



Effect of Some Antioxidant Foliar Sprays on Growth and Productivity of Olive Trees in North Coast Region

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Abstract

THIS investigation was carried out on Picual olive trees during the two successive seasons of 2022 and 2023 at Matrouh Governorate, Egypt. The purpose of this study is to evaluate the effect of glycine betaine, glutathione, and chitosan foliar sprays on improving vegetative growth characteristics, blooming, yield properties, and fruit physical and chemical properties of Picual olive trees under a rain-fed production system. The results indicated that foliar spray of glycine betaine at 50, 100 ppm, and glutathione at 50 and 100 ppm are suggested to increase yield and quality. With higher percentages of flowering, perfect flowers, initial and final fruit set increase productivity. Also, they ameliorated fruit's physical properties (fruit weight, length & diameter, flesh weight & flesh dry matter) and fruit oil content. Application of chitosan at 10 g/l produced less fruit oil content while having the same impact on increasing fruit yield and quality. However, chitosan at 2.5 g/l and control trees exhibited low yield and fruit physical and chemical properties under the rain-fed conditions in both seasons, respectively. According to the data, proline and glycine accumulation and plant stress are positively correlated, which reinforces the idea that quantitative measurement of proline and glycine is used as an indicator of stress sensitivity and the extent of its relationship to physical and chemical characteristics.

Keywords: Olive, Picual, Glycine betaine, Glutathione, Chitosan, Rain-fed, Fruit set, Yield.

Introduction

Rain-fed agriculture is a type of farming that depends on rainfall. Rain-fed lands, particularly those in the world's dry and semiarid agro-ecological zones, are vulnerable, marginal, wasteful, problematic, threatened, low potential, or less favored fields when there is no irrigation (Johan et al., 2010). Since 80% of agriculture depends on rain, rain-fed agriculture has been essential to the world's food production and accounts for more than 50% of global food production. Poverty and malnutrition are major problems in places that receive rainfall. In addition to contributing to the world's food supply, raising these regions' production will stabilize them through improved food availability and rural development. In dry regions, agricultural development is undoubtedly at the core of sustainable

development. Increasing the productivity of rain-fed areas is the biggest problem of the twenty-first century, particularly in the world's semi-arid and arid regions with limited water supplies and inadequate soil resource bases (Khadse and Deoli 2018).

Recent studies show that soils in rain-fed regions are nutrient-deficient in addition to being thirsty (water-deficient). However, recent attention to rain-fed agriculture and the increased investment in this sector, which is necessary to increase productivity in rain-fed areas sustainably, have accelerated research in these areas and led to the development of technology packages that simultaneously address the nutrient disturbances and water shortages that limit the potential of rain-fed areas (Trivedi et al., 2022)

One of the most important crop production systems in Mediterranean agriculture is olive. This crop is valued in agricultural policy because of its

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socioeconomic significance and capacity for environmental adaptation, particularly drought (Sofa et al., 2008). The quantity of olives produced is determined by several variables, including soil management, soil water content, and the quality of the soil the trees are planted in (El-Taweel and Farag 2016). However, because of drought, olive yields in rain-fed orchards are still very low and inconsistent, and fruit yields hardly ever surpass 3 tons per hectare because of the restricted amount of soil water available owing to summer storms and fall and winter rains (Connor, 2005).

Different plant species and cultivars have varying capacities for nutrient absorption and transmission (Visioli et al., 1998). From a horticulture perspective, the productivity and vegetative development traits of various cultivars are compared to ascertain their success under certain environmental conditions. In arid and semi-arid areas, the environmental adaptability of species and cultivars has a major impact on climate change adaptation and sustainable agricultural production. The olive fruit is a drupe that is used as a table food and for its oil (Michelakis, 2002). Olive oil is a staple of the traditional Mediterranean diet, and its use is growing worldwide. It is produced unpurified and contains a lot of antioxidants and unsaturated fatty acids that are thought to have preventive and therapeutic effects on cancer and cardiovascular disease (El-Badawy et al., 2019).

The Picual olive arrived in Egypt from Spain. One of the best and most popular cultivars is this one. It is used for both table fruit and oil (Crane, and Nelson, 1972). Due to their high oil production, early maturity, ease of cultivation, and superior oil, Picual olive trees are commonly grown. Adapted to many soil conditions, the Picual tree is a robust tree with short branches (El-Sherkawy et al., 2010).

An amino polymer called chitosan is produced by alkalinely deacetylating chitin that has been taken from the cell walls of certain fungi or the exoskeleton of crustaceans like shrimp and crabs (Dzung et al., 2011). It can be employed in a variety of applications, including coating, preservative, antioxidant, antimicrobials, and soil modifiers, thanks to its intriguing qualities, which include biocompatibility, non-toxicity, low allergenicity, and biodegradability (Katiyar et al., 2015). Furthermore, chitosan foliar sprays promote growth and enhance the quality and production of numerous fruit crop modifications (Cheung et al., 2015). For plant defense, chitosan has been utilized extensively (Bautista-Baños et al., 2003). Additionally, plants treated with chitosan may avoid senescence, decrease transpiration and reactive oxygen species (ROS) production, and be less vulnerable to stress brought on by adverse environmental factors like

drought, salinity, and low or high temperatures (Esraa Hussein, 2017). Chitosan protects plants from senescence by promoting important activities at all levels of biological organization, including single cells and tissues, physiological and biochemical processes, and molecular alterations linked to gene expression and reactive oxygen species (ROS) (Nguyen Van et al., 2013).

An amphoteric chemical, glycine betaine (GB) "N, N, N-trimethylglycine" is electrically neutral throughout a broad range of physiological pH values. Despite having three methyl groups in its non-polar hydrocarbon component, it is very soluble in water. The molecular characteristics of GB enable it to interact with macromolecules' hydrophilic and hydrophobic regions, including protein complexes and enzymes (Kanu et al., 2017).

