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Inceptive Assessment of a New Olive Mill Waste–derived Biostimulant for Mitigating Water Stress in Pomegranate Trees

Darine Tlili
National Agronomic Institute of Tunisia (INAT), University of Carthage
Samia Abboud
Chott Mariem-Sousse
Sahar Ben Abdelwaheb
University of Carthage
Azhar Ouni
Higher Agronomic Institute of Chott Mariem-Sousse, University of Sousse
Soumaya Dbara
soumayadbara@yahoo.fr
Chott Mariem-Sousse

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Abstract

Water scarcity is a major constraint to the pomegranate (Punica granatum L.) cultivation. A need to find a new sustainable strategy to emphasize the tolerance of trees to water stress is necessary. In this context, the present study shows an initial evaluation of a novel biostimulant, prepared by a mix of phenolic extract (PE) and lignin nanoparticles (LNPs) extracted from olive mill solid waste (OMSW), applied on pomegranate trees submitted to deficit irrigation (ST) which compared to control (C) and deficit irrigation (S) treatments. Various parameters were evaluated. In treated leaves, the biostimulant has enhanced the content in total chlorophylls, decreased the malondialdehyde and starch, but the total phenolic and flavonoids content didn't show any significant variation. With treatment (ST), the weight of fruits was ameliorated and the juice content was enriched by soluble solids, phenols, flavonoids and lycopene. In conclusion, this study opens the horizon of the validity use of bioactive compounds with a biostimulant action by recycling and valorizing bio-residues from olive oil chain production.

1. Introduction

The climate change and water scarcity are increasingly threatening the agricultural production in arid and semi-arid regions like Tunisia (FAO 2022). In fact, the water availability is a key important and determinant of plant development, and water deficit induced by a lack of water resources lead to physiological and biochemical alterations that negatively affect plant growth and yield.

Pomegranate (Punica granatum L.) is one of the strategic fruits crops in Mediterranean areas, it is well adapted to dry environments, however, its productivity and fruit quality are highly sensitive to prolonged water stress (Porras-Jorge et al. 2025). Researchers now are aiming to improve plant tolerance to abiotic stress while reducing the environmental impact for a better crop management and sustainable agricultural practices. Among these practices, the use of biostimulants that has been revealed as an effective and eco-friendly approach to enhance plant resilience, optimize nutrient intake and ameliorationg yields under stressed and limited conditions (Du Jardin 2015; Rouphael & Colla 2020). Indeed, biostimulants are generally bio-substances and microorganisms that can stimulate plant processes and improve nutrient uptake and abiotic stress tolerance (Nardi et al. 2016). Their mechanisms of action aim to enhance the plant metabolism, the activation of antioxidant response and the improvement of the water-use efficiency (Battacharyya et al. 2015).

Recently, an increasing attention has been focused on the valorization of agro-industrial by-products as a source of bioactive compounds and some of them have a biostimulant potential. Among them, olive mill solid waste that represents a major by-product of olive oil chain production. This waste is rich in organic matter, polyphenols, and other bioactive molecules (Dermeche et al. 2013; Mekki et al. 2013). Tunisia, one of the world's major olive oil producers, has industries that generate substantial volumes of olive mill waste each year. These wastes are representing an environmental challenge due to their pollution impact and offer an opportunity for the circular economy by the valorization of bioactive substances to develop sustainable bio-solutions and innovative biostimulant formulations.

The present study reports some primary results of the evaluation of a new biostimulant derived from olive mill solid waste, applied to pomegranate trees subjected to controlled water stress conditions in Tunisia. The main objectives were to assess the effect of this bioproduct by evaluating its impact on the fruit quality and biochemical responses of the tree. Consequently, this research explores its potential in mitigating drought impact on growth and productivity. In overall, this work aims a contribution to develop sustainable solutions for managing water stress in the Mediterranean fruit crops through the valorization of locally available organic resources.

2. Materials and methods

2.1. Experimental site and orchard characteristics

This study was conducted in the semi-arid centre-east region of Tunisia exactly in an organic pomegranate orchard located in 'Sidi Elheni' (35°38'02.5"N 10°18'25.0"E). Trees were 10 years old, spaced at 2 m × 3 m and drip irrigated. The soil had a sandy loam texture with a medium level of organic matter (4.63%). The pH and the electric conductivity were respectively 8.90 and 522 µs/cm.'Sidi El Heni' region has a Mediterranean climate, characterized by hot summers and mild, low rainfall winters. May through August are the sunniest months, and July is generally the brightest month of the year. The average annual temperature in Sidi el Heni is around 19.9°C and the annual rainfall is around 301.8 mm. In summer, maximum temperatures can reach 33°C, while in winter, the average temperature of the coldest month is 12.7°C.

2.2. Treatments

Trees were irrigated by a drip irrigation system with two lines per row of trees. Each tree was irrigated with a total of 2 emitters with 4 L/h for the control (C) and 2 emitters with 2 L/h for the sustained deficit irrigation (S). The average water requirement of the pomegranate tree was evaluated using the Penman–Monteith formula (Allen et al. 1998) at 4900 m3ha-1during the growing season. Trees subjected to S treatment were divided in two groups one of which received foliar treatment with the biostimulant (ST). A total of 15 trees were used for the experiment distributed as 5 trees per treatment.

The biostimulant was prepared using the phenolic extract (PE) and lignin nanoparticles (LNPs) extracted from the olive solid mill waste (OMSW). The PE was produced by the hydroalcoholic extraction method using the procedure cited by Tlili et al. (2024) and Abboud et al. (2024). The extract of lignin nanoparticles LNPs was performed using an ionic liquid [Et3NH][HSO4]. The details of the extraction were explained by Cequier et al. (2019) and Tolisano et al. (2023). The phenolic extract and the lignin nanoparticles were mixed and diluted in water later at a concentration of 300 ppm. The physicochemical characteristics and chemical composition of the olive mill solid waste (OMSW), as well as those of the phenolic extract examined in this work, have been previously described by Tlili et al. (2026). Moreover, according to the FT-IR results reported in the same study, the formation of lignin nanoparticles involved mainly physical reorganization rather than notable chemical degradation, while leading to an enrichment of functional groups at the particle surface.

2.3. Studied parameters

2.3.1. Biochemical analysis of leaves

For biochemical analysis, the extraction and the measurements of compounds were made by the Rainbow protocol reported by Lopez-Hidalgo et al. 2021. Leaves samples are collected from all plants of the three treatments (C, S and ST) and they are lyophilized and homogenized on a fine powder. Then, for each sample, an extract (E) was prepared by mixing 25 mg of the leaf powder with 1 mL of ethanol (80%). This mixture was homogenized by a vortex and centrifuged at 4 C and 10000 rpm for 10 min. All compounds are quantified by spectrophotometer.

