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The Utilization of Olive Oil Mills Wates (OMW) in Wheat (*Triticum aestivum L.*) Breeding

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Abstract
In parallel with the increase in the world's population, agricultural industry wastes increase from year to year. The olive oil mills come to the forefront among the industrial wastes based on the agriculture. In particular, these wastes are rich in nutrients and can be used as a source of organic matter. In the present study, we tried to determine the usability of olive oil mills including olive pomace, olive pomace oil and olive mill wastewater (OMW) in wheat breeding. In the study, germination rate (%), plant height (cm), root length (cm), fresh and dry weight of plant were investigated. The highest germination rate was 84% for olive pomace oil and 30.67% for olive pomace. All parameters in olive pomace application showed low values, and the highest plant height (22.07 cm) was obtained in OMW application. The results of the study showed that olive pomace oil and olive mill wastewater can be used effectively in wheat farming.
Increasing global population leads to a rise in agricultural industry waste, with olive oil mills being a prominent contributor. This study explores the potential use of olive pomace, olive pomace oil, and olive mill wastewater in wheat breeding.

Key words
Wheat, Olive mill wastewater (OMW), Olive pomace, Olive oil, Plant breeding.

Introduction
Cereal and cereal products constitute the basic food sources of humanity from past to present. For this reason, cereals constitute a group of plants that are cultivated and produced in large areas around the world. In addition to human and animal nutrition, the use of cereals in the industry has led people who are engaged in agriculture to seek new opportunities. The importance of wheat, which maintains its strategic importance among cereals is increasing day by day. In the world, wheat cultivation areas cover 1/7 of the cultivated land in each succeeding year. Wheat is planted in the world in 22 million ha area in 2021 and, 770 tons production has been realized. The wheat cultivation area in Türkiye in 2020 was 6 million ha and 20 million tons were produced (FAO, 2023).
Along with the rapidly growing population, adequate and balanced nutrition is becoming more difficult every day. For this reason, growing wheat with high value in terms of yield and quality is of great importance in supplying the growing nutritional needs. In this context, more plant feeding methods need to be determined to obtain efficient wheat cultivation. For this purpose, various fertilizer and fertilizer doses on wheat are tried to determine the most suitable conditions. Research to determine the sources of fertilizer is important for increasing wheat yield in the unit area. The importance of organic fertilizers has increased as a result of the soil pollution based on irregular chemical fertilizers use for years, the formation of harmful substances for human health in food products, and a decrease of quality and durability.
In the world, olive production was made in 10 million hectares and total production became 23 million tons in 2021. According to 2020 data, olive area in Türkiye is 8 million decares and production is 1.3 million tons. Türkiye ranks 5th in olive oil production with 217 bin tons after Spain, Italy, Greece and Tunisia (FAO, 2023). In Türkiye, the share of agricultural land allocated to cultivated crops, including olive trees, in total agricultural land increased over the years, reaching 8.8% in 2017, while the share of the total area covered by olive trees reached 2.2% (Anonymous, 2024). In the Türkiye, olive production was made in 889 bin ha and total production 1,7 million tons in 2021 (FAO, 2023)
The amount and properties of the wastes revealed during olive oil production vary depending on the olive oil manufacturing stages, the type of olives to be processed, the pesticide and fertilizer types used during olive cultivation, and the climate of the cultivated region (Andreozzi et al., 1998).
Olive pomace, olive pomace oil and olive mill wastewater (OMW) are the by-product of olive oil production. After the olive oil is extracted from olives in factories, the remaining residue is called as olive pomace. Also, olive pomace oil is oil that has not undergone the reesterification process, obtained by extractions with organic solvents, being mixed with other oils and mixtures. Olive pomace oil cannot be called olive oil under any circumstances and is used in soap, paint and similar industries. OMW is the material of the fine structure in the sedimented or suspended form in the pools where the wastewater is stored after the oil is obtained.
The wastewater from olive oil factories is currently being used as irrigation water in agricultural areas. Olive mill wastewater emerges as a waste during the process of squeezing the olive in factories. If given directly to the soil without participating in irrigation water, it negatively affects the development of plants.
The application of olive mill wastewater (OMW) in agriculture has been limited because it contains toxic phenolics that negatively affect biological activity. However, polyphenols are important in agri-food as they reduce oxidative stress by neutralizing free radicals. (El Moudden et al., 2022).
OMW adversely affects photosynthesis by preventing plants from taking advantage of sunlight. Again, because of the oil it contains, it creates a film layer on the surface of the water and prevents oxygen transport from the air to the water. Olive oil processing units, however, play an important role in the agricultural industry in all Mediterranean countries. Olive oil, which contains organic acids and phenolic substances, show a weak acid reaction and is also rich in dispersible or colloidal substances. Organic compounds in the structure of olive mill wastewater include sugar, nitrogen compounds, volatile acids, polyalcohol, pectin, oil, polyphenols, tannins that give the colour to olive mill wastewater (Cassano et al., 2013; Sygouni et al., 2019). The main crop of agricultural areas irrigated with oil wastewater is wheat and soybeans (Aybeke et al., 2000).
Olive cultivation is widespread in the world, so the wastes obtained after olive oil production have the potential to be used as organic fertilizer in plant farming. Cabrera et al. (1996) stated that, by directly irrigating the soil with olive oil mills, the water will be preserved making the fertilization possible and that it is useful for areas less than 800 m^3/ha. Altındal and Altındal (2016) investigated the effect of OMW on the germination of sainfoin seed showing the natural spread widely in Türkiye and having great importance in agriculture and animal husbandry. In the study, olive mill wastewater was applied in 6 doses [control, direct (pure olive oil wastewater), 1/1, 1/2, 1/3, 1/4 (olive oil wastewater/water)]. In general, increased wastewater concentration reduced the rate of germination. The highest germination rate was obtained from the concentration of 1/1 (30.01%) (Olive oil wastewater/water). Olive mill wastewater was applied to seeds in studies on different plant species, direct and 1/10.000 applications and increased the germination rate in wheat seeds, whereas, at different concentrations (1/10, 1/100 and 1/1000), germination was either lower or not present (Aybeke et al., 2000).
In this research, the effect of olive mill wastewater at different concentrations (control, 1/1, 1/2, 1/3, 1/4 [OMW/distilled water] and undiluted OMW) on the seed germination of sainfoin (Onobrychis vicifolia Scop.) was studied. According to treatments, germination in the shortest time occurred in 1/4 (OWM/distilled water) treatment. As a result, olive oil wastewater (OMW) mixed to irrigation water in certain doses may increase seed germination and can be used as fertilizer in agriculture (Altındal and Altındal 2018).
Dakhli et al. (2021), OMW produced in Tunisia contains phototoxic, acid and salinity content in its content and therefore toxic in the soil. In order to determine the availability of OMW in faba bean cultivation and to develop ways to utilize it in agriculture, researches applied different 0-15-30-45 m3/ha OWV in a three-year study on sandy soil. The study reported that 15 m3/ha OWV application increased bean growth and yield and soil microorganism activity