To protect the plant from oxidative damage, glycine betaine activates the activity of several antioxidant enzymes, including peroxidase (POX), catalase (CAT), and superoxide dismutase (SOD). GB's stimulation of antioxidant machinery in many plant species under a variety of stressful situations has been thoroughly investigated and documented in oxidative damage research (Yao et al., 2018). Additionally, by using a far superior photosynthetic system in plants, GB not only enhances the plant's growth and development but also boosts production (Chen and Murata 2008).

Various amino acids build up as antioxidants in plant cells when plants are stressed, and they have crucial physiological roles in helping plants adapt or in enhancing their growth and production. They function as osmolytes, alter ion transport and stomata opening, detoxify heavy metals, and regulate redox balance, enzyme activity, and the redox buffering capacity—all of which are essential for effective photosynthesis (Rai, 2002). One of the most significant amino acids that builds up in plant tissues under stress is proline (Hayat et al., 2012). Glutathione is a polypeptide consisting of three amino acids: glycine, cysteine, and glutamic acid. Under stressful conditions, it protects plant tissues from free radical damage by acting as an antioxidant and enzyme (Tandogan and Ulusu 2006).

Glutathione increases a plant's resistance to a variety of abiotic stressors, such as drought, high temperatures (HT), cold temperatures, salt, and toxic metal stress. Plants' ability to withstand abiotic stress can be enhanced by exogenously supplied GSH (Chen et al. 2012). Additionally, it offers a perfect viewpoint for removing harmful metals from the environment using phytoremediation and bioremediation. The production of particular chelators and the sequestration of metal complexes are the two methods used to lower

the free metal content achieved within the plant cell (Cuypers et al., 2010)

In this paper, the main goal is to investigate how Glycine Betaine, Glutathione and Chitosan affect the production of olive cv "Picual" groves under a rain-fed production system at specific times of the year and their effect on fruit physical and chemical properties.

Material and Methods

This study was conducted on healthy "Picual" olive trees that were planted at 7 x 7 meters apart and were around 12 years old throughout two consecutive seasons in 2022 and 2023. Trees at a private olive grove in the Marsa Matrouh Governorate (El Halazeen Valley 31°43'23.33"N26°86'21.94'E) - Egypt, are grown on sandy loam soil that receives rainfall (279.1 and 200.4 ml) for the 1st and 2nd season respectively. Table (1) shows some properties of the experimental soil.

Using the same horticultural practices, thirty healthy trees, nearly the same size, shape, and production were put through ten treatments as control (Water spray), Glycine Betaine (GB) at 25, 50 and 100 ppm, Glutathione (G) at 25, 50 and 100 ppm and Chitosan (Ch) at 2.5, 5.0 and 10.0 g/l. All treatments were sprayed three times (before the beginning of flowering (end of February), at full bloom (at the beginning of April), and a month later the second spray (at fruit set) in the two seasons. Treatments are described as follows:

1. Control water spray
2. Glycine Betaine at 25 ppm
3. Glycine Betaine at 50 ppm
4. Glycine Betaine at 100 ppm
5. Glutathione at 25 ppm
6. Glutathione at 50 ppm
7. Glutathione at 100 ppm
8. Chitosan at 2.5 g/l
9. Chitosan at 5.0 g/l
10. Chitosan at 10.0 g/l

Trees were given winter horticultural treatments in mid-November in the 2021 and 2022 seasons before the arrival of rain. A combination of chemical fertilizers (1 kg/tree super calcium phosphate (45% P₂O₅) + 250 g/tree potassium sulfate + 500 g/tree sulfur (95% S) + 250 g/tree sulfur coated urea (36.5% urea and 16% S) and sheep manure fertilizer (20 kg/tree sheep manure) was applied to each studied tree. Table (2) presented chemical analysis of the tested sheep manure

In early March for each season, twenty healthy uniformed one-year-old shoots from each tree's canopy

were chosen at random and labeled (five shoots from each direction) to perform the following measurements:

1-Vegetative growth characteristics

During the last week of September, the following vegetative development features were identified according to Ahmed and Morsy (1999) description:

Shoot length: New shoots on one-year-old shoots for each replicate were labelled in March. Using a ruler, the shoot lengths were measured and recorded in centimeters (cm) the last week of September.

Number of leaves /shoot: The leaves were counted on shoots.

Leaf area (cm²): In the last week of September, samples of 20 adult leaves were randomly picked from the middle of each year's growth of selected shoots to measure the area of the leaf blader, according to the equation cited by Shaheen *et al.*, (2011). Leaf area = 0.53 (leaf length x leaf width) + 1.66

Total chlorophyll: Leaf chlorophyll content (mg/100g F. W) was measured as described by Moran and Porath (1985)

2 -Blooming and Yield characteristics

Blooming characteristics

Number of inflorescences/shoot: For every replicated tree, inflorescences for every one year old shoot were counted in the 3rd week of March.

Number of flowers/inflorescences was counted.

Perfect flowers percentage: calculated according to the next equation

$$\text{Perfect flowers \%} = \frac{\text{No of perfect flowers}}{\text{No of total flowers}} \times 100$$

Yield properties

Initial fruit set percentage: After twenty days of pollination, the initial fruit set was calculated using the following formula

$$\text{Initial fruit set \%} = \frac{\text{No of initial fruits}}{\text{No of perfect flowers}} \times 100$$

Final fruit set percentage: The final fruit set percentage prior to harvest was determined by the following formula

$$\text{Final fruit set \%} = \frac{\text{No of fruits before harvest}}{\text{No of perfect flowers}} \times 100$$

Yield: The fruits of each tree were taken separately at the maturity stage (mid-October), weighed, and the yield was assessed in kilograms per tree.

