim et al., 2014; Zubair et al., 2017; Calahorra et al., 2018).

Egypt is considered one of the major producers of olives around the world. Furthermore, it is the pioneer in cultivating olives in desert areas (Yacout et al., 2016).

Arbosana is one of the most popular and widely utilized cultivars in newly planted olive orchards using the intensive farming approach used in modern farms in Egypt. Medium-low vigor of trees, early bearing, a high constant yield of fruits, and good olive oil quality are the traits of these cultivars (Camposeo et al., 2021; Lo Bianco et al., 2021). Improved plant tolerance to adverse climatic circumstances increases the yield of trees and economic yield to farmers, so it must be studied and fully exploited.

Chitin, a cationic polymer with a large molecular mass obtained from crustacean exoskeletons, fungi, insects, some algae, is deacetylated to create chitosan. Among the most often used biodegradable polymers is chitosan. Where it has been utilized in various agricultural and horticultural applications because of its exceptional capacity to form an anti-bacterial and antifungal film that is biocompatible, biodegradable, and nontoxic to humans (Muzzarelli et al., 2012; Suhag et al., 2020). Chitosan treatments may make plants less susceptible to stress brought on by unfavorable conditions including drought, salt, low or high temperatures (Shao et al., 2015; Liu et al., 2011). Treatment of the olive trees with Chitosan led to increase productivity, enhance product quality, and reduce negative environmental effects (Zhang and Brown, 1999). Spraying chitosan (500 ppm) on Picual olive trees after berry setting increased growth, yield and enhanced the chemical and physical properties of both the fruit and oil (Kasem and Fawzy, 2020). Utilizing chitosan on mungbean (*Vigna radiate*) resulted in increased activity of crucial enzymes involved in nitrogen metabolism, as well as improved nitrogen (N) transportation in functional leaves, both of which boosted vegetable growth, fruit yield, and oil % (Mondal et al., 2013). The chemical structure of chitosan showed in Figure 1a.

N-acetyl thiazolidine 4-carboxylic acid (N-ATCA) is a brand-new plant bio-regulator that can promote the uptake of major and micronutrients; enhances enzymatic activity, and photosynthesis; and encourages protein synthesis since it contains the amino acid, increasing valine and glutamine content, also raising resistance in plants to drought and temperature stress. Furthermore, triggers the production of hormones including IAA and gibberellins, which aid in fruit set and flowering (Ramteke and Khot, 2015; Hota et al., 2017). N-ATCA is a blend of organic amino acids that are utilized as activators in fruit trees, and other crops. It enhances the metabolic processes of the crops, as a fruit improvement, a bio-stimulant germination enhancer, to boosts vegetative growth and the production of chlorophyll in the leaves, and helps increase the yield potential of plants, causing the synthesis of hormones and amino acids in plants, which are necessary for their healthy growth and development (Hota et al., 2019). The chemical structure of N-acetyl thiazolidine-4-carboxylic acid showed in Figure 1b.

Several investigations were being made to evaluate the dangers that N-ATCA might pose to people, animals, and the environment. N-ATCA didn't record any negative consequences in harmful to the growth, composition, and blood flow of the liver, or reproductive system, currently no evidence that ATCA use causes mutagenic effects (Chauhan et al., 2018; Hota et al., 2019).

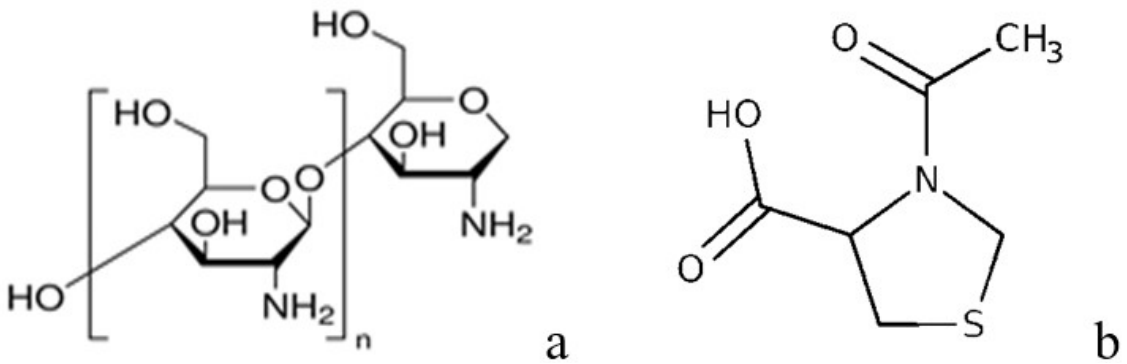

Figure 1. The chemical structure of chitosan (a) and N-acetyl thiazolidine-4-carboxylic acid (b).

