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The Efficacy of Melatonin on Flower Characteristics and Fruit Formation in Some Olive Cultivars Exposed to Low Irrigation

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ABSTRACT

The purpose of this study was to address the influence of melatonin foliar application on the flowering and fruit formation of olive cultivars of Sevillana and Roughani at the Dalaho Olive Research Station conditions of Kermanshah province. The experiment was conducted in a randomized complete block design in a factorial arrangement and three factors (MEL concentration, irrigation regime and olive cultivar) with 3 replicates and implemented in the crop year 2021-2022. The trees were sprayed with melatonin [0 (as a control), 50 and 100 µM] before flowering and during rapid fruit growth (two growth stages) on leaves. Irrigation treatments included 100% (control), 75 and 50% evapotranspiration. In order to investigate the state of flowering and fruit formation of olive cultivars under drought stress and melatonin foliar application, flower and fruit formation were recorded. Statistical analysis of variance and comparison of averages was done by Duncan for multiple comparisons between pairs of treatment as a post-hoc test. The results showed that flower traits and fruit formation were significant under the impact of variety, various levels of melatonin and irrigation regime at the probability level of 1%. The decrease in the amount of irrigation water affected flower characteristics and fruit formation. Melatonin foliar spraying improved the condition of flowering and fruit formation in Sevillana and Roughani cultivars.

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1. Introduction

Olive (*Olea europaea* L.) is a drought-resistant tree (Karamatlou *et al*., 2023). Olive is considered one of the most economically important fruits of the Mediterranean region; it is often cultivated for oil extraction and canning in regions with limited water sources (Fraga *et al*., 2020). Due to the existence of suitable conditions to cultivate olives and the country's need to produce oil, this product is very important from an economic point of view. Considering the serious risk of drought, the supply of water for trees was recognized as one of the main constraints for the development of olive cultivation. The use of mulch (Liao *et al*., 2021) and plant growth regulators reduces transpiration (Brito *et al*., 2020) and decreases water consumption. Meanwhile, more recently the use of drought stress modifiers such as melatonin (MEL) has proven to be a promising method in olive orchards to mitigate drought stress effects. The synthesis of this low molecular weight substance starts from the protein amino acid tryptophan, which is initially decarboxylated obtaining tryptamine hydroxylated, which is then converted to the indamine MEL (N-acetyl-5-methoxytryptamine) (Hassan *et al*., 2022). MEL is widely distributed in

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plants, but its levels vary from one specie to another, showing also differences between cultivars within the same species (Imran *et al*., 2021). Its accumulation in stressful conditions is still debated; however, thanks to its interesting amphiphilic properties, it can be important during stress response and adaptative strategies. MEL may freely cross cellular membranes and acts as an osmolyte, redox potential buffer, ROS scavenger, membrane stabilizer, signal transducer and activator of transcription of oxidative stress-responsive genes (Hassan *et al*., 2022).

In olive trees, flowering depends on environmental factors and plant internal factors (Maniriho, 2022). In comparison with deciduous fruit trees, which have a short cycle from flowering to the beginning of flowering, in olive trees, the time from flowering to the beginning of flowering is about eight months. Obviously, food and internal factors such as carbohydrates and hormones affect flowering (Erel *et al*., 2016).

The shortage of irrigation during winter has no efficacy on the formation of flowers in olive trees, but the lack of water affects the flowering parameters and formation of inflorescences during the development of flowers and inflorescences, and water deficit in this period reduces the number of flowers in each inflorescence as well as the number of complete flowers (Ghasemnezhad *et al*., 2019). In order not to affect fruit formation and size, from the stage of the emergence of flowers in olive trees until the beginning of hardening of the kernel, and also during fertilization and fruit formation, it should be avoided reducing the amount of irrigation water (Hueso *et al*., 2021). However, in an experiment carried out on olives of the Koroneiki variety at the Tarem Zanjan research station by applying four levels of irrigation: 25, 50, 75 and 100% of evapotranspiration (ET) from the beginning to the end of the no rain season, the percentage of complete flowers significantly decreased only under the 25% ET treatment (Nikbakht *et al*., 2011).

In this context, we studied the beneficial effect of exogenous foliar spray of MEL (0, 50 and 100 µM) on flower characteristics and fruit formation in 2 olive cultivars, Sevillana and Roughani, cultivated in fields under three different water regimes (100%, 75%, and 50% ET). The new knowledge on the MEL-dependent optimization of water consumption under low water conditions and the consequent improvements in flower characteristics and fruit formation will be useful to design new agronomic strategies to support oil production in Iran, projected to have warmer conditions (+5°C) and receive up to 10%–20% precipitation reduction at the end of the century (Zittis *et al*., 2022).

