



Full Length Article

Changes in Yield and Forage Quality of Six Alfalfa Cultivars in Response to Sustainable Irrigation with Treated Wastewater

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Abstract

Sustainable usage of water resources becomes a necessity in the recent years because of the increased pressure on these resources resulted from climate change events *e.g.*, intensive droughts. Irrigation of forage crops consumes vast amounts of ground water in arid and semi-arid areas. Therefore, vast research efforts are being invested to find alternative sources of freshwater suitable for irrigation. The aim of this study was to examine the potential of using treated wastewater in sustainable irrigation of alfalfa for forage production and the effect of this type of water on productivity and forage quality of alfalfa plants comparing to irrigation with normal water. Six different cultivars (Hassawi, Egyptian local, Cuf 101, Server, WG CAL 10 and Saltine) were grown in two different seasons (2013–2014 and 2014–2015). Regardless of cultivar and/or cultivation season, irrigation with treated wastewater did not adversely affect the productivity of alfalfa plants. Moreover, irrigation with treated wastewater did not show any adverse effects on forage quality of alfalfa plants with slight decrease in protein and fat contents in comparison with plants irrigated with normal water. In conclusion, alfalfa forage crop can be successfully grown with treated wastewater. © 2020 Friends Science Publishers

Keywords: *Medicago sativa* L.; Sustainability; Water reuse; Forage crops; Protein contents

Introduction

In arid and semi-arid regions, renewable water resources witness a vast reduction as a result of climate change leading to frequent extreme weather events *i.e.*, intensive drought (IPCC 2014). Furthermore, demand of freshwater significantly increased in these regions due to rapid population growth, changes in lifestyle and increased urbanization (Karimi *et al.* 2018; Khanpae *et al.* 2020). It is expected that water supply problem will transform into serious challenge to sustainability and food security in such areas (Reed *et al.* 2013; The World Bank 2017). Therefore, sustainable utilization of freshwater resources in arid and semi-arid regions areas such as Saudi Arabia becomes a necessity (Hussain *et al.* 2019).

Globally, the agricultural sector is considered as the major consumer of freshwater reserves (FAO 2015). In arid and semi-arid *e.g.*, Saudi Arabia, agriculture consumes more than 90% of total freshwater usage (Khanpae *et al.* 2020) leading to increased depletion of deep non-renewable freshwater reserves. Furthermore, irrigation with saline

underground water maximizes the problem of topsoil salination. Therefore, in 2015 and 2016, decrees to limit and control cultivation of low-value and high-water demand crops including alfalfa have been issued by the Saudi cabinet (Kim and van der Beek 2018). Finding alternative water resources for irrigation of forage crops that consumes freshwater reservoirs becomes mandatory strategy to save water. Irrigation with treated wastewater could be one of the available alternatives. However, the potential adverse effects of using this type of water on productivity and quality of forage crops should be carefully examined. One of the main alternatives is producing forage crops *e.g.*, alfalfa (*Medicago sativa* L.) depending on irrigation with treated wastewater (Malki *et al.* 2017). Indeed, several countries adopted irrigation with treated wastewater, especially developing countries in both Africa and Asia (Scott *et al.* 2004; Gosain *et al.* 2006; Misra 2014; D'andrea *et al.* 2015). Currently, the main application of treated wastewater is to irrigate ornamental crops in the streets, gardens, or parks. Recently, treated wastewater is being used to irrigate certain crops under strict governmental control and supervision (Al-

A'ama and Nakhla 1995; Aljaloud 2010; (Hussain *et al.* 2019).).