The N-ATCA compound's beneficial effects on plants are related to the positive effects of L-proline and L-cysteine in biochemical reactions at the cellular level N-ATCA, allowing it to successfully cross metabolic barriers and slowly release the amino group of acids into cells. Through a series of biochemical reactions, N-ATCA is gradually converted to thioproline (TCA), formyl cysteine, and finally to L–cyeteine (Hota et al., 2019). In addition to Hota et al. (2017) found that the application of N-ATCA at 50-100 ppm on apricot trees increased fruit set, fruit retention, decrease fruit drop percentage, increased fruit size, and increased yield. Foliar spray of (N-ATCA) on Starking Delicious apple at the petal fall stage (1.0 ml/L) recorded the maximum fruit set, the color of fruit, lowest fruit drop, highest yield, and fruit weight (Chauhan et al., 2018). N-ATCA amazingly increases different growth parameters and crop yield. Their utilization may continue to remodel achievement by upgrading the development of moderately developing nearby plants and transplanted seedlings and cuttings.

Recently as a result of bad climatic changes and the exposure of olive trees to many stresses, it is led to a decline in the yield of olive trees, particularly oil varieties. So, the study's main is to improve plant tolerance to stress and increase the trees' yield of fruits as well as the oil percentage and oil quality by applying some materials has not previously been studied that affect olives yield and the percentage of oil and oil quality under the conditions of the Arab Republic of Egypt.

2. Material and Methods

2.1. Materials

2.1.1. Chemicals

All chemicals were purchased from Sigma-Aldrich (USA). Every single reagent and solvent was of analytical grade.

2.1.2. Olive samples

The research was carried out in a private olive grove on eight-year trees (*Olea europaea* L.) of Arbosana cv., located at Wadi Al-Natrun region (30°24'01.7"N 30°02'20.6" E), El- Behera Governorate, Egypt, planted in sandy soil at 4 ×6 m, under a drip irrigation system, the weather data were recorded under location of study in 2021 and 2022 seasons by Central Laboratory for Agricultural Climate as shown in Figure 2.

2.2. Preparation methods

2.2.1. Preparation of chitosan nanoparticles (CHNPs)

To prepare chitosan nanoparticles (CHNPs) chitosan was dispersed in 1% aqueous acetic acid. The pH was then adjusted to 5.5 using NaOH. Sodium tripolyphosphate solution (1%) was added to the chitosan solution. The resultant suspension was stirred for 40 minutes (Algarni et al., 2022). Analyses using transmission electron microscopy (TEM) were carried out.

2.2.2. Experimental design and preparation of treatments

Chitosan nanoparticles (CHNPs), N-acetyl thiazolidine-4-carboxylic acid (5% w/v), and mixes of them at different levels were sprayed on olive trees on the 15th September, the 1st of October, and the 15th of October. Three replicates for each treatment and three trees were chosen randomly per replicate, seven liters of spray solution for every tree, and a mini drops of tween 20 were added to all spray solution to reduce the surface tension of the droplets and facilitate absorption beside citric acid to decrease the pH of the solution to 5.5% in both seasons as follows (Table 1):

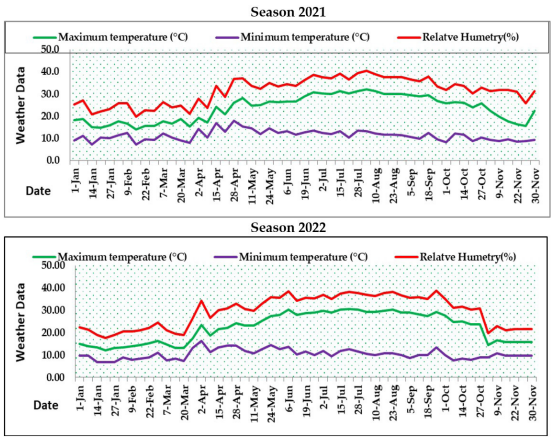

Figure 2. Prevailing temperatures °C and relative humidity % under location of study during the period from January to November 2021 and 2022 seasons.

Table 1. Pre-harvest foliar sprays of Arbosana olive cultivar.

Foliar spray material	Concentration	Symbol
Water (control)	0.0	T0
CHNPs	500 ppm	T1
CHNPs	1000 ppm	T2
CHNPs	1500 ppm	T3
N-ATCA	50 ppm	T4
N-ATCA	100 ppm	T5
N-ATCA	150 ppm	T6
CHNPs + N-ATCA	500 ppm+50 ppm	T7
CHNPs + N-ATCA	1000 ppm+100 ppm	T8
CHNPs + N-ATCA	1500 ppm+150 ppm	T9
CHNPs + N-ATCA	1000 ppm +50 ppm	T10
CHNPs + N-ATCA	1000 ppm +100 ppm	T11
CHNPs + N-ATCA	1000 ppm +150 ppm	T12
CHNPs + N-ATCA	1500 ppm +50 ppm	T13
CHNPs + N-ATCA	1500 ppm +100 ppm	T14
CHNPs + N-ATCA	1500 ppm +150 ppm	T15

2.3. Analytical methods

2.3.1. Chitosan nanoparticles describe

2.3.1.1. Size and shape

Using a transmission electron microscope with a 120 kV acceleration voltage (JEOL, JEM-1230, Tokyo, Japan), chitosan nanoparticles (CHNPs) were analysed for size and shape.

