

Effect of Application of Treated Wastewater on Seed Yield and Heavy Metals Content of Safflower Cultivars

A. A. Yazdani^{1*}, M. Saffari¹, and G. Ranjbar²

ABSTRACT

Treated wastewater could be a valuable source of water for recycling and reuse in arid regions. Two one-year field experiments were carried out to determine the effects of municipal treated wastewater on seed yield and seed heavy metals content of safflower cultivars, in Research Farm of Yazd Municipal Wastewater Purification Station, during 2015 and 2016. The experiments were arranged as split plot based on a randomized complete block design with three replicates. Irrigation treatments were in the main plot, consisting of three irrigation strategies (irrigation with only treated municipal wastewater, irrigation with treated wastewater/fresh water alternatively, and irrigation with only fresh water) and three safflower (*Carthamus tinctorius*) cultivars (Sofeh, Isfahan native, and Goldasht) in the subplots. Results showed that yield and yield components increased by treated wastewater treatment compared to the other irrigation treatments. Application of treated wastewater caused increase in safflower grain (40%) and biological (9%) yield as compared to fresh water treatment. Treated wastewater application led to accumulation of trace elements (Fe, Mn, Cu, Cd and Pb) in safflower seeds; however, the content of all the metals were below the permissible limits recommended by World Health Organization.

Keywords: *Carthamus tinctorius*, Irrigation, Municipal water, Permissible limits, Trace elements.

INTRODUCTION

Approximately seventy percent of the world water use, including all the water diverted from rivers and pumped from underground, is used for agricultural irrigation. Ground water resources in most areas of the world are shrinking at an alarming rate and may not meet the ever-increasing demands from agriculture and industry in the future. In this regard, reuse of treated municipal wastewater for agricultural and landscape irrigation reduces the amount of water that needs to be extracted from natural water sources and reduces discharge of wastewater to the environment. Thus, treated municipal

wastewater could be a valuable water source for recycling and reuse in arid and semi-arid regions, which are confronting increasing water shortages (Hanjra *et al.*, 2012). Treated municipal wastewater for agricultural reuse is increasingly recognized as an essential management strategy in areas of the world where water is in short supply. Wastewater has also been considered as low price fertilizer because of its high Nitrogen (N), Phosphorus (P), and potassium (K) content (Chaw and Reves, 2001; Mohammad and Mazahreh, 2003; Rattan *et al.*, 2005). Therefore, wastewater has great potential as manure when used for irrigation of crops. Use of wastewater for crop

¹ Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahid Bahonar University, Kerman, Islamic Republic of Iran.

² National Salinity Research Center, Agricultural Research, Education and Extension Organization (AREEO), Yazd, Islamic Republic of Iran.

*Corresponding author; e-mail: yazdani.abas@gmail.com



irrigation results in significant increase in soil organic matters compared to soils irrigated with fresh water (Osaigbovo *et al.*, 2006; Rusan *et al.*, 2007; Dheri *et al.*, 2007; Zhang *et al.* 2008). In fact, wastewater irrigation could be a good means of carbon sequestration in soil and can thus be referred to as a soil quality sustaining practice. Wastewaters also contain valuable plant nutrients and thus its reuse in agriculture serves as an important source of nutrients and irrigation water for crops (Aghabarati *et al.*, 2008). Results of many studies on the use of wastewater for long period have recapitulated significant increase in crop yields compared to fresh-water irrigated fields. Application of wastewater generally leads to increased concentrations of trace elements in the soil as well as plants (Arora *et al.*, 2008; Rusan *et al.*, 2007; Mapanda *et al.*, 2005). The widespread contamination with heavy metals occurring in the last decade has raised public and scientific concern due to its serious health effects on humans. This has encouraged researchers to study the pollution levels of heavy metals in the air, water, and foods, aiming to avoid their harmful effects and to determine their permissibility for human consumption (Amiri *et al.*, 2008). Safflower (*Carthamus tinctorius* L.) is a valuable oil crop from the economic viewpoint. In recent years, safflower has become an increasingly important source of vegetable oil and biomass, usefully employed for food, chemical, energy, and industrial purposes.

The objective of this investigation was to determine the effects of treated wastewater irrigation on yield of safflower and on the heavy metals contents of safflower seeds.