Ranking the first among all forage crops in the world, alfalfa is gaining increased attention regarding productivity and forage quality. According to FAO, alfalfa is the first forage crops around the world in terms of production and area cultivated (FAO 2015). However, it is classified among the high water consuming crops (Lloveras 2001). Fortunately, alfalfa can tolerate a moderately low-quality water supply (Helalia *et al.* 1996). Therefore, more investigation to examine the potential effects on alfalfa productivity and quality resulted from irrigation with treated wastewater is needed before adoption. Irrigation with treated wastewater has been examined on several crops, and positive and/or negative effects have been reported. Enhancement of production, improvement of soil fertility, reduction of groundwater consumption and lessening of wastewater discharge are the main positive effects of irrigation with treated wastewater (Toze 2006; Hamilton *et al.* 2007; Muyen *et al.* 2011; Chávez *et al.* 2012). On the other side, the negative effects of irrigation with treated wastewater relate mainly to presence of heavy metals, pathogens, and pollutants (Gibson *et al.* 2010; Muyen *et al.* 2011; Pereira *et al.* 2011; Muller *et al.* 2012).

Until now, there is no clear information regarding the efficiency and safety of using treated wastewater in irrigation of alfalfa. Nevertheless, we found in a previous study that irrigating alfalfa with treated wastewater did not lead to increased levels of heavy metals in neither forage nor soil with non-significant changes in nutrient contents (Soufan *et al.* 2019). Application of treated wastewater in irrigation of alfalfa is faced by the unacceptance among farmers who think that it has adverse effects and are not aware about its potential beneficial effects (Dikinya and Areola 2010; Leonard *et al.* 2015; Makropoulos *et al.* 2018). Therefore, further studies are needed to confirm the efficiency and safety of treated wastewater application and to increase the farmers' awareness regarding beneficial effects of irrigation with treated wastewater. In the present study, the changes in yield and forage quality of alfalfa plants resulted from sustainable irrigation with treated wastewater was examined comparing to irrigation with normal water. The results of the current study would provide the required information to support the adoption of treated wastewater in irrigation of alfalfa forage crop to alleviate the increased consumption pressure on groundwater in arid and semi-arid areas such as Saudi Arabia.

Materials and Methods

Experiment design

In the current study, sustainable irrigation of alfalfa with treated wastewater was examined. In this regard, the effects of irrigating 6 different alfalfa cultivars with treated wastewater on fresh yield and forage quality were compared

to irrigating those cultivars with normal water. The whole experiment was carried out in the fields of Agricultural Research and Experiment Station in Dirab (24°25'34.43" N, 46°39'10.86" E, 571 m a.s.l.), College of Food and Agricultural Sciences, King Saud University (KSU), Riyadh, Saudi Arabia, during two different seasons *i.e.*, 2013–2014 and 2014–2015. The studied alfalfa cultivars were Hassawi (Saudi Arabian local), Egyptian local, Cuf 101 (USA), Server (Australian), WG CAL 10 (USA) and Saltine (USA). The seeds of these cultivars were obtained from the Agricultural Research and Experiment Station in Dirab, College of Food and Agricultural Sciences, KSU, Riyadh, Saudi Arabia. Normal water used for typical irrigation processes at Dirab Agricultural Research and Experiment Station was utilized and considered as the control. The tertiary-treated wastewater was obtained from Riyadh water treatment plant. Treated wastewater typically reaches the area *via* pipeline networks connecting the treatment plant with the fields in the Agricultural Research and Experiment Station. Treated wastewater reaches the experiment daily *via* the pipelines without any special transportation or storage conditions.

This experiment was laid out following randomized complete block design under split-plot arrangement keeping alfalfa genotypes in main plots while water types were kept in sub-plots. Each treatment was replicated three times. Distribution of cultivars on plots and assignment of irrigation types for each cultivar were performed randomly. Seeds of each cultivar were planted at a seeding rate of 40 kg ha⁻¹ in plots (3 m × 4 m) with 12 cm spacing between lines and 5 cm seeding depth on 10th November in both years (2013 and 2014). Recommendations of the Saudi Ministry of Environment, Water and Agriculture (MEWA; <https://www.mewa.gov.sa/en/>) were followed for all irrigation and fertilization processes. Plots were irrigated until reaching field capacity with respected water type by surface irrigation method once weekly in winter and twice in summer. Regarding fertilization, diammonium phosphate (DAP; 18% N, 46% P₂O₅) was applied before seeds sowing at a rate of 120 kg ha⁻¹. After sowing, fertilizers were applied three times throughout the growing season. Urea (46% N) was applied as N source (50 kg ha⁻¹), DAP was applied as phosphorus source (120 kg ha⁻¹), and potassium sulfate (50% K₂O; 18% S) was applied as K and S source (50 kg ha⁻¹). Weeds were removed manually according to need.

