

Impact of mixing treated industrial water with olive mill wastewater on vetch (*Vicia sativa L.*) germination and early seedling growth

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Abstract

Vetch is a forage crop that is widely grown in high rainfall areas in Jordan for forage production and grazing. Growing vetch species out of the season will need irrigation, which is very expensive. In here, we propose application of wastewater or olive mill wastewater as alternative to the fresh water. An experiment was carried out to evaluate the effect of diluting the industrial water (IND) with olive mill wastewater (OMWW) on the vetch germination (GR) and early seedling growth under controlled conditions. The water treatments consisted of T1 (pure IND_{100%}), T2 (mix of IND_{75%} + OMWW_{25%}), T3 (IND_{50%} + OMWW_{50%}), T4 (IND_{25%} + OMWW_{75%}) and T5 (pure OMWW_{100%}) and tap water (control). This experiment was designed in a CRD with three replications for each treatment. Several measurements on the morphological and biochemical analysis were taken during this study such as seed germination (GR), as well as the seedlings fresh weight of shoot (FWS), dry weight of shoot (DWS), fresh weight of root (FWR), dry weight of root (DWR), shoot length (SL), root length (RL) and the ratios of DWS/DWR and SL/RL. The water treatments had a significant influence on data taken, in which the highest mean values of morphological characteristics were obtained under the T1, while the lowest were obtained under the T5 due to high phenols concentrations. The OMWW treatment showed an adverse effect on GR, DWS, DWR, SL, and RL due to low pH values (4.5) and high phenols concentration (1700 mg/L). In contrast, the industrial water showed low to moderate effect on the vetch seed germination. The means of DWS and DWR under T3 and T4 were not significantly different from each other but were different comparing with control. It is recommended that the use of a mixture of OMWW and the Industrial water will have a positive effect on Vetch growth.

Keywords: blue water; seed germination; wastewater dilution; water quality.

Introduction

The agro-industrial activity of olive oil production is one of the most important agri-based activity in many countries of the Mediterranean Region. This industry is estimated to generate revenues of around 100 million JOD, as stated by the Amman Chamber of Commerce (DOS, 2016) and supports the livelihood of approximately 180,000 families in Jordan. The olive oil production industry is associated with the generation of the large quantities of by-products, such as wastewater (olive mill wastewater – OMWW). Management, treatment, and safe disposal of OMWW raise serious environmental concerns especially that the olive mills are considered as family-run businesses and olive mills are located in the olive production area, which makes individual on-site treatment unfeasible. In general, it is estimated that the amount of wastewater produced ranges from 1.0-1.2 m³ per ton of product depending on the type of production process (Azbar, 2009). Jordan is the 8th among top ten producers of the olive oil production in the world with the production of

approximately 26,000 ton of olive oil for the production season of 2015-2016 (JOPEA, 2016). Currently, the only method available for the disposal of OMWW in Jordan is transporting the OMWW without any treatment from the mill to the landfill designed for receiving the effluent (MWI, 2013). The untreated OMWW has the number of characteristics, which include dark color, specific smell, acidic pH, and high organic content (Ayoub, 2014). The organic content consists mainly of classes of pollutants such as polyphenols known to exhibit antimicrobial, eco-toxic and phytotoxic properties (Paraskeva, 2007). Discharge of olive waste into soil may cause a number of environmental issues, such as leaf and fruit abscission (Erel, 2008) and inhibition of seed germination, enhancement of microbial fermentation with the production of methane (Azbar, 2004) and proliferation of the phosphorous content (Segal, 2011).

The recent study carried out by Rusan et al. (2015) evaluated the impact of treated and untreated OMWW on seed germination of barley (*Hordeum vulgare L.*). This study showed

that 100%, 75% and 50% OMWW displayed phytotoxic properties and completely prohibited seed germination. However, 25% OMWW dilution showed some enhancement in Germination Rate Index (GRI), which proves that the treated or diluted OMWW could be used for irrigation. Komilis (2005) studied the effect of OMWW on seed germination after various pretreatment techniques. The results of the study showed that phytotoxicity of OMWW was decreased with increased dilution with water, after aeration of OMW and without pH adjustment. The unprocessed OMWW cause modification on physicochemical features of soils, in which the phenolic compounds were detected at a depth of 1.2 m four months after the application of OMWW (Mekki, 2006). Zenjari (2001) found that spreading of OMWW on soils resulted in the increase of organic carbon, Kjeldahl N, total phosphorus, potassium in addition to the increase of electrical conductivity, phenol concentration and salinization level in soils which could negatively impact soil fertility. In general, the irrigation by the reclaimed wastewater demonstrates a positive impact on the plant growth due to the increased water availability and soil fertility (Palese, 2009). On the other hand, accumulation of salt in the root area shows a negative effect on the plant (Biggs, 2009) and potentially can be a source of groundwater contamination due to leaching below the root zone (Aragues, 2004).