Fruit Quality (Physical and chemical properties of fruits)

At harvest time, twenty fruits were picked from each tree (replicate) for the following measurements.

Fruit physical properties:

Fruit weight: The average of fruit weight (g) was determined by using a digital balance.

Fruit dimensions (cm): The average of fruit length (cm) and diameter (cm) was measured by a Verner Caliper.

Seed weight (g) and flesh weight (g): A digital balance weighed the seed and flesh.

-Flesh weight/fruit weight percentage was calculated .

-*Flesh dry matter percentage:* calculated according to the following equation

$$\text{Flesh dry matter \%} = \frac{\text{Flesh dry weight}}{\text{Flesh fresh weight}} \times 100$$

Fruit chemical properties

Flesh fruit oil percentage: The percentage of oil in the fruit flesh was calculated using a dry weight according to Banat et al. (2013).

Fruit oil acidity percentage: The determination was made in accordance with Dieffenbacker and Pocklington (1992).

Leaf chemical determination: Proline amino acid content and Glycine betaine content

Proline amino acid : Leaf proline content ($\mu\text{g/g}$ dry weight) was determined according to Bates et al., (1973)

Glycine betaine : Leaf Glycine betaine content ($\mu\text{g/g}$ dry weight) was determined according to Camilo et al.,(2019)

Statistical Analysis

The layout of the experiment was a completely randomized block design (RCBD). The experiment included 10 treatments. Each treatment included three replicates; each replicate one tree. The data were statistically analyzed as analysis of variance according to Snedecor and Cochran (1990). Duncan's multiple range test (Duncan, 1955) at a 5% level was used to compare treatments mean values

Results and Discussions

Vegetative growth characteristics:

Shoot Length (cm) and Leaf number

Table (3) demonstrates statistical differences between all tested glycine betaine, glutathione, and

chitosan treatments in increasing shoot length and leaf number of the Picual olive trees compared with the control treatment of both studied seasons. Generally, glycine betaine at 50 and 100 ppm , glutathione at 50 and 100 ppm, and chitosan at 10g/l raise shoot length and leaf number of the Picual olive trees in both studied seasons. On the contrary, the control trees and chitosan at 2.5 g /l distinguished a lower shoot length and leaf number. Other treatments produced an intermediate-enhancing effect in this respect.

Leaf area(cm^2)

The results in Table (3) indicated significant differences between the treatments sprayed on the leaf area (cm^2) of Picual olive trees during the experiment seasons. Trees sprayed with glycine betaine at 25 ppm, glutathione at 25 ppm, and chitosan at 2.5 and at 5 ppm showed the highest leaf area in this concern. In contrast, trees treated with glycine betaine at 100 and 50 ppm and untreated trees (control) showed less leaf area compared to other treatments in both seasons.

Total Chlorophyll: Leaf Chlorophyll content (mg/100g F. W)

Table (3) illustrated that the tested concentrations of glycine betaine , glutathione, and chitosan positively affected the leaf chlorophyll content of the Picual olive tree compared with the control treatment in both seasons .In general glycine betaine at 50 and 100ppm exceeded the equivalent ones of glutathione and chitosan treatments to improve the leaf chlorophyll content of the Picual olive tree and recorded (2.89 and 2.78 mg/100g F. W in the 1st season and 3.07 and 2.91 mg/100g F. W in the 2nd season) respectively followed by glutathione at 50 and 100 ppm and chitosan at 10g/l in both seasons. On the contrary, controlled trees and trees treated with chitosan at 2.5g/l recorded less leaf chlorophyll content. Other treatments ranged in intermediate values in both studied seasons.

In general, drought stress results in the destruction and decomposition of chloroplasts, decreased chlorophyll content, and enzyme activity. Plants cope with drought in dry conditions through various methods such as stomata closure, osmoregulation, and accumulation of drought-compatible soluble substances. As an osmoregulatory, proline stabilizes membranes and the protein synthesis system, maintains water balance, keeps proteins stable, stores carbon and nitrogen for growth after stress, and lowers the generation of reactive oxygen species (ROS). (Samah and Ghada 2020)

Drought stress conditions resulted in a decrease in plant growth and chlorophyll pigments. The breakdown of chlorophyll pigments and associated chemicals or defective production could be the cause of the decrease in photosynthetic pigments under

stressful circumstances like drought (Bhuiyan, et al., 2019). When there was a water deficiency, the foliar application of GB enhanced the chlorophyll pigments. This increase in pigment concentrations might have resulted from GB's function in shielding the photosynthetic machinery and maintaining the membrane and Rubisco structures in plants when there is a water deficit (Rezaei et al., 2012).

Additionally, because water scarcity can impair water relations and gas exchange characteristics, it decreases the amount of leaf area per plant (Kapoor et al., 2020). Underwater deficiency conditions, leaf area increased when given GB supplements, which was attributed to GB's ability to boost photosynthetic activities in stressed plants (Kurepin et al., 2015).

Many physiological functions, including pH regulation, the production of metabolic energy, or redox power, and stress tolerance, depend on glutathione (Gibson et al., 2009). Both nitrogen and sulfur, which are required at distinct phases of plant growth, are present in the structure of glutathione. A crucial component in the synthesis of proteins, chlorophyll, and several enzymes is nitrogen. Significant differences in the compositions of essential oils are caused by the sulfur ion (Deponete, 2017).

Blooming and yield characteristics

Blooming characteristics

Number of inflorescences/shoot and number of flowers/inflorescence

Tabulated data in Table (4) demonstrated that all tested treatments of glycine betaine, glutathione chitosan, and control of Picual olive trees are statically insignificant differences for number of inflorescences /shoot and number of flowers/inflorescence in both seasons of study.