2.3.1.1. Chlorophyll and carotenoid concentrations

From a mixture of 300 µL of the supernatant of (E) with 300 µL of ethanol 80%, we took a volume of 150µl that was placed in the microplate with 3 repetitions for each treatment. Concentrations of chlorophyll a, chlorophyll b and carotenoids are measured at 664, 649 and 470 nm and calculated on (µg/mL) with equations of Lichtenthaler (1987):

Chlorophyll a = (Chla) = 13.36 A664–5.19 A649

Chlorophyll b = (Chlb) = 27.43 A649–8.12 A664

Carotenoids = (1000 A470–2.13 Chla – 97.63 Chlb)/209.

2.3.1.2. Phenolic compounds and Flavonoids content

From a mixture of 50 µl of the supernatant extract (E) and 450 µl of ethanol 80%, we take 100 µl and we add it to 200 µl of Folin –Ciocalteu reagent (10%) in an Eppendorf tube. After two minutes, we add 800 µl of Na_2CO_3 to this tube that was incubated later for 2h in the darkness and centrifuged 1 min at 10000 rpm. For all samples the absorbance of spectrophotometer was 720 nm to determinate the total phenolic compounds referring to gallic acid standards.

However, to determinate the total flavonoids content, another mixture was prepared by mixing 100 µl of the supernatant extract (E) with 300 µl of methanol, 20 µl $AlCl_3$ (10%), 20 µl of potassium acetate (1M) and 700 µl methanol. This mixture was incubated 30 min in the darkness at room temperature. The absorbance of measurement was 415 nm and the calculation was made referring to quercetin standards.

2.3.1.3. Malondialdehyde and Free amino-acids

The concentration of malondialdehyde MDA was determined by preparing at first a basic mixture from 500 µl of the supernatant of the extract (E) and 500 µl of ethatnol 80% µl. From this mixture, two screw cup tubes were prepared: in the first 250 µl of the mixture + 250 µl TBA + and in the second 250 µl of the mixture + 250 µl TBA-. In bath water (95°C), all tubes were incubated for 30 min and then immediately were cooled down for 10 min to be after that centrifuged 10 min at 4°C and 10000 rpm. For measurement, the absorbances were 660, 532 and 440 nm and for calculating the MDA content 3 equations were used:

A= [(A532 TBA+ - A600 TBA+) – (A532 TBA- - A600 TBA-)]; B= [(440 TBA+ - A600 TBA+) 0.0571]

MDA equivalents (nmol/mL) = (A –B)/157.000)106

To determinate the free amino acids content FAA, a mixture of 150 µl of the supernatant of the extract (E) and 75 µl ninhydrin reagent were incubated at 100°C for 10 min in a water bath, then cooled down for 10 min. Later, a volume of 375 µl of ethanol 95% were added to this mixture. Light absorbances of reading were 570, 520 and 440 nm and the calculation made according to L-Proline + L-Glycine standards.

2.3.1.4. Sugars

To determinate the total content of soluble sugars TSS, at first an ethalonic mixture was prepared with 450 µl of ethanol (80%) and 50 µl of the supernatant of the extract (E). From this mixture, 50µl was taken and added to 750µl of anthrone reagent and later heated at 95°C for 30 min in a bath water and then cooled down in ice.

For insoluble sugars (Starch STA), the pellet of the extract tube (E) was used and the supernatant was eliminated. 1 mL of perchloric acid was added to the pellet, mixed and incubated for 1h at 60°C in a thermobloc. From this mixture, in a screw cup tube, 50 µl was added to 450 µl of perchloric acid, incubated for 30 min at 95°C and then cooled down. Finally, to determinate starch content, 75 µl of the screw cup tube was mixed with 750 µl of anthrone reagent to be measured with the spectrophotometer.

The calculation was made according to D-glucose standards and the absorbance of measurements was 625 nm for SS and starch.

2.3.2. Fruit characterization

Fruit samples were subjected to a series of external measurements. The fruit weight (FW, in grams) and fruit diameter and length (FD and FL, in millimeters) were measured using a digital caliper.

2.3.3. Juice Analysis

* pH, Titratable Acidity (TA), Total Soluble Solids (SS) and Flavor index (SS/TA) determination

The pH of the juice was measured using digital pH-meter (Laqua PC220, Horiba, Japan). Titrable acidity was determined using NaOH (0.1N). In addition, soluble solids expressed in degrees Brix (°Brix) was determinate using a digital refractometer (HI96802, Hanna Instruments, Romania) then the flavor index was calculated by the rapport (TSS/TA).

*Total Phenolic Content (TPCJ) and Total Flavonoid Content (TFCJ)

Total Phenolic Content was performed by the Folin-Ciocalteu method, based on the optimized conditions established by Singleton et al. (1999) which expressed in mg GAE (gallic acid equivalent)/L of pomegranate juice. The Total Flavonoid Content determination was accomplished according to Yang et al. (2009) and results were expressed as mg CE (catechin equivalent)/L of pomegranate juice.

TPC (mg/mL) = Absorbance *2.916

TFC (mg/mL) = Absorbance *5.1403

* Lycopene

1mL of juice was mixed in 10 mL of solvant, then, the mixture was homogenized by vortex. Later, the mixture was left to settle to allow phase separation (the lower phase containing the plant material particles, and the upper phase consisting of the solvent containing extracts). The samples were then measured by spectrophotometer reading at different wavelengths (453, 505, 654, 663). The content is then measured using the following formulas:

Lycopene (mg/100 mL) = (-0.0458×A663) + (0.204×A645) + (0.372×A505) – (0.0806×A453)

2.4. Statistical analysis

All parameters were expressed as the mean of three replicates. Data were analyzed using one-way analysis of variance (ANOVA) with SPSS software (v.21.0, SPSS Inc., Chicago, IL), and mean differences were compared using Duncan's multiple range test at a 5% significance level. The correlation matrix was performed using GraphPad Prism software 10.4.1.

3. Results and discussion

3.1. Biochemical profile of leaves

3.1.1. Chlorophyll and carotenoid concentrations

Fig.1 shows a significant decrease in total chlorophyll, chlorophyll a and chlorophyll b and carotenoids contents in pomegranate plants under water stress (S) compared to those in control (C). This decrease may be due to an alteration of the photosynthetic system or chlorophyll degradation related to a reduction in nutriment uptake especially which are essential for chlorophyll synthesis as it was clarified by Hepaksoy et al. 2015. Similar results related to chlorophyll a and b decrease in pomegranate under deficit irrigation were observed by Adiba et al. (2023). Also, it was noted that water stress reduced total chlorophyll and carotenoids in pomegranate (Abboud et al. 2025). However, this alteration was restored by the application of the biostimulant in the treatment (ST). This result suggests that this phenol-rich biostimulant, containing bioactive compounds, may play a protective role in antioxidant activity and pigment stability. As it was demonstrated by Ertani et al. (2016) that an application of exogenous phenol on blueberries, hawthorn, and red grape skin has improved photosynthetic pigments. Other results were approved by Tolisano et al. 2023 and Del Buono et al. 2021 which demonstrated a positive effect of lignin nanoparticles on pigment synthesis and its antioxidant proprieties applied on maize plants. In general, the application of phenolic molecules ameliorates the nitrogen assimilation and enhances plant nutrition (Tanase et al. 2014), also improve the rise of carotenoids which have a protective and antioxidant role under abiotic and biotic stress (Bartucca et al. 2020).