Mekki (2007) studied effects of treated and untreated OMWW on seed germination, plant growth, and soil fertility and showed beneficial effects of using treated olive mill wastewater in irrigation of different crops. Tomato (*Lycopersicon esculentum*), chickpea (*Cicer arietinum*), fava bean (*Vicia faba*), wheat (*Triticum durum*), and barley (*Hordeum vulgare*) were tested for germination index and growth. In general, the plants irrigated with treated wastewater showed an improvement in seed biomass, spike number, plant growth, and improved dry productivity compared to plants irrigated by normal water. Also, an increase in the germination index from 110 to 115 % was observed. The study has concluded that treated OMWW can be used for ferti-irrigation of the crops. However, diluted untreated OMW causes leaf necrosis and low productivity.

In Jordan, the possibility of using treated OMWW in irrigation of some types of crops is considered significant for agriculture since Jordan is recognized as the 4th poorest country in the world in terms of the water resources (USAID, 2012). Therefore, the re-use of the large amounts of OMWW generated during the olive-pressing season could potentially reduce the use of potable quality water in agriculture (Chartzoulakis, 2005).

Vicia sativa L. is a vine annual forage legumes crop belonging to *Fabaceae* family known as common vetch, native to the Mediterranean and southern Europe growing in spring. It has rapid growth and characterized by rapid re-growth after harvesting. Vetch stem is weak, leaves compound consist of several leaflets, purple flowers and seeds are black and round (The Forage Information System, 2009).

In Jordan, common vetch is known as Beekia and is grow for decades. It is a known crop for the local community as highly nutritive valued and seed production (Reeve, 1990). Vetch is being useful for grazing, hay production, for forage crop

mixture, green manuring, fix nitrogen and can be used in cropping rotations to disrupt the life cycle of cereal root pathogens (Reeve, 1990; Saoub et al., 2012).

The main goal of this research paper is to study the impacts of industrial water and olive wastewater on germination of vetch seeds under controlled conditions with different dilution treatments. In this study we investigated the possibility of using OMWW on vetch cultivation as it is produced in huge quantities in Jordan. This was tested as another alternative resource of irrigation water. The vetch was selected since it is used as well-known forage source and a nitrogen-fixer. As a result of this research, the main outcomes will contribute to the preservation of precious drinking water resources by using the treated wastewater for irrigation of non-edible vegetation.

Results and Discussion

Water quality characteristics

The water quality showed that the dilution percentage of industrial water (IND) in olive mill wastewater (OMWW) increases as the pH increases, which leads to decrease the soil acidity (Table 1). In the mix and dilution of water treatments, the minimum pH value was 4.6 at OMWW₁₀₀ % and the maximum was 8.8 at IND₇₅ %+OMWW₂₅ %. While, the EC was the highest (8.4 dS/m) for OMWW₁₀₀ % and the lowest (3.9 dS/m) for IND₁₀₀%. However, other water quality parameters such as EC, Na, K, TSS, PO₄, COD, POD, turbidity, and phenols were decreased as the percentage of IND water increased in the mix with OMWW. The water quality concentrations of Ca, Mg, and NO₃ were increased as the percentage of IND water increased. The OMWW has higher concentration of K, Ca, oil grease, TSS, PO₄, POD, COD, phenols and lower concentration of Na and NO₃ compared to industrial water. The results of the OMWW analysis in general are similar to the results reported by Ayouba (2014) and Rusan (2015). Similarly, Zenjari (2001) showed that OMWW increase the organic carbon, N, P, K as well as increase the EC and phenolic compounds.