MATERIALS AND METHODS

Experimental Site and Climate

Field experiment was conducted in Research Farm at Yazd Municipal Wastewater Purification Station (31° 96' N/ 54° 30' E) in 2015 and 2016. Urban wastewater resources

were mostly residential, commercial, institutional and recreational. The climate of the experimental region is hot and arid according to Koppen Climate Classification System (Dastorani *et al.*, 2011). Annual precipitation averages for 2014-2015 and 2015-2016 were 51 and 25 mm, respectively.

Experimental Design and Treatments

Experiment was arranged as split plot based on a randomized complete block design with three replicates. Treatments were three irrigation water strategies under surface irrigation system comprising: (1) Irrigation with only treated municipal WasteWater (WW), (2) Irrigation with Fresh Water and treated municipal WasteWater alternatively during growing seasons (FW/WW), and (3) Irrigation with only Fresh Water (FW); in the main plot; and three safflower cultivars, namely, Sofeh, Isfahan native, and Goldasht in the subplots. Prior to planting, fertilizer was applied according to soil analysis results. Fresh water plots received 50 kg ha⁻¹ triple superphosphate (46% P₂O₅) and 50 kg ha⁻¹ potassium sulphate (48-52% K₂O) mixed with the top soil before sowing. Nitrogen fertilizer was top-dressed in two equal doses (one at sowing and the other after thinning on 20th of April) at the rate of 75 kg N ha⁻¹. Since WW treatment contained plenty of nutrients, and application of WW sequentially or alternatively could lead to increase in nutrients in the soil, no mineral fertilizer was used in WW and WW/FW treatments. In both years, safflower seeds were sown on 1st of March. The seeds were planted in plots having 6 rows, 5 m length, with 0.6 m interrow spacing and 7 cm interplant spacing within rows.

The treated wastewater and fresh water samples were analysed for pH, Electrical Conductivity (EC), and heavy metals contents based on standard APHA (1998) methods. Two monthly water samples were taken for analysis during Mar and Aug from Yazd Purification Station (Table 1). Average values of the water analyses (WW and FW) and quality standards for WW application in

agriculture according to World Health Organization (WHO), Food and Agriculture Organization (FAO) and Iranian Department Of Environment (IRNDOE) are shown in Table 1. To measure the physico-chemical properties and heavy metals concentration, soil samples were taken before sowing. The total concentrations of Fe, Mn, Cu, Zn, Cd, Pb and As were determined by inductively coupled plasma optical emission spectrometry (ICP-MS, Agilent series 4500, made in USA) and

four acids method were used (Baker and Amacher, 1982). Physical characteristics and heavy metal content of the soil are shown in Table 2.

Plant Sampling, Harvesting, and Data Analysis

In both years, the plots were harvested on August 1st. Observations on yield and yield components of safflower were taken

Table 1. Averages of selected properties of fresh water and treated wastewater and maximum permissible limits of heavy metals content for treated wastewater application in agriculture according to WHO and IRNDOE.

Parameter	Unit	Fresh water	Treated wastewater		Wastewater standards for agriculture	
			2015	2016	WHO	IRNDOE
EC	ds m ⁻¹	0.8	1.6	1.67	< 3	-
pH	-	7.2	7.5	7.6	6 – 8.4	6 – 8.5
Nitrate	mg l ⁻¹	7.21	14.5	12.5	5-30	10
Nitrite	mg l ⁻¹	0.003	1.2	1.5	5-30	10
Ammonium	mg l ⁻¹	-	8.5	5.7	-	1
P	ppm	-	2	2.1	4	6
K	ppm	-	0.42	0.44	-	-
Fe	ppm	<0.02	0.186	< 0.1	5	3
Zn	ppm	<0.01	0.157	< 0.1	2	2
Cu	ppm	<0.01	< 0.1	< 0.1	0.2	0.2
Mn	ppm	<0.01	< 0.1	< 0.1	0.2	1
As	ppm	Not detected	0.17	< 0.1	0.1	0.1
Pb	ppm	Not detected	0.11	< 0.1	5	1
Cd	ppm	Not detected	0.023	< 0.01	0.01	0.05

Table 2. Chemical characteristics of soil due to irrigation with WW, WW/FW and FW at the beginning and end of growing seasons. ^a