2.3.1.2. Chemical composition

The chemical structure of pure olive fruit in comparison to those modified with chitosan and N-acetyl thiazolidine-4-carboxylic acid employing a Nicolet Nexus 8700 FT-IR spectrometer (Nicolet, USA) in 500 to 4000 cm^{-1} range wavenumber by accumulating 32 scans where the resolution was 4 cm^{-1}.

2.3.2. Vegetative growth

2.3.2.1. Leaf area cm^2

A sample of fifteen mature leaves per tree was abscised after harvesting fruits only the fifth leaf per shoot on the 15th and 7th of November for the Arbosana olive variety in both seasons respectively to determine leaf area (cm^2) according to Ahmed and Morsy (1999).

2.3.2.2. Total chlorophyll (SPAD unit)

Nine mature olive leaves from each tree only the fifth leaf per shoot was picked in order to measure the amount of total chlorophyll at 15th Sept.; 21st Sept.; 7th Oct. and 15th Nov. Two positions were calculated for every lamina of the leaf by Minolta SPAD-502 chlorophyll meter, and total chlorophyll was calculated as (SPAD unit) according to Rosado et al. (2002).

2.3.2.3. Proline µmol/gm f.w

Proline content of leaf samples was determined at 15ath Seb; 21st Seb; 7th Oct and 15th Oct. A 0.5 g sample of leaf and mashed and mixed with sulfosalicylic acid 3% (w/v) then in a water bath to be heated at 100 °C. Then add the reagent mixture (glacial acetic acid with ninhydrin) and for 1 h boil at 100 °C, then added toluene after cooling the solution. Toluene-containing chromophore was isolated, and absorbance at 520 nm was measured using toluene as a blank. The concentration of proline was determined according to Bates et al. (1973).

2.3.3. Fruit characteristics

Harvesting Arbosana olive fruits were carried out on the 15th and 7th of November in both seasons respectively at the ripening stage and end of color change according to Vidal et al. (2019). Only healthy fruits that had not been physically harmed or infected were chosen and covered after being harvested. The samples of olive fruit were delivered right away to the laboratory of the Horticulture to determine the fruit characteristics (fruit appearance, fruit weight (g) and flesh weight (%)).

2.3.4. Oil characteristics

2.3.4.1. Preparation and extraction of olive oil

Oils were extracted from fruits during the first 24 hrs after harvest. The fruits were extracted in an Abencor system that included a hammer crusher, malaxer, and centrifuge. The olive fruits were cleaned and then slowly blended for 30 minutes at 25 °C. The resulting paste was then centrifuged for one minute at a speed of 3000 rpm. It allowed for the oil phase to naturally decant into the sample. The top oil layer was taken off and kept until analysis in dark glass vials at -18 °C.

2.3.4.2. Oil color

Using the Minolta colorimeter CR400, oil color was measured (Konica, Osaka, Japan). 10 ml of the oil sample was placed into a 25 ml glass cup, and recorded, chroma (C) and hue (h). $C = (a^*2+b^*2)^{1/2}$ and $h = b^*/a^*$ (Xylia et al., 2019).

2.3.4.3. Free fatty acids (%)

The percentage of free fatty acids (as oleic acid) in the sample oils was calculated using a method adapted from European Union Commission (2013), where the oil samples were diluted in neutralized ethanol-diethyl ether solvent (1:1 v/v) prior to titration against 0.1 (M) NaOH.

2.3.4.4. Peroxide value (PV)

Based on the European Union Commission approach (2013), the peroxide value (PV) was calculated. In a nutshell, a 5 g sample was weighed, mixed with 2/3 isooctane / acetic acid, and agitated until dissolved. 1 ml of potassium iodate solution was added, and the sample was agitated for 1 minute. Then 25 ml of water had been added and the sample was titrated with a 0.01 M sodium thiosulfate solution. PV was expressed as milliequivalents of active oxygen per kilogram of oil (mEq O_2/Kg oil).

2.4. Statistical analysis

A three-replicate, fully randomized strategy was used to collect the data. Using SPSS software, the variance examination (ANOVA) was performed (version 22.0) at the level of $p<0.05$, and Duncan's multiple range test was employed to determine mean separation. The results are presented in terms of mean value and standard deviation (SD).