Seed germination of vetch under different mixture of water qualities

The main concern in using of OMWW in irrigation is the availability of phenolic acids. The phenols has an impact on the seed germinations, crop growth, and soil properties as explained by Mekki (2007). One solution is to enhance the quality of OMWW by treating physically, chemically, or by diluting with other types water qualities. This study found that mixing IND with OMWW has a significant effect on the germination percentage of vetch seeds. The results of germination characteristics of vetch in response to mixing IND with OMWW with different percentages showed that the germination percentage were not significantly different between IND₁₀₀% and IND₇₅% + OMWW₂₅% but they were different from that at IND₅₀% + OMWW₅₀%, IND₂₅% + OMWW₇₅%, and OMWW₁₀₀% (Fig 1). A trend of similar results was reported by Mesad (2014) and Tadros et al. (2012) showing that germination was increased with treated OMWW. Compared to seeds germinated under Tap water (98.72%), the decrease in

germination percentage was 8.83%, 11.67%, 40.44%, 45.06%, 62.00% at IND_{100%}, IND_{75%} + OMWW_{25%}, IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%}, OMWW_{100%}, respectively. Our results showed that the diluting INDW + OMWW delay but did not constrain the seed germination similar to results obtained by Krogmeier and Bremner (1989). The increase in the percentage of OMWW affect germination percentage. The results of this study showed that using the OMWW is adversely affect the crop production due to toxicity of high phenols concentration. Mas'ad (2014) showed that decreasing the phenolic compounds as results of treated OMWW cause positive effect on vetch. In contrast, the industrial water showed light to moderate effect on the crop growth or in seed germination as well as the seedling growth (Al-tabbal and Amamry, 2014).

Effect of mixing water qualities on the fresh weight shoot and root of vetch germination

The fresh shoot and root weight was investigated in the seed germination (Fig 2). The effect of mixing of industrial water and olive mill wastewater had a significant effect on the fresh weight of root and shoot. The IND_{100%} scored the highest shoot fresh weight per plant (138.91gm) and the lowest (57.44 gm) under OMWW_{100%}. The nutrient solution in the IND water is rich with nutrients compared to the other treatments, which positively affected the growth and fresh weight of shoot as well. In shoot, from physiological view, the cell content is increased with nutrients causing an increase in solute of the cells and then increase the movement of water toward the cell based on the gradient. The mean of the shoot fresh weight per plant under IND_{50%}+ OMWW_{50%} was not different from IND_{50%} + OMWW_{25%} and from IND_{25%} + OMWW_{75%}, but they were different from the mean at OMWW_{100%}. With respect to the fresh weight shoot (169 gm) under the tap water, the decrease percentage of fresh weight shoot was 14.20%, 30.86%, 41.36%, 45.69%, 64.82% at IND_{100%}, IND_{75%} + OMWW_{25%}, IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%}, OMWW_{100%}, respectively. While, the fresh weight of roots was 4.85%, 17.48%, 29.13%, 32.04%, 53.40% at IND_{100%}, IND_{75%} + OMWW_{25%}, IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%}, OMWW_{100%}, respectively. The means of the fresh root weight at IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%} were not different from each other, but they were different from those means at IND_{100%} and OMWW_{100%}.

Effect of mixing water on vetch germination and dry weight of shoot and root

The results of mixing of industrial water with olive mill wastewater at different ratios showed a significant effect in seed vetch morphological parameters of dry weight shoot and dry weight root in the early growth stages (Fig 3). Significant differences ($p < 0.05$) indicated that the mixing ratio of IND_{100%} scored the highest dry weight shoot per plant (12.54 gm) followed by 9.96, 8.11, 7.30, 4.60 gm under water treatments of IND_{75%} + OMWW_{25%}, IND_{50%} + OMWW_{50%}, IND_{25%} +

OMWW_{75%}, OMWW_{100%}, respectively. The means of dry weight shoot per plant under IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%} were not different from each other but they were different from other treatments. With respect to the shoot dry weight (15.42 gm) using tap water, the decrease percentage of shoot dry weight was 18.68%, 35.41%, 47.41%, 52.59%, 70.23% under treatments of IND_{100%}, IND_{75%} + OMWW_{25%}, IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%}, OMWW_{100%}, respectively. Root dry weight was significantly different under all the treatments of mixing the industrial water with olive mill wastewater. The highest (7.11 gm) root weight was observed under IND_{100%} and the lowest (3.13 gm) under OMWW_{100%}. Compared to the root dry weight (8.02 gm) at tap water, the rate of decrease was 11.24%, 25.09%, 40.82%, 44.69%, 60.92%, respectively, at IND_{100%}, IND_{75%} + OMWW_{25%}, IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%}, OMWW_{100%}. Mixing OMWW with IND may reduce the effect of phenolic compounds and nutrients. Also, Mekki et al (2007) showed that the diluted OMWW with tap water caused low productivity and leaf necrosis.

