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Effect of Different Olive Mill Wastewater Treatments on Seed Germination

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Abstract

Untreated olive mill wastewater (OMW) has generally detrimental effects on seed germination. In the present study, the effect of treated OMW by filtration using some different media types on the germination of barley was investigated. Ten media types with a depth of 0.5 m were used. All media types, except hay and sawdust, increased the low pH value of OMW. The highest pH values and the lowest turbidity values were obtained when (rubber and zeolite) were used as filter media. The best removal efficiencies of COD and phenols were obtained in (rubber and clay loam) and (zeolite) filters, respectively. Untreated OMW has completely prevented the germination and the growth of the seedlings. However, treated OMW, using column filters of clay loam, loamy sand, rubber granules, zeolite, and rubber and zeolite mixtures, improved the germination percentage and seedling growth especially effluent generated from clay loam and loamy sand soil filters.

Keywords: *Wastewater reuse, Land application of OMW, Filtration, Industrial wastewater, Barley germination.*

1. Introduction

Olive mill wastewater (OMW) is the main residual product of the olive oil industry, and its disposal may cause many environmental problems, especially in Mediterranean countries, where olive oil production is large and concentrated in a short time. If not managed properly, OMW may cause soil and water contamination, which may affect human health and the environment. About 30 million m^3 of OMW is generated worldwide (mainly in the Mediterranean region) every year from olive oil production (Niaounakis and Halvadakis 2006).

OMW effluent is rich in nutrients such as organic matter, N, P, K, and Mg, which are favorable for agriculture (Mechri et al., 2011) in addition to water content (Ammar et al., 2005). In semi-arid areas, soils usually have a deficiency in nutrients and organic matter content (Garcıa et al., 1994). OMW contains various phenolic compounds, which have antimicrobial and phytotoxic effects (Hachicha et al., 2009). The Polyphenolic fraction is gradually degraded in soil with time and is partially transformed into humic substances. Thus, polyphenol degradation and incorporation into the soil humic fraction depend on environmental conditions (Sierra et al., 2007).

The direct application of organic fertilizers to cropland is the most common method nowadays (Mekki et al., 2006; Ben-Rouina et al., 1999). Adding OMW to cropland as irrigation water in the Mediterranean countries will balance both water shortages and low soil fertility. Many studies pointed out the beneficial effects of spreading fresh OMW, stored or treated, on the cultivated soil (Ayoub et al., 2014), emphasizing the positive effects of utilization of OMW in agriculture..

Crop response to OMW application is variable. For example, olive fruit yields and quality were not affected by the OMW application (Chartzoulakis, et al, 2010). Ryegrass and proteic pea yields were increased with untreated OMW application, whereas clover yield was negatively affected (Montemurro et al., 2011). In another study, maize growth was not affected, while plant stress parameters increased following the application of untreated OMW (Belaqziz et al., 2008). Hanifi and El Hadrami (2008) found an increase in maize yield following moderate and progressive OMW application. Al-Tabbal and Al-Zboon (2019), used olive mill cake along with stone-cutting sludge as a soil amendment. They found that the addition of olive cake negatively impacted the growth of maize. However, the addition of stone-cutting sludge to olive mill cake on clayey soil resulted in an improvement in maze growth parameters. Germination problems were also observed due to the phytotoxic effects of the phenolic compounds contained in the OMW (Mekki, et al, 2007; Massoudinejad, et al, 2014).

Several treatment methods have been used to treat OMW. These methods include advanced oxidation processes (Alrousan, 2021; Al-Bsoul et al., 2020), anaerobic and aerobic biological treatment (Shabir et al., 2023; Ammary, 2007), liquid extraction, filtration, flotation, adsorption, flocculation, coagulation, dilution, centrifugation electrohydrolysis, electrocoagulation, photocatalytic membrane reactor, Fenton and Fenton-like processes, combustion, pyrolysis, co-digestion and composting (Al-

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Hmoud et al., 2020; Shabir et al., 2023). Ojodale et al. (2022) used bacteria and fungi for the bioremediation of cassava, cocoa, and palm oil industry effluents. They found that bioremediation represents a promising method for the bioremediation of these effluents.

The present study evaluated the effect of treated OMW on barley germination. OMW was treated using filtration. Different filter media were used and the results were compared.