Khalil et al. (2021), in the study investigating the effect of olive factory wastewater doses on durum wheat (*Triticum aestivum* var. Douma1) yield and soil organism activity under Syrian conditions, OMW was applied to potted vertisol soil at a dose of 0-5-10-15 L/m. Germination rate (%), plant height (cm), spike length (cm), number of grains and grain yield (g/m^2) increased compared to the control. In addition, microbial activity increased in parallel with the wastewater dose. Researchers reported that studies on pre-application and bacterial activities in vertisol soils and Syrian climate should be carried out.

In the study investigating the effect of olive mill wastewater (OMW) on the yield parameters and essential oil composition of *Mentha aquatica* var. citrata, 9 ml/100 g, 22.5 ml/ dose of OMW did not affect the amount of leaves, stems, roots and chlorophyll of bergamot-mint cultivated for 110 days in pot soil, but phenol content in leaves increased. It was also reported that OMW affected secondary metabolite production and essential oil content (El Hassani et al. 2022). Organic matter obtained by drying waste sludge (OMWS) from olive oil with solar energy (SDy) was grown in greenhouse to evaluate the agronomic yield of *Zea mays* (Youness et al., 2022). In this study, SDy increased soil fertility, decreased soil pH and delayed germination and plant growth in the early stages of OMWS application. In the present study olive pomace, olive pomace oil and OMW were applied to the soil to determine their usefulness.

Materials and Methods

To determine the effect of olive oil mills on wheat, this research was carried out in pots in 4 repetitions as 1/3 olive pomace/soil, 1/3 olive pomace oil/soil, 99 ml olive oil wastewater/650 kg soil and only soil according to the random plots trial design in greenhouse conditions. Organic wastes (olive mill wastewater (OMW), olive pomace, and olive pomace oil) used in the study were obtained from local olive oil factories located in Muğla region. The data from the plants in each pot was obtained separately and the averages were taken and examined as one repetition in each pot.

Germination rate (%): Germination rates were calculated according to this formula; (number of germinated seeds/ number of seeds placed in germination environment) x 100.