Perfect flowers %

Table (4) claims that in both study seasons, every tested treatment had a significantly positive impact on perfect flowers as compared to the control treatment. Moreover, 50 and 100 ppm glycine betaine treatments in both seasons were more effective than other treatments in recording the highest percentage of perfect flowers (59.0, and 56.5 in the first season and 68.8, and 61.2 % in the second season respectively. Additionally, glutathione at 50, 100 ppm and chitosan at 10.0 g/l followed the previous treatments in affecting the percentage of perfect flowers, which ranged between 52.9 to 56.7 in both seasons. The lowest concentrations of glycine betaine and glutathione at 25 ppm moderately affect the perfect flowers percentage.

Lastly, the chitosan at 2.5g/l and control had less effect on the perfect flowers percentage, which was recorded (37.8 and 35.2 % in the first season and 34.4 and 33.0% in the second season respectively.

Stress can affect the development of flowers or the mobilization and synthesis of photosynthetic chemicals, which can lead to abnormalities in flower functioning (Sonoike, 1998). The reduced gamete function in stressed plants or their flowers may be attributed to the supra-optimal amounts of abscisic acid, since it has been discovered that this substance controls the fertility status of flowers (Nayyar et al., 2005).

Application of glutathione led to improvements in morphological and chemical properties. The addition of glutathione to plants that grow under stress conditions leads to an increase in growth speed. It stimulates the detoxifying composition of methylglyoxal, helping the plant withstand stress (El-Shabrawi et al., 2010). Glutathione's functions as an antioxidant component (enzymatic, non-enzymatic or suitable organic soluble or osmo protectors) have been confirmed and act as the final component of signal transmission pathways under stress conditions (Salama and Al-Mutawa 2009). Glutathione has been observed to improve the growth of stress-prone plants by improving the light pigment content. Glutathione reduces stomata opening rate and breathing (Kattab, 2007).

Yield characteristics

Initial fruit set percentage

Table (5) illustrated that the tested concentration of glycine betaine, glutathione, and chitosan excited a higher favorable impact on the initial fruit set % of Picual olive trees compared to the control during both study seasons. Moreover, 50 and 100 ppm glycine betaine surmounted the corresponding ones of glutathione and chitosan treatments in enhancing initial fruit set% in both seasons under study. In contrast, chitosan at 2.5g/l and untreated trees (control) were recorded (7.4 and 6.1 % in the first season and 7.1 and 5.7 %) in both seasons respectively.

Final fruit set percentage

Table (5) states that all treated trees with glycine betaine, glutathione, and chitosan concentrations significantly exerted a high positive effect on the final fruit set % of Picual fruit trees compared with the untreated trees (control) 2022 and 2023 seasons. Moreover, glycine betaine at 50 and 100 ppm and glutathione at 50 ppm raised the final fruit set percentage in both investigated seasons. Glycine betaine at 50 and 100 ppm and glutathione at 50 ppm were recorded (6.9, 6.6, and 5.9 % in the 1st season

and 8.4 , 7.0 , and 7.3 % in the 2nd season) respectively. On the other hand, the control treatment showed less final fruit set and exhibited (2.7 and 2.1 %) in both experimental seasons.

Yield/tree (kg)

Data in Table (5) show the effect of glycine betaine, glutathione, and chitosan on the yield/tree (kg) of Picual olive trees. Olive trees sprayed with glycine betaine at 50 and 100 ppm recorded the highest values of Yield/tree in the two seasons under study. In contrast to untreated ones, all applied materials were successful in raising the yield/tree values.

The treatments of glycine betaine at 50 and 100 ppm produced (16.60 & 17.23 kg/ tree in the 1st season and 17.94 and 15.88 kg/tree in the second season) respectively. Also, glutathione at 50 and 100 ppm and chitosan at 10g/l were more pronounced in this respect. Olive trees treated with the 2.5g/l chitosan and control trees as the lowest values showed no significant differences. Other examined concentrations of all mentioned materials induced intermediate values in both investigated seasons.

Chitosan helps reduce the water stress effect on yield, it could return to an increase in stomata conductance under water stress. Furthermore, a proposal for participation in physiological mechanisms that stop water loss by transpiration (Yang et al., 2009). Since stomata closure has been shown to occur when plants are dusted with chitosan, this suggests that the growth-stimulating impact following stomata closure may be connected to an antiperspirant effect on the substrate (Kuyyogusuy et al., 2018).

Chitosan reduces the buildup of dangerous free radicals by triggering antioxidants and enzymes, which alleviates drought stress and promotes growth (Malekpoor et al., 2016). Moreover, chitosan is discussed about pathways involving the manufacture of jasmonic acid, which is crucial for controlling how much water plants utilize.

The negative effects of stress on growth, yield, and productivity in the plants and stress led causing a reduction in the leaves' area and quantity, overall yield, length, and diameter (Kaya et al., 2013). GB is a particularly effective protectant (osmoregulatory substance) against abiotic stress. Exogenous GB application has the potential to swiftly pass through leaves and reach other organs, where it can enhance stress resistance. Additionally, the mechanism of foliar application of GB is transferred to meristematic tissues specifically, flower buds and shoot apices when applied to leaves, and thereafter transferred to tissues that are actively growing and expanding. Thus, it improves the chlorophyll contents, stomata conductance, relative water content, water use

efficiency, and membrane stability, this may result in improved crop performance under stressful circumstances. (Annunziata et al., 2019).