2. Material and Methods

A germination experiment was conducted to determine the effects of OMW generated from various filters at Al-Huson University College. Twenty seeds of barley were sterilized with sodium hypochlorite solution (Khan et al., 1992) and, after drying, were grown on Petri dishes that contained a double layer of filter paper. Ten ml of OMW taken from the effluent of various filters were placed on each petri dish. The filter paper was changed daily to prevent contamination. The seeds were supported to sprout at 26°C ± 1°C for 10 days. The seeds were considered as germinated seeds when radicle reached 2 mm long.

2.1 Experimental layout

OMW effluent, taken from various filters with different media (zeolite, hay, pomace, rubber, clay loam soil, loamy sand, fruit peel, sawdust, coal,, and rubber + zeolite), in addition to control (tap water), were used for the germination experiments. Figure 1 shows the various filters used for OMW treatment with different media types and depths. Each germination experiment was made in triplicate.

2.2 Germination Parameter Measurements

The number of germinated seeds was recorded daily from the beginning of the experiment until the 10th day when the germination process was assumed to be completed. At the end of this experiment, shoot and root, lengths were measured and, then, the roots and shoots were separated from the seeds, and their weights were taken separately using the digital balance. The ratio of root to shoot was also calculated from the data obtained. The germination percentage at the end of the experiment was calculated by dividing the number of germinated seeds by the total number of used seeds using the formula below (Al-Tabbal and Al-Zboon, 2019):

$$\text{Germination Percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \text{ X } 100\%$$

Germination speed index, relative germination rate, and germination energy were also calculated. (Al-Tabbal and Al-Zboon, 2019):

$$\text{Germination Speed Index} = \frac{\text{Gt}}{\text{Dt}} \quad (1)$$

$$\text{Reletive Germination Rate} = \frac{\text{a}}{\text{b}} \quad (2)$$

where

Gt: germinated seed in time days

Dt: the number of germination days

a: germinated percentage of treatment

b: germination percentage of control.