3. Results and Discussion

3.1. Characterization results

The Transmission Electron Microscope (TEM) imaging of the chitosan nanoparticles sample revealed their morphological characteristics, which include a nearly spherical shape, a smooth surface, and an average diameter of 20.3 nm (Figure 3).

3.2. The chemical composition

As shown in Figure 4a the main IR bands for the surface of olive fruit are attributed as follows: the band at 700 and 758 m^{-1} were observed which related to a

mono substituted aromatic ring vibration. Other ring vibrations are responsible for the bands at 841 and 905 cm^{-1} (Fahmy and Friedrich, 2013). Additionally, bands of aromatic phenyl groups were seen in the range of 1660 to 2000 cm^{-1}, with the band at 1600 cm^{-1} (ring) and bands at 1450 and 1490 cm^{-1} (doublet, ring) being connected to vibrations of the aromatic rings CH and CC, indicating their dominance. Additionally, the bands correlated to aliphatic and aromatic (symmetric and asymmetric) C–H stretching vibrations were detected. The aliphatic ones (υC–H ali) are found in the range of 2850–3000 cm^{-1} wave numbers where the aromatic C–H stretching vibrations of phenyl ring were observed at 3000–3100 cm^{-1} (υC–H arom. ring) (Fahmy et al., 2022).

In case of N-ATCA coated a broad band that might be generated from OH-stretching band (υOH) of -COOH groups in proline from 2500 to 3600 cm^{-1} was also observed (Figure 4b). However, the intensity of C=O that was observed in the range from 1650 to 1780 is weak indicating that the layer of N-ATCA was deposited on the olive fruit as a thin film. On the other hand, the spectra of olive fruit that was coated by CHNPs is presented in Figure 4c. It was observed that the main peaks those related to the chitosan structure such as the broad band ranging from 3600-3200 cm^{-1} which corresponded to the OH-stretching are shown (Kamari et al., 2011; Pandey and De, 2017). It can be indicated that the most peaks those related to the olive fruit were overlapped with those corresponded to the CHNPs.

3.3. Vegetative growth

3.3.1. Leaf area (cm^2)

Leaf area is one of the most essential variables influencing photosynthesis. Spraying were overlapped with those corresponded to the CHNPs and N-ATCA leads to increased leaf area of Arbosana olive as compared to the control, as demonstrated in (Figure 5). Furthermore, the data showed that (T15 and T14) recorded significant increments in leaf area cm^2 compared to other treatments especially T0 in both seasons of the study. The data agree with Ramteke and Khot (2015) who indicated that the treatment of N-ATCA along with GA3 and CPPU increased the leaf area and total chlorophyll in Sonaka grapes. Chitosan Foliar on Picual olive trees at 250, 500 and 1000 ppm significantly promotes the vegetative growth, leaf area (cm^2), shoot length (cm.), and leaves number per shoot rather than the control (Kasem and Fawzy, 2020). In diverse crops, chitosan operates as a growth regulator by enhancing, enzymatic and antioxidant activity, as well as providing some amino compounds essential for plant growth which is reflected in the vegetative growth (Kamari et al., 2011; Salachna and Zawadzinska, 2014; Pandey and De, 2017).

3.3.2. Total chlorophyll content (SPAD unit)

The application of CHNPs and N-ATCA significantly affected the amount of total chlorophyll in olive leaves compared with untreated trees (Figure 6). Significantly rising total chlorophyll was observed in olive trees treated by T15 and T14 followed by T12 and T11 respectively during all times of measuring in both studied seasons.

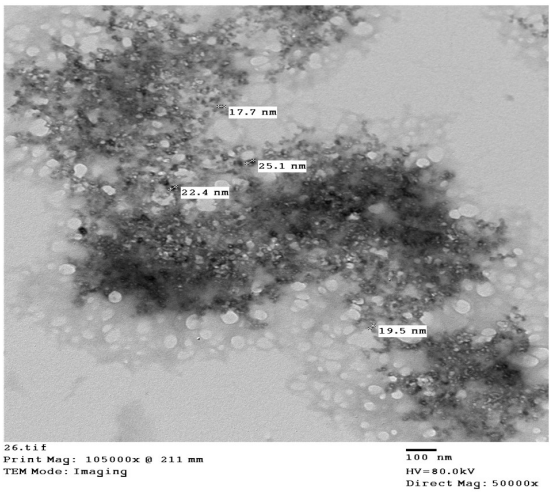

Figure 3. TEM of chitosan nanoparticles.