The availability of water with GB in plant tissues, which improves the solubility of nutrients in tissue cells, may be the cause of the higher yield from GB treatment under stress. These nutrients allowed the plant to maximize its metabolic processes and photosynthesis (Rezaei et al., 2012). Also, Masoumi et al., (2010) points out this compound's beneficial function in improving resistance to drought stress by upregulating processes related to growth and production under stress. Akram et al., (2017) explained that foliar application of GB enhances total phenolic under drought conditions, and ascorbic acid, is a potent antioxidant that enhances plant growth and productivity and is crucial for drought stress tolerance.

Fruit quality (Fruit physical and chemical properties)

Fruit physical properties

Fruit weight (g)

Table (6) shows that all tested glycine betaine, glutathione, and chitosan sprays were surmounted in raising fruit weight of Picual fruit in comparison to the control treatment for both seasons. Anyway, glycine betaine at 50ppm is seen better in this aspect followed by glutathione at 50 ppm, glutathione at 100 ppm, and glycine betaine at 100 ppm in the first season. Statically, Glutathione at 50 ppm appeared to show better amelioration in fruit weight followed by glycine betaine at 25 ppm and chitosan at 10g/l with a slight significant difference in the 2nd season. However, in both of the seasons under study, the control treatment showed less recording data.

Fruit length (cm)

Table (6) clears that all examined sprayed glycine betaine, glutathione, and chitosan treatments enhanced fruit length of Picual olive compared with the untreated trees in both investigated seasons. Meanwhile, glycine, glutathione at 25 and 50 ppm, and chitosan at 10 g /l arise fruit length with glycine betaine at 50 ppm in the 1st season and glycine betaine at 25 ppm in 2nd season. On the other hand, other treatments exhibited fruit length in descending values. On the contrary, control trees exhibited lower fruit length values in this concern.

Fruit diameter (cm)

Arranged data in table (6) illustrate that all sprayed treatments had a higher beneficial impact on the Picual olive fruit diameter in both seasons of study compared to the control (unsprayed tree). Moreover, glycine betaine at 50 ppm demonstrated the most efficient treatment at this point and recorded

(2.36 cm in the 1st season and 2.45 cm in the 2nd season) respectively. However, Control, glycine betaine at 25 ppm and chitosan at 5 ppm showed less tabulated data.

Seed weight (g)

Data in Table (7), showed that all examined glycine betaine, glutathione, and chitosan sprays exhibited a statistically insignificant effect with values ranging from 0.94 to 1.11 g in the 1st season and from 0.93 to 1.26 g in the 2nd season respectively.

Flesh weight (g)

Table (7), shows that all trials concentrations of glycine betaine, glutathione, and chitosan sprays exhibited a beneficial effect on the fruit flesh weight of Picual olive in comparison to the control in the 1st and 2nd seasons. Moreover, glycine betaine at 50 ppm showed 6.89 (g) followed by a slight significant difference with glutathione at 50 ppm 6.48 (g) in the first season. However, glutathione at 50 ppm recorded 6.55 g followed by glycine betaine at 50 and 100 ppm, glutathione at 100 ppm, and chitosan at 10g/l in the 2nd season. However, the control exhibited 3.76 g in the 1st season and 3.95 g in the 2nd season respectively. The rest of the treatments were obvious in intermediate values.

Flesh weight/fruit weight percentage

Table (7) illustrates that all evaluated treatments have a beneficial effect on flesh weight/fruit weight % of Picual olive than the control trees in 2022 and 2023 seasons. Moreover, glutathione at 50 and 100 ppm, glutathione at 50 and 100 ppm, and chitosan at 10g/l proved superior compared with other treatments and control treatments. However, glutathione at 50 ppm recorded 87.33% in the 1st season and glycine betaine at 100 ppm exhibited 86.42 % in the 2nd season. In contrast, untreated trees (control) showed (79.49 % in the 1st season and 77.32 % in the 2nd season) respectively with no significant difference with chitosan at 2.5g/l.

Flesh dry matter percentage

As it is shown in table (7) the flesh dry matter % of "Picual" olive fruits was greatly affected by glycine betaine, glutathione, and chitosan treatments. All applied treatments increased dry matter % than control and the highest flesh dry matter % (59.7 % in the 1st season and 57.1 % in the 2nd season) was recorded by glycine betaine at 50 ppm. On the other hand, chitosan 2.5g/l and control trees exhibited less recorded data in the 2022 and 2023 seasons.

Glutathione is a significant antioxidant that has been linked to improved plant development and the buildup of dry matter in the leaves, roots, and stems as well as in

the overall dry matter (Fronto et al., 2013). Pinheiro et al. (2008) discovered that plant growth was enhanced by the dry matter accumulation of leaves, roots, and stems as well as the overall dry matter. The plant benefits from glutathione's ability to form conjugates with xenobiotics. (Coleman et al., 1997).

Chitosan has been shown to be effective in stimulating protective reactions to biotic and abiotic stresses and plant growth-promoting activities. Additionally, using chitosan offers a potential remedy for crop plants' ability to respond to stress (Hidangmayum et al., 2019). It has proven to be an effective antiperspirant in agriculture, increasing resistance to oxidative damage and water stress without lowering production. (Almeida et al. 2020). Additionally, chitosan is involved in the activation of protein kinases and phosphatases, the regulation of gene expression, and the development of systemic acquired resistance. In order to prime defense systems, it also initiates the generation of ROS and activates signaling pathways involving hydrogen peroxide (H₂O₂), nitrous oxide, salicylic acid, and jasmonic acid (Cataldo et al., 2022)

Fruit chemical characteristics

Flesh fruit oil percentage

Table (8) reveals a noticeable rise in the proportion of fruit oil obtained with the applied treatments in both seasons under study as compared to the control. Glycine betaine at 50 and 100 ppm succeeded in enhancing flesh fruit oil percentage compared to other treatments and recorded (27.5 and 26.3 % in the 1st season and 25.3 and 24.8% in the 2nd one) respectively. However, glutathione at 50 and 100 ppm and chitosan at 10 g/l followed the previous glycine betaine concentrations with slight differences among them. On the contrary, chitosan at 2.5 g/l and control trees showed a low level of flesh fruit oil percentage without significant differences between them and recorded (20.1 and 19.3 % in the 1st season and 18.9 and 18.7 % in the 2nd one) respectively. All treatments showed the same trend of data in seasons 2022 and 2023.