Plant height and root length (cm): the roots and bodies of seedlings germinated in accordance with ISTA rules were cut with a razor from the joints, and the average root and plant height of each plant were determined as cm with the help of a millimeter ruler.

The fresh and dry weight of plants (g): Plant fresh weight was determined as gram by taking the total weights of the body and roots of the plants that represent the repetition for each application. The plants and roots were then dried at 105°C for 48 hours. After this process, dry weights were calculated as gram.

Statistical analysis was performed by taking the mean values and standard errors of the data obtained from all the applications (mean±SE). The data obtained from morphological observations were analyzed using the SAS package program to determine the effects of olive pomace, olive pomace oil, OMW, and control applications. Significant differences were determined using the LSD and Duncan multiple comparison tests in the MSTAT program to ensure control.

Results and Discussion

The highest germination rate in the study was obtained from olive pomace oil with 84.00% and the lowest rate from olive pomace with 30.67%. All the parameters examined in the application of olive pomace showed low values and the highest plant height (22,07 cm) was obtained from olive mill wastewater (Table 1).

Table 1: Effect of olive oil mills on germination rate, plant height, root length, fresh and dry weight of wheat

Treatment	Germination rate (%)	Plant height (cm)	Root length (cm)	Plant fresh weight (g)	Plant dry weight (g)
Olive pomace	30,67 ± 2,31	7,14 ± 1,23	6,53 ± 0,55	0,78 ± 0,00	0,16 ± 0,02
Olive pomace oil	84,00 ± 4,00	16,43 ± 0,78	9,23 ± 0,73	2,98 ± 0,14	0,39 ± 0,21
OMW	81,33 ± 2,31	22,07 ± 1,08	14,25 ± 0,65	4,19 ± 1,16	0,46 ± 0,02
Control	77,33 ± 2,31	18,70 ± 1,01	19,37 ± 0,63	4,38 ± 1,34	0,48 ± 0,15

Germination rate (%):

The negative effect of the application of olive pomace on the germination rate was determined through the study. It reduced the germination rate (30,67%). According to obtained results, the germination rate obtained by olive pomace oil and OMW application was higher than the control (Table 1; Figure 1).

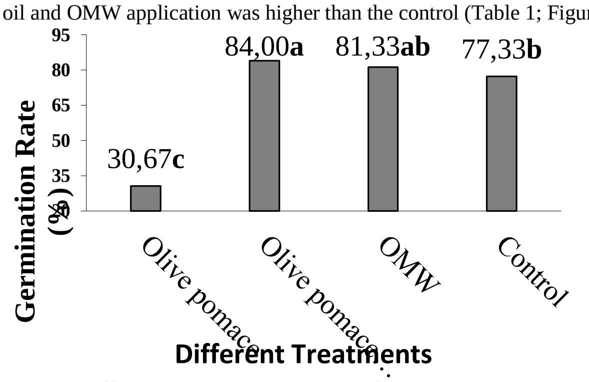

Figure 1. Effect on germination rate (%) of olive pomace, olive pomace oil, OMW and control on wheat. Data are mean ±SD of four replicates The same letters are not significantly different by Duncan's test (p> 0.05)

We believe that the adverse effect of olive pomace on seed germination is due to the acidic nature of the olive pomace and the phenolic compounds it contains.

Plant Height (cm)

While olive mill wastewater application significantly increased plant height in wheat, olive pomace application reduced plant height (7,14 cm). This value was followed by the olive pomace oil application with 16.43 cm (Table 1; Figure 2).

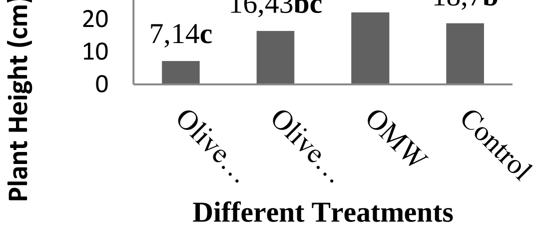

Figure 2. Effect on plant height (cm) of Olive pomace, Olive pomace oil, OMW and Control on wheat. Data are mean ±SD of four replicates The same letters are not significantly different by Duncan's test (p> 0.05)

Root Length (cm)

While the shortest root length (6.53 cm) was obtained from the olive pomace application, the longest root length was obtained from the control group with 19,37 cm (Table 1; Figure 3). In the study, in general, root length increased parallel to the plant height. Some of the carbohydrate requirements of plants are obtained from the seed's reserves and from photosynthesis, immediately after germination. As a result of this, photosynthesis increases with increasing plant height, thus root development is positively affected.