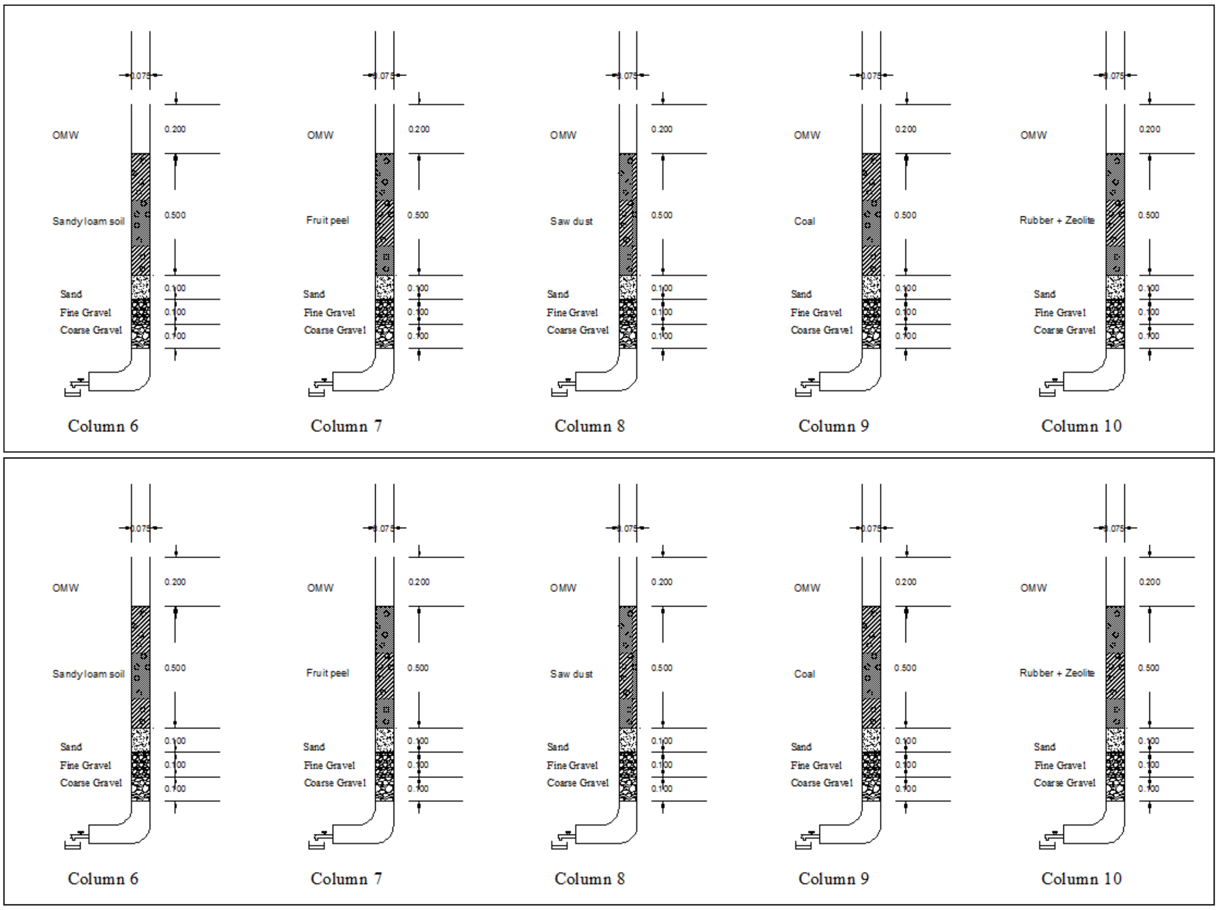

Figure 1. Schematic of experiment layout.

All columns filter from bottom: coarse gravel (10 cm), fine gravel (10 cm), sand (10 cm), filter media (50 cm), and then 20 cm of OMW. Column diameter = 7.5 cm.

Column #1: Zeolite, Column #2: Hay, Column #3: Pomace, Column #4: Rubber, Column #5: Clay loam soil,
Column #6: Loamy sand soil Column #7: Fruit peel, Column #8: Saw dust, Column #9: Coa Column #10: Rubber + Zeolite

$$\text{Germination Energy} = \frac{a}{b} \quad (3)$$

where

a: total germinated seed in OMW in fourth days

b: total number of seeds to germinate

The germination rate was measured using mean germination time (Younsheng and Sziklai, 1985).

At the end of the experiment, the promptness index (PI) and various stress tolerance indexes including germination stress tolerance indexes (GSTI), shoot length stress tolerance indexes (SlSI) (%), root length stress tolerance indexes (RLSI) (%), shoot dry weight stress tolerance indexes (SDSI) (%), root dry weight (RDSI) (%), and seedling vigor index (SVI) (%) were estimated using the following formulas (Al-Tabbal and Al-Zboon, 2019)):

$$\text{Promptness Index (PI)} = nd2(1) + nd4\ (0.75) + nd6(0.5) + nd8(0.25) \quad (4)$$

where nd2, nd4, nd6 and nd8 = Number of seeds germinated on the 2nd, 4th, 6th and 8th day, respectively.

$$\text{Germination Stress Tolerance Index (\%)} = \frac{\text{PI of Stressed Seeds}}{\text{PI of Contro Seeds}} \text{ X } 100\% \quad (5)$$

$$\text{Shoot length Stress Index (\%)} = \frac{\text{shoot length of stressed plant}}{\text{shoot length of control plants}} \text{ X } 100\% \quad (6)$$

$$\text{Root length Stress Index (\%)} = \frac{\text{Root length of stressed plant}}{\text{Root length of control plants}} \text{ X } 100\% \quad (7)$$

$$\text{Shoot dry weight Stress Index (\%)} = \frac{\text{shoot dry weight of stressed plant}}{\text{shoot dry weight of control plants}} \text{ X } 100\% \quad (8)$$

$$\text{Root dry weight Stress Index (\%)} = \frac{\text{root dry weight of stressed plant}}{\text{root dry weight of control plants}} \text{ X } 100\% \quad (9)$$

$$\text{Seedling vigour index} = \text{germination percentage} \times \text{seedling length} \quad (10)$$

$$\text{Seedling length} = \text{shoot length} + \text{root length}$$

Measurements were analyzed with SAS program for data analysis using statistical analyses with analysis of variance and the means were compared using LSD ($P < 0.05$) (SAS, 2004).