Effect of mixing water qualities on plant height and root length of vetch germination

Mixing industrial water and olive mill wastewater with different percentages showed a significant effect on vetch morphological parameters of plant such as height and root length (Fig 4). Our results are in agreement with Mekki (2007) findings. The plant height was the highest (5.50 cm) at IND_{100%} and the lowest (2.93 cm) at OMWW_{100%}. The means of the plant height between any two sequences treatments were not significant from each other. The decrease percentage of plant height was 11.42%, 15.92%, 21.45%, 32.18%, 39.10%, respectively, at IND_{100%}, IND_{75%} + OMWW_{25%}, IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%}, OMWW_{100%}, compared with the plant height (6.42 cm) at tap water. For the root length, we observed 14.49%, 22.59%, 33.02%, 45.33%, 54.36%, respectively, at IND_{100%}, IND_{75%} + OMWW_{25%}, IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%}, OMWW_{100%}, compared with the root length (1.76 cm) at tap water. However, decreasing the ratio of mixing OMWW/IND can be used for irrigation and it can enhance the germination index and vice versa. Rusan et al. (2015) showed that 100%, 75% and 50% of OMWW displayed phytotoxic properties and completely prohibited seed germination. The phytotoxicity of OMWW was decreased with increased dilution with water, after aeration of OMW and without pH adjustment (Komilis, 2005).

Effect of mixing water qualities on dry weight of shoot/root ratio and root/shoot length ratio

Mixing the industrial water and olive mill wastewater with different percentages had a significant effect on dry weight of shoot/root ratio (SDW/RDW) (Fig 5). However, it had a significant effect on the shoot/root length ratio (SL/RL). The mean of the highest dry shoot weight to dry root weight

Table 1. Water quality analysis for the different mixing percentage of water types was conducted in the laboratories of Jordan University of Science and Technology (JUST).

Parameters	Tap water	OMWW _{100%}	Treatments			
			IND _{100%}	IND _{25%} +OMWW _{75%}	IND _{50%} +OMWW _{50%}	IND _{75%} +OMWW _{25%}
pH	7.9	4.6	9.2	6.1	7.6	8.8
EC (dS/m)	1.1	8.4	3.9	7.4	6.15	5.8
Na (mg/L)	225	124	330	140	155	170
K (mg/L)	78	2500	45	1885	1240	640
Ca (mg/L)	390	200	120	230	265	290
Oil-grease(mg/L)	0	1900	5	1450	960	450
Turbidity	0	340	130	291	235	185
TSS (mg/L)	14	3100	370	2420	1735	980
NO ₃ (mg/L)	45	11	39	18	25	32
PO ₄ (mg/L)	30	65	16	54	40	28
BOD(mg/L)	200	40000	496	30125	20248	10370
COD (mg/L)	490	53000	960	39990	26980	13970
Phenols(mg/L)	0	1700	0	85.2	66	3.5