Parameter	Beginning of the growing season	Irrigation					
		FW		WW/FW		WW	
		Year		Year		Year	
		2015	2016	2015	2016	2015	2016
pH	7.9	7.7	7.8	7.6	7.7	7	6.8
Organic carbon (%)	0.101	0.11	0.13	0.165	0.145	0.257	0.31
N (%)	0.009	0.008	0.009	0.014	0.016	0.022	0.027
P (ppm)	13	11	12.01	12.1	13.3	15.3	14.33
K (ppm)	92	92	80	100	121	221	242
Fe (ppm)	6.14	5.55	5.2	6.12	6.3	7	7.21
Cu (ppm)	0.66	0.6	0.65	0.81	0.9	1.11	1
Zn (ppm)	1.73	1.65	1.38	1.88	1.92	2.2	2.73
Mn (ppm)	1.32	1.31	1.25	1.65	1.45	2.81	2.77

^a WW: WasteWater, FW/WW: Fresh Water/WasteWater and FW: Fresh Water.



randomly for five safflower plants from an area of 1.5×1.5 m at the centre of each plot. Seed numbers per head, number of head per plant, weight of 1000 seeds (g), seed yield (kg ha^{-1}) and biological yield (kg ha^{-1}) were recorded. To measure heavy metals content of safflower seeds, samples were digested using the 4-acid digestion procedure, which is carried out in open vessels on a hot-plate. The method uses a combination of nitric, hydrochloric, hydrogen peroxide and perchloric acids. Heavy metal analyses were carried out after mineralization using acids for seed samples. Heavy metal contents (Fe, Cu, Mn, Cd and Pb) were recorded by the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technique (Avula *et al.*, 2010). ICP-MS has been usually applied for rather limited number of elements compared to its capabilities, the reason being, most probably, the research aims in the respective studies. All data were subjected to combined Analysis Of Variance (ANOVA) using the General Linear Models (GLMs) procedures of the Statistical Analyses System (SAS, 9.2). Treatment Means were also compared by Duncan's multiple range test ($P < 0.05$).

RESULTS

Grain Numbers per Head

Combined variance analysis showed that interaction between irrigation water strategies and cultivars was significant on grain numbers per head (Table 3). The highest grain number per head was obtained in Sofeh, which was irrigated with WW. In contrast, the lowest grain number per head was observed in Goldasht irrigated with FW (Table 4). The grain numbers per head of safflower was significantly affected by irrigation water strategy (Table 3). Generally, Sofeh and Goldasht irrigated by WW treatment had the highest grain number per head compared to the FW (Table 4).

Head Number per Plant

The results showed that simple effects of treatments on head number per plant were significant, but, their interaction effects were not (Table 3). The number of head per plant increased significantly in WW treatment. The lowest number of head per plant was observed in FW and FW/WW treatments (Table 5). In fact, irrigation with WW treatment improved head number per plant by 17% compared to the FW treatment. Sofeh had also the highest head number per plant (Table 6).

Thousand-Grain Weight

The highest thousand-grain weight (47.58 g) was obtained in Goldasht cultivar, which was irrigated with WW. The lowest thousand-grain weight (29.27 g) was also obtained in Isfahan native cultivar, which was irrigated with FW (Table 4). In all cultivars, the wastewater treatment significantly increased thousand-grain weight as compared to FW treatment (Table 4). Results showed that WW treatment had the highest effect on the Goldasht cultivar compared to other cultivars. In fact, irrigation with WW improved thousand-grain weight by about 16% compared to the FW treatment in Goldasht cultivar.

Biological and Seed Yield

Interactions between irrigation water strategies and cultivars on grain and biological yield were significant (Table 3). The mean of irrigation water strategies effects showed that the highest grain ($2,991 \text{ kg ha}^{-1}$) and biological yield ($7,522 \text{ kg ha}^{-1}$) of safflower was obtained in WW treatment (Table 5). Application of treated wastewater caused increase in safflower grain (40%) and biological (9%) yield compared to fresh water treatment. Results also showed that the highest biological and grain yield were obtained in Sofeh cultivar (Table 6).