2. Materials and methods

The present research was done in Dalaho Olive Research Station Sarpole-Zahab City, (34º30′ N, 45º51′ E, 581 m up sea level), Kermanshah Province, in the crop year 2021-2022. Two olive cultivars, Sevillana and Roughani, by moderate canopy density and stand growth habit were used for the experiments, being the former superior Iranian local olive cultivar for oil extraction, and the latter a table Spanish olive cultivar (Gholami *et al*., 2022). Mature trees of the two olive cultivars (Table 1 and Fig. 1) were chosen in the randomized complete block with three replicates under three irrigation regimes as a factorial experiment. Complete foliar spraying of mature olive trees with MEL in concentrations of 0, 50 and 100 mM was done in two growth stages, before the beginning of flowering and during rapid fruit growth. Irrigation regimes included 100% as a control, 75% and 50% ET, which were carried out immediately after the last rain (May 1st to late October) by the Penman Mantis method. Irrigation was done by drip irrigation method once three days by considering daily evapotranspiration and the volume of water needed, considering olive plant coefficients (FAO, 2008). The amount of water used in the treatment of 100, 75 and 50% of water requirement during the growing season was 8649.01, 6486.75 and 4324.5 m^3/hectare, respectively. The climate of the region was semi-tropical with an average minimum and maximum temperature of 9 °C and 43.3 °C respectively, and an average annual temperature of 23.5 °C.

In field conditions, to assess the status of the flowering in the two olive cultivars, five branches in different directions for each tree were selected in May 2022, and based on this, the number of inflorescences per branch, the number of flowers in the inflorescence, the length of the inflorescence, the number of perfect flowers in an inflorescence, the length of the flowering branch, the number of fruits per branch, the number of perfect flowers per branch and the percentage of fruit formation (one month before harvest) were measured based on perfect flowers per branch (I.O.O.C., 2002).

Statistical analysis was performed using MSTATC software, and for multiple comparisons of averages, Duncan's method was used as a post hoc test. Due to the large number of tables, variance analysis tables were not given in the results section and only average comparisons were presented.

Table 1. Stages of flower phenology in tested olive cultivars.

Name of the variety	The date of the beginning of flowering	The date of full flowering	The date of petal fall
Sevillana	May 6	May 10	May 13
Roughani	May 2	May 7	May 10

Sevillana

Roughani

Figure 1. Branches of Sevillana and Roughani cultivars identified for the study.

3. Results and discussion

Variance analysis showed that all the measured traits of the variety, number of inflorescences per branch, number of flowers in an inflorescence, inflorescence length, number of perfect flowers in an inflorescence, length of branch flowering, the number of fruits per branch, the number of perfect flowers per branch and the percentage of fruit formation, were all significantly influenced by melatonin concentration and irrigation regime ($p<0.01$).

3.1. The number of inflorescences in a branch

The number of inflorescences in a branch under the influence of variety, MEL concentration and irrigation regime significantly differed ($p<0.01$). In the studied olive cultivars, in terms of the number of inflorescences in the branch, the highest value was in the Sevillana (12.23) and the lowest number was recorded in the Roughani variety (9.38). Drought stress decreased the number of flowers in the branch (Table 2).

3.2. The number of flowers in an inflorescence

The number of flowers in an inflorescence was significantly ($p<0.01$) impacted by cultivar, MEL concentration and irrigation regime, but their interaction was not significant. The number of flowers in the inflorescence was higher in the Sevillana cultivar (10.64) than in the Roughani one (6.06) (Table 2). There was a significant difference between the irrigation regimes in terms of the number of flowers in the inflorescence, besides the 100% ET treatment enhanced the number of flowers in the inflorescence (9.31), while the 50% ET one decreased it (8.21) (Table 2).

3.3. Inflorescence length

Inflorescence length was significantly affected by the variety, MEL concentration and irrigation regime ($p<0.01$). The comparison of the averages of the above traits showed that the highest amount of inflorescence length was obtained in the Sevillana cultivar (2.01cm) and 100% ET (1.91cm). Meanwhile, MEL foliar spraying elevated the length of inflorescences in both cultivars under study. Among the irrigation regimes, the highest amount of inflorescence length was found in the 100% ET treatment, while the 50% ET treatment decreased it (Table 2).