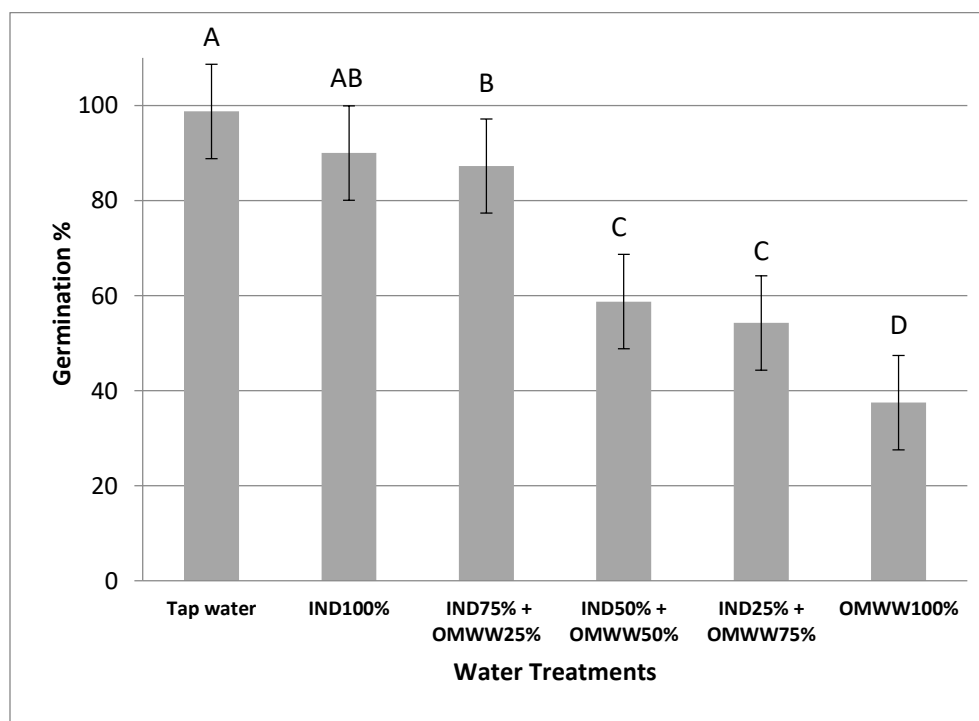


Fig 1. Germination characteristics of vetch (*Vicia sativa*) in response to different wastewater under controlled environment conditions. Values with the same letters are not significantly different based on least significant difference (LSD = 11.03% for the seed germination, P = 0.05).

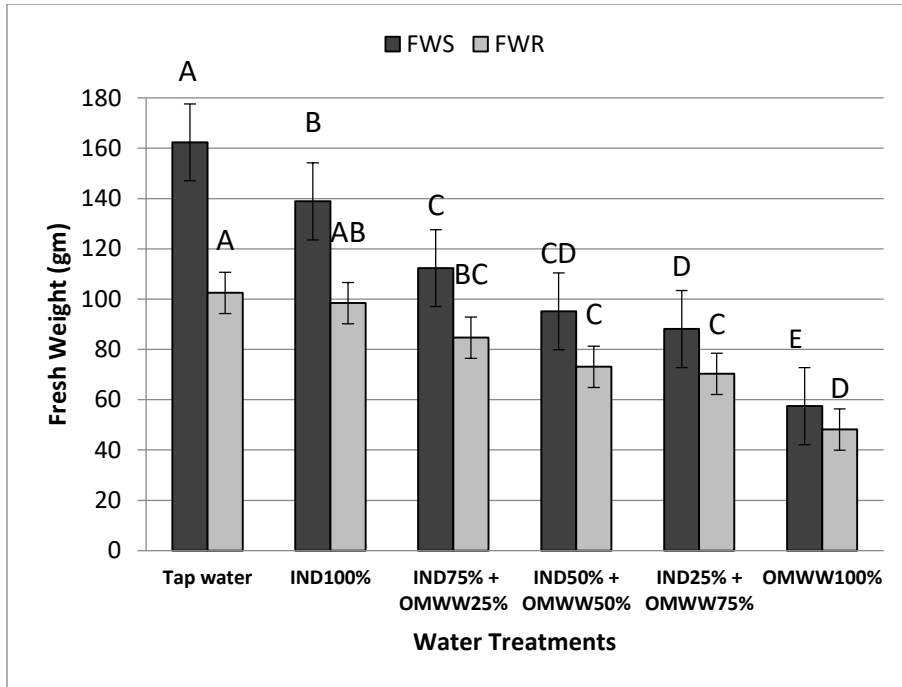


Fig 2. Fresh weight of shoot and weight root (gm) of vetch seeds as affected by mixing different water qualities: tap water, IND_{100%}, IND_{75%} + OMWW_{25%}, IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%}, OMWW_{100%}. Values with the same letters are not significantly different based on least significant difference (LSD = 19 and 18 for fresh weight shoot and dry root, respectively, P < 0.05).