3. Results

A germination experiment was conducted to determine the effects of OMW generated from various filters at Al-Huson University College. Twenty seeds of barley were sterilized with sodium hypochlorite solution (Khan et al., 1992) and, after drying, were grown on Petri dishes that contained a double layer of filter paper. Ten ml of OMW taken from the effluent of various filters were placed on each petri dish. The filter paper was changed daily to prevent contamination. The seeds were supported to sprout at 26°C ± 1°C for 10 days. The seeds were considered as germinated seeds when radicle reached 2 mm long.

The characteristics of treated and untreated OMW are summarized in Table 1.

Table 1. Physicochemical Properties of Treated and Untreated OMW Using Filters Filled with Various Materials.

Parameter	Unit	Untreated OMW	OMW generated from various filters								
			Clay Loam soil	Zeolite + Rubber	Saw dust	Rubber	Hay	Zeolite	Loamy Sand soil	Pomace	Coal
EC	dS/m	10.5	6.08	7.3	6.7	5.5	4.7	5.9	6.5	6.5	6
pH		4.22	5.72	5.2	4.5	5.95	4.34	5.8	5.6	5	4.7
Turbidity	NTU	14420	6380	4120	11300	4270	5300	4215	8120	*	*
TSS	mg/l	23.8	19.3	21.2	23.1	*	*	15.8	*	*	*
COD	g/l	44.8	10	16.8	35	10	25	19.7	12.5	29	31
Phenol	g/l	8.2	4.6	5.6	6.7	5.3	6.2	4.5	4.8	6	6.1

* Not measured

Table 1 shows that untreated OMW is an acidic waste. Utilizing various materials in the filters to filter OMW increased the pH of OMW, compared to untreated water except for hay and sawdust filters. The highest pH values were obtained by rubber and zeolite filters followed by loamy sand and clay loam soil. Untreated OMW has the highest value of EC (10.5), followed by the waste generated from rubber + zeolite while the lowest value was obtained from hay filter. Turbidity was the highest for untreated olive wastewater followed by sawdust while the lowest turbidity was obtained from zeolite + rubber. The COD values were the highest for the untreated OMW, while the lowest COD was obtained from rubber and clay loam filters followed by sandy soil. Phenols were the highest for the untreated OMW while the lowest value was obtained by zeolite followed by clay loam soil then loamy sand soil.

3.1 Effects of Irrigation with Treated OMW with Various Materials on Germination

The present study was conducted to decide the impacts of irrigation with OMW, treated with various low-cost filters on germination and seedling establishments of barley. The results indicated that the investigated traits after irrigation with different treated OMW were significantly ($P < 0.01$) different. The highest germination percentage was recorded after irrigation with tap water (96.7%) compared to 66,7%, 25%, 23%, 18%, 16%, 8.4%, 6.7%, and 5% after irrigation with OMW that was filtered with clay loam , rubber, loamy sand, rubber+ zeolite, pomace, fruit peel, zeolite, and coal, respectively (Figure 2).

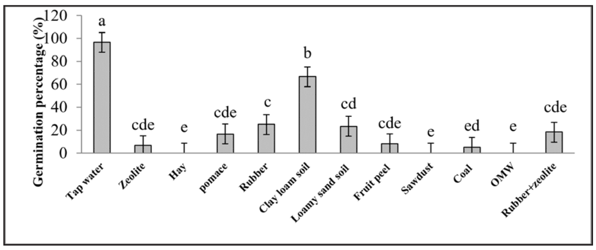

Figure 2. Germination percentage of barley seeds irrigated with treated OMW.

Irrigation with olive oil wastewater filtered with hay and sawdust without filtration led to the failure of the process of germination.

Relative germination rate (Figure 3) was also significantly different within the treatments. The relative germination rate was higher in tap water (1) than it is in the other treatments. The lowest relative germination rate was observed after irrigation with OMW and OMW filtered with either hay or sawdust filter.

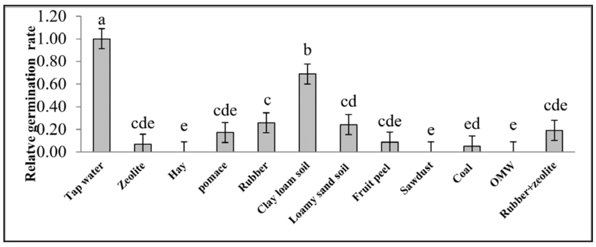

Figure 3. Relative germination rate of barley seeds irrigated with treated OMW.

