

(RESEARCH ARTICLE)



The effect of olive pomace and seaweed extract on the growth of pepper seedling

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Abstract

In recent years, environmental pollution problems have increased due to organic wastes. The use of organic wastes as fertilizer plays an important role in reducing environmental pollution and improving the physical, chemical and biological properties of the soil. This study was carried out to determine the effect of unprocessed olive pomace (OP) which is a by-product of olive oil industry and seaweed (SW) on the growth of pepper seedlings. In the study, 4 different pomace ratios (5% OP + 95% peat, 10% OP + 90% peat, 15% OP + 85% peat and 20% OP + 80% peat V:V) and 4 different SW extract doses (0% 0.5%, 1% and 2%) were used. In the study, parameters such as plant height, stem diameter, leaf area, plant fresh and dry weights, and chlorophyll index were investigated. According to the results of the study, OP applications significantly decreased the stem diameter, leaf area, leaf number, leaf dry weight, stem dry weight, root dry weight, chlorophyll content, stem-root ratio and total plant dry weight, while root length increased. On the other hand, SW applications had no an important effect on plant growth but partially imporoved in the zero OP application.

Keywords: Olive Mill Waste; Algea Extract; Development; *Capsicum Annum*

1. Introduction

Vegetables are propagated by two methods as vegetative and generative. However, the majority of vegetables are propagated by generative method, namely by seeds. The generative propagation has two methods. One of them is direct seed sowing (carrot, parsley, spinach, radish), and the other is seedling cultivation (tomato, pepper, eggplant, cucumber, watermelon, etc.). There are many advantages of growing vegetables with seedlings such as obtaining healthy seedlings, saving from seeds, field and energy, earliness and high yield. Although all its advantages are important, the most important advantage is earliness [1]. Due to the early release of the products to the market, they can find buyers at higher prices and the income to be obtained increases. In order to be able to produce successfully in vegetable growing, the first step is to start with a healthy and high-quality seedling. While seedling production was previously preferred only in greenhouse cultivation, it is now preferred in open field cultivation as well. Modern nurseries established in recent years have a great impact on the spread of cultivation with seedlings. The producer does not spend additional time, energy and effort for seedling production and can provide more convenient access to healthy seedlings.

Unconscious agricultural practices for many years have led to the exploitation of the organic matter content of the soil [2]. In order to maintain the productivity of the soils under the intensive agricultural production system, it is inevitable to add enough organic matter to the soil [2]. Depending on the species grown in each region and processing methods, varying amounts of organic waste are brought out. Evaluating wastes in a way that does not harm the environment and reusing them for nature is an inevitable necessity in terms of optimum use of scarce resources and prevention of environmental pollution [3, 4]. For a sustainable agricultural production, natural resources must be protected and used effectively [2]. Vegetable wastes and by-products produced by the agri-food industry have high organic matter and

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nutrient content. It has a great potential in the application of waste as fertilizer and in soil improvement [2, 4, 5]. One of these organic wastes is OP. The largest olive oil producers are located in the Mediterranean countries. Olive pomace and olive liquid waste generated by the olive milling industry are an important environmental problem [4]. Olive pomace can be used mostly as fuel, animal feed or soil conditioner [6]. However, it is necessary to be careful when using them for agricultural purposes. Because, OP and by-products have a phytotoxic and antimicrobial effect due to their high phenol, organic and fatty acid contents [7]. Altier and Esposito [8] reported that composting is an environmentally friendly and economical technology in the management of organic wastes such as OP, and it turns into a non-toxic product by reducing the polyphenol ratio with composting. Composted pomace, which does not contain toxic substances and has a high organic matter content, can be used in growing horticultural crops and strengthening the soil [9].

There is a great increase in the use of pesticides and inorganic fertilizers in agricultural production. This increase is mostly due to unconscious practices. More fertilizers or more pesticides does not mean more crops. These chemicals, which are used in excessive amounts especially in the long term, harm the organisms living in the soil and the physical, chemical and biological properties of the soil are adversely affected. For this reason, some researchers have adopted an environmentally friendly and sustainable production technique by feeding plants only organically. Organic fertilizers are important in terms of plant growth and conversion of nutrients into available form. Increasing the amount of organic matter in the soil can increase the population and activities of beneficial organisms by improving the physical, chemical and biological properties of the soil, such as the aeration and water holding capacity, the cation exchange capacity, and the nutrient content of the soil [10]. Cattle, sheep and poultry manures, compost, vermicompost, blood meal, bone meal (etc.) and the mixing of green plants directly into the soil can give as example the organic fertilizers. In addition to these, SW can also be used as organic fertilizers. Seaweed is a fertilizer that has been used for many years. However, the popularity of its use has increased in recent years. At the same time, SW extracts have growth promoting hormones, macro and micro elements, vitamins and amino acids [11], growth regulators such as auxins, gibberellins and cytokinin. In addition, it has been reported that SW accelerates germination, promotes the growth of roots and shoots [12], increases the development and yield of plants, provides resistance to environmental stress [13], increases the uptake of nutrients and improves antioxidant content [14, 15]. Seaweed is also used in organic agriculture, especially in developed countries [16].