Table 3. Irrigation strategies and cultivars effects on head number per plant, grain number per head, 1,000 grain weight, grain and biological yield of safflower (2015-2016).^a

SOV	d f	Mean squares				
		Head number per plant	Grain number per head	1000 Grain weight	Grain yield	Biological yield
Year	1	1.85 ^{ns}	54 ^{**}	3.861 ^{ns}	1072834 ^{ns}	2533700 ^{**}
Block (Year)	4	0.53 ^{ns}	0.592 ^{ns}	0.685 ^{ns}	19510 ^{ns}	109928 ^{ns}
Water	2	8.38 ^{**}	104.24 ^{**}	56.88 ^{**}	3565075 ^{**}	4460678 ^{**}
Water (Year)	2	0.24 ^{ns}	2.05 ^{ns}	10.8 ^{ns}	42597 ^{ns}	240178 ^{ns}
W×B×Y	8	0.425 ^{ns}	3.7 ^{ns}	1.23 ^{ns}	56563 ^{ns}	186555 ^{ns}
Cultivar	2	60.16 ^{**}	139 ^{**}	811.89 ^{**}	6495987 ^{**}	18158298 ^{**}
(C×W)	4	0.805 ^{ns}	22.01 ^{**}	16.82 ^{**}	117830 ^{ns}	415330 ^{ns}
Cultivar (Year)	2	0.796 ^{ns}	6.5 ^{ns}	10.14 ^{ns}	52101 ^{ns}	428904 ^{ns}
(C×W×Y)	4	1.435 ^{ns}	3.05 ^{ns}	13.24 ^{ns}	121627 ^{ns}	241067 ^{ns}
Error	24	0.407	3.972	1.109	55504	193524

^a SOV= Source Of Variation, df= Degrees of freedom, W×B×Y= Water×Block ×Year; C×W= Cultivar ×Water; C×W×Y= Cultivar×Water×Year. * Significant at 5% level, ** Significant at 1% level and ^{ns} Not significant.

Table 4. Mean comparisons of interaction effects of irrigation water types and cultivars on grain number per head and 1,000 grains weight of safflower.^a

		Means of 2016	
Irrigation	Cultivar	Grain number per head	1000 Grain weight (g)
WW	Sofeh	36 a	34.85 d
	Isfahan native	31 b	31.91 f
	Goldasht	29 bc	47.58 a
FW/WW	Sofeh	31 b	33.04 ef
	Isfahan native	28 bc	32.59 ef
	Goldasht	27 c	43.16 b
FW	Sofeh	28 bc	33.35 e
	Isfahan native	30 b	29.27 g
	Goldasht	23 d	41.06 c

^a Each mean values followed by the same letters are not significantly different for $P \leq 0.05$ according to the Duncan's test. WW: WasteWater, FW/WW: Fresh Water/WasteWater and FW: Fresh Water.

Table 5. Effects of irrigation strategies on grain number per head, head number per plant, 1,000 grains weight, grain yield and biological yield of safflower.^a

Irrigation	Trait		
	Head number per plant	Grain yield (kg ha ⁻¹)	Biological yield (kg ha ⁻¹)
WW	8.66 a	2991 a	7522 a
FW/WW	7.61 b	2379 b	6536 c
FW	7.38 b	2125 c	6912 b

^a Each mean values followed by the same letters are not significantly different for $P \leq 0.05$ according to the Duncan's test. WW: WasteWater, FW/WW: Fresh Water/WasteWater and FW: Fresh Water.

**Table 6.** Means of grain number per head, head number per plant, 1,000 grains weight, grain yield and biological yield in safflower cultivars. ^a

Cultivar	Trait		
	Head number per plant	Grain yield (kg ha ⁻¹)	Biological yield (kg ha ⁻¹)
Sofeh	9.94 a	3177 a	7903 a
Isfahan native	7.27 b	2036 c	7152 b
Goldasht	6.44 c	2282 b	5914 c

^a Each mean values followed by the same letters are not significantly different for $P \leq 0.05$ according to the Duncan's test.

Seed Heavy Metals Contents

Irrigation with WW treatment had significant effects on all measured heavy metals content of safflower seeds (Table 7). Results showed that treated municipal wastewater application increased Cd and Pb in safflower seeds (Table 8). There were little differences in the concentrations of seed heavy metals between FW and WW/FW treatments. Interaction between irrigation water strategies and cultivars significantly affected Fe, Cu and Mn content in seeds (Table 9). Means comparison showed that the highest Fe and Cu content was in Sofeh cv and Mn in Goldasht cv which were irrigated with WW treatment (Table 9). Generally, all cultivars irrigated by FW treatment had the lowest contents of Fe, Cu, and Mn. Concentrations of Fe, Cu,

Mn, Pb, and Cd in safflower seeds that were harvested from plants exposed to treated wastewater were not found to be above the standard of WHO and Institute of Standards and Industrial Research of Iran (ISIRI) for human consumption (Pb= 0.1 ppm, Fe= 400 ppm, Cu= 75 ppm, Mn= 450 ppm, and Cd= 0.1 ppm). However, Fe, Cu, Mn, Pb and Cd were within the acceptable limits.