The germination speed index (Figure 4) was significantly different within the olive oil mill wastewater treatments where the highest speed index was scored by tap water (22.44) compared to 12.46%, 7.65%, 5.25%, 5%, 4.95%, 3.5%, 3%, 2.71% after irrigation with OMW that was filtered with clay loam soil, rubber, loamy sand soil, pomace, rubber + zeolite, fruit peel, zeolite and coal, respectively.

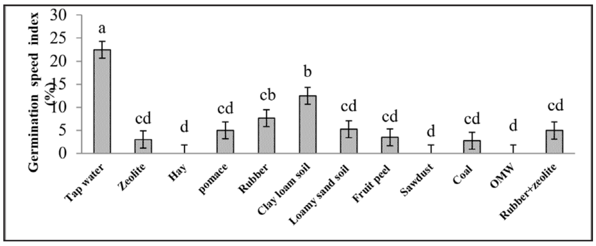

Figure 4. Germination speed index of barley seeds irrigated with treated OMW.

Significant effects of the different types of treated OMW resulted from the filtration process on morphological parameters in the seedling stage. The results indicated that the OMW produced from clay loam filters scored the highest shoot length (Figure 5) as well as root lengths (7.55 and 10.40 cm, respectively) (Figure 6) among the various filtrated OMW. These values were not significantly different from tap water (7.59 and 10.56 cm, respectively). Although they scored lower values than clay loam filters did, there was no significant differences between shoot length of seeds, irrigated with OMW and produced from rubber, loamy sand, rubber + zeolite, fruit peel, and pomace filters. These were, however, significantly higher than shoots irrigated with OMW filtered with coal, hay, sawdust, or without filtration.

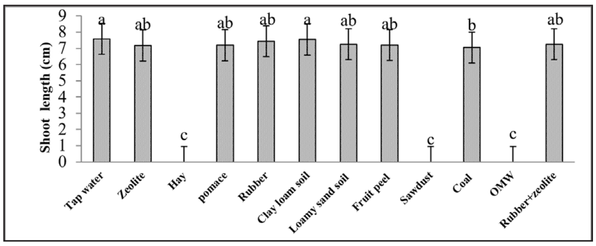

Figure 5. Shoot length of barley seeds irrigated with treated OMW.

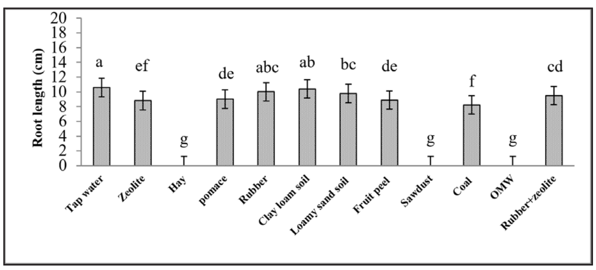

Figure 6. Root length of barley seeds irrigated with treated OMW.

The significant effect of filtered OMW on shoot and root length was reflected on the shoot dry weight (SDW) (Figure 7) and root dry weight (RDW) (Figure 8). The impact of various filtration media was highly significant on the shoot and root dry weight. OMW, discharged from clay loam, rubber, loamy sand, coal, zeolite and mixture of rubber and zeolite filters, scored the highest shoot dry weight that was not significantly different from tap water. The root dry weight was significantly different from the other filter media treatments ($p = 0.05$).

OMW, discharged from clay loam and rubber filters, produced the highest root dry weights, which were not significantly different from tap water.

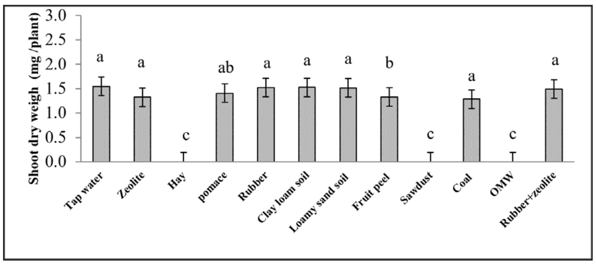

Figure 7. Shoot dry weigh of barley seeds irrigated with treated OMW.