3.1.2. Phenolic compounds and Flavonoids content

For polyphenols content in leaves of pomegranate trees, no significant difference was observed between treatments C, S and ST as shown in fig. 2. This result may suggest that the applied deficit irrigation induced moderate stress or/and the application of the nanobiostimulant at this experimental stage didn't modify highly biosynthesis of phenolic metabolites in leaves. As for TPC, the content of flavonoids in leaves remain relatively stable across all treatments. Contrary, Abboud et al. (2025) affirmed that drought stress caused significant decrease in the polyphenols and flavonoids contents of leaves comparatively to plants normally irrigated. In addition, soil application of 150 or 250 ppm of phenolic extract improved the phenol contents relative to control.

Generally, the stability of these compounds may indicate a certain degree of metabolic resilience and tolerance of the variety studied. These results align with those of Dermeche et al. (2013) who demonstrated that phenols derived from olive mill wastes are implicated in the direct reduction of oxidative stress more than in the stimulation of foliar metabolites.

3.1.3. Malondialdehyde and Free amino-acids

Figure 3 shows that ST treatment markedly reduced MDA levels compared to plants under water stress (S) and control (C) treatments. As MDA is a by-product of lipid peroxidation and a classic indicator used to assess oxidative stress, this result may indicate that this nanobiostimulant has a protective effect from oxidation and membrane deterioration. Tolisano et al. 2023 presented the ability of lignin nanoparticles to stabilize the MDA in treated maize plants.

Water stress (S) has induced a decrease in free amino acids compared to control plants (C) and in (ST) plants, FAA level was moderated. However, an accumulation of free amino acids is a typical response aimed at ensuring osmotic adjustment and protein stabilization under water stress (Du Jardin 2015). This result may suggest that the water stress was less severe and this input extracted from OMW has improved the metabolism of plants under water deficit conditions (Mekki et al. 2013).

3.1.4. Content of sugars and starch

Stressed plants in treatment (S) showed a significant accumulation of soluble sugars as typical mechanism of osmotic adjustment and ROS detoxification (Tolisano et al. 2024) compared to the plants in control treatment (C). In treated plants (ST), there is a slight increase of TSS level that can suggest an improvement in cellular water balance and a decrease in metabolic compensation caused by stress conditions.

Water stress increased starch content; however, in stressed and treated plants (ST), starch levels decreased. This response can be explained by an active metabolic adjustment and suggests that the treatment may alleviate the effects of water stress by reducing the accumulation of insoluble sugars, which are involved in osmoprotection and stress management (Martinez-Lorente et al. 2024). In addition, maltose, a product of starch degradation, plays a protective role in the chloroplast electron transport chain, thereby contributing to stress tolerance (Martinez-Lorente et al. 2024).

3.2. Fruit and juice characteristics

As shown in Tab.1 water stress (S) caused a significant reduction in fruit weight compared with fruits in the control treatment (C). However, the application of the nanobiostimulant (ST) has partially mitigated the negative effect of drought by producing fruits heavier than in the treatment (S). Previously, it presented that the pomegranate weight was significantly reduced under deficit irrigation and the reduction intensity was related to the cultivar and the season (Adiba et al. 2021). This is may be explained by the restriction of water availability in (S) that has limited cell expansion and fruit growth as response of pomegranate plants to drought (Mellisho et al. 2012). The nanobiostimulant applied has contributed to a better water status or a better metabolic activity, which is in line with previous studies showing that a biostimulant can improve fruit weight under water-limited conditions (Rouphael & Colla 2020).

Similarly, fruit diameter has decreased under water stress (S) as an effect of deficit irrigation (Hassan et al. 2025) on fruit development and ST treatment didn't show a strong improvement over S for restoring the diameter like in the control treatment (C).

Water stress significantly affected fruit growth, whereas the biostimulant treatment alleviated some of the negative effects but did not fully restore control conditions. This result agrees with Ertani et al. 2021 who indicated some enhancement using biostimulant like plant nutrition but without completely eliminating drought impact.

Tab.1 Fruit characteristics: Fruit weight (FW), fruit diameter (FD) and fruit length (FL).

According to Tab.2, no significant difference between treatments was noted. The juice pH ranged between 3.86 and 4.13. In contrast, the TA was reduced by water stress and water stress combined to LN-PE treatment resulting in a higher flavor index (SS/TA) comparatively to control. Results are in accordance with Porras-Jorge et al. (2025) which affirmed that the sustained deficit irrigation of pomegranate raised the SS, however, no significant difference in TA leading to constant maturity index. Similarly, it was confirmed that water stress increases the concentration of soluble solids by accumulation of sugars in fruits (Ripoll et al. 2016). In fact, the present results indicated that the nanobiostimulant has enhanced sugar accumulation in juice by improving water efficiency and carbohydrate metabolism.

A similar result has been found by using a biostimulant based on humic substances (Nardi et al. 2016). Biostimulants often reduce acidity by improving carbohydrate metabolism and respiratory balance (Rouphael & Colla 2020).

As for phenols content, water deficit has slightly decreased phenolic content even with LN-PE application. Whereas, no difference in flavonoids concentration was recorded. This is may suggest that the biostimulant treatment has improved defense and antioxidant responses. Overall, flavonoids levels are correlated with stress priming induced by natural biostimulants and those based on phenolic compounds biosynthesis are consistent to increase phenols levels on plant organs and to boost their metabolism like in fruits (Gatti et al. 2025) or leaves (Tlili et al. 2024 ; Tolisano et al. 2024).

Lycopene increased under stress (S) and was highest in ST. Drought often elevates carotenoid levels as part of a photoprotective strategy (Zhang et al. 2021), while biostimulants can further enhance carotenoid

biosynthesis through improved redox balance and metabolic activity.

Tab.2 Juice biochemical parameters: pH, titratable acidity (TA), soluble solids (SS), flavor index (FI), phenols (TPCJ), flavonoids (TFCJ) and lycopene (LYC).