This study aims to reduce the need for imported peat, to prevent/reduce environmental pollution by using OP in seedling production, and to encourage seedling development with SW extract.

2. Material and methods

2.1. Material

The experiment was carried out in the unheated plastic greenhouse of Harran University Faculty of Agriculture, Department of Horticulture in the spring production period of 2021. Afat F₁ pepper variety was used as plant material in the study. Afat F₁ has bitter, early, highly productive and vigorous plants and forms a very dense leaf surface. It is a variety suitable for outdoor cultivation. In our study, the effects of 4 different amounts of OP and peat mixture (0% OP + 100% peat, 5% OP + 95% peat, 10% OP + 90% peat, and 20% OP + 80% peat) and 4 different doses of SW fertilizer (0%, 0.5%, 1.0%, 2.0%) on the development of pepper seedlings were investigated. Olive pomace was provided from Harran University, Ebrulim Olive Oil Processing Facility.

2.2. Method

The seeds of Afat F₁ pepper variety were sown in germination trays filled with peat on 13 April 2021 and transferred to viols filled with a mixture of untreated OP and peat at different rates before the true leaves formed (23 April 2021). After the first true leaves were seen, SW fertilizer applications were started and continued at 7-day intervals. In addition, semi-strength Hoagland solution was applied once a week until the end of the experiment. During the experiment, diseases and pests were controlled both mechanically and chemically.

2.3. Features examined in the trial

Plant height was determined by measuring the part from the growing medium to the growth tip with a ruler. Stem diameter was measured with a digital caliper from 1-2 cm above the substrate level. Leaf number was determined by counting the leaves on plant. The area of all leaves on the plant was determined by the "ImageJ" program [17]. At the end of the experiment, the leaves, roots and stems of plants from were weighed separately with precision scales and their fresh weights were determined, then these samples were placed in a paper bag and dried in an oven at 70 °C until they reached a constant weight, and again weighed on a precision scale and leaf, roots and stems dry weight were determined. Total plant dry weight was calculated by summing the stem, leaf and root dry weight values. Stem/root

weight ratio was determined by dividing the stem dry weights by the root dry weights. In order to determine how long the roots could grow in the medium, the length of the longest root was measured with the help of a ruler.

The chlorophyll index values of the leaves were determined by measuring with a portable chlorophyll meter (Minolta SPAD-502).

2.4. Statistical analysis

The experiment was established with 4 replications according to the randomized plot design and 9 plants were included in each replication. Mstat -C package program was used to evaluate the data obtained from the experiment and LSD test was applied to compare the averages.

3. Results

The results obtained from this study, which was carried out to determine the effects of OP and SW applications on the development of pepper seedlings, are given below.

3.1. Plant height

The effect of OP and SW treatments on plant height was significant, while the effect of OP x SW interaction was non-significant. Mixing 5% OP into the growing medium reduced plant height by approximately 16%, while mixing 20% OP reduced plant height by approximately 59% compared to the control. While the tallest plants were seen in 0% pomace application, the shortest plants were obtained from the medium containing 20% OP. Application of SW at low doses produced plant heights close to control plants. However, high dose application of SW (2%) reduced plant height by approximately 9.41% compared to the control. In the OP x SW interaction, the longest plant height was measured in the OP 0% and SW 1% application, while the shortest plant height was measured from the OP 20% and SW 0% application.

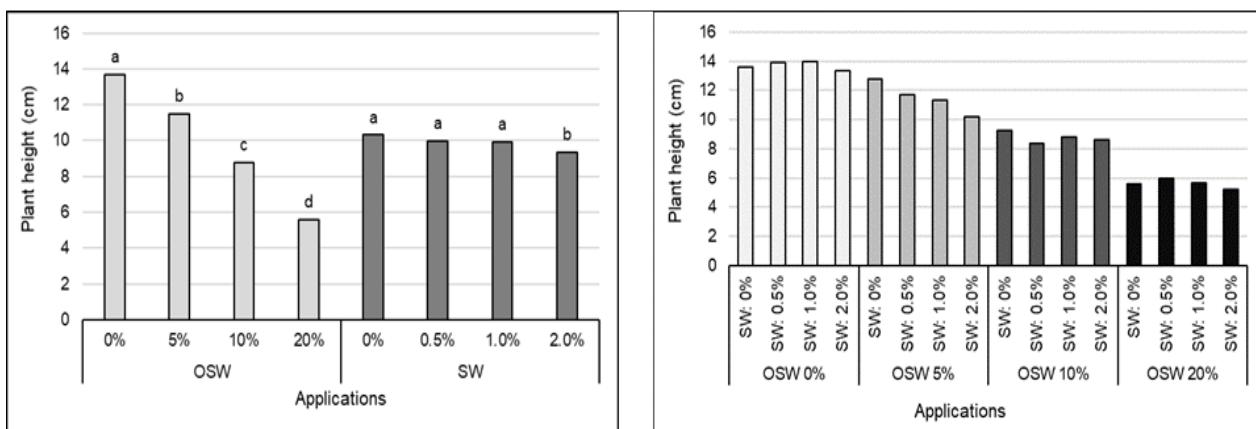
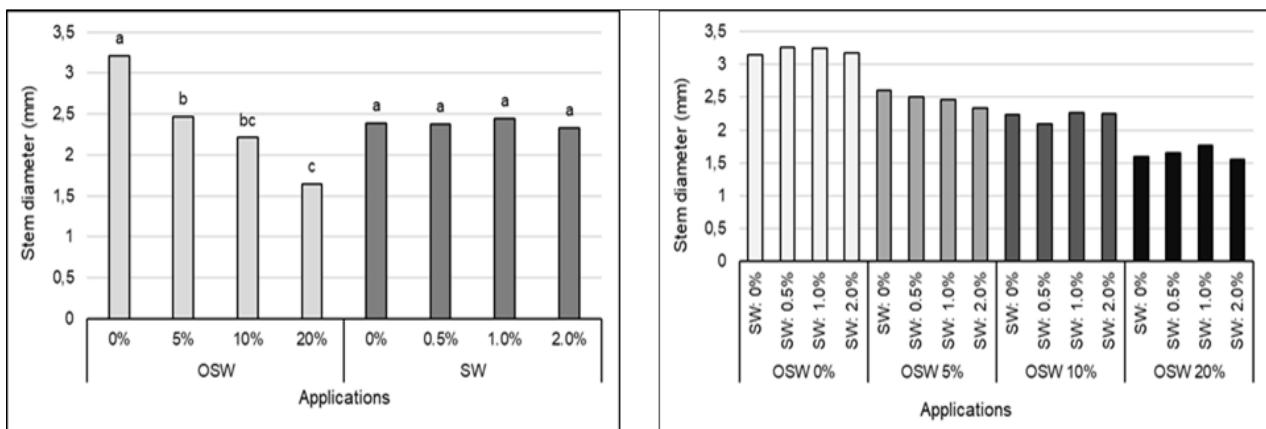


Figure 1 Main and interaction effects of OP and SW on plant height

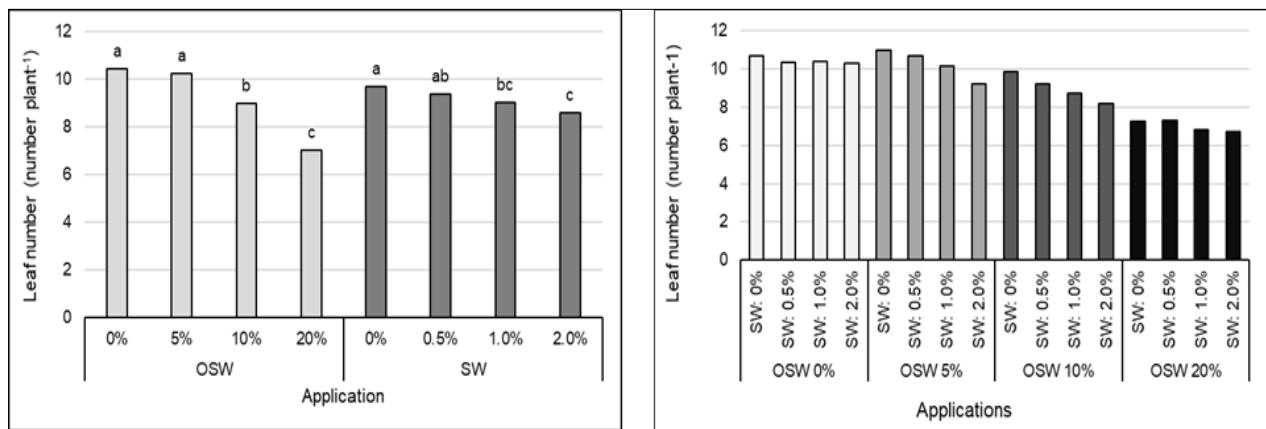
3.2. Stem diameter

Effects of OP on stem diameter was significant but, the effect of SW and OP x SW interaction was non-significant. Adding 5% OP to the growing medium decreased the stem diameter by 23.05%, while increasing the pomace rate to 20% caused a 48.91% decrease in stem diameter compared to the control. Seaweed doses had no significant effect on stem diameter, and all doses were in the same statistical group. The highest stem diameter value of OP x SW interaction was obtained from OP 0% and SW 0.5% application, while the lowest stem diameter value was obtained from OP 20% and SW 2% application.

**Figure 2** Main and interaction effects of OP and SW on stem diameter

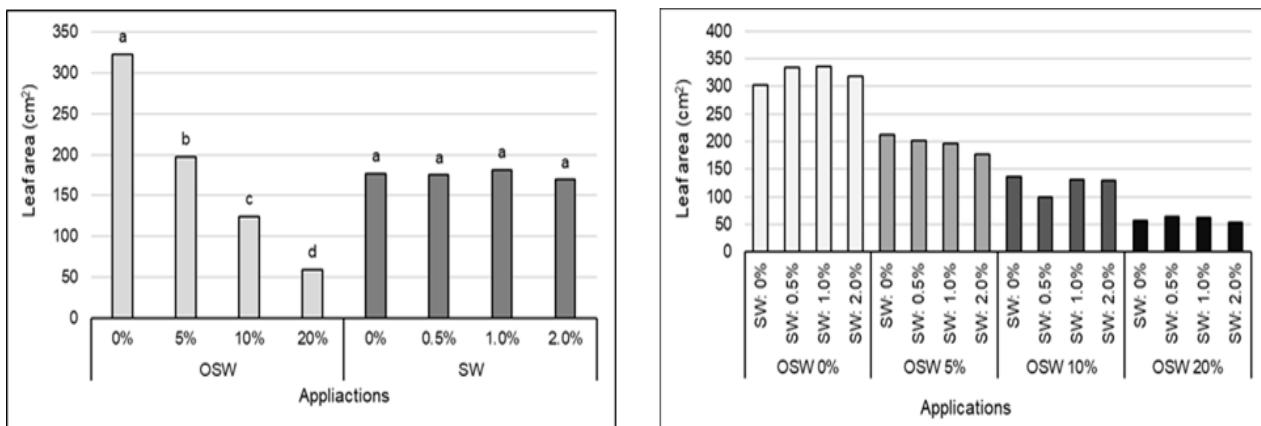
3.3. Number of leaves

The effect of OP and SW applications on the number of leaves was significant, while the effect of OP x SW interaction was non-significant. Adding 5% OP to the growing medium did not change the number of leaves, but the OP ratio 20% decreased the number of leaves by approximately 32.53%. Also, increasing the doses of SW decreased the number of leaves. While the number of leaves decreased with the increase in SW doses, especially in the applications where 5% and 10% OP was used, no effect of SW was observed in 0% and 20% OP applications. The maximum number of leaves was obtained from 5% OP and 0% SW application, while the least number of leaves was obtained from 20% OP and 2% SW application.

**Figure 3** Main and interaction effects of OP and SW on leaf number

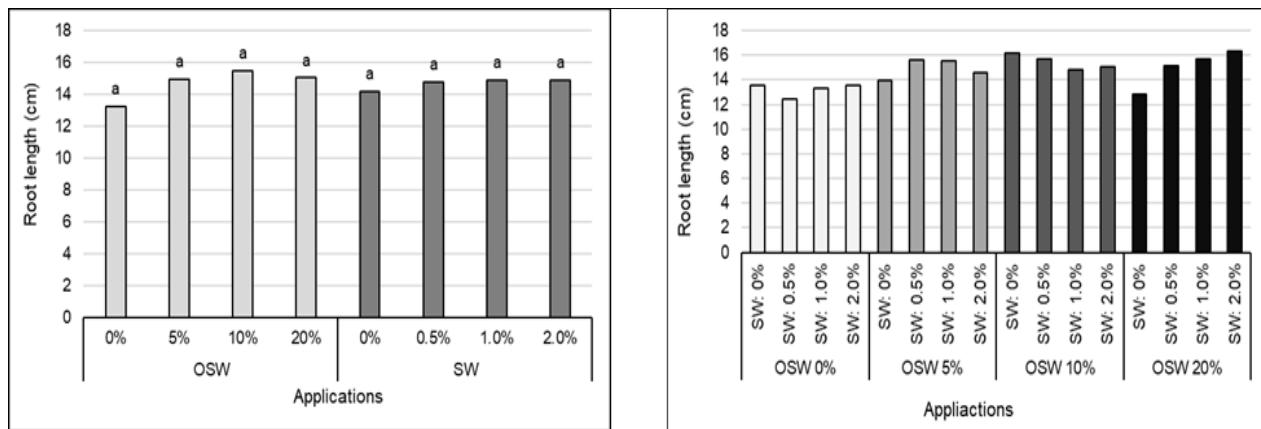
3.4. Leaf area

It has been observed that with the increase in the ratio of OP added to the growing medium, there was a significant decrease in the leaf area. When 20% OP was added to the growing medium, the leaf area decreased by 81.65% compared to the control. The effects of SW and OP x SW interaction applications on leaf area were found non-significant, and all applications were in the same statistical group. Seaweed applications in the medium without OP slightly increased the leaf area, while the applications of SW in the medium containing 5% OP slightly decreased the leaf area. While the narrowest leaf area was detected in 20% OP and 2% SW application, the widest leaf area was determined in 0% OP and 1% SW application.

**Figure 4** Main and interaction effects of OP and SW on leaf area

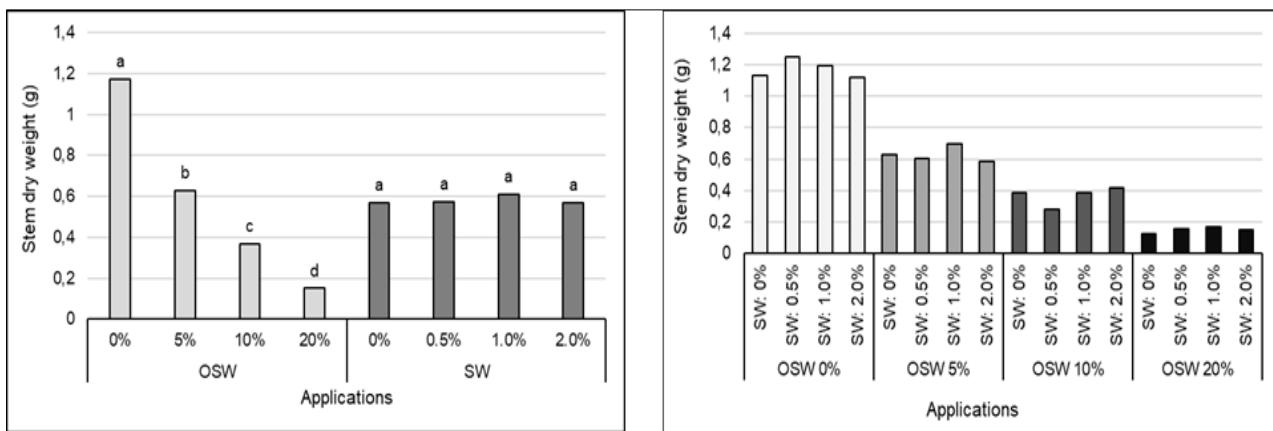
3.5. Root length

According to the results of the analysis of variance, the effect of OP, SW and OP x SW interaction on root length was non-significant. It was observed that root lengths slightly increased as the dose increased in OP and SW applications. It was observed that the SW applied to the medium containing 20% OP slightly increased the root length in the OP x SW interaction.

**Figure 5** Main and interaction effects of OP and SW on root length

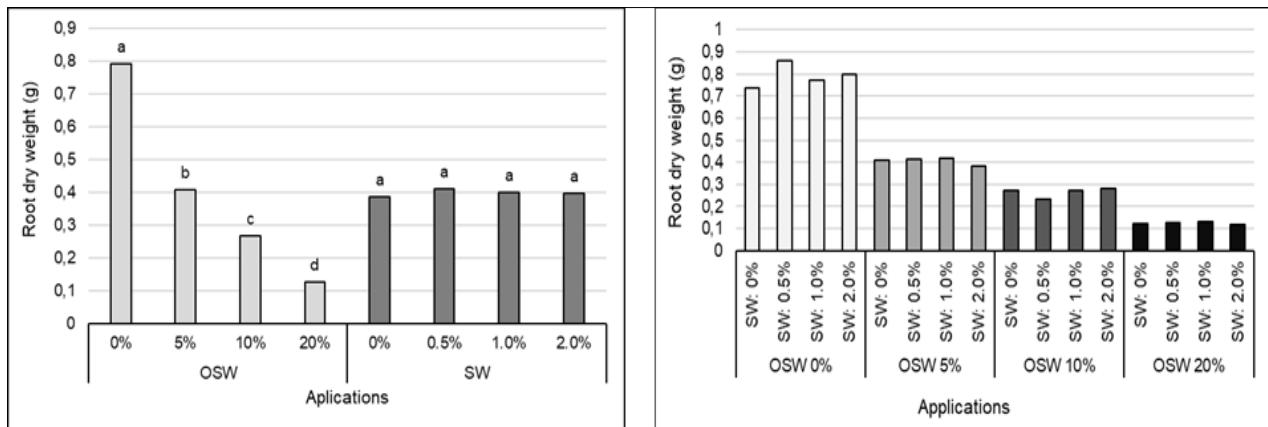
3.6. Stem dry weight

When the effects of the treatments on the stem dry weight were examined, it was found that the effect of OP was significant, while the effect of SW and OP x SW interaction was non-significant. In the study, the stem dry weights of the grown plants were examined and it was determined that there was a significant decrease in the stem dry weights as the pomace ratios increased. It was determined that when the pomace added to the growing medium was 20%, the dry stem weight decreased by 87.16% compared to the control. As the SW application rate increased, the stem dry weights increased slightly, but this increase was found non-significant. As seen in the figure, it was determined that the application of SW at the rate of 0.5% and 1% in the applications where the OP was 0% and 20% increased the stem dry weight compared to the control, but this increase was non-significant.

**Figure 6** Main and interaction effects of OP and SW on stem dry weight

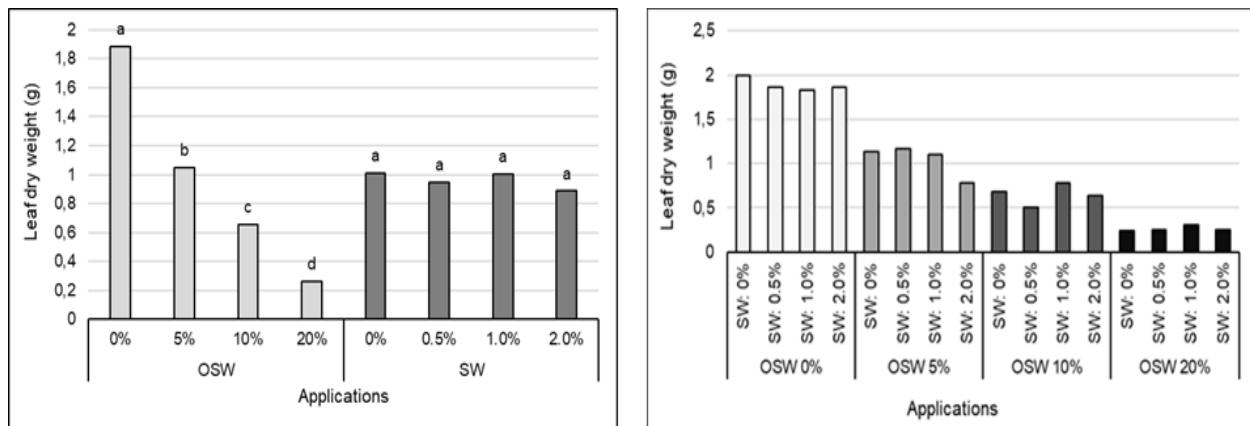
3.7. Root dry weight

As the proportions of OP added to the growing medium increased, root dry weights decreased significantly. This decrease was approximately 84.22% compared to the control in the medium in which 20% pomace was added. Seaweed applications increased root dry weight slightly, but this increase was statistically non-significant. Prina x SW interaction did not affect root dry weight. In general, 0.5% and 1% SW doses slightly increased root dry weights in all OP ratios.

**Figure 7** Main and interaction effects of OP and SW on root dry weight

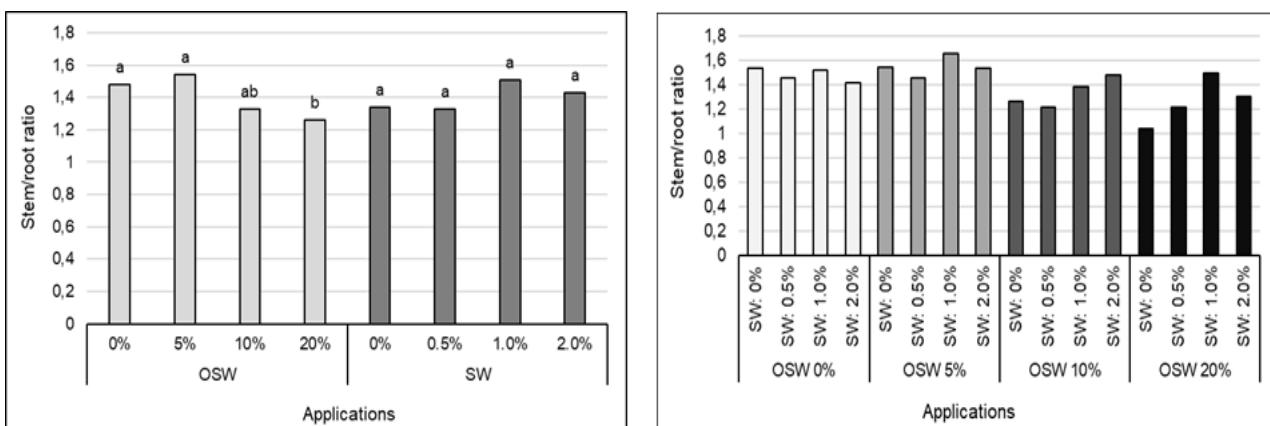
3.8. Leaf dry weight

The effect of OP applications on leaf dry weight was significant, the effect of SW and OP x SW interaction were non-significant. Olive pomace applications significantly reduced leaf dry weight. Increasing the OP ratio to 20% decreased the dry leaf weight by 86.03%. In SW applications, all doses were located in the same statistical group. In the OP x SW interaction, the highest leaf dry weight value was obtained from OP 0% and SW 0.5% application, the lowest leaf dry weight was obtained from pomace 20% and 2% SW application.

**Figure 8** Main and interaction effects of OP and SW on leaf dry weight

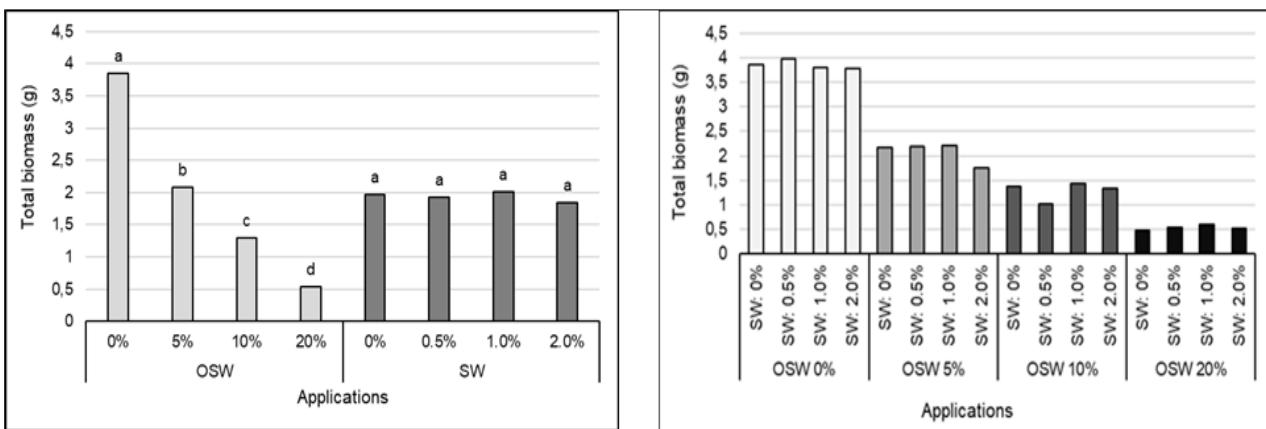
3.9. Stem/root ratio

According to the results of the statistical analysis performed to determine the effect of the applications on the stem/root ratio, the effect of the OP applications was found significant, while the effect of the SW and OP x SW interaction was found non-significant. While the highest stem/root ratio was determined in 5% OP application, it was in the same statistical group with 0% OP application. The lowest stem/root ratio was obtained from media containing 20% OP. Among SW applications, the highest stem/root ratio was obtained from 1% SW application, while the lowest ratio was obtained from control and 0.5% SW applications. However, all SW applications were in the same statistical group. In the interaction of OP x SW, the stem/root ratio decreased with the increase in OP ratios. Seaweed applications in 5%, 10% and 20% of OP increased the stem/root ratio non-significantly in general. According to the application results, the highest stem/root ratio was obtained from 5% pomace and 1% SW application.

**Figure 9** Main and interaction effects of OP and SW on stem / root ratio

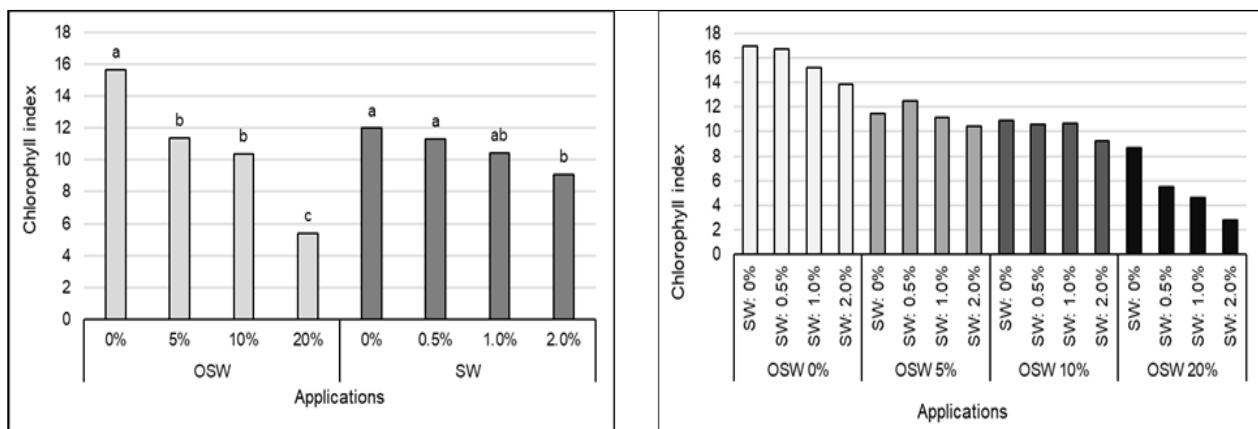
3.10. Total plant dry weight (Biomass) (g plant⁻¹)

The effect of OP applications on the total plant biomass was significant, the effect of SW and OP x SW interaction were non-significant. The increase in OP ratios caused a decrease in the total biomass. Adding 20% OP to the growing ratio reduced the total biomass by 86.11% compared to the control. Doses in SW applications formed values close to each other. In the OP x SW interaction, the highest total biomass value was obtained from OP 0% and SW 0.5% application, while the lowest value was obtained from pomace 20% and SW 0% application.

**Figure 10** Main and interaction effects of OP and SW on total biomass

3.11. Chlorophyll content index

The effects of SW and pomace treatments on the chlorophyll content index were found significant, while the effect of OP x SW interaction was non-significant. A decrease in the chlorophyll content index was observed in parallel with the dose increases in both OP and SW applications. Increasing the percentage of pomace from 0% to 20% in the growing medium decreased the chlorophyll content index by 65.54%. High dose application of SW (2%) caused a decrease in the chlorophyll content index value by 24.17% compared to the control.

**Figure 11** Main and interaction effects of OP and SW on chlorophyll content index

4. Discussion

In this study, the effects of OP and SW applications on the growth of pepper seedlings were investigated. According to the results obtained from the study, it was observed that the raw (unprocessed) OP added to the growing medium negatively affected the seedling growth. Raw (unprocessed) pomace is thought to inhibit plant growth by making a toxic effect on plants. Indeed, Cayuela et al. [18] reported that phytotoxic properties of OP are associated with high concentrations of phenols (catechol, hydroxytyrosol, tyrosol, oleuropein). Similarly, Killi and Kavdir [4], have determined that the direct application of large amounts of OP to the soil has adverse effects on seed germination and plant growth due to this high phenol and organic acid content of untreated OP. Plant height, chlorophyll content, plant fresh and dry weights, root dry weight and root length values of tomatoes grown by applying composted and uncomposted OP to sandy and loamy soils increased in composted OP, and decreased significantly in uncomposted OP [4]. Olive mill wastes and by-products from the edible olive oil industry have been reported to be unsuitable for direct agricultural application because they contain an unstabilized high organic load, including organic acids, phenolic compounds [19], and oils with antimicrobial and phytotoxic properties [5]. However, in the composting process, toxic compounds are broken down and olive mill wastes are completely detoxified [5, 18, 20].

Assimakopoulou et al. [21] investigated the effects of zeolite and OP applications (0, 2.5% and 5%) on plant growth and yield of pepper. As a result of the study, although it had no effect on the number of fruits per plant, it was stated that fruit fresh and dry weights, stem fresh and dry weights, leaf dry weight and aboveground parts fresh and dry weights decreased significantly with increasing OP. Similarly, Ilay et al. [7] determined that the application of OP to the soil improved the properties of the soil and the organic matter content of the soil increased, but negatively affected the growth of beans and sunflowers. It has been expressed that the increase in OP ratios decreases the plant height, stem diameter, number of leaves, fresh and dry weight values in beans and sunflowers. In parallel with this study, Papafotiou [22] determined that plant height, number of side branches and root length decreased with the increase in OP ratios in the growing medium of poinsettia.

Also, the C:N ratio of the growing medium has an important role in plant development. As a result of the microbial activity occurring during the degradation and decomposition phase of OP, a decrease in the amount of nitrogen available for the plant in the soil is observed [23]. Since the carbon source increases with the OP given to the environment, a large amount of nitrogen (N) immobilization may be in question, and this may have a negative effect on the nitrogen uptake required for the plants [24]. By composting of the OP, the C:N ratio is balanced and the nutrients are converted into more available forms for plants [7]. The findings of our study are in line with the above studies. On the other hand, Cegli et al. [25] examined the effect of mixing pomace and green waste compost with peat at different rates on the development of tomato seedlings, and reported that 20-45% compost can be mixed into the growing medium. Researchers found that plant height, plant fresh and dry weights were negatively affected by the increase in pomace ratios in the medium, but increased when compared to only peat usage. Chilosi et al. [26] used composted OP in the cultivation of the Photinia fraseri plant and reported that the addition of 33% and 66% to the medium increased plant growth. In a study conducted with OP compost and grape waste compost, it was determined that the yield of radish increased by 10% [27]. It has also been observed that pomace phytotoxicity differs depending on the growing mediums also, toxic symptoms are more severe in hydroponic culture or in sand-grown plants than in soil-grown plants [28, 29]. In addition, Ouzounidou et al. [29] examined the responses of tomatoes exposed to olive mill wastewater (OMWW) to growing in sand and soil. Soil experiments lasted 3 months, sand experiments only 10 days due to intense lethal symptoms. In both cases, the roots were reported to be more susceptible to OMWW than the upper of tomato plants grown in sand or soil. Pinho et al. [30], in the study where they evaluated the phytotoxic effect of two waste samples in two-phase and three-phase centrifugation olive oil production processes; They determined that 2-phase OMW was more phytotoxic than 3- phase OMW, although both OMWs had similar total phenolic content.

A healthy plant development can be ensured by the availability of water and nutrients needed by the plant in sufficient and available forms in the growing medium, as well as climatic factors [31]. Due to the beneficial properties of SW, its application in horticultural crops is increased rapidly. In many studies, it has been reported that seaweeds increase plant growth. Seaweeds increase the quality and performance of plants by containing cytokinins that plants need [32]. Mohammed et al. [33], in their study on lettuce, reported that the number of leaves and leaf area decreased depending on the dose increase of SW applications, had no effect on stem diameter and chlorophyll content, however increased head weight and yield. Demir et al. [34] observed that SW extracts were effective in seedling growth and accelerated growth. Cassan et al. [35] stated that as a result of spraying SW on the leaves of the spinach plant, an increase in the fresh weight of the spinach plant occurred. Allwright [36] found that SW application in wheat increased plant height and dry weight. Khan et al. [37] and Craigie [38] expressed that SW fertilizer reduced the effects of abiotic stress factors on plants. Whapham et al. [39] reported that by applying proton SW to grass, tomato and cucumber plants, the amount of chlorophyll in the leaves increased, making the leaves appear darker green. El- Sheekh [40] reported that extracts from some green algae and red algae increased the protein content of roots in legumes, the amount of total soluble sugar and chlorophyll in leaves, and the development of bean sprouts was stimulated at different levels. However, according to the results of our study, the effects of SW applications on seedling growth were generally found ineffective. This may be due to the difference in plant species, SW and doses. In addition, the nutrients needed by the plant were met by the semi-strength Hoagland solution given to the plants, so SW may have been ineffective.

5. Conclusion

Mediterranean countries are the first rank in olive and olive oil production in the world. The solid product formed as a result of processing olives into oil can be used as fuel, animal feed or soil conditioner. Olive pomace is a product of rich in organic and mineral matter. By mixing this product with the soil, the organic and mineral matter content of the soil increases so the physical and chemical properties of the soil improve. However, the application of pomace to the soil without processing can have a phytotoxic effect due to the phenolic substances it contains. Studies have reported that pomace must be composted before use in order to remove the phytotoxic substances in it. Untreated pomace inhibits plant growth, while plant growth improves after composting. The use of pomace in agricultural production after

composting, that is, after removing the phenols in it, will both prevent environmental pollution and increase the amount of organic and inorganic matter in the soil, thus promoting plant growth.

Seaweed fertilizer non-significantly increased seedling growth in medium without OP. We believe that applying SW to soil or leaves instead of peat would be more effective. The preference of organic fertilizers in plant production is indispensable for a sustainable agricultural production.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared no conflict of interest.

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