DISCUSSION

Results of the present study showed that yield and yield components in all cultivars increased with treated wastewater. Since treated municipal wastewater contains large amounts of nutrients (Table 1), application of WW increased soil nutrients (Table 2). Therefore, no nutrition deficiency was

Table 7. Irrigation water strategies and cultivars effects on seed heavy metals content (2015-2016).^a

SOV	df	Mean squares of heavy metals				
		Fe	Mn	Cu	Cd	Pb
Year	1	121 ^{ns}	0.166 ^{ns}	29 ^{**}	0.000129 ^{ns}	0.000515 ^{**}
Block (Year)	4	58 ^{ns}	10.75 ^{ns}	1.7 ^{ns}	0.0001 ^{ns}	0.000018 ^{ns}
Water	2	61730 ^{**}	157 ^{**}	102 ^{**}	0.0134 ^{**}	0.00194 ^{**}
Water (Year)	2	113 ^{ns}	11.55 ^{ns}	0.796 ^{ns}	0.000279 ^{ns}	0.000089 ^{ns}
W×B×Y	8	43 ^{ns}	11.14 ^{ns}	2.23 ^{ns}	0.00071 ^{ns}	0.000213 ^{ns}
Cultivar	2	289 ^{**}	32 ^{ns}	2.72 ^{ns}	0.000157 ^{ns}	0.000294 ^{**}
(C×W)	4	170 [*]	69 ^{**}	12.86 ^{**}	0.000178 ^{ns}	0.000039 ^{ns}
Cultivar (Year)	2	60 ^{ns}	1.72 ^{ns}	0.35 ^{ns}	0.000266 ^{ns}	0.000114 ^{ns}
(C×W×Y)	4	137 ^{ns}	6.44 ^{ns}	0.76 ^{ns}	0.000326 ^{ns}	0.000039 ^{ns}
Error	24	43	7.99	2.138	0.000355	0.000111

^a SOV= Source Of Variation, df= Degrees of freedom, W×B×Y= Water×Block× Year; C×W= Cultivar×Water; C×W×Y= Cultivar×Water×Year. * Significant at the 5% level, **. Significant at the 1% level and ^{ns} Not significant.

Table 8. Effects of irrigation water strategies on seed heavy metals content of safflower.^a

Irrigation	Heavy metal (mg kg ⁻¹)	
	Cd	Pb
WW	0.09 a	0.046 a
FW/WW	0.063 b	0.028 b
FW	0.035 c	0.028 b

^a Mean values followed by the same letters are not significantly different at $P \leq 0.05$ according to the Duncan's test. WW: WasteWater, FW/WW: Fresh Water/WasteWater and FW: Fresh Water.

Table 9. Mean comparisons of interaction effects of irrigation water strategies and cultivars on seed heavy metals content.^a

Irrigation	Cultivar	Mean		
		Fe	Mn	Cu
WW	Sofeh	160 a	38 b	19 a
	Isfahan native	154 a	32 c	19 a
	Goldasht	145 b	42 a	16 b
WW/FW	Sofeh	53 c	32 c	16 b
	Isfahan native	57 c	34 c	14 c
	Goldasht	55 c	32 c	15 bc
FW	Sofeh	56 c	32 c	12 d
	Isfahan native	45 d	32 c	14 c
	Goldasht	45 d	32 c	14 c

^a Mean values followed by the same letters are not significantly different at $P \leq 0.05$ according to the Duncan's test. WW: WasteWater, FW/WW: Fresh Water/WasteWater and FW: Fresh Water.

observed in the sensitive periods of vegetative and reproductive growth of the plant. As shown earlier, due to enrichment of the nutrients in WW treatment, generally, head number per plant, number of fertilized florets, grains number per head and number of filled seeds increased compared to WW/FW and FW treatments. These results were in agreement with Safi-naz and Shaaban (2015), who reported that application of treated wastewater enhanced the yield and yield components of sunflower.