In general, drought has affected the quality of juice and the nanobiostimulant treatment has improved juice quality such as higher sugars, phenols, flavonoids, lycopene and a lower acidity, indicating enhanced antioxidant metabolism, improved stress tolerance, and metabolic stability.

3.3. Correlations among leaf, fruit, and juice quality traits under deficit irrigation and biostimulant application

The correlation heatmap revealed clear relationships among leaf biochemical traits, fruit morphology, and juice quality parameters under deficit irrigation and biostimulant application. Photosynthetic pigments (Chl a, Chl b, total chlorophyll, and carotenoids) showed strong positive correlations with fruit size attributes (FW, FD, FL), juice pH, and soluble solids (SS), indicating that improved photosynthetic capacity was closely associated with enhanced fruit development and sweetness. These pigments were negatively correlated with malondialdehyde (MDA) and starch (STA), highlighting that oxidative damage and carbon immobilization increased as photosynthetic efficiency declined under water stress. MDA, a marker of lipid peroxidation, exhibited pronounced negative correlations with chlorophylls, carotenoids, and fruit biometric parameters, while showing positive associations with starch accumulation. This pattern confirms that drought-induced oxidative stress restricts carbon export toward fruits, promoting starch retention in leaves and limiting growth. Total soluble sugars (TSS) were positively correlated with free amino acids (FAA) and fruit quality traits, suggesting coordinated osmotic adjustment and metabolic reprogramming in response to stress. Conversely, starch showed negative correlations with TSS and most fruit parameters, supporting the view that effective remobilization of carbohydrates is essential for maintaining fruit filling under deficit irrigation. Leaf total phenolics (TPC) and flavonoids (TFL) were positively associated with MDA and negatively with chlorophylls, reflecting their accumulation as part of the antioxidant defense response under stress. In contrast, juice phenolics (TPCJ), flavonoids (TFCJ), and lycopene (LYC) were positively inter-correlated and strongly associated with soluble solids and flavor index, indicating that the biostimulant promoted preferential allocation of secondary metabolites toward fruits rather than leaves. Notably, lycopene content showed strong positive correlations with SS, TPCJ, and TFCJ, and negative correlations with MDA and starch, highlighting that improved antioxidant status and carbohydrate partitioning favor carotenoid biosynthesis in fruits. Overall, the matrix demonstrates that application of the PE–LNPs biostimulant mitigated drought-induced oxidative stress (lower MDA), preserved photosynthetic machinery, enhanced carbon mobilization, and redirected metabolic fluxes toward fruit growth and nutraceutical quality. These coordinated responses underline the capacity of olive-waste–derived bioactive compounds to improve both stress tolerance and fruit functional traits in pomegranate under limited water availability.

4. Conclusion

Results of this research work revealed that the nanobiostimulant (PE+LNPs) prepared using OMSW can improve some biochemical parameters and fruit quality of pomegranate trees under water stress. These bioactive molecules have led to a positive effect by showing a greater level of total chlorophyll pigment in leaves, enhancing the weight and content in soluble solids of fruits. Nonetheless, they didn't improve other parameters and this can be explained by the moderated stress applied. Overall, the present biobased product needs further investigation and optimization to explore more its potential under other levels of stress conditions and for adapting its dose and the method of application.

Declarations

Competing Interests:

The authors declare no conflict of interest.

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Author Contribution

Conceptualization: Darine Tlili: Formal analysis, Investigation, Data curation, Writing – review & editing, Writing – original draft. Samia Abboud: Formal analysis, Data curation. Sahar Abdelwahab: Formal analysis. Azhar Ouni: Formal analysis. Soumaya Dbara: Supervision, Resources, Methodology, Conceptualization, review.

Data Availability

Dataset available from the corresponding author upon reasonable request.

References

1. Abboud S, Ouni A, Aydi Ben Abdallah R, Bchir A, Ben Abdelwaheb S, Tlili D, Dbara S (2024) Unraveling the effect of phenolic extract derived from olive mill solid wastes on agro-physiological and biochemical traits of pomegranate and its associated rhizospheric soil properties. Journal of Hazardous Materials, 470, 134234, https://doi.org/10.1016/J.JHAZMAT.2024.134234.
2. Abboud S, Tlili D, Ouni A, Ben Abdelwaheb S, Lahmar K, Jellali M, Sghaier N, Aydi Ben Abdallah R, Dbara S (2025) Ameliorating the deleterious effect of water deficit stress on pomegranate through soil

application of polyphenols-enriched extract from olive mill solid wastes. Journal of Environmental Chemical Engineering, 13(3), 11644.

3. Adiba A, Hssaini L, Haddioui A, Hamdani A, Charafi J, El Iraqui S, Razouk R (2021) Pomegranate plasticity to water stress: attempt to understand interactions between cultivar, year and stress level. Heliyon 7(6).
4. Adiba A, Haddioui A, Boutagayout A et al. (2023) Growth and physiological responses of various pomegranate (Punica granatum L.) cultivars to induced drought stress. Vegetos 37, 887–899. https://doi.org/10.1007/s42535-023-00625-1
5. Allen R.G, Pereira L.S, Raes D, Smith M (1998) Crop evapotranspiration: guidelines for computing crop water requirements. Irrigation and Drainage Paper 56. FAO, Rome, pp. 1–15. https://doi.org/10.1016/j.eja.2010.12.001
6. Bartucca M.L, Guiducci M, Falcinelli B, Del Buono D, Benincasa P (2020) Blue: Red LED Light Proportion Affects Vegetative Parameters, Pigment Content, and Oxidative Status of Einkorn (Triticum Monococcum L. Ssp. Monococcum) Wheatgrass. Journal of Agricultural and Food Chemistry, 68, 8757–8763.
7. Battacharyya D, Babgohari M.Z, Rathor P, Prithiviraj B (2015) Seaweed extracts as biostimulants in horticulture. Scientia Horticulturae 196: 39–48.
8. Cequier E, Aguilera J, Balcells M, Canela-Garayoa R (2019) Extraction and Characterization of Lignin from Olive Pomace: A Comparison Study among Ionic Liquid, Sulfuric Acid, and Alkaline Treatments. Biomass Conversion And Biorefinery, 9, 241–252.
9. Del Buono D, Luzi F, Puglia D (2021) Lignin nanoparticles: A promising tool to improve maize physiological, biochemical, and chemical traits. Nanomaterials, 11 (846), http://dx.doi.org/10.3390/nano11040846. 2021
10. Dermeche S, Nadour M, Larroche C, Moulti-Mati F, Michaud P (2013) Olive mill wastes: biochemical characterizations and valorization strategies. Process Biochemistry 48(10): 1532–1552.
11. Du Jardin P (2015) Plant biostimulants: definition, concept, main categories and regulation. Scientia Horticulturae 196: 3–14.
12. Ertani A, Pizzeghello D, Francioso O, Tinti A, Nardi S (2016) Biological activity of vegetal extracts containing phenols on plant metabolism, Molecules 21 (2) 205, https://doi.org/10.3390/MOLECULES21020205
13. Ertani A, Nicola S, Petrini A, Bulgari R (2021) Biostimulants and their role in improving the nutrition of plants in hydroponic conditions. In III International Symposium on Soilless Culture and Hydroponics: Innovation and Advanced Technology for Circular Horticulture 1321 pp. 185-190.
14. FAO (2022) The state of the world's land and water resources for food and agriculture: Systems at breaking point. Food and Agriculture Organization of the United Nations, Rome.
15. Gatti N, Maghrebi M, Serio G, Gentile C, Bunea V.V, Vigliante I, Mannino G (2025) Seaweed and yeast extracts as sustainable phytostimulant to boost secondary metabolism of apricot fruits. Frontiers in Plant Science, 15, 1455156.