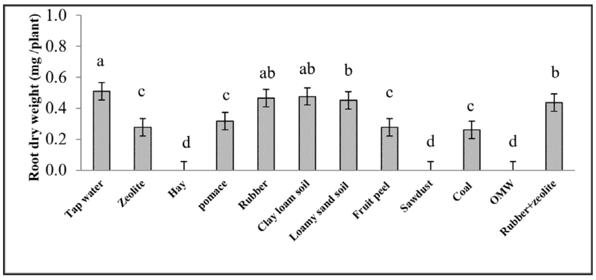

Figure 8. Root dry weight of barley seeds irrigated with treated OMW.

Root to shoot dry weight was variably affected by OMW, discharged from various filters. It was indicated that OMW, discharged from clay loam and rubber, gave the highest root to shoot ratio that has insignificant difference from tap water (Figure 9).

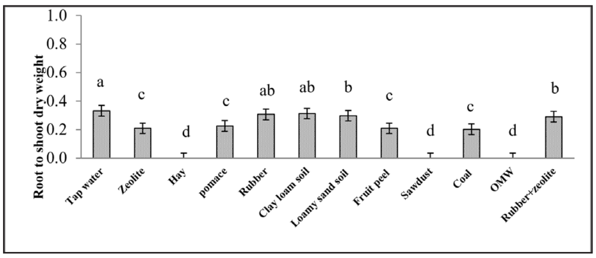

Figure 9. Root to shoot dry weight of barley seeds irrigated with treated OMW.

The percentage of decrease in germination energy is a useful parameter to assess the negative effect of waste containing phenol compound on seed germination. Germination energy was severely damaged for seeds irrigated with untreated OMW as well as for seed irrigated with treated OMW and contained high quantity of phenol compound. OMW, treated with clay loam, gives the highest germination energy after the control while OMW, generated from sawdust and hay filters, gave the lowest germination energy (figure 10). Promptness Index (Figure 11) was calculated to estimate germination stress tolerance index (identify the negative type of treated OMW on seeds) (Figure 12). Germinated seeds, irrigated with OMW and generated from clay loam soil, produced highest promptness index and germination stress tolerance index after the control (Tap water) while seeds, irrigated with untreated OMW or treated by sawdust and hay filters, gave the lowest values.

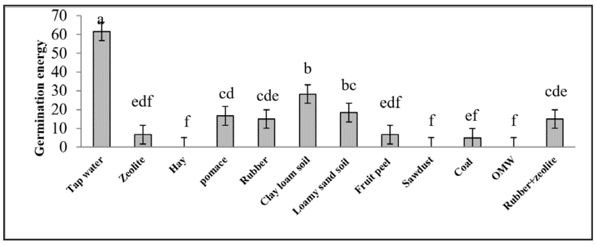

Figure 10. Germination energy of barley seeds irrigated with treated OMW.

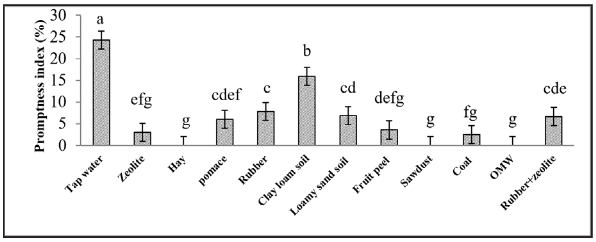

Figure 11. Promptness index of barley seeds irrigated with treated OMW.

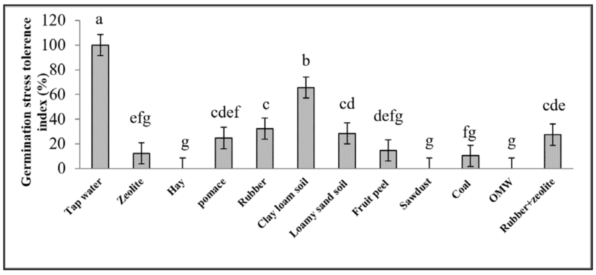

Figure 12. Germination stress tolerance index of barley seeds irrigated with treated OMW.