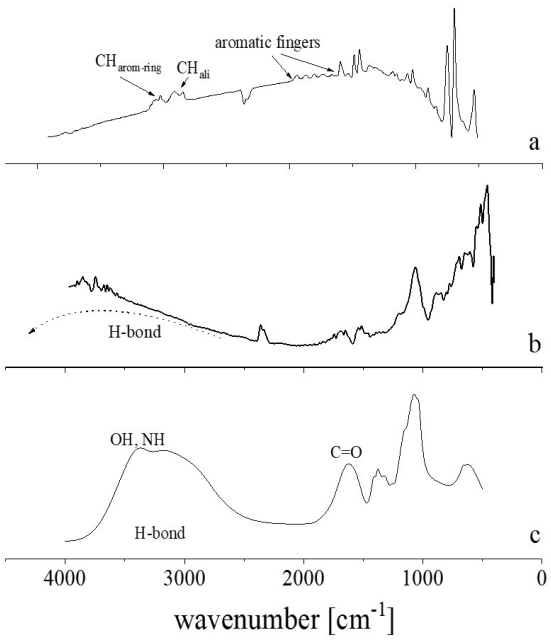

Figure 4. FTIR spectra for the surface of olive fruit (a) compared to that coated with N-ATCA (b) and CHNPs (c) layers.

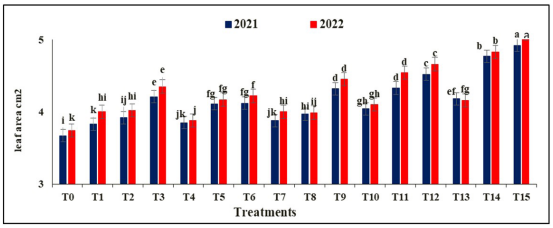

Figure 5. Effect of foliar spraying with chitosan and N-ATCA on the leaf area cm^2 of Arbosana olive in 2021 and 2022 seasons. Bars indicate mean values ± SE (n = 9). Different letters above columns indicate Duncan's Multiple Range Test at $P = 0.05$).

Meanwhile, the control had the lowest level of total chlorophyll. There were no significant variations in the total chlorophyll of the studied samples in the 15th Sep., due to the collecting of leaf samples before applying foliar treatments on Arbosana olive leaf in both seasons. A similar trend was observed by Kasem and Fawzy (2020) who find that spraying chitosan at 500 ppm on Picual olive trees significantly increased total carotenoid and total chlorophyll compared with the control. When chitosan is applied, important nitrogen metabolism enzymes perform more actively and nutrients are transported more efficiently in functioning leaves, which increases the amount of total chlorophyll and boosts plant development (Mondal et al., 2013).

Moreover, chitosan has an effect on the expression of chloroplast genes and thus develops the chloroplast enlargement and size, which is an agent that leads to the rise in the total chlorophyll content (Limpanavech et al., 2008). Moreover, Hota et al. (2017) found that, the spray of N-ATCA on apricot trees increased the leaf chlorophyll content and leaf area. Exogenous amino acids sources like N-ATCA that are absorbed by the leaves increase the activity of enzymes (phosphatase, nitrate reductase, phosphorylase and peptidase), which increases the amount of chlorophyll and the rate of photosynthetic activity.

3.3.3. Proline content

The accumulation of proline in the olive tree is an adaptive mechanism to tolerate stress. Spraying olive trees with varying concentrations of CHNPs and N-ATCA produced and caused a significant rise in proline content in the leaves compared with control trees (Figure 7). Due to the collecting of leaf samples before applying foliar treatments on olive trees in both studied seasons, no significant deviations in the proline of the studied samples in the 15th Seb. In both seasons, the largest proline contents were found at (T15; T14; T12, and T11) respectively, while the lowest proline content in treated trees was recorded in (T4; T3; T2; T1 and control) in all times of measurement. The findings support those of Hota et al. (2019) who found that N-ATCA has its active sites adequately protected, letting it transit metabolic barriers adequately and start releasing the amino acids slowly into the cells as an effect of the enzymatic modification. N-ATCA has gradually transformed into L-proline and L-cysteine. The different concentrations of chitosan did not have a significant and clear effect on proline content as in N-ATCA, It may be because chitosan works to increase the concentration of other amino acids, like glycine and glutamine. In the same direction Sheikha and Al-Malki (2015) reported that being treated with chitosan led to increasing amino acid concentrations,

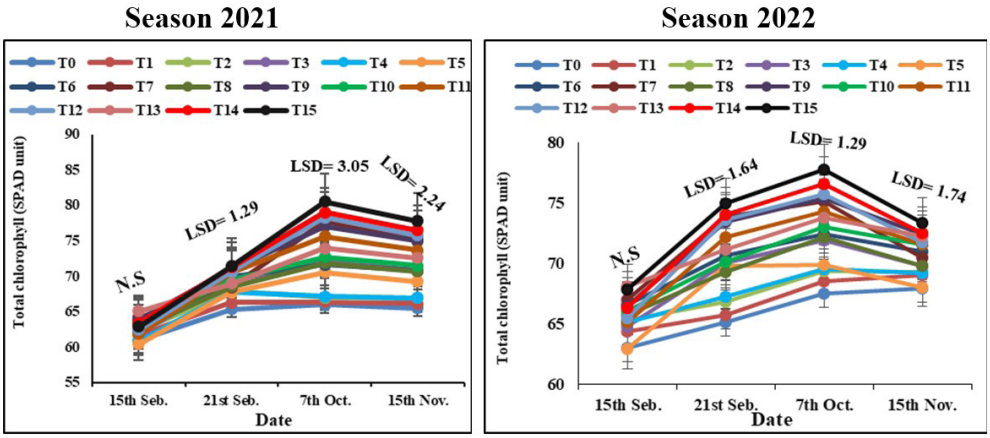

Figure 6. Chitosan and N-ATCA foliar spraying's impact on the amount of total chlorophyll.