Fruit oil acidity percentage

It is clear from Table (8) that all examined glycine betaine, glutathione, and chitosan treatments reduced fruit oil acidity % of Picual olive in comparison to the control in the two experimental seasons. Generally, glycine betaine at 50 and 100 ppm showed a lower acidity percentage and was recorded (0.24 and 0.26 % in the 1st season and 0.22 and 0.25 % in the 2nd one) respectively. Conversely, the control showed the highest acidity percentage (0.34 % in the 1st season and 0.35 % in the 2nd season).

Leaf chemical determination

Leaf Proline content ($\mu\text{g/g d.wt}$)

Data clearly show in Table (8) that all of the applied treatments resulted in a considerable decrease in proline content compared to the untreated ones. Plants respond to biotic and abiotic stressors by accumulating proline, which varies from species to species and can be 100 times higher in situations with water scarcity than in those with adequate irrigation. (Szabados and Savoure 2010). Proline amino acid content values were lower in those treated with glutathione at 100 and 50 ppm (78.1 & 73.8 $\mu\text{g/g d.wt}$ in the 1st season and 69.8 & 65.8 $\mu\text{g/g d.wt}$ in the 2nd season) respectively with slight significant difference with chitosan at 10g/l. Whereas, maximum values 124.2 $\mu\text{g/g d.wt}$ in the 1st season and 112.6 $\mu\text{g/g d.wt}$ in the second season) were viewed with control trees

Leaf Glycine content ($\mu\text{g/g d.wt}$)

As shown in Table (8) Picual olive trees sprayed with glycine betaine, glutathione, and chitosan treatments produced lower glycine betaine values than untreated trees in the two studied seasons. The treatment of glycine betaine at 100 and 50 ppm were superior to other treatments or the control in both seasons in terms of lowering glycine betaine levels (27.4 & 29.3 $\mu\text{g/g d.wt}$ in the 1st season and 29.6 & 31.1 $\mu\text{g/g d.wt}$ in the second season) respectively. In contrast, control trees exhibited higher values of glycine betaine recorded (42.5- $\mu\text{g/g d.wt}$ in the 1st season and 44.0 $\mu\text{g/g d.wt}$ in the 2nd season) respectively, followed by chitosan at 2.5g/l treatment with significant differences between them, which clarified the connection between glycine betaine component buildup and stress.

Plants manufacture and accumulate vital glycine when exposed to various environmental factors that cause stress (Rhodes and Hanson, 1993). An alternative explanation proposed that osmotic stress triggered GB production through the transmission of the jasmonics signal, which is crucial for both tolerance and osmotic stress resistance. (Xu et al., 2018). Additionally, by maintaining a faster rate of light representation, GB improved the plant's reaction to low-phosphate stress by loading the bark and boosting sucrose production and transportation. (Li et al., 2019).

Tiequan et al., (2021) explained how exogenously administered GB minimized oxidative stress in woody plants under drought stress by inhibiting ROS

formation, lowering lipid peroxidation, increasing enzymatic antioxidant capacities, and protecting plants against oxidative drought, which was linked to increased endogenous GB aggregation in pears.

Since the osmolytes Proline and Glycine Betaine aid in water conservation and shield proteins and biological membranes, they play a crucial part in higher plants' responses to osmotic, hydric, and oxidative stress. Under stressed conditions, free Proline accumulates more and plays a significant impact in many plant species (Bhaskara et al., 2015). Many species have been the subject of extensive research on the functions of Proline and its metabolism under stressful conditions, and it is now widely acknowledged that Proline plays a variety of roles in how plants react to stress. Apart from its role as an osmolyte, Proline also scavenges reactive oxygen species (ROS) and stabilizes subcellular structures, which helps to regulate the cell's redox homeostasis. During times of stress, it also functions as a signal molecule that interacts with other metabolic pathways and provides energy (Camilo et al., 2019)

Conclusion

The results indicate that foliar spraying with glycine betaine at 50 and 100 ppm are recommended to improve olive trees' productivity with higher percentages of flowering , perfect flowers, initial fruit set , and final fruit set, increasing productivity and fruit quality under a rain-fed irrigation system. Also , they ameliorated fruit's physical properties (fruit weight, length & diameter, flesh weight & flesh dry matter) and fruit oil content. Finally , measurement of proline and glycine after Glycine betaine , Glutathione and chitosan applications is used as an indicator of stress sensitivity and the extent of its relationship to physical and chemical characteristics.

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Conflict of interest

The author declares that he has no competing interests.

TABLE 1. Analysis of experimental soil

Soil Depth (cm)	Texture Class	PH Soil Paste	E.Ce (dSm ⁻¹)
0-30	Sand Loam	7.7	0.9
30-60	Sand Loam	7.4	0.6
Soluble cations			
Ca (Meq/l)	Mg (Meq/l)	Na (Meq/l)	K (Meq/l)
3	1	5.5	0.5
2	0.8	4.04	0.16
Soluble anions			
CO ₃ (Meq/l)	HCO ₃ (Meq/l)	Cl (Meq/l)	SO ₄ (Meq/l)
-	2	5	3
-	1	3.7	2.3

TABLE 2. Chemical analysis of sheep manure

N(%)	P(%)	K (%)	Ca (%)	Mg(%)	Fe ppm	Mn ppm	Zn ppm	Organic matter content (%)
1.70	0.62	0.63	0.32	0.37	869	161	16	51.0

TABLE 3. Effect of Glycine Betaine, Glutathione, and Chitosan foliar sprays on shoot length (cm), leaf number/shoot, leaf area (cm²), and leaf chlorophyll content (mg/100g F.W) of rain-fed "Picual" olive tree during 2022 and 2023 seasons.