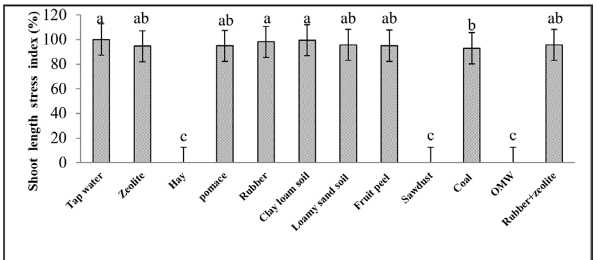

Figure 13. Shoot length stress index of barley seeds irrigated with treated OMW.

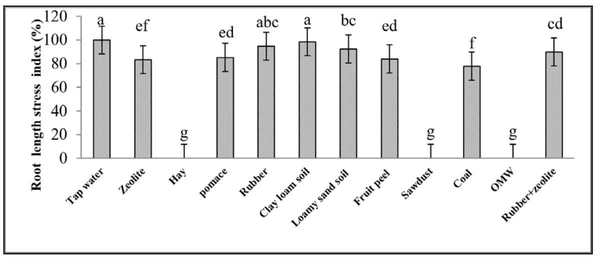

Figure 14. Root length stress index of barley seeds irrigated with treated OMW.

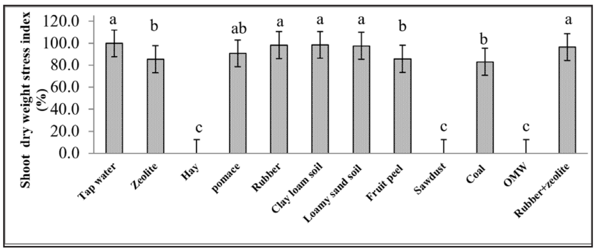

Figure 15. Shoot dry weight stress index of barley seeds irrigated with treated OMW.

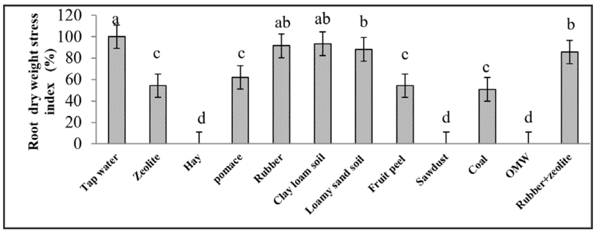

Figure 16. Root dry weight stress index of barley seeds irrigated with treated OMW.

It was found that there were significant ($P < 0.05$) differences between treatments on the shoot and root dry index (Figures 15 and 16). The highest values were observed for seeds irrigated with tap water followed by OMW generated from filters containing clay loam soil, rubber, sandy soil, and rubber + zeolite while the lowest value were obtained for untreated wastewater as well as for OMW generated from sawdust filter and hay filter. Results represented in Figure 17 showed that the seedling vigour index was the highest for the control treatment followed by olive oil mill wastewater generated from clay loam filter. The lowest seedling vigor index value was observed for the untreated OMW treatment as well as for OMW generated from hay and sawdust followed by coal filter and zeolite filter.

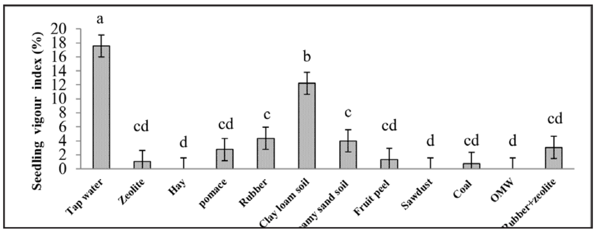

Figure 17. Seedling vigour index of barley seeds irrigated with treated OMW.