Water and soil analysis

Before starting the experiment, physical and chemical characteristics of the normal water and treated wastewater samples were analyzed in the laboratories of KSU and MEWA in Saudi Arabia. Table 1 shows the physical characteristics (pH, electrical conductivity "EC"; total dissolved solids "TDS") and contents of N, P, and K of the two different water types used in the current study.

Both before starting the experiment and at the end of experiment, soil samples were collected at 4 different depths (ranging from 0 to 70 cm beneath soil surface). After pooling all the soil samples based on water type used for their irrigation, physical and chemical characteristics of the soil were analyzed. Analyses were performed in the laboratories of KSU and MEWA in Saudi Arabia using Inductively Coupled Plasma-Mass Spectrometer (NexION 300D, Perkin Elmer, USA). Table 2 shows the studied soil physical characteristics (pH, EC, soil texture) and contents of P and K.

Forage productivity and quality

Plants in each replicate of the 12 different treatments (6 cultivars \times 2 water types) were harvested for the first time after 3 months of sowing. Afterwards, harvesting was conducted when plants reach 45 cm or at the start of flowering stage. Plants were harvested to the height of 5 cm above soil surface in each harvest (Shen *et al.* 2013).

Total productivity was calculated as the total fresh matter produced by alfalfa cultivar throughout the whole season. Fresh matter was weighed directly after harvesting and expressed as $t\ ha^{-1}$. Weights of all harvestings along the season were summed and the average of three replicates was reported.

Dry matter production was expressed as the percentage of dry matter production in relation to fresh matter. A random sample were taken from each replicate in each treatment and weighed. Fresh weight of the samples was recorded. Thereafter, samples were air-dried in an oven for 48 hours at 60°C before weighing the dried samples and recording the dry weight. Dry matter percentage was then calculated. Moreover, dry matter productivity along the season was calculated based on these percentages.

Forage quality was estimated in grinded samples. After drying, an electrical grinder (IKA® MF 10.1 cutting-grinding head) was used to grind samples at a speed of 5500 rpm. The resulted grinded plant material was then passed through 1-mm-diameter sieves and the passed-through fine powder was collected for further analysis.

All chemical analyses were performed in the laboratories of the College of Food and Agricultural Sciences, KSU and Verband Deutscher Landwirtschaftlicher Untersuchungs-und Forschungsanstalten (VDLUFA) e. V., Speyer, Germany. Samples were air-shipped to Germany in sealable plastic bags. An amount of 500 g grinded powder was shipped from each sample. Crude ash (CA) content was measured by combusting a known amount of powdered material for 6 h at 550°C. Near-infrared spectroscopy (NIRS) instrument (Technicon 500, Technicon Industrial Systems, NY, USA) was used to determine percentages of crude protein (CP), crude fiber (CF), crude fat (CFA), WSC (WSC), neutral detergent fiber (NDF), acid detergent fiber (ADF) and digestibility (DIG) in alfalfa plants.

Table 1: Chemical and physical properties of the water types used for irrigation

| Parameters | Normal water | Treated wastewater |
|-------------------------------|--------------|--------------------|
| pH | 7.15 | 7.82 |
| EC* (dS m ⁻¹) | 3.56 | 2.48 |
| TDS (ppm) | 1773 | 1253 |
| Total N (mg L ⁻¹) | 5.8 | 9.2 |
| P (%) | 2.2 | 1.7 |
| K (%) | 4.4 | 1.5 |

*EC: Electrical conductivity, TDS: Total dissolved solids

Table 2: Chemical and physical properties of soils at the beginning and end of the experiment

| Parameters (%) | Beginning of the Experiment | End of the experiment | |
|--------------------------|-----------------------------|-----------------------|--------------------|
| | | Normal water | Treated wastewater |
| pH | 8.28 | 7.98 | 8.07 |
| EC (dS m ⁻¹) | 0.25 | 0.49 | 0.22 |
| Sand (%) | 62 | 60 | 54 |
| Silt (%) | 28 | 30 | 34 |
| Clay (%) | 10 | 10 | 12 |
| Soil texture | Sandy loam | Sandy loam | Sandy loam |
| Phosphorus (%) | 8.2 | 4.1 | 4.8 |
| Potassium (%) | 12.7 | 7.8 | 8.3 |

