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Effect of Irrigation with Contaminated Water by Cloth Detergent on Seed Germination Traits and Early Growth of Sunflower (*Helianthus annuus* L.)

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Abstract

One of the sources for irrigation is sewage. Contaminated water may affect seed germination and plant growth. A laboratory experiment and a pot experiment were conducted in 2012 to determine the effect of different doses of detergent on seed germination traits and early growth of sunflower (*Helianthus annuus* L.). The experiments included eight doses of cloth detergent (0, 0.00002, 0.0002, 0.002, 0.02, 0.2, 2, 20 g/L). Results showed that 20 and 2 g/L of detergent severely reduced seed germination, plant height, leaf number per plant, total biomass and stem weight. 20 g/L of detergent reduced shoot length, root length, seedling weight and seed vigor. Seed germination stage was more sensitive to contaminated water than early growth stage. The results demonstrated that irrigating sunflower by contaminated water with household cleaning products at high concentration should be avoided.

Keywords: biomass, detergent, growth parameters, seed vigor, sunflower

Introduction

Irrigation is an increasingly important practice for crop production in west of Iran. In this region, irrigation water supplies are mainly from ground water sources that are being depleted. Iran is located in arid and semiarid area that its annual average precipitation (273 mm) is less than average precipitation of the world (IMO, 2012) One of the sources for irrigation is sewage. Sewage contains great deal of household cleaning products. People are worried about effect of the products in the environment. Contaminated water can affect seed germination and growth of crop plant. Increasing concentrations of chlorpyrifos (pesticide) in the soil decreased seed germination percentage of ryegrass (*Lolium multiflorum*) (Korade and Fulekar, 2009). Seed germination of peanut (*Arachis hypogea*) was more sensitive to high doses of spent diesel fuel than maize (*Zea mays*) (Ehiagbonare *et al*., 2011). Some elements such as heavy metals may have a positive or negative effect on plant growth for example lower concentration of heavy metals increased root, shoot and biomass of sunflower (*Helianthus annuus*) (Jadia and Fulekar, 2008). Cr (VI), Cu (II), Ni (II), and Zn (II) increased alfalfa (*Medicago sativa*) shoot size by 14.0%, 60.0%, 36.0%, and 7.7%, respectively compared to control, but 5 ppm of Cd (II) reduced shoot size by 16% compared to the control (Peralta *et al*., 2000). Sunflower is an important oil crop in Iran. There is little information about effect of detergent on seed germination and plant growth of sunflower, so the objective of this study was to determine sunflower seed germination traits and early growth at different doses of detergent powder.

Materials and methods

Experiment 1

A laboratory experiment was conducted to test sunflower seed germination traits under different doses of detergent powder. The experiment included eight doses of detergent powder (T1=20, T2=2, T3=0.2, T4=0.02, T5=0.002, T6=0.0002, T7=0.00002, T8=0 g/L). Major chemical ingredient of studied cloth washing powder included sodium alkyl benzen sulfonate, nonyl phenol ethoxylate, sodium silicate, sodium carbonate, sodium sulphate, sodium toluene sulfonate, acrylate polymer, optical brightener, bleach, builder and essence.

The study was conducted as a randomized complete block design with three replications in 2012. Seeds of sunflower (*Helianthus annuus,* cv. 'Songhuri') were gathered from maternal plants harvested in 2011. After harvesting seeds from maternal plants, they were stored at 20-25°C for six months.

Before trial beginning, seeds were sterilized by sodium hypochlorite solution (1% active chlorine) for 10 minutes to avoid fungal contamination. Then each petri dish received 20 seeds and 8 cc of solution was added to them. The control solution (0 g/L) used in this study was distilled water. The petri dishes were categorized by dose and each category was sealed with plastic wrap to keep moisture in. The temperature during experiment period was kept at 26 ± 1°C. Two millimeters growth of coleoptile and radical was the criterion for germination.

Seed vigor was estimated by these equations (Abasian *et al*., 2010; Sharifzadeh *et al*., 2006):

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Seed vigor (% cm) = [(Radicle length (cm) + Caulicle length (cm)) * (Germination percentage (%))]

Seed vigor (% g) = [(Radicle weight (g) + Caulicle weight (g)) * (Germination percentage (%))]

Trial period was 7 days and plant samples were dried in a forced-air oven at 65°C for 24 hours.

Experiment 2

Plant materials, experimental design and treatments: The pot experiment was conducted in 2012 at Faculty of Agriculture, University of Razi, Kermanshah, Iran. Sunflower seeds (*Helianthus annuus,* cv. 'Songhuri') were planted in 24 pots (7 cm in diameter, 7.5 cm in depth) on Jun 26, 2012. The pots were filled with clay soil. Seeds were densely sown 1 cm deep but after emergence seedling were thinned to five plants per pot. Plants were initially well-watered and treatments of irrigation with contaminated water were only imposed 8 days after sowing. 336 mg nitrogen per 1 kg of soil as urea was used for nourishing plants grown in pots. The study was a randomized complete block design (RCBD) with three replications. The treatments were different detergent doses. There were eight doses of contaminated water with cloth detergent for irrigation (T1=20, T2=2, T3=0.2, T4=0.02, T5=0.002, T6=0.0002, T7=0.00002, T8=0 g/L). Control (T8=0 g/L) was tap water.