16. Hassan I.F, Ajaj R, Abd El-Khalek A.F, Alam-Eldein S.M, Gaballah M.S, Athar H.U.R, Hatterman-Valenti H.M (2025) Effects of deficit irrigation on growth, yield, and quality of pomegranate (Punica granatum) grown in semi-arid conditions. Horticulturae, 11(1), 101.
17. Hepaksoy S, Bahaulddin A, Kukul Kurttas Y.S (2015) The effects of irrigation on leaf nutrient content in pomegranate'İzmir 1513'. In III Balkan Symposium on Fruit Growing 1139 (pp. 581–586)
18. Lichtenthaler H.K (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology 148: 350–382.
19. López-Hidalgo C, Meijón M, Lamelas L, Valledor L (2021) The rainbow protocol: A sequential method for quantifying pigments, sugars, free amino acids, phenols, flavonoids and MDA from a small amount of sample. Plant, Cell and Environment, Vol. 44, No. 6, pp. 1977-1986.
20. Martínez-Lorente S.E, Martí-Guillén J.M, Pedreño M.Á, Almagro L, Sabater-Jara A.B (2024) Higher plant-derived biostimulants: mechanisms of action and their role in mitigating plant abiotic stress. Antioxidants, 13(3), 318.
21. Mekki A, Dhouib A, Sayadi S (2013) Effects of olive mill wastewater application on soil properties and plant growth. International Journal of Recycling of Organic Waste in Agriculture 2(1): 15.
22. Mellisho C.D, Egea I, Galindo A, Rodríguez P, Rodríguez J.B, Conejero W, Romojaro F, Torrecillas A (2012) Pomegranate (Punica granatum L.) fruit response to different deficit irrigation conditions. Agricultural Water Management, 114, 30-36.
23. Nardi S, Pizzeghello D, Schiavon M, Ertani A (2016) Plant biostimulants: physiological responses induced by protein hydrolysate-based products and humic substances in plant metabolism. Scientia Agricola 73(1): 18–23.
24. Porras-Jorge R, Aguilar J.M, Baixauli C, Bartual J, Pascual B, Pascual-Seva N (2025) The Effect of Deficit Irrigation on the Quality Characteristics and Physiological Disorders of Pomegranate Fruits. Plants, 14, 720. https://doi.org/10.3390/plants14050720
25. Ripoll J, Urban L, Brunel B, Bertin N (2016) Water deficit effects on tomato quality depend on fruit developmental stage and genotype. Journal of Plant Physiology, 190, 26-35.
26. Rouphael Y, Colla G (2020) Biostimulants in agriculture. Frontiers in Plant Science 11: 40.
27. Singleton V.L, Orthofer R, Lamuela-Raventos R.M (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 299: 152–178.
28. Tanase C, Boz I, Stingu A, Volf I, Popa V.I (2014) Physiological and biochemical responses induced by spruce bark aqueous extract and deuterium depleted water with synergistic action in sunflower (Helianthus annuus L.) plants. Industrial Crops and Products, 60, 160-167. http://dx.doi.org/10.1016/j.indcrop.2014.05.039.
29. Tlili D, Abboud S, Abdelwaheb S.B, Ouni A, Dbara S (2024) Palliative effect of phenolic extract derived from olive mill solid wastes on pomegranate plants submitted to water stress. Waste and Biomass Valorization 1–14. https://doi.org/10.1007/s12649-024-02736-5
30. Tlili D, Abboud S, Ouni A, Abdelwaheb S.B, Bchir A, Dbara S (2026) Eco-innovative biostimulant derived from olive mill solid wastes enhances agro-physiological performance and biochemical function in

drought-stressed pomegranate (Punica granatum L.). Journal of Environmental Science and Health, Part B.

31. Tolisano C, Luzi F, Regni L, Proietti P, Puglia D, Gigliotti G, Di Michele A, Priolo D, Del Buono D (2023) A way to valorize pomace from olive oil production: lignin nanoparticles to biostimulate maize plants. Environmental Technology & Innovation 31: 103216. https://doi.org/10.1016/j.eti.2023.103216
32. Tolisano C, Priolo D, Brienza M, Puglia D, Del Buono D (2024) Do Lignin Nanoparticles Pave the Way for a Sustainable Nanocircular Economy? Biostimulant Effect of Nanoscaled Lignin in Tomato Plants. Plants, 13(13), 1839. https:// doi.org/10.3390/plants13131839
33. Yang L, Iglesias P.A (2009) Chemotaxis: Methods and Protocols, Methods in Molecular Biology, vol. 571, pp. 489-505. PUBMED: 19763987 doi: 10.1007/978-1-60761-198-1_32
34. Zhang R.R, Wang Y.H, Li T, Tan G.F, Tao J.P, Su X.J, Xu Z.J, Tian Y.S, Xiong A.S (2021) Effects of simulated drought stress on carotenoid contents and expression of related genes in carrot taproots. Protoplasma, 258(2), 379-390.

Tables

Tab.1 Fruit characteristics: Fruit weight (FW), fruit diameter (FD) and fruit length (FL).

Treatment	**FW** (g)	**FD** (mm)	**FL** (mm)
C	148.52±18.54 a	70.574±3.89 a	33.21±0.31 a
S	89.33±10.89 c	68.64±7.52 b	29.34±5.11 b
ST	109.648±16.83 b	66.63±3.95 b	29.45±4.01 b

Tab.2 Juice biochemical parameters: pH, titratable acidity (TA), soluble solids (SS), flavor index (FI), phenols (TPCJ), flavonoids (TFCJ) and lycopene (LYC).