EC: Electrical conductivity

Statistical analysis

Three-way analysis of variance (ANOVA) was used to examine the effects of irrigation water type, cultivars, and season on different studied parameters of alfalfa plants using IBM SPSS Statistics 20. Values are reported as mean of three replicates and the differences between means were separated by Duncan's Multiple Range Test ($P \leq 0.05$).

Results

The effects of irrigation water type, cultivar, and season on productivity and forage quality were examined. Three-way ANOVA showed that overall interaction between the three studied factors did not lead to any significant changes in any of the studied indicators of forage productivity and quality (Table 3). Therefore, effects of pair-wise interaction between the studied factors and effects of each single factor were considered. Interaction between irrigation water type and season of cultivation did not significantly affect alfalfa productivity (Table 4). On the other hand, CP, CF, CFA and WSC were affected by the interaction between irrigation water type and season. Irrigation with normal water in the first season enhanced crude protein contents by 16.7% as compared to the second season; however, there was no change in this trait under irrigation with treated wastewater. It is noteworthy that irrigation with normal water showed the highest crude protein content in the first season, but in the second season no difference was observed as a result of irrigation with different water types. The lowest crude fibers content was in plants irrigated with treated wastewater in the first season. However, this content increased in the second season with no statistical difference compared to other

Table 3: Effects of irrigation water type, cultivar and/or season on alfalfa productivity and forage quality

| Factors | FM (t ha ⁻¹) | DM (t ha ⁻¹) | CA (%) | CP (%) | CF (%) | CFA (%) | WSC (%) | NDF (%) | ADF (%) | DIG (%) |
|------------------------------|--------------------------|--------------------------|---------|----------|-----------|---------|---------|---------|---------|----------|
| Water type (W) | | | | | | | | | | |
| NW | 207.18 | 46.64 | 11.61 | 23.31 a | 26.78 a | 2.36 | 2.38 | 41.66 | 34.05 a | 73.50 |
| TW | 207.14 | 46.62 | 11.28 | 21.92 b | 26.05 b | 2.34 | 2.12 | 40.96 | 33.31 b | 72.75 |
| Significance $P \leq 0.05$) | ns | ns | ns | * | * | ns | Ns | ns | * | ns |
| Cultivar (C) | | | | | | | | | | |
| Hassawi | 207.76 b | 47.09 ab | 11.73 | 21.53 c | 27.08 ab | 2.18 c | 3.41 a | 40.37 | 33.67 | 71.99 b |
| Egyptian local | 203.98 c | 45.94 ab | 11.63 | 22.28 bc | 27.36 a | 2.25 bc | 2.36 b | 41.45 | 34.25 | 72.04 b |
| Cuf 101 | 201.93 c | 45.73 ab | 11.19 | 22.47 b | 26.43 a-c | 2.35 ab | 1.91 bc | 41.64 | 33.81 | 73.37 ab |
| Server | 202.43 c | 45.16 b | 11.47 | 23.51 a | 25.47 c | 2.50 a | 1.62 c | 41.40 | 33.11 | 74.51 b |
| WG CAL 10 | 211.40 ab | 47.59 ab | 11.28 | 22.95 ab | 26.16 bc | 2.40 ab | 2.19 bc | 41.36 | 33.70 | 73.30 ab |
| Saltine | 215.44 a | 48.25 a | 11.38 | 22.97 ab | 26.00 c | 2.41 ab | 2.03 bc | 41.65 | 33.52 | 73.51 ab |
| Significance $P \leq 0.05$) | * | * | ns | * | * | * | * | ns | ns | * |
| Season (S) | | | | | | | | | | |
| 2013-2014 | 192.00 b | 43.57 b | 12.21 a | 23.11 a | 27.70 a | 2.41 a | 3.04 a | 43.17 a | 35.08 a | 74.32 a |
| 2014-2015 | 222.32 a | 49.68 a | 10.69 b | 22.13 b | 25.13 b | 2.29 b | 1.46 b | 39.45 b | 32.28 b | 71.93 b |
| Significance $P \leq 0.05$) | * | * | * | * | * | * | * | * | * | * |
| Interactions | | | | | | | | | | |
| W × C | ns | ns | ns | ns | ns | ns | Ns | ns | ns | ns |
| W × S | ns | ns | ns | * | * | * | * | ns | ns | ns |
| C × S | * | * | ns | ns | ns | ns | Ns | ns | ns | ns |
| W × C × S | ns | ns | ns | ns | ns | ns | Ns | ns | ns | ns |