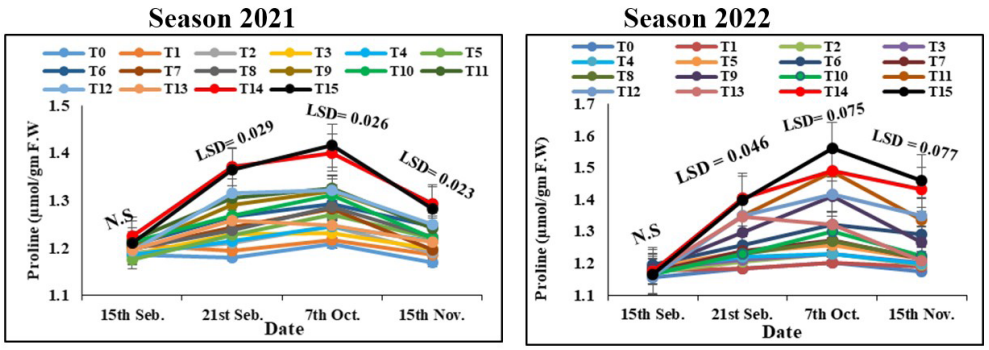

Figure 7. Chitosan and N-ATCA foliar spraying impact on the proline content µ mol/gm F.W of Arbosana olive leaves in 2021 and 2022 seasons.

especially aspartate, alanine, and glutamate glycin, betain, glutamin and aspargin in Phaseolus vulgaris Super stryke

3.4. Fruit characteristics

3.4.1. Fruit appearance

It was clear from the data in Figure 8 that the appearance quality of Arbosana olive fruits, is affected by spraying by CHNPs and N-ATCA also fruit color and volume were affected positively regardless of treatments or concentration. Among all treatments in this study, (T14, T15, and T12) showed the best appearance of Arbosana olive fruits in the 2021 and 2022 seasons it also agrees with the previous data, applying CHNPs and N-ATCA (T14, T15, and T12) resulting in raised leaf area, total chlorophyll, and proline content and leads to increased fruit weight, flesh weight % reflected in increased the fruit appearance in Figures 9 and 10.

3.4.2. Fruit weight (g) and flesh weight (%)

The data clearly show that the various concentrations (CHNPs and N-ATCA) considerably enhanced the weight of the Arbosana olive fruit in the 2021 and 2022 seasons as indicated in Figure 9. Among all treatments under study (T15, T14, T12 and T11) recorded the highest weight and flesh weight (%) for Arboasna olive fruit, on the other hand, the minimum fruit weight and flesh weight (%) was recorded in T0 and T1 in both seasons. In the same vein spraying Picual olive trees with chitosan at 500 ppm significantly increased fruit weight and volume and flesh weight (%), spraying chitosan leads to stimulates nutrient uptake, biosynthesis of the natural hormones, plant pigments, stimulates photosynthesis, and sugars, finally enhancing the cell division process (Savvas et al., 2002; Kasem and Fawzy, 2020).

The application of N-ATCA affected apple fruit weight, maximum fruit weight was recorded at 1 and 1.5 ml/L (Chauhan et al., 2018). N-ATCA application increase amino acids, folic acid, and promoting chemicals which leads to fruit growing, carbohydrate sink strength and increase cell enlargement caused fruit development (Dokoozlain, 2000; Chauhan et al., 2018).

3.5. Oil characteristics

3.5.1. Fruit oil (%)

As shown in Figure 10 CHNPs and N-ATCA foliar application on Arbosana olive trees Led to improved fruit oil (%) in all treatments compared with untreated trees, while the spraying with (T15 and T14) showed a substantial increase in fruit oil (%) compared to all treatments, while the minimum of fruit oil (%) observed in (T0; T1; T2 and T4) respectively in both seasons. The results agree with Kasem and Fawzy (2020) who indicated that spraying chitosan on Picual olive with concentration of 500 ppm had a significant relative enhancement of leaf pigments and nutrients which leads to the increased oil % of Picual olive fruit. Due to the insufficient research in this part, the increase in fruit oil (%) may be due to the CHNPs and N-ATCA working on increasing the concentration of amino acids, plant growth promoter, total chlorophyll fruit weight, flesh weight.