Application of wastewater also increased soil organic carbon compared to FW treatment (Table 2). Similar results were reported by Brar *et al.* (2000), Osaigbovo *et al.* (2006), Rusan *et al.* (2007), Dheri *et al.* (2007), and Zhang *et al.* (2008). It seems that wastewater could be considered as low-price fertilizer for crop nutrition because of its high N, P, and K content (Chaw and Reves, 2001; Mohammad and Mazahreh, 2003; Rattan *et al.*, 2005). As shown in the

section on result, WW significantly increased grain (2,991 kg ha⁻¹) and biological (7,522 kg ha⁻¹) yield in safflower. These results are in agreement with Nasri *et al.* (2012), who reported that application of treated wastewater enhanced the yield and yield components of safflower. Results of other studies also showed that use of WW treatment for long period have significant effect on the crop yields compared to the FW treatment. (Fonseca *et al.*, 2005; Fonseca *et al.*, 2007; Shahandeh and Hossener, 2002; El-Hady, 2007; Aghabarati *et al.*, 2008; Safi-naz and Shaaban, 2015).

Based on the results, application of treated wastewater leads to increase in heavy metals concentration in safflower seeds. However, the contents of these metals were lower than the maximum permissible limits. Leblebici and Kar (2018) and Alikhasi *et al.* (2012) also reported similar results. Generally, accumulation of heavy metals depends on different factors such as soil pH, such that reduction in soil pH could increase the



uptake of heavy metals by the plants. It seems that application of treated wastewater could reduce soil pH by increasing soil organic matters, especially as chelates (Mutengu *et al.*, 2007; Kiziloglu *et al.*, 2007). Therefore, increasing of organic matters and decreasing of soil pH led to increase in the uptake of heavy metals by plants and reduced leakage of the metals of root zone (Mojiri and Aziz, 2011). It has been documented that heavy metals uptake by plants is strongly pH dependent (Bolan *et al.* 2003). In particular, soils with pH values below 7 are very prone to heavy metal migration from soil solid components into the soil solution (Wang *et al.* 2011; Zeng *et al.*, 2011). Also, decreasing of soil pH led to higher availability of heavy metals in soil and organic matters and increases the uptake by plants. However, as reported by Tsadilas and Vakalis (2003), Jimenez (2005) and Lucia-Helena *et al.* (2011), these amounts of absorbed heavy metals had no negative effects on yield and yield components in wastewater treatments.

CONCLUSIONS

The present study showed that irrigation with treated municipal wastewater could have a positive influence on yield and growth of safflower in all growth stages. Irrigation with wastewater increased heavy metals content in safflower seeds, but the contents of trace elements were below the permissible limits recommended by WHO and ISIRI. According to these results, the use of treated wastewater could be suggested for irrigating of safflower in arid and semi-arid regions where the amount of fresh water is scarce.

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اثر کاربرد فاضلاب تصفیه شده بر عملکرد و مقدار فلزات سنگین دانه ارقام گلرنگ

ع. ا. یزدانی، م. صفری، و غ. رنجبر

چکیده

فاضلاب تصفیه شده می تواند یک منبع آب ارزشمند برای بازیافت و استفاده مجدد در مناطق خشک باشد. به همین منظور جهت تعیین اثرات فاضلاب تصفیه شده شهری بر عملکرد و میزان فلزات سنگین در دانه گلرنگ، آزمایش دو ساله در مزرعه تحقیقاتی ایستگاه تصفیه خانه فاضلاب شهری یزد در سال ۹۴ و ۹۵ اجرا شد. آزمایش به صورت کرت های خرد شده و در قالب طرح بلوک کامل تصادفی و در سه تکرار انجام شد. تیمار های این آزمایش شامل سه نوع آب آبیاری (آبیاری با آب فاضلاب تصفیه شده، آبیاری با آب شیرین و فاضلاب تصفیه شده به صورت متناوب و آبیاری با آب شیرین) به عنوان کرت اصلی و سه رقم گلرنگ (صفه، بومی اصفهان و گلدشت) به عنوان کرت فرعی بودند. نتایج نشان دادند که آبیاری با فاضلاب تصفیه شده در مقایسه با دیگر روش های آبیاری باعث افزایش اجزاء عملکرد گلرنگ شده است. به طور کلی کاربرد فاضلاب تصفیه شده در مقایسه با آب شیرین باعث افزایش ۴۰ درصد عملکرد دانه و ۹ درصد عملکرد بیولوژیک شد. مصرف آب فاضلاب تصفیه شده همچنین باعث تجمع فلزات کمیاب (آهن، منگنز، مس، کادمیوم و سرب) در بذر گلرنگ شد هر چند مقادیر این فلزات کمتر از حد مجاز توصیه شده توسط سازمان بهداشت جهانی بود.