Means followed by different letter are statistically different from each other according to Duncan's Multiple Range test at $P \leq 0.05$

NW: normal water, TW: treated wastewater, FM: fresh matter, DM: dry matter, CA: crude ash, CP: crude protein, CF: crude fibers, CFA: crude fat, WSC: water-soluble carbohydrates, NDF: neutral detergent fiber, ADF: acid detergent fiber, DIG: digestibility, ns: non-significant, *: significant at $P \leq 0.05$

Table 4: Interactive effect of seasons × water types, and seasons × cultivars on forage quality and yield of alfalfa

| Interacting variables | | CP (%) | CF (%) | CFA (%) | WSC (%) |
|-----------------------|----------------|----------|----------|---------|---------|
| Water type | | | | | |
| Season | | | | | |
| NW | 2013-2014 | 24.19 a | 27.83 a | 2.45 a | 2.78 b |
| | 2014-2015 | 22.43 b | 27.57 a | 2.28 b | 1.47 c |
| TW | 2013-2014 | 22.02 b | 25.73 b | 2.36 ab | 3.31 a |
| | 2014-2015 | 21.82 b | 27.83 a | 2.45 a | 1.44 c |
| Season | | | | | |
| Cultivar | | | | | |
| 2013-2014 | Hassawi | 189.62 e | 43.41 f | | |
| | Egyptian local | 182.62 f | 41.31 g | | |
| | Cuf 101 | 188.80 e | 43.00 f | | |
| | Server | 190.53 e | 42.85 f | | |
| | WG CAL 10 | 197.42 d | 45.06 e | | |
| | Saltine | 203.02 c | 45.80 d | | |
| | 2014-2015 | Hassawi | 225.90 a | 50.77 a | |
| | Egyptian local | 225.35 a | 50.57 a | | |
| | Cuf 101 | 215.06 b | 48.45 b | | |
| | Server | 214.33 b | 47.47 c | | |
| | WG CAL 10 | 225.38 a | 50.12 a | | |
| | Saltine | 227.87 a | 50.70 a | | |

Means followed by different letters are statistically different from each other according to Duncan's Multiple Range test at $P \leq 0.05$

NW: normal water, TW: treated wastewater, FM: fresh matter, DM: dry matter, CP: crude protein, CF: crude fibers, CFA: crude fat, WSC: water-soluble carbohydrates

plants irrigated with normal water. Plants irrigated with normal water and treated wastewater in the first and second seasons, respectively showed the highest crude fat contents. Interestingly, content of water-soluble carbohydrates was the highest in plants irrigated with treated wastewater in the first season; however, a drop in this content was observed in the second season. Contrarily, the interaction between season and cultivar significantly affected the productivity of alfalfa plants regardless of irrigation water type. Hassawi, Egyptian local, WG CAL 10 and Saltine cultivars cultivated in the second season showed the highest FM and DM as compared to other cultivars in both seasons (Table 4). All the studied parameters with regard to forage quality were not significantly affected by the interaction between

cultivation season and cultivar. Furthermore, no significant effects were resulted from the interaction between water type and cultivar (Table 3).