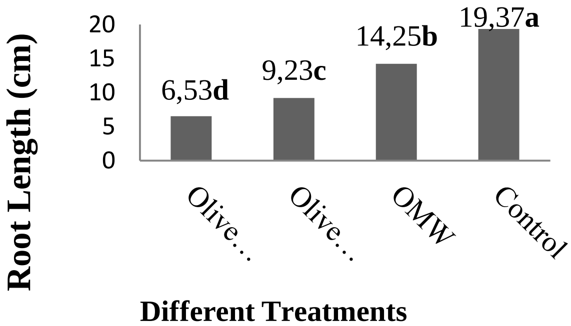

Figure 3. Effect on root length (cm) of Olive pomace, Olive pomace oil, OMW and Control on wheat. Data are mean ±SD of four replicates The same letters are not significantly different by Duncan's test (p > 0.05)

Plant Fresh Weight (g)

The plant fresh weight (4.38 g) was high in the control pots without olive oil wastes, followed by OMW application (4.19 g). The lowest fresh weight (0.78 g) was also detected in the application of olive pomace (Table 1; Figure 4).

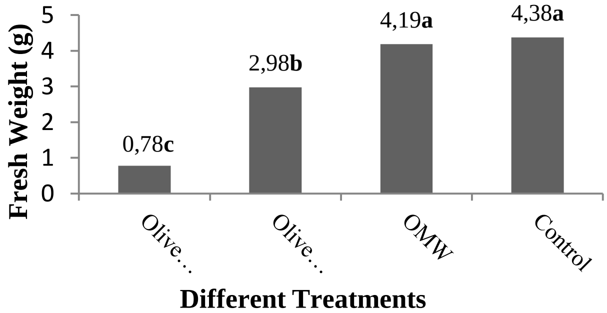

Figure 4. Effect of on fresh weight (g) of olive pomace, olive pomace oil, OMW and control on wheat. Data are mean ±SD of four replicates The same letters are not significantly different by Duncan's test (p> 0.05)

Plant Dry Weight (g)

In the study, plant dry weight increased parallel to plant fresh weight. According to the results obtained, the lowest dry weight was obtained from olive pomace administration (0.16 g) and the highest was obtained from the control group (0.48 g) (Table 1; Figure 5). Again, dry weight was determined as low for all olive oil waste applications.

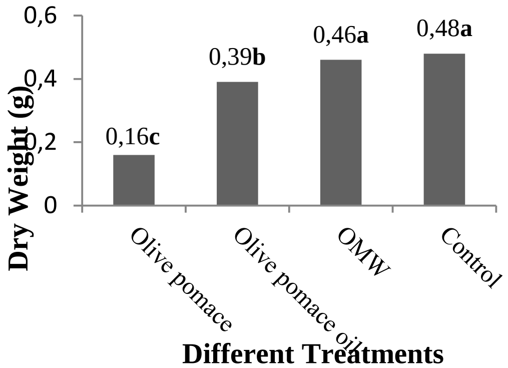

Figure 5. Effect on dry weight (g) of olive pomace, olive pomace oil, OMW and control on wheat. Data are mean ±SD of four replicates The same letters are not significantly different by Duncan's test (p> 0.05)

The present study, we determined that all the parameters were affected variously by the application of olive pomace, olive pomace oil and OMW. As can be seen from the results, the germination rate was high in olive pomace oil application, and the plant-height was high in olive pomace wastewater application. The reason for this is thought to be plant nutrients, which are present in a high proportion in olive pomace oil and OMW. They were not effective in the germination rate of olive pomace, plant and root lenght, plant wet and dry weight. The negative effect of olive pomace on seed germination and plant growth can be attributed to the acidic structure of olive pomace, the antimicrobial properties caused by phenolic compounds it contains and the unbalanced C/N ratio. Because the C source given to the environment with olive pomace increases, there may be a large amount of N immobilization, which can have a negative effect on the N intake of plants (Chapman, 1997). Olive pomace and olive pomace oil have negative effects on plants due to its high ratio of crude cellulose, tannins and phenolic compounds. Therefore, it should be made useful for agriculture by using physical, chemical, biological and natural refinement methods. In addition, OMW contains high amounts of organic (polyphenol etc.) and inorganic matter, and its acid properties and salt ratio is high (Hocaoğlu et al., 2017). For this reason, when diluted with water and used as a controlled and suitable volume, it can be evaluated as a liquid fertilizer because of the plant nutrient elements, minerals and organic matter it contains.

To increase the use of olive oil mills in agriculture, further research is needed to determine the effects of these wastes on plant development and also on the physical and chemical properties of soils, as well as the use of them as fertilizers and soil improving the organic matter.

Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

The contribution of the authors is equal

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