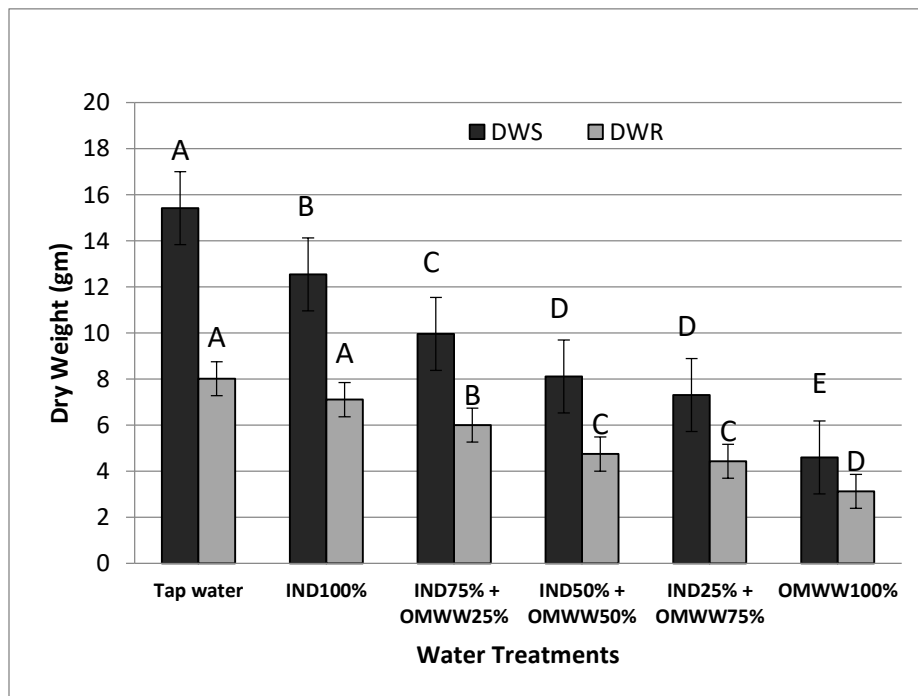


Fig 3. Dry weight shoot and dry weight root (gm) of vetch seeds as affected by mixing different water qualities: tap water, IND_{100%}, IND_{75%} + OMWW_{25%}, IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%}, OMWW_{100%}. Values with the same letters are not significantly different based on least significant difference (LSD = 1.72 and 1.08 for dry weight shoot and dry root, respectively, P < 0.05).

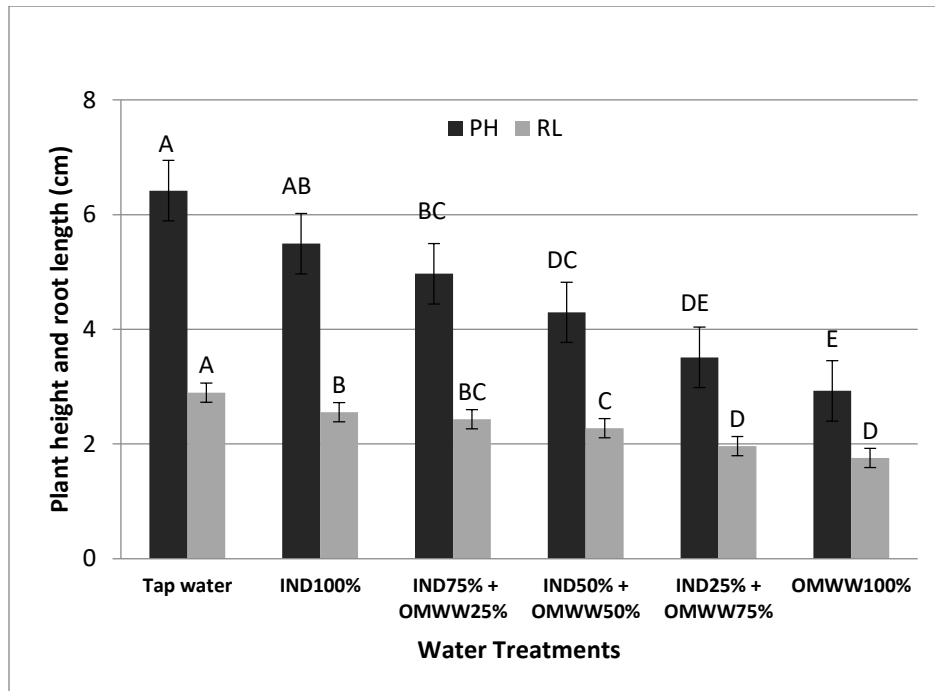


Fig 4. Plant height and root length (cm) of vetch seeds affected by mixing different water qualities: tap water, IND_{100%}, IND_{75%} + OMWW_{25%}, IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%}, OMWW_{100%}. Values with the same letters are not significantly different based on least significant difference (LSD = 1.00 and 0.28 for plant height and root length, respectively, at P < 0.05).