Our results showed that irrigation with treated wastewater did not show any adverse effects on all the studied alfalfa cultivars in both seasons as compared to plants irrigated with normal water (Table 3). Furthermore, our results showed a slight decrease in CP, CF and ADF contents in alfalfa plants irrigated with treated wastewater as compared to those plants irrigated with normal water. Alfalfa plants cultivated in the first season showed lower productivity as compared to plants cultivated in the second season. However, plants cultivated in the first season produced forage with higher value in terms of all the studied

Table 5: Effects of irrigation water type, cultivar and season on alfalfa productivity and forage quality

| Factor | FM ^a (t ha ⁻¹) | DM (t ha ⁻¹) | CA (%) | CP (%) | CF (%) | CFA (%) | WSC (%) | NDF (%) | ADF (%) | DIG (%) |
|----------------|---------------------------------------|--------------------------|---------|----------|-----------|---------|---------|---------|---------|----------|
| Water type | | | | | | | | | | |
| WW | 207.18 a | 46.64 a | 11.61 a | 23.31 a | 26.78 a | 2.36 a | 2.38 a | 41.66 a | 34.05 a | 73.50 a |
| TW | 207.14 a | 46.62 a | 11.28 a | 21.92 b | 26.05 b | 2.34 a | 2.12 a | 40.96 a | 33.31 b | 72.75 a |
| Cultivar | | | | | | | | | | |
| Hassawi | 207.76 b | 47.09 ab | 11.73 a | 21.53 c | 27.08 ab | 2.18 c | 3.41 a | 40.37 a | 33.67 a | 71.99 b |
| Egyptian local | 203.98 c | 45.94 ab | 11.63 a | 22.28 bc | 27.36 a | 2.25 bc | 2.36 b | 41.45 a | 34.25 a | 72.04 b |
| Cuf 101 | 201.93 c | 45.73 ab | 11.19 a | 22.47 b | 26.43 abc | 2.35 ab | 1.91 bc | 41.64 a | 33.81 a | 73.37 ab |
| Server | 202.43 c | 45.16 b | 11.47 a | 23.51 a | 25.47 c | 2.50 a | 1.62 c | 41.40 a | 33.11 a | 74.51 b |
| WG CAL 10 | 211.40 ab | 47.59 ab | 11.28 a | 22.95 ab | 26.16 bc | 2.40 ab | 2.19 bc | 41.36 a | 33.70 a | 73.30 ab |
| Saltine | 215.44 a | 48.25 a | 11.38 a | 22.97 ab | 26.00 c | 2.41 ab | 2.03 bc | 41.65 a | 33.52 a | 73.51 ab |
| Season | | | | | | | | | | |
| 2013/2014 | 192.00 b | 43.57 b | 12.21 a | 23.11 a | 27.70 a | 2.41 a | 3.04 a | 43.17 a | 35.08 a | 74.32 a |
| 2014/2015 | 222.32 a | 49.68 a | 10.69 b | 22.13 b | 25.13 b | 2.29 b | 1.46 b | 39.45 b | 32.28 b | 71.93 b |

^aFM: fresh matter, DM: dry matter, CA: crude ash, CP: crude protein, CF: crude fibers, CFA: crude fat, WSC: water-soluble carbohydrates, NDF: neutral detergent fiber, ADF: acid detergent fiber, DIG: digestibility. Means followed by the same letter are not significantly different ($P \leq 0.05$)

parameters (Table 3). Cultivation of different cultivars significantly affected the studied parameters except CA, NDF and ADF contents regardless of irrigation water type or cultivation season (Table 3). WG CAL 10 and Saltine cultivars showed the highest FM productivity among all the studied cultivars (Table 5). However, less variation was observed in DM production among the studies cultivars with Server cultivar showed the lowest DM values. Hassawi local Saudi cultivar showed the highest WSC values regardless of irrigation water type and cultivation season. On the other hand, the same cultivar showed the lowest CP and CFA among all the studied cultivars.