3.5.2. Oil color (l, c, h^{o})

As shown in Table 2 spraying CHNPs and N-ATCA on Arbosana olive trees improves oil color where the value of (h) decreased which means an increase in color clarity and (l) was decreased which improves the brightness of the fruits and (c) was increased It is an indicator of chroma of color, while (T15 and T14) recorded a significant increase (l and h) and decrease (c) compared to all treatments, while the maximum of (l and h) values was observed in (T0; and T1) respectively in both seasons. In the same vein Elkarmout et al. (2022) reported chitosan applied at 1% (w/v) as a spray that chitosan successfully prevented carotenoid and other pigment losses under salinity stress, chitosan forms a semipermeable membrane that regulates gas exchange, lowering transpiration and leading to ripening (Dutta et al., 2009). Furthmore, Chauhan et al. (2018), found that sprayed apple cv. Starking Delicious with 1 and 1.5 (ml/L) of N-ATCA increased the content of dry matter, carbohydrate metabolism, ripening process, fruit color, and their quality

Figure 8. Effect of foliar spraying with chitosan and N-ATCA on the appearance of Arbosana olive fruit in 2021 and 2022 seasons.

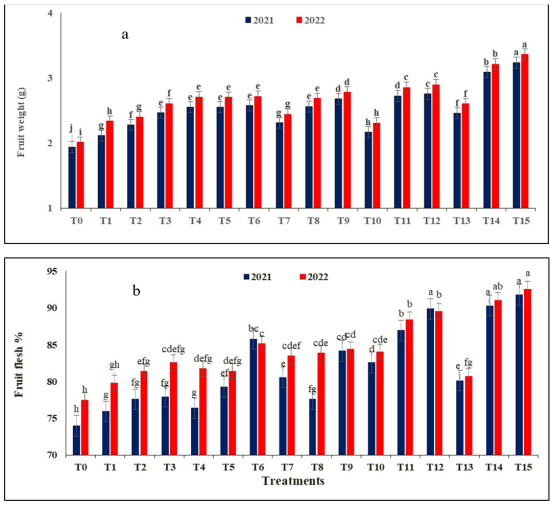

Figure 9. Effect of foliar spraying with chitosan and N-ATCA on the fruit weight (a) and flesh weight (b) of Arbosana olive in 2021 and 2022 seasons. Bars indicate mean values ± SE (n = 9). Different letters above columns indicate Duncan's Multiple Range Test at P = 0.05).

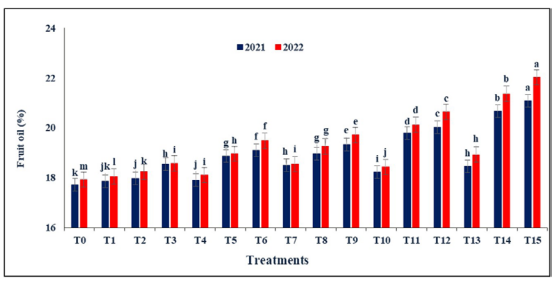

Figure 10. Effect of foliar spraying with chitosan and N-ATCA on the fruit oil % of Arbosana olive in 2021 and 2022 seasons. Bars indicate mean values ± SE (n = 9). Different letters above columns indicate Duncan's Multiple Range Test at P = 0.05).

3.5.3. Physicochemical properties

Olive oil quality grades can be evaluated using physicochemical criteria. The free fatty acids (FFA) and peroxide values (PV) were measured. The fresh olive oils were characterized as extra virgin olive oil (EVOO) based on the parameters of the experiments (IOC, 2021) as shown in Table 2.

3.5.4. Free fatty acids

Free fatty acid values (FFA) are used as a quality indicator for olive oil, olive types, producing areas, processing techniques, and procedures used (Konuşkan, 2020). FFA values (expressed as percentage of free oleic acid) of our oils vary between 0.332 and 0.530% (Table 3). These acidity index readings in both of the analysed seasons are below the IOC (2021), 0.8% EVOO limit, demonstrating the high caliber of the oil produced from healthy olives under less-than-ideal but closely monitored conditions. These findings concur with the FFA estimate made by Rodrigues et al. (2021). Moreover, Arafat et al. (2022) reported FFA were 0.19 and 0.25% for Arbosana olive oil cultivars during two consecutive seasons. The difference in the degree of phosphatide and triglyceride hydrolysis and the liberation of free fatty acids may be the cause of the variance in free fatty acid value. In addition, the breaking and oxidation of double bonds during oxidation results in the generation of free fatty acids (Arafat et al., 2022).