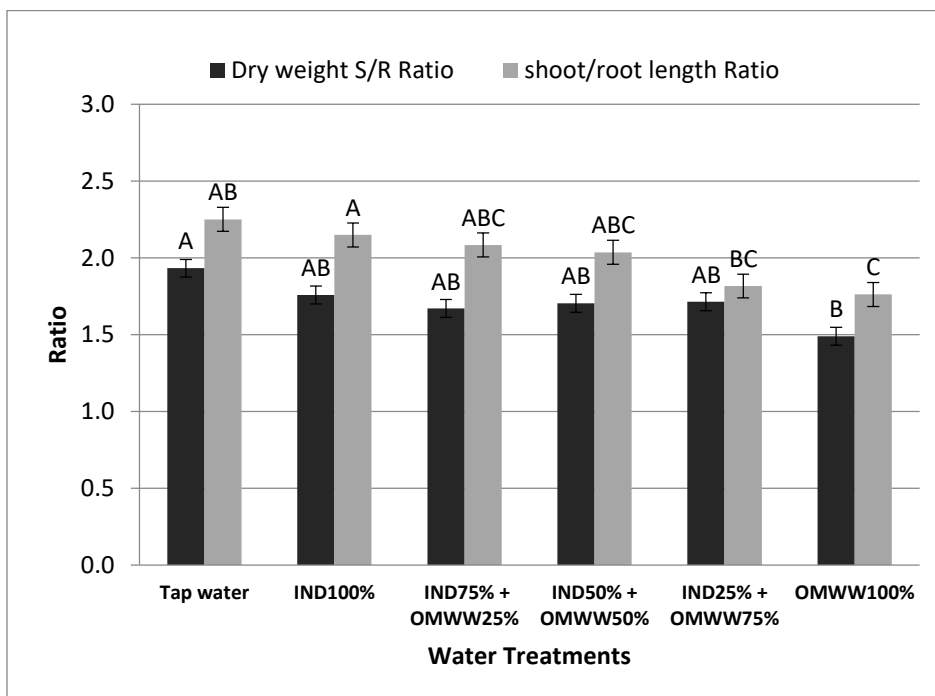


Fig 5. Dry weight ratio (shoot/root) and length ratio (shoot/root) of vetch germination as affected by mixing different water qualities: tap water, IND_{100%}, IND_{75%} + OMWW_{25%}, IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%}, OMWW_{100%}. Values with the same letters are not significantly different based on least significant difference (LSD = 0.45 and 0.30 for dry weight Soot/Root ratio and length ratio of root /shoot, respectively, at P < 0.05).

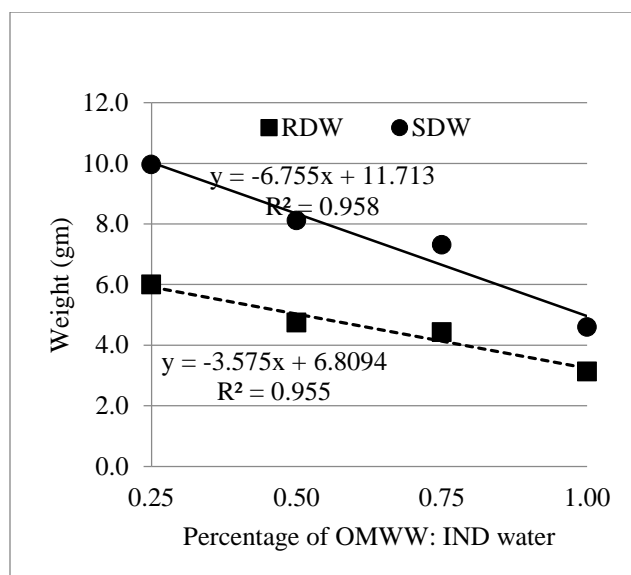


Fig 6. The relationship of the shoot and root dry weights as a function of the percentage of OMWW to the industrial water.

(DSW/RDW) was 1.76 for IND_{100%} and the lowest was 1.49 for OMWW_{100%}. The means of the DSW/RDW at different water mixing treatments were not different from each other. The highest SL/RL ratio (2.45) was obtained at IND_{100%} and the lowest (1.76) at OMWW_{100%}. The means of SL/RL ratio at IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%}, and OMWW_{100%} were not different from each other. Our results are in accordance with the results of Tadros et al. (2012) and Mas'ad (2014) reported that non-treated IND water decreased the ratio (SL/RL).

Effect of mixing ratio OMWW/IND on the shoot and root dry weight

The relationships were observed between the shoot dry weights and increase of IND water and decrease of OMWW (Fig 6). These results reflect that the phenols concentration in OMWW increases when the germination parameters of vetch seeds decrease. This study found that when the industrial water increases in the mixture the germination parameters are increased as well. Similarly, Tadros et al. 2012 declared that the industrial water had a positive effect on germination of vetch seeds. Mas'ad (2014) reported that no significant differences were observed between dry weight of *Vetch* under raw and lime-treated OMWW compared to the significant decrease in *Sorghum* and *Leucaena* dry weight.