Treatments	Shoot Length (cm)		Leaf Number/shoot		Leaf Area (cm ²)		Leaf Chlorophyll content (mg/100g F. W)	
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	13.4ef	11.4e	18.9e	17.3f	4.16f	4.44f	2.22f	2.18f
GB 25	17.7cd	18.3cd	26.9cd	27.1de	5.88ab	6.19ab	2.49de	2.58cd
GB 50	25.4a	24.7a	37.6a	35.9a	4.76e	4.95e	2.89a	3.07a
GB100	23.2ab	21.5b	32.7b	30.2cd	5.04de	5.32de	2.78ab	2.91a
G25	16.1de	17.4d	23.8d	24.8e	5.94ab	6.37a	2.42de	2.51d
G50	22.8ab	22.7ab	31.7b	32.4abc	5.34cd	5.61cd	2.69bc	2.76b
G100	21.8b	23.6ab	33.8ab	34.7ab	5.56bc	5.72cd	2.64bc	2.71bc
Ch2.5	12.4f	13.5e	17.9e	19.5f	6.12a	5.89bc	2.27f	2.34e
Ch5	17.5cd	16.9d	25.7cd	24.5e	5.80ab	5.92bc	2.36ef	2.45de
Ch10	20.3bc	21.1bc	29.8bc	31.7bc	5.28cd	5.46d	2.56cd	2.66bc

Means having the same letter (s) in each column is not significantly different at 5% level

TABLE 4. Effect of Glycine Betaine, Glutathione, and Chitosan foliar sprays on number of inflorescences/shoot, number of flowers /inflorescence, and perfect flowers % of rain-fed "Picual" olive tree during 2022 and 2023 seasons

Treatments	No of inflorescences /shoot		No of flowers /inflorescence		Perfect flowers %	
	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	14.67a	15.34a	16.00a	15.34a	35.2f	33.0e
GB 25	10.34a	10.67a	14.00a	13.34a	47.6de	45.0d
GB 50	12.67a	12.00a	12.34a	10.67a	59.0a	68.8a
GB100	12.34a	12.34a	13.00a	12.00a	56.5ab	61.2b
G25	13.34a	12.67a	14.67a	11.34a	44.8e	50.0cd
G50	12.00a	11.00a	12.34a	14.00a	56.1ab	53.2c
G100	11.34a	11.00a	13.67a	12.34a	53.3bc	56.7bc
Ch2.5	12.00a	14.00a	15.00a	15.34a	37.8f	34.4e
Ch5	12.67a	12.67a	12.34a	14.00a	50.0cd	46.2d
Ch10	13.00a	12.00a	14.34a	10.67a	52.9bc	56.1bc

Means having the same letter (s) in each column is not significantly different at 5% level

TABLE 5. Effect of Glycine Betaine, Glutathione, and Chitosan foliar sprays on initial fruit set %, final fruit set%, and yield /tree (kg) of rain-fed "Picual" olive tree during 2022 and 2023 seasons.

Treatments	Initial fruit set %		Final fruit set %		Yield /tree(kg)	
	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	6.1f	5.7f	2.7e	2.1f	6.7f	7.29f
GB 25	9.2cd	9.8de	4.3d	5.8cd	10.45e	11.14de
GB 50	12.2ab	14.1a	6.9a	8.4a	16.60ab	17.94a
GB100	12.4a	12.6ab	6.6a	7.0b	17.23a	15.88b
G25	8.5de	9.4e	4.3d	4.7e	11.86de	10.42e
G50	11.8ab	11.0cd	5.9ab	7.3b	12.25de	13.96bc
G100	11.1ab	11.7bc	5.6bc	6.9b	13.10cd	14.15bc
Ch2.5	7.4ef	7.1f	3.4e	2.7f	8.43f	8.18f
Ch5	8.4de	8.8e	4.7cd	5.3de	11.43de	12.74cd
Ch10	10.7bc	10.9cd	5.4bc	6.5bc	14.8bc	13.35c

Means having the same letter (s) in each column is not significantly different at 5% level

TABLE 6. Effect of Glycine Betaine, Glutathione, and Chitosan foliar sprays on fruit weight (g), fruit length (cm), and fruit diameter (cm) of rain-fed "Picual" olive tree during 2022and2023 seasons.

Treatments	Fruit weight (g)		Fruit length (cm)		Fruit diameter (cm)	
	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	4.73f	5.11e	2.27d	2.19e	1.87e	1.92e
GB 25	6.30cd	7.11ab	2.40c	2.64a	1.91 e	1.97e
GB 50	7.90a	6.94bc	2.61a	2.53bc	2.36a	2.45a
GB100	7.20b	6.80bc	2.42c	2.49c	2.02cd	1.91e
G25	6.19cd	6.31cd	2.57ab	2.68a	2.08c	2.21cd
G50	7.42b	7.75a	2.54ab	2.58ab	2.22b	2.32b
G100	7.28b	6.87bc	2.48bc	2.2.37d	2.21b	2.11d
Ch2.5	5.36e	5.67de	2.24d	2.25e	2.02cd	2.17cd
Ch5	6.09d	6.12d	2.41c	2.36d	1.97de	1.91e
Ch10	6.64c	7.05ab	2.51ab	2.60ab	2.19b	2.28bc

Means having the same letter (s) in each column is not significantly different at 5% level

TABLE 7. Effect of Glycine betaine, glutathione, and chitosan foliar sprays on seed weight (g), flesh weight (g), flesh weight/fruit weight % and flesh dry matter % of rain-fed "Picual" olive tree during 2022 and 2023 seasons.