3.4. The number of perfect flowers in an inflorescence

The number of perfect flowers in an inflorescence was significantly ($p<0.01$) influenced by cultivar, MEL concentration and irrigation regime. The number of perfect flowers in the inflorescence was higher in the Sevillana cultivar (4.03) than in the Roughani one (3.09). In both studied cultivars, with the decrease in the amount of irrigation water, the number of perfect flowers in the inflorescence also decreased (Table 2).

3.5. Length of flowering branch

The length of the flowering branch was significantly ($p<0.01$) influenced by cultivar, MEL concentration and irrigation regime. In the Sevillana cultivar, the length of the flowering branch was longer (12.23cm) than that of the Roughani cultivar (6.97cm). In both studied cultivars, the length of the flowering branch decreased with the decrease in the amount of irrigation water, while in both cultivars, the length of the flowering branch increased with the increase in MEL concentration (Table 2).

Table 2. Effect of variety, levels of melatonin concentration and irrigation regime on flower traits of olive cultivars.

Treatment	Number of inflorescences in a branch	Number of flowers in an inflorescence	Inflorescence length (cm)	Number of perfect flowers in an inflorescence	Flowering branch length (cm)
Olive cultivars					
Sevillana	12.23[a]	10.64[a]	2.01[a]	4.03[a]	12.23[a]
Roughani	9.38[b]	6.06[b]	1.54[b]	3.09[b]	6.97[b]
Melatonin concentration					
Zero	10.23[c]	7.52[b]	1.68[c]	3.37[c]	8.65[b]
50 µM	10.71[b]	8.21[ab]	1.76[b]	3.53[b]	9.44[ab]
100 µM	11.48[a]	9.31[a]	1.89[a]	3.78[a]	10.71[a]
Irrigation regimes					
100% ET	11.62[a]	9.52[a]	1.91[a]	3.83[a]	10.95[a]
75% ET	10.82[b]	8.37[ab]	1.78[b]	3.57[b]	9.62[ab]
50% ET	9.97[c]	7.15[c]	1.64[c]	3.29[c]	8.22[b]

Different letters in each column show significant differences according to Duncan's multiple-range test. Data are expressed as mean, n = 3.

3.6. Number of fruits per branch

The number of fruits per branch was significantly ($p<0.01$) influenced by variety, MEL concentration and irrigation regime. There was no significant difference between 0 and 50 µM MEL use in terms of the number of fruits per branch (Table 3). In both studied cultivars, the number of fruits per branch also decreased with the decrease in the amount of irrigation water (Table 3).

3.7. The number of perfect flowers per branch

The number of perfect flowers in the branch was significantly ($p<0.01$) affected by cultivar, MEL concentration and irrigation regime. With the increase in MEL concentration, the number of perfect flowers in the branch increased (Table 3). Whereas, in both studied cultivars, the number of perfect flowers in the branch decreased with the reduction in the irrigation water content (Table 3).

3.8. The percentage of fruit formation

The percentage of fruit formation was significantly ($p<0.01$) affected by variety, MEL concentration and irrigation regime. In terms of the percentage of fruit formation, no significant difference was recorded between the two cultivars, although numerically, the percentage of fruit formation was higher in the Sevillana cultivar (7.43%) than in the Roughani one (7.16%) (Table 3).

In the two studied cultivars, the percentage of fruit formation increased with the enhancement in MEL concentration, although there was no significant difference between 0 and 50 µM MEL application (Table 3). In both studied cultivars, by decreasing the amount of irrigation water, the percentage of fruit formation also decreased, although, in terms of the percentage of fruit formation, no significant difference was observed between the 75% and 100% irrigation regimes (Table 3).

Table 3. Effect of variety levels of melatonin concentration and irrigation regime on flower traits of olive cultivars.

Treatment	number of fruits per branch	number of full flowers per branch	percentage of fruit formation
Olive cultivars			
Sevillana	3.82[a]	49.69[a]	7.43[a]
Roughani	2.15[b]	29.30[b]	7.16[a]
Melatonin concentration			
Zero	2.28[b]	35.39[c]	6.23[b]
50 µM	2.63[b]	38.68[b]	6.79[b]
100 µM	4.04[a]	44.42[a]	8.88[a]
Irrigation regimes			
100% ET	3.96[a]	45.44[a]	8.51[a]
75% ET	3.26[b]	39.36[b]	8.17[a]
50% ET	1.74[c]	33.68[c]	5.51[b]

Different letters in each column show significant differences according to Duncan's multiple-range test. Data are expressed as mean, n = 3.