Discussion

Results of this two-year field study showed that treated wastewater characterized by better chemical parameters as compared to normal water with higher N contents and lower EC values (Table 1). Higher concentration of nutrients available in treated wastewater significantly improves soil fertility and crop growth and productivity (Table 3; El-Nahhal *et al.* 2013). Soils irrigated with treated wastewater showed higher contents of P and K (Table 2). This agrees with the results of previous reports (Boruah and Hazarika 2010; Elfanssi *et al.* 2018). In the current study, the results showed that irrigation with treated wastewater did not show any negative effects on the productivity of all the studied alfalfa cultivars in both seasons as compared to plants irrigated with normal water. In another study, yields of maize (*Zea mays* L.) and vetch (*Vicia sativa*) plants was increased after irrigation with treated wastewater (Mohammad and Ayadi 2004). Furthermore, sorghum (*Sorghum bicolor* L.) plants showed significant increase in productivity of plants irrigated with treated wastewater as compared to those irrigated with normal water (Galavi *et al.* 2009). In barely (*Hordeum vulgare*), application of treated wastewater effectively increased the productivity of green and dry fodder as compared to irrigation with tap water and mix of treated wastewater and tap water (Al-Karaki 2011). It is well established that fodder productivity in alfalfa plants has high correlation with N contents in irrigation water (Azevedo *et*

al. 2006). Our results showed higher levels of N in treated wastewater as compared to normal water. Maintenance of productivity under irrigation with treated wastewater could be attributed to high amount of nutrients available in soil irrigated with treated wastewater (Ghanbari *et al.* 2007; GhassemiSahebi *et al.* 2020). Therefore, soil irrigated with treated wastewater showed higher P and K levels (Table 2). Furthermore, irrigation of treated wastewater may provide other means for growth enhancement such as increasing organic matter contents in the soil and improving soil physical properties (Shahalam *et al.* 1998; Schalscha *et al.* 1999; Gori *et al.* 2000). In the current study soil irrigated with treated wastewater has lower EC values indicating less salinity levels. Results indicated a slight decrease in CP, CF and ADF contents in alfalfa plants irrigated with treated wastewater as compared to those plants irrigated with normal water. These results agree with findings of previous studies. For example, Galavi *et al.* (2009) found that irrigation with treated wastewater throughout the season led to reduction in CP and ADF contents in alfalfa forage as compared to alternate irrigation with treated wastewater and normal water. In our previous study (Soufan *et al.* 2019), treated wastewater showed higher amounts of Cd. Although protein content was anticipated to increase in plants irrigated with treated wastewater because of increased N levels, slight reduction in protein content may be attributed to presence of heavy metals *e.g.*, Cd in treated wastewater. Cadmium may lead to nutrient deficiency in plants *via* competing with other essential nutrients on the same receptors (Nazar *et al.* 2012).

Alfalfa plants cultivated in the first season showed lower productivity as compared to plants cultivated in the second season. However, plants cultivated in the first season produced forage with higher value in terms of all the studied parameters (Table 5). Variation in productivity of alfalfa plants in different seasons could not be attributed to only one factor. However, changes in productivity and forage quality of alfalfa plants may be attributed to changes in temperature, CO₂ and precipitation levels (Andresen *et al.* 2001; Sanz-Sáez *et al.* 2012). Regardless of water type and season, saltine cultivar showed the highest forage productivity. This could be attributed to high salinity

tolerance of this cultivar (Al-Farsi *et al.* 2020; Bhattarai *et al.* 2020). High CP observed in this cultivar approved its potential adaptability to irrigation with treated wastewater among other cultivars. Further examination of long-term effects of irrigation with treated wastewater on alfalfa and other forage crops is needed to validate the safety and profitability of large-scale application of this type of irrigation water in production of forage crops.

Conclusion

Irrigation with treated wastewater did not adversely affect the productivity and forage quality of six different cultivars of alfalfa plants as compared to irrigation with normal water regardless of cultivar and/or cultivation season. Irrigation with treated wastewater has no negative effects on productivity of alfalfa plants with slight reduction in quality. Therefore, alfalfa forage crop can be grown with treated wastewater.

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Author Contributions

WS and NA planned the experiment, WS and EA implemented the experiment, MS obtained the fund, WS, PT and MO analyzed and illustrated the results, WS and EA made the manuscript first draft, NA, MO and PT revised the manuscript first draft.

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