Juice	pH	**TA (%)**	**SS (°Brix)**	**Fi (SS/TA)**	**TPCj** (mg GAE/mL)	**TFCj** (mg GAE/mL)	**lyc** (mg/mL)
C	4.14±0.11 a	0.35±0.05 a	14.83±0.55 b	43.08±7.42 c	5.30±1.46 a	2.23±0.08 a	0.41±0.06 b
S	3.86±0.16 a	0.27±0.02 b	15.13±0.55 b	57.28±7.56 b	0.1±0.41 b	2.06±0.029 a	0.48±0.05 ab
ST	3.93±0.06 a	0.21±0.01 b	17.03±0.4 a	81.44±6.46 a	5.81±1.87 a	2.46±0.45 a	0.51±0.02 a

Figures

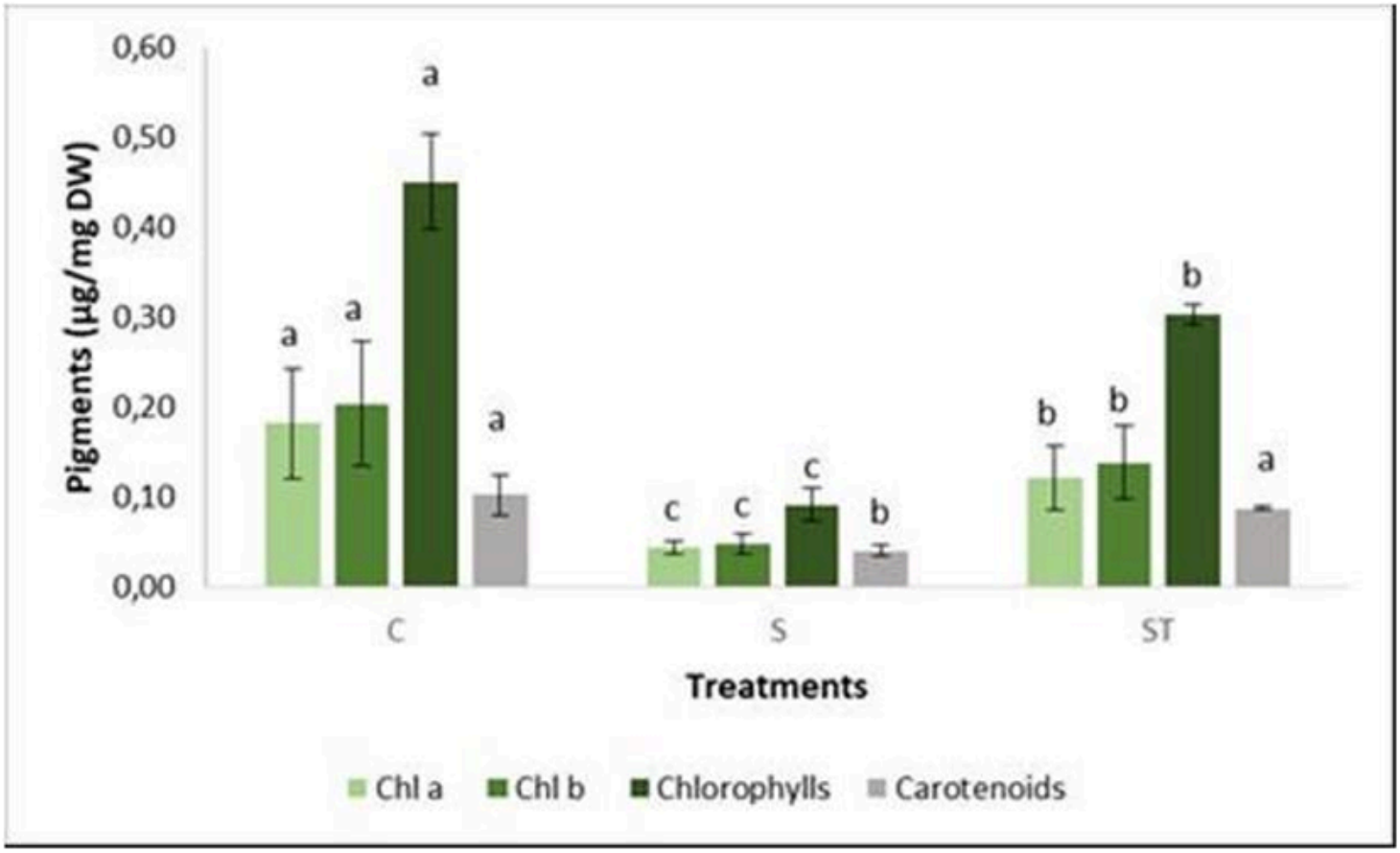


Figure 1

Photosynthetic pigments contents in leaves (chlorophyll a (Ch a), chlorophyll b (Ch b), total chlorophylls and carotenoids) in pomegranate plants under control (C), water stress (S), and water stress treated with the nanobiostimulant (ST). Data are means of 3 repetitions and error bars belongs to the standard deviation. Different letters indicate significant differences at $p \leq 0.05$ (Duncan test) separately for each parameter.