Treatments	Seed weight (g)		Flesh weight (g)		Flesh weight/ fruit weight %		Flesh dry matter %	
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	0.97a	1.16a	3.76f	3.95e	79.49f	77.32f	52.3f	49.7f
GB 25	1.03a	1.26a	5.27cd	5.85b	83.64cd	82.29cd	54.2de	52.4de
GB 50	1.01a	0.97a	6.89a	5.97b	87.22a	86.11a	59.7a	57.1a
GB100	0.98a	0.93a	6.22b	5.87b	86.39ab	86.42a	58.1ab	56.4ab
G25	1.07a	1.19a	5.12cd	5.12c	82.71cde	81.23d	54.9cd	53.8cd
G50	0.94a	1.20a	6.48ab	6.55a	87.33a	84.61ab	57.4b	56.1ab
G100	1.02a	1.12a	6.26b	5.75b	86.00ab	83.76bc	55.5cd	53.9c
Ch2.5	1.01a	1.20a	4.35e	4.47d	81.16ef	78.94ef	53.1ef	51.1ef
Ch5	1.11a	1.07a	4.98d	5.04c	81.76d	80.45de	55.0cd	54.2c
Ch10	1.02a	1.06a	5.62c	5.99b	84.64bc	85.09ab	56.6bc	55.1bc

Means having the same letter (s) in each column is not significantly different at 5% level

TABLE 8. Effect of Glycine Betaine, Glutathione, and Chitosan foliar sprays on flesh fruit oil (%), fruit oil acidity (%), leaf proline content, and leaf Glycine content of rain-fed "Picual" olive tree during 2022 and 2023 seasons

Treatments	Flesh fruit oil (%)		Fruit oil acidity (%)		Proline (µg /g d.wt)		Glycine (µg /g d.wt)	
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	19.3f	18.7f	0.34a	0.35a	124.2a	112.6a	42.5a	44.0a
GB 25	22.3cd	21.7d	0.30bc	0.31bc	89.5cd	83.4d	37.6c	38.4bc
GB 50	27.5a	25.3a	0.24e	0.22f	63.7g	57.9g	29.3f	31.1fg
GB100	26.3a	24.8ab	0.26de	0.25ef	67.9g	62.1g	27.4f	29.6g
G25	22.0d	21.1de	0.30bc	0.32bc	87.5d	91.6c	38.2bc	39.2b
G50	24.2b	23.5b	0.27cd	0.25ef	73.8f	65.8f	31.8e	33.5ef
G100	23.7bc	23.4bc	0.28cd	0.26de	78.1ef	69.8ef	33.7de	34.9de
Ch2.5	20.1ef	18.9f	0.32ab	0.32bc	116.3b	104.2b	39.8b	40.1b
Ch5	21.1de	20.2e	0.31ab	0.34ab	94.7c	87.4cd	36.9c	38.1bc
Ch10	22.5cd	22.1cd	0.28cd	0.29de	81.4e	74.2e	34.1d	36.8cd

Means having the same letter (s) in each column is not significantly different at 5% level

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تأثير الرش ببعض مضادات الأكسدة علي نمو و إنتاجية اشجار الزيتون في منطقة الساحل الشمالي

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الملخص

أجري هذا البحث علي أشجار زيتون بيكوال خلال الموسمين المتتاليين 2022 و 2023 بمحافظة مطروح – مصر. تهدف هذه الدراسة الي تقييم تأثير الرش الورقي بالجليسين بيتاين، الجلوتاتيون و الشيتوزان في تحسين خصائص النمو الخضري، التزهير خصائص المحصول و الخواص الفيزيائية و الكيميائية لثمرة الزيتون "بيكوال" في ظل نظام الري المطري.

أشارت النتائج الي أن الرش الورقي بالجليسين بيتاين بمعدل 50 و100 جزء في المليون و الجلوتاتيون بمعدل 50 و100 جزء في المليون يوصي به لتحسين إنتاجية و الجودة. يعزى تحسين الإنتاجية الي زيادة نسبة التزهير، نسبة الأزهار الخنثى، نسبة العقد الأولي و النهائي للثمار بالإضافة الي تحسين الخصائص الفيزيائية للثمرة (وزن الثمرة، الطول و القطر، وزن اللحم و الوزن الجاف للحم) و محتوى الثمرة من الزيت. كان لتطبيق الشيتوزان بمعدل 10 جم/لتر نفس التأثير علي تحسين المحصول و جودة الثمار، في حين أنه نتج محتوى أقل من زيت الثمار. من ناحية أخرى أظهرت الأشجار المعاملة بالشيتوزان 2.5 جم/لتر و الأشجار الغير معاملة إنتاجية منخفضة و كذلك صفات فيزيائية و كيميائية منخفضة تحت ظروف الري المطري في كلا الموسمين علي التوالي.

تشير البيانات الي وجود علاقة إيجابية بين تراكم البرولين و الجليسين و اجهاد النبات مما يعزز فكرة أن القياس الكمي للبرولين و الجليسين يستخدم كمؤشر لحساسية الاجهاد ومدى علاقته بالخصائص الفيزيائية و الكيميائية.

الكلمات الدالة: الزيتون، بيكوال، جليسين بيتاين، جلوتاتيون، شيتوزان، الري المطري، عقد الثمار، المحصول.