Plant sampling and measurements: Measurement of total dry weight, leaf to stem ratio, leaf dry weight and stem dry weight was carried out by five plants per pot, while plant height and leaf number per plant were measured by random selection of three plants per pot. Harvest time for total dry weight was 23 days after sowing and plant samples were dried in a forced-air oven at 65 °C for 24 hours.

Statistical analysis

Analysis of variance (ANOVA) was used to determine significant differences. The Multiple Range Test of Duncan performed the separation of means ($p<0.05$). Correlation coefficients were calculated for the relationship between several crop parameters. All statistics were performed with the program MINITAB (version 14.0), SAS (version 9.1) and SPSS (version 16.0).

Results and discussion

Experiment 1

Seed germination percentage: The highest dose of detergent (T1) reduced seed germination severely compared to other treatments except T2 (Tab. 1). T1 and T2 reduced seed germination compared to control (distilled water). Seed germination percentage had a positive and significant correlation with all traits except root length (Tab. 2).

Shoot length and root length: The highest dose of detergent (T1) reduced shoot length and root length severely compared to control (Tab. 1). Shoot length had a positive and significant correlation with all traits except seed vigor (Tab. 2). Root length had a positive and significant correlation with shoot length (Tab. 2).

Seedling weight and seed vigor: T1 had the lowest seedling weight except compared to T2, T3 and T5 (Tab. 1). Seedling weight had a positive and significant correlation with shoot length and seed germination percentage (Tab. 2). Seed vigor severely reduced under the highest doses of detergent (Tab. 1).

Seed germination traits of sunflower showed that the highest dose of detergent reduced seed germination, shoot length, root length, seedling weight and seed vigor compared to control. The results are compatible with findings of many researchers (Ashraf and Ali, 2007; Barua *et al*., 2011; Ehiagbonare *et al*., 2011). Reduction in seed germination traits under high dose of detergent can be attributed to induced oxidative stress, resulting in lipid peroxidation and increase in cell membrane permeability to toxic ions (Hejazi Mehrizi *et al*., 2012). Jadia and Fulekar (2008) reported that increasing doses of cadmium to sunflower reduced root and shoot length compared to control. High dose of detergent around a germinated seed reduces water uptake due to high osmotic potential, so seed germination metabolic activities delayed and probably production of oxygen free radical at the condition can damage cell membrane (Sharifzadeh *et al*., 2006). Ultimately, reduction in seed germination traits such as vigor due to high doses of detergent can be described by higher osmotic water potential, salinity and heavy metal stresses (Jadia and Fulekar, 2008).

Tab. 1. Effect of detergent doses on sunflower seed germination traits

Treatments [x]	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling weight (g/plant)	Vigor based on weight (% g)	Vigor based on length (% cm)
T1	6.67 c [y]	2.590 c	2.667 d	0.0080 b	0.0004 c	0.457 c
T2	17.50 bc	4.333 bc	3.493 cd	0.0140 ab	0.0066 ab	3.394 bc
T3	50.00 a	6.960 ab	9.953 ab	0.0153 ab	0.0078 ab	8.236 a
T4	33.33 ab	8.410 a	11.533 a	0.0210 a	0.0027 bc	2.563 bc
T5	35.00 ab	7.733 a	9.937 ab	0.0173 ab	0.0067 ab	6.085 ab
T6	52.50 a	9.450 a	7.263 abc	0.0220 a	0.0116 a	8.556 a
T7	35.00 ab	7.043 ab	6.457 bcd	0.0180 a	0.0037 bc	2.710 bc
T8	43.33 a	6.540 ab	7.307 abc	0.0213 a	0.0112 a	8.312 a

x T1=20, T2=2, T3=0.2, T4=0.02, T5=0.002, T6=0.0002, T7=0.00002, T8=0 g/L

y Means followed by the same letter within each column are not significantly different at $p<0.05$ as determined by Duncan's Multiple Range Test

Tab. 2. Pearson's correlation coefficients among studied traits in sunflower under different doses of cloth detergent

	Germination percent	Shoot length	Root length	Seedling weight	Vigor based on weight	Vigor based on length
Germination percent	1.000	0.849**	0.673	0.781*	0.779*	0.912**
Shoot length	0.849**	1.000	0.791*	0.887**	0.525	0.633
Root length	0.673	0.791*	1.000	0.637	0.163	0.429
Seedling weight	0.781*	0.887**	0.637	1.000	0.656	0.629
Vigor based on weight	0.779*	0.525	0.163	0.656	1.000	0.917**
Vigor based on length	0.912**	0.633	0.429	0.629	0.917**	1.000

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level

Experiment 2

Plant height and leaf number per plant: High doses of detergent (T1 and T2) reduced plant height and leaf number per plant (Tab. 3). Plant height and leaf number per plant had a positive and significant correlation with most traits (Tab. 4).

Leaf weight and leaf to stem ratio: Contaminated water even under high doses had a lower effect on sunflower leaf weight than other traits (Tab. 3), while traits such as stem weight was severally affected by high doses of detergent. This is a reason for higher leaf to stem ratio of T1. As shown in Tab. 3, high doses (T1 and T2) of contaminated water increased leaf to stem ratio.