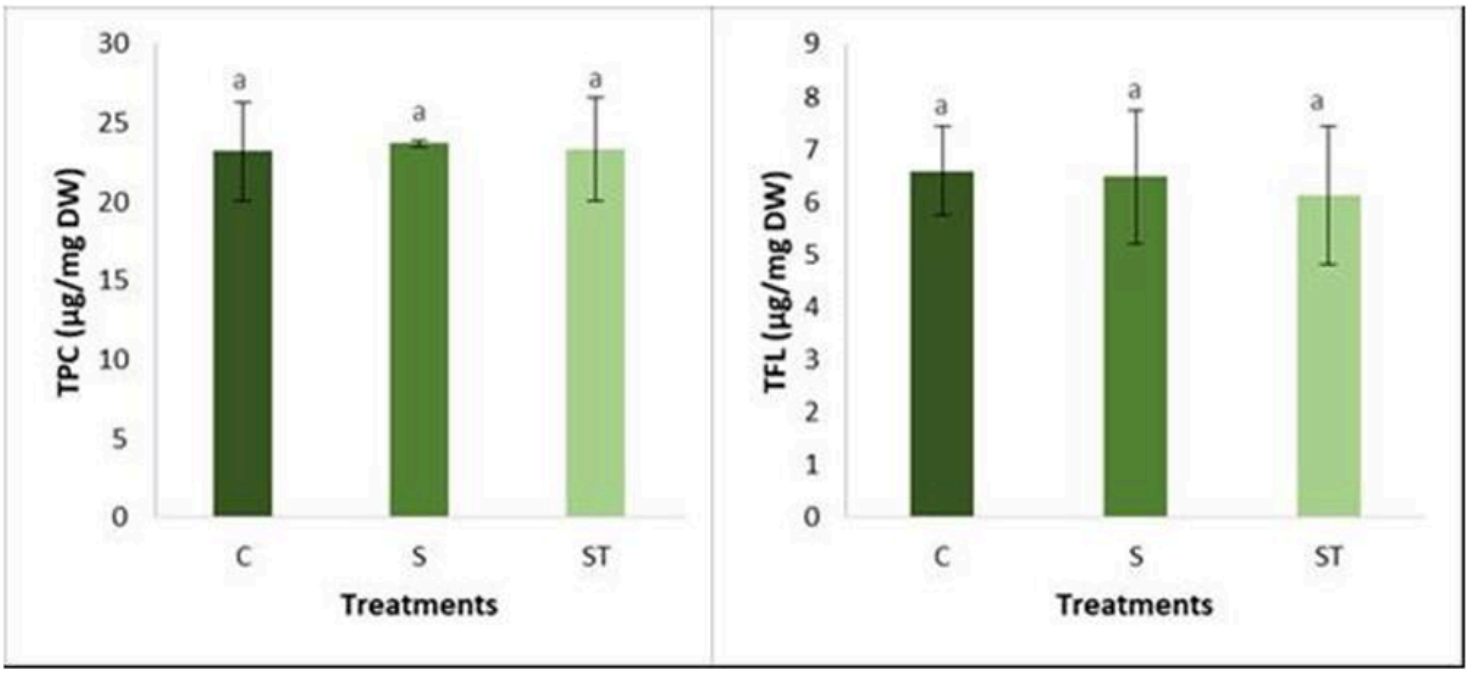


Figure 2

Contents of polyphenols (TPC) and flavonoids (TFL) in leaves of pomegranate plants under control (C), water stress (S), and water stress treated with the nanobiostimulant (ST). Data are means of 3 repetitions and error bars belongs to the standard deviation. Different letters indicate significant differences at $p \leq 0.05$ (Duncan test).

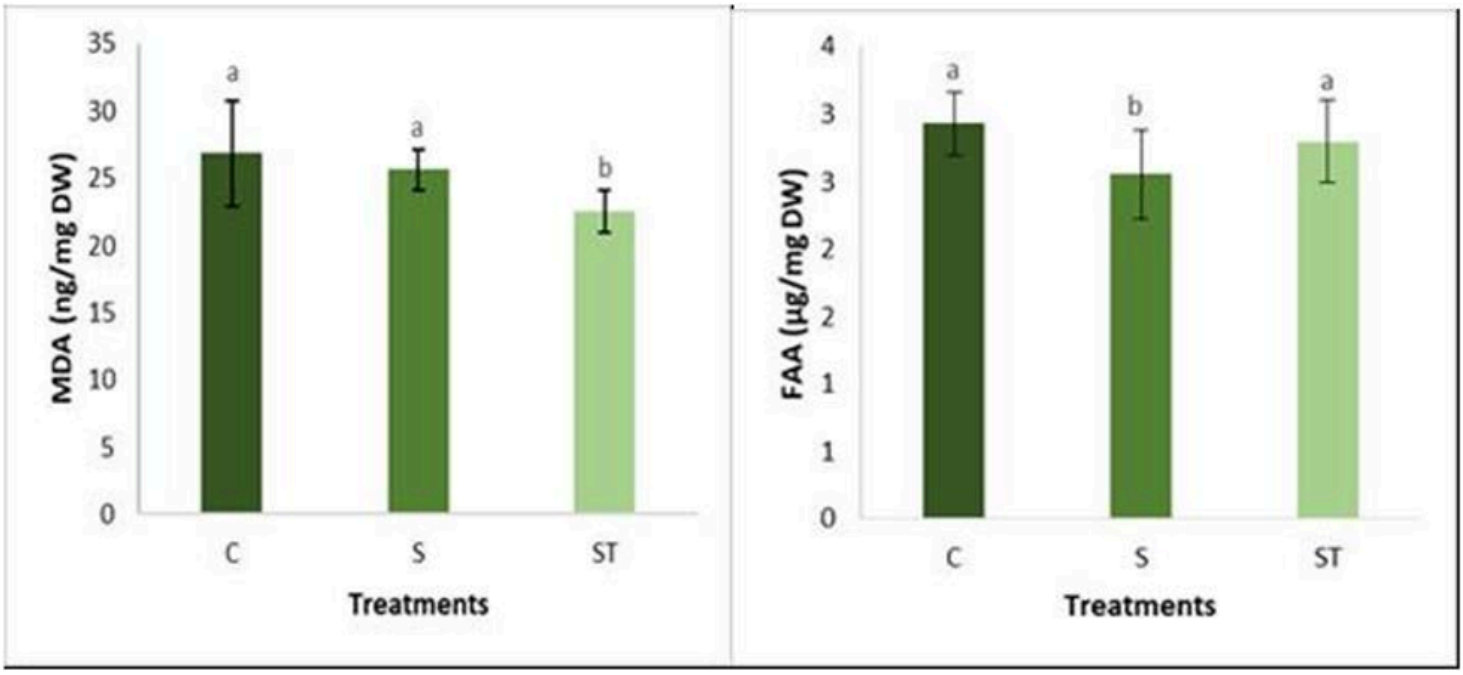


Figure 3

Contents of malondialdheyde (MDA) and free amino-acids (FAA) in leaves of pomegranate plants under control (C), water stress (S), and water stress treated with the nanobiostimulant (ST). Data are means of 3 repetitions and error bars belongs to the standard deviation. Different letters indicate significant differences at $p \leq 0.05$ (Duncan test).

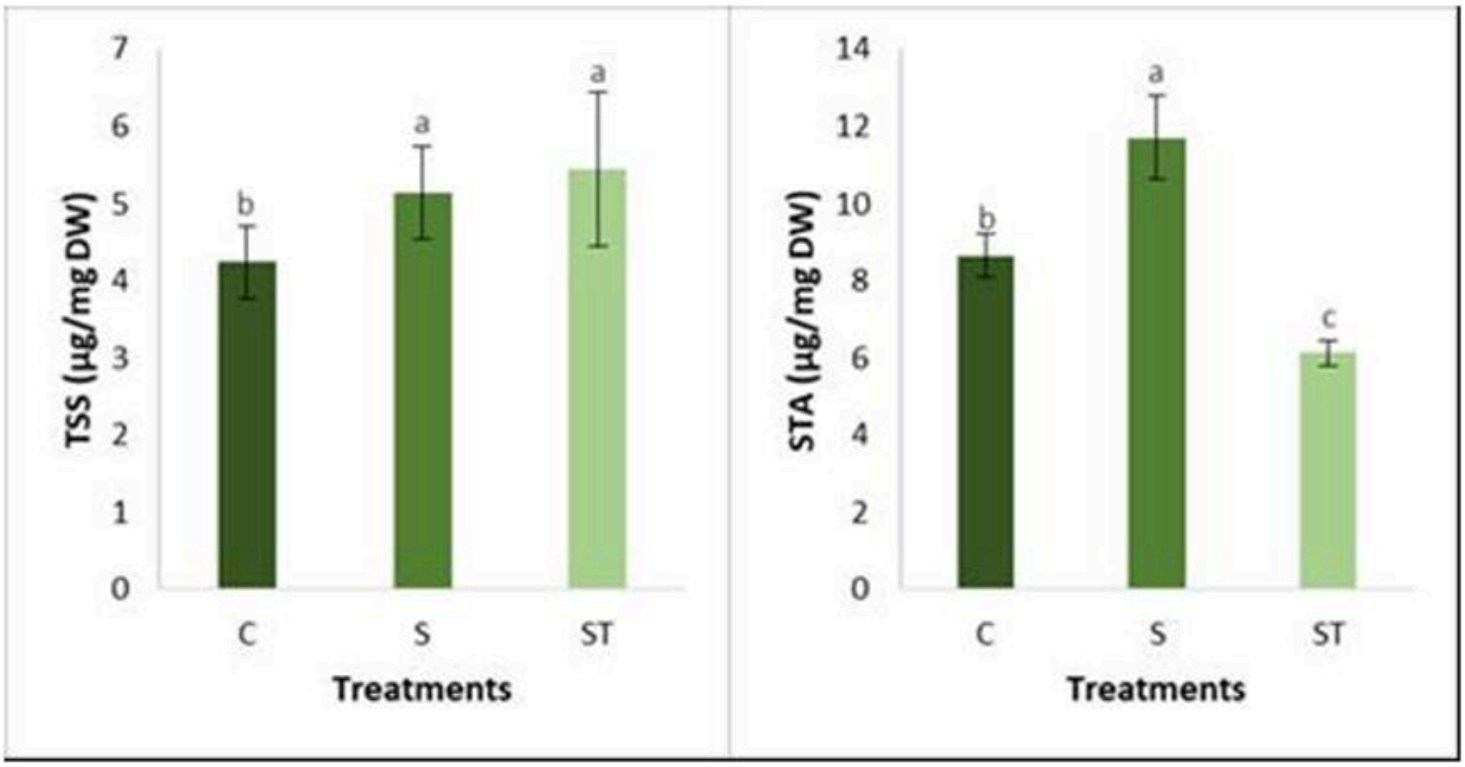


Figure 4

Contents of total soluble sugars (TSS) and starch (STA) in leaves of pomegranate plants under control (C), water stress (S), and water stress treated with the nanobiostimulant (ST). Data are means of 3 repetitions and error bars belongs to the standard deviation. Different letters indicate significant differences at $p \leq 0.05$ (Duncan test).

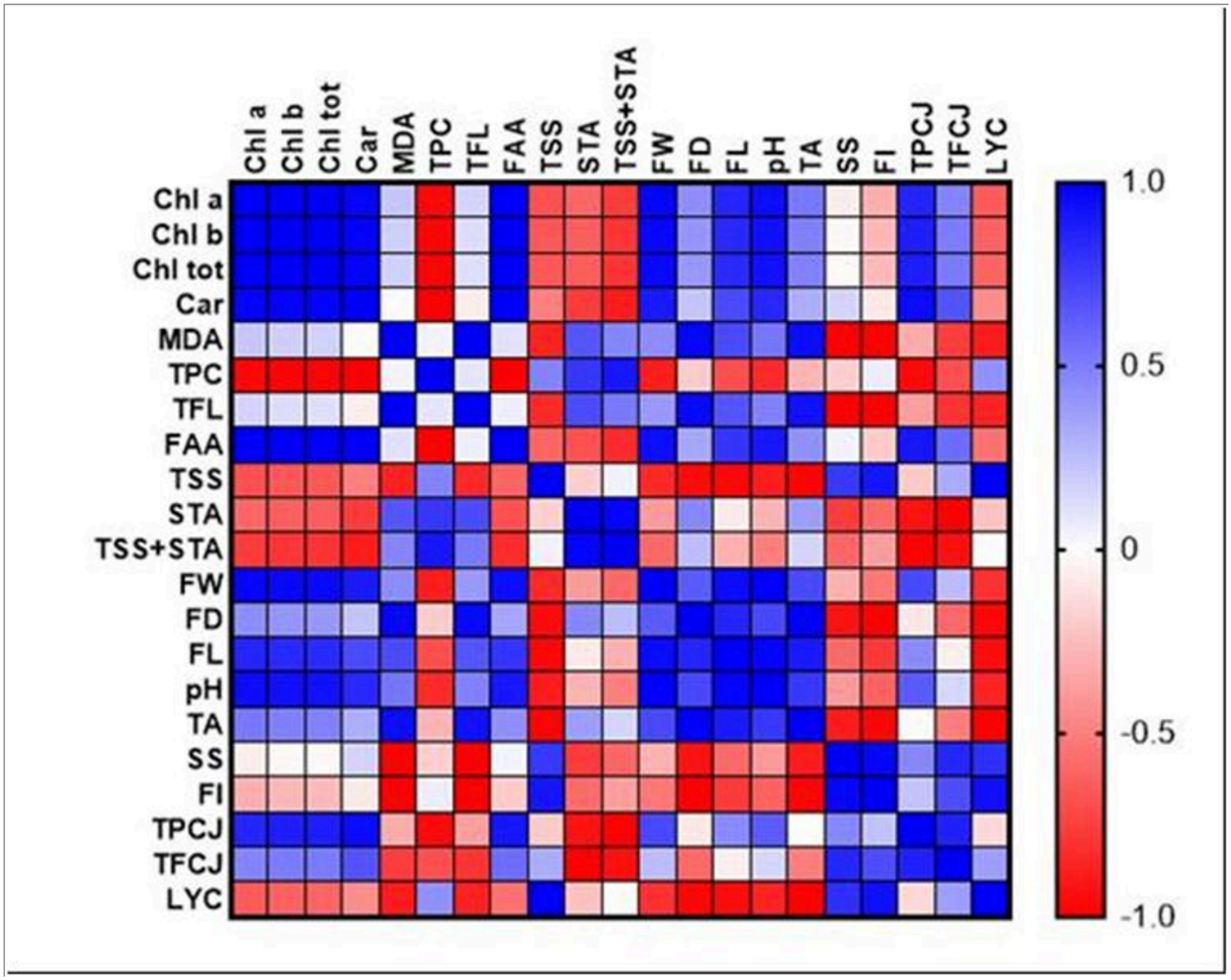


Figure 5

Pearson correlation matrix among leaf biochemical traits, fruit morphology, and juice quality parameters in pomegranate under deficit irrigation and PE–LNPs biostimulant application.