Materials and Methods

Plant materials

The vetch seeds (*Vicia sativa* L.) were obtained from farmer fields located close to Marrow Agricultural Research Station (about 80 km north of Amman, Jordan). The vetch seeds were grown in several areas, especially close to wastewater treatment plants for forage production.

Experimental site and experimental design

This study was conducted at the Jordan University Science and Technology, Faculty of agriculture in the laboratory of the Department of Plant Production in a controlled growth chamber (20°C). The impact of different water qualities on the germination of vetch seeds as well as the early seedling stage was investigated under the control conditions. The water types of industrial and olive wastewater (OMWW) mixed together with different percentages. The water treatments consisted of T1 (pure IND_{100%}), T2 (mix of IND_{75%} + OMWW_{25%}), T3 (IND_{50%} + OMWW_{50%}), T4 (IND_{25%} + OMWW_{75%}) and T5 (pure OMWW_{100%}) and tap water (control). The experimental design used in this research was complete randomize design with three replications.

Data collection

Germination percentage, shoot length, root length, shoot fresh and dry weight, root fresh and dry weight, shoot to root length ratio (SL/RL) and shoot to root dry weight ratio (SDW/RDW) were determined according to Al-Tabbal (2017). The total number of seeds that germinated in each treatment after final count to the number of seeds that were planted was expressed as germination percentage. Shoot length and root length were taken daily after germination with ruler. Seedlings length that could be considered as a vigor test has potential to give information to the germination test and makes possible to estimate the potential of emergence of seedlings in the field. This character was measured by adding root length to shoot length. Shoot and root fresh weights were taken at the end of experiment after 21 days from the beginning of germination. Shoot and root dry weights were recorded after oven drying at 60°C for 48 hr. The ANOVA analysis was run for the data collected by using the SAS software version 9.0 (SAS, 2002). Means were tested by using Fisher's least significant differences (LSD) at probability level of 0.05.

Industrial and olive mill wastewater collecting and analyzing

The treated industrial was brought from the textile factory at Prince Hassan Industrial City that situated north of the Jordan University of Science and Technology (JUST) Campus. The olive wastewater was brought from olive mill wastewater that was located in Irbid (about 70 km north of Amman, Jordan) and was operated by centrifugation system. Samples were collected in plastic containers and maintained at 4 °C. The industrial water treated physically and chemically only. The wastewater was treated physically, chemically, and biologically. The water quality parameters (pH, EC, Na, K, Ca, N%, and phenols) analyzed in the laboratory of Wadi Al-Arab Waste Water Treatment Plant according to APHA (1995).

Germination procedure

Vicia sativa L. seeds were treated with mercuric chloride (0.1%) to prevent surface fungal/bacterial contamination. Seeds were washed several times with water to remove the mercuric chloride particles. The germination test based on ISTA from 21 days was performed in wet autoclaved filter paper in sterile petri dishes (ISTA, 2003; Al-Tabbal and Ammary, 2014). Germination was distinct by radical emergence (Crowe et al., 2002).

Conclusions

We examined the effect of different percentages of mixing industrial water (IND) and olive mill wastewater (OMWW) on vetch germination during the early stages of germination. This study consisted of mixing IND with OMWW with different percentages as: tap water, IND_{100%}, IND_{75%} + OMWW_{25%}, IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%}, and OMWW_{100%}. The increase in percentage of industrial water on OMWW caused a positive effect on germination characteristics mainly due to decrease in the acidity of water. On the contrary, seed germination characteristics were affected by increasing the percentage of OMWW due to the increase in the acidity and phenols concentrations. However, mixing the unconventional water such as IND and OMWW will decrease the stress on the fresh water, which is used for irrigation. The results of this research showed that the IND_{100%}, IND_{75%} + OMWW_{25%}, IND_{50%} + OMWW_{50%} were the best treatments as a results of the means of shoot dry weight, root dry weight, shoot length, and root length that were not different under IND_{50%} + OMWW_{50%}, IND_{25%} + OMWW_{75%} treatments. Generally, the results of this study recommended that further research needs to be carried out in the field to study the effect of different percentages of water qualities on the seed vetch germination and seedling growth.

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