Flowering in olive trees is promoted by environmental and genetic factors. Usually flowering in olives happens in the June-July period before the beginning of kernel hardening. The formation and development of the flower bud occur in the early stage of flowering by modifications of the expression in the genes to form the reproductive structure. The next stage is the initiation of flowering, when the budding structure can be observed under a microscope, and this stage takes place during fall. Finally, the differentiation stage in which the bud grows to form flower organs takes place at the end of winter (Zare *et al*., 2023).

The amount of irrigation strongly and significantly affected the formation of flowers and inflorescences, and therefore, the attributes related to flowers. In particular, in our research by decreasing the amount of irrigation the number of inflorescences in a branch, the number of flowers in an inflorescence, an inflorescence length, the number of perfect flowers in an inflorescence, the length of a flowering branch, the number of fruits per branch, the number of perfect flowers per branch, and the percentage of fruit formation remarkably decreased in comparison with control. Accordingly, Mezghani et al. (2012) found that irrigation regimes (20, 50, and 100% ET) significantly affected the flowering and fruit formation of native and foreign olive cultivars (Winter, Shamlali, Coratina, Picual, and Manzanilla) in Tunisia.

Martín-Palomo et al. (2020) researching regulated low irrigation in olive trees, showed that the stage of flowering and fruit formation, due to high cell division, are really sensitive to drought stress, while the kernel hardening stage is the most resistant stage against drought stress. Rapopert et al. (2012) in a study on the influence of low irrigation on three-year-old olive trees of the variety Picual grown in 50-liter pots, showed that the lack of irrigation during winter did not affect the formation of flowers or inflorescences in olive trees, but it reduced the percentage of fruit setting and fruit production. Moreover, different periods of water shortage had different effects on the measured traits. In particular, they found that the lack of water during the development of flowers and inflorescences affected the number of flowers and inflorescences, the percentage of complete flowers and the development of ovules, and caused the absence of inflorescences and the reduction of the number of flowers. In our study, the values of flower traits measured in the two olive cultivars were different from each other depending on the cultivar type, elucidating that the response of olive cultivars to drought stress depends on genetic characteristics. Olive is known for its tolerance to drought, however, there is a difference between cultivars in terms of response to water shortage (Gholami and Zahedi, 2019). In this regard, in an experiment on olives of the Koroneiki variety at the Tarem Olive Research Station in Zanjan, where four irrigation levels (25, 50, 75 and 100% ET) were applied, the percentage of perfect flowers showed a significant decrease under 25% ET treatment. Instead, the percentage of fruit formation in control plants (100% ET) did not differ from those at lower irrigation treatments (50 and 75% ET) (Nikbakht *et al*., 2011). Therefore, the results of this latter research are in agreement with the achievements of Rapopert et al. (2012) and Nikbakht et al. (2011). The stress effect on flower and/or fruit formation is relevant only when the irrigation decrease is consistent (25% ET) but varies according to plant genetic traits.

MEL treatment at 50 µM was already able to significantly increase the number of inflorescences in a branch, the inflorescence length, the number of perfect flowers in an inflorescence and of full flowers per branch. Whereas at 100 µM, it positively affected all the studied traits. Accordingly, previous results had already shown that MEL can be a suitable tool to reduce environmental stress, especially water shortage (Gholami *et al*., 2022). In tomato, (Ibrahim *et al*., 2020), apple (Wang *et al*., 2022), soybean (Imran *et al*., 2021), coffee (Cherono *et al*., 2021) and *Phoebe sheareri* (Hemsl.) Gamble (Li *et al*., 2023) the impact of MEL on decreasing oxidative damage caused by dehydration has been proven. The use of MEL can help plants survive in abiotic stress conditions by improving their regeneration capacity (Erland and Saxena, 2018). The several beneficial properties of MEL allow this substance to increase the plant's ability to deal with environmental stress (El-Yazied *et al*., 2022). The improvement of drought stress resistance in the treatment by MEL has also been observed in maize roots, tobacco seedlings, and alfalfa (Wang *et al*., 2023; Liu *et al*., 2021; Roy *et al*., 2021).

4. Conclusion

According to the obtained results, the tested olive cultivars had different reactions to MEL and irrigation treatments. Indeed flowering status of olive cultivars was highly impacted by drought stress. The effects of drought stress in the treatment of investigated cultivars with MEL showed a significant reduction. Therefore, this study provides further evidence that MEL is an inexpensive and eco-friendly low-weight bioactive molecule able to ameliorate olive trees' flower characteristics and fruit formation even under severe drought stress.

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed Consent

The authors declare not to use any patients in this research.

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