3.5.5. Peroxide value

When oils are exposed to oxygen in the air, they often oxidise or autoxidize. This is un-desirable since it has an impact on the sensory attributes of the oil because oxidation results in the production of rancid aromas. Hydroperoxides are the cause of the peroxide value (PV) (first stage of oxidation). Hence, PV is yet another crucial test that must be carried out on the oil.

Table 2. Effect of foliar spraying with chitosan and N-ATCA on the oil color of Arbosana olive fruit in 2021 and 2022 seasons.

Season \ Treatments	2021			2022		
	Lightness (L)	Chroma (C)	hue angle (hº)	Lightness (L)	Chroma (C)	hue angle (hº)
T0	84.71	0.64	86.28	83.24	0.63	89.09
T1	84.74	0.67	86.12	84.32	0.66	88.94
T2	84.83	0.69	85.87	84.25	0.69	88.60
T3	84.75	0.77	84.56	84.19	0.75	87.33
T4	84.93	0.70	84.97	83.86	0.71	87.75
T5	85.27	0.76	84.10	83.51	0.76	86.08
T6	86.04	0.80	83.81	85.40	0.78	85.05
T7	85.51	0.81	84.30	84.54	0.82	86.31
T8	85.62	0.79	84.12	84.42	0.80	86.09
T9	86.83	0.82	83.73	85.67	0.81	84.97
T10	84.73	0.78	84.90	84.60	0.78	86.92
T11	86.78	0.83	83.50	85.99	0.84	84.79
T12	86.29	0.83	82.99	85.92	0.84	84.46
T13	84.85	0.79	83.90	84.91	0.79	85.63
T14	87.08	0.85	82.39	85.90	0.86	83.72
T15	87.36	0.87	82.34	86.08	0.87	83.66
LSD at 5%	0.33	0.045	0.77	0.87	0.017	0.71

Table 3. Quality indicators (free fatty acids and peroxide value) in fresh Arbosana varietal extra virgin olive oils.

Season \ Treatments	2021		2022		IOC Standard 2021	
Samples	Peroxide value	FFA	Peroxide value	FFA	Peroxide value	FFA
T0	7.11	0.479	7.00	0.445	≤ 20***	≤ 0.8***
T1	7.11	0.388	6.95	0.394		
T2	9.35	0.510	8.55	0.451		
T3	9.40	0.521	8.60	0.473		
T4	9.48	0.407	8.45	0.425		
T5	9.37	0.372	8.50	0.382		
T6	9.50	0.357	8.64	0.355		
T7	9.21	0.349	8.43	0.362		
T8	8.58	0.437	8.00	0.421		
T9	8.75	0.530	8.20	0.515		
T10	7.20	0.408	6.94	0.393		
T11	6.17	0.390	6.12	0.410		
T12	7.46	0.439	7.30	0.435		
T13	6.67	0.401	6.78	0.410		
T14	6.62	0.334	6.70	0.350		
T15	6.58	0.332	6.63	0.341		

Peroxide value (meq O2/kg), FFA (% of oleic acid).
***Actual criteria for the category of extra virgin olive oil (IOC, 2021).

The PV values of olive oil tested samples were determined, and the obtained results were shown in Table 2. According to the findings, the PV (meq O_2/kg oil) ranged between 6.17 and 9.50 in the first season and 6.12 to 8.64 in the second season, which is consistent with a high-quality EVOO and is much lower than the limit of 20 mEq O_2/kg, so it is included in the extra virgin olive oil classification (IOC, 2021). This result is consistent with that of Arafat et al. (2022) who observed that the peroxide value ranged from 1.20 to 3.20 meq O_2/kg for oils derived from the Arbosana cultivar.

In our experiment, the peroxide value was considerably higher in T2 to T9 samples (ranged from 8.58 to 9.50 meq O_2/kg in the 2021 season and 8.20 to 8.64 in 2022) compared to other tested samples (ranging from 6.17 to 7.46 meq O_2/kg in 2021 and from 6.95 to 7.30 in season 2022). Given that PV values stayed below the limit established by the IOC standards for the EVOO group (IOC, 2021), this increase should be regarded as mild. The minor increase in PV is mirrored in the high oxidation of olive oils, caused by factors like processing circumstances, transport, and storage. Because of oxygen's role in the lipoxygenase cascade events and subsequent favorable sensory notes, this quality criterion is extremely important. In fact, too much of it can cause unfavorable flaws (Zanoni, 2014; Krichene et al., 2010).

4. Conclusion

Applying (CHNPs) and (N-ATCA) only or a combination of them on Arbosana olive trees three times on 15th Sept., 1st Oct. and 15th Oct. resulted in raising the efficiency of olive trees against various stresses as it increased (leaf area, total chlorophyll and proline content) and increased the fruit quality represented in the (fruit weight, flesh weight, oil color and oil %) moreover improvement oil properties (peroxide value and free fatty acids), especially combination between (CHNPs at 1500 ppm and N-ATCA at 150 ppm).

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