4. Discussion

Germination percentage and seedling establishments are considered important parameters during germination, which decide the victories of plant foundation beneath antagonistic environment conditions (Peralta et al., 2001). Untreated OMW leads to complete destruction of germinated seeds. The negative effect of the untreated OMW on seed germination of barley was probably attributed to the high phenolic compounds contained in the OMW (Rusan et al., 2015). The negative impact of phenolic compound in the higher plants is highly severe during germination process (Krogmeiewr and Bremmer, 1989). Various works have recommended that phenolic compounds are involved in the OMW germinability suppression or lessening which they can influence the germinability (Muscolo et al., 2001; Muscolo et al., 2002). For this reason, it is necessary to remove this compound from OMW in order to utilize this waste in agriculture. Filtration of OMW using various materials leads to the reduction of the phenolic compound from OMW (Table 1). The lowest amount of phenolic compounds was found in OMW generated from clay loam filters. In this treatment, the germination percentage was the second after the control (Figure 2). Looking at other results, germination speed index, relative germination rate, shoot length root length shoot and root dry weight, and root to shoot ratio, indicated that OMW generated from clay loam filter has the highest values compared to other filters. Germination rate index can be utilized as a pointer of phytotoxicity with the higher values representing a more rapid rate of germination.

In the present study, decreased germination energy in OMW can be describe due to seed deterioration and degradation of cell membranes (Falleri, 1994). Other investigators stated that the increase in the osmotic concentration decreased the energy of seed germination (Ahmadloo et al, 2011). In previous experiments, the promptness index and germination stress tolerance index were used as indications to identify drought-tolerant genotypes (Partheeban et al., 2017). Higher values indicate the tolerance of those genotypes to drought stress. In this experiment, the promptness index and germination stress tolerance index were used to identify the negative impact of OMW and OMW generated from various filters compared to tap water. Promptness index and germination stress tolerance index (GSTI) decreased with increasing concentration of stress levels. The highest promptness index and germination stress tolerance index were found for seeds irrigated with tap water followed by OMW generated from clay loam filters. This means that OMW generated from clay loam filters has less negative impact on barley seeds compared with other filters especially untreated OMW as well as OMW generated from sawdust and hay filters.

The germination stress tolerance index was used to interpret differences in the rate of germination due to environmental stress (Bouslama and Schapaugh, 1984). A higher value of the germination stress tolerance index indicates a high rate of germination, which was inversely related to environmental stress. Reduction in shoot length stress index, root length stress index, shoot dry weight stress index, and root dry weight stress index reflect the impacts of the phenolic compound effect generated from OMW. The highest reduction was observed for seeds irrigated with untreated OMW as well as seeds irrigated with OMW and generated from sawdust and hay filters. The highest values were observed for seeds irrigated with tap water followed by OMW generated from filters containing clay loam soil, rubber, sandy soil and rubber + zeolite.

OMW treatment with various filters has dissimilar and statistically significant effects on seedling vigor index. Therefore, we concluded that this variation would be attributed to the phenolic stress variation of the treated OMW by different filters (Table 1). A similar assumption was drawn by other reports stating that the vigor index is reduced by environmental stress effects (Motamedi et al. 2013; Khodarahmpour 2011; Mensah et al. 2006).

It has been stated that OMW results in a strong prohibition of seeds and seedling growth (Casa et al. 2003 and Komilis et al. 2005). These researchers have reported a decrease in OMW toxicity and the inhibition effect of seed germination with various processes. In this study, it was concluded that utilization of various naturally made filters can be used to decrease the negative impact of phenolic compounds that prevent seed germination and seedling growth. Investigators have reported that the germination rate index was significantly reduced by OMW application due to the phenolic compounds contained in OMW (Aggelis et al. 2003). They found that reducing phenolic compounds enhanced the germination rate index.

5. Conclusions

This study reveals the following conclusions:

1- The low pH value of OMW increased when it passed through the different media, except hay and sawdust. When rubber in combination with zeolite was used as the filter media, the pH of OMW increased to the extreme.

2- Rubber and zeolite in combination as filter media produced the lowest turbidity values as compared to the other types of used media.

3- The best removal efficiencies of COD and phenols were obtained in (rubber and clay loam) and (zeolite) filters, respectively.

4- The use of untreated OMW has completely prevented the germination process of barley seeds.

5- It was found that the use of clay loam and loamy sand along with granular rubber, zeolite, and rubber and zeolite mixtures as filter media was efficient in the treatment of OMW.

6- The use of treated OMW has a negative effect on the germination process of barley as compared to germination when tap water was used.

7- Treated OMW, using most filter media types used in this research, improved the germination percentage and seedling growth, as compared to untreated OMW, especially effluent generated from clay loam and loamy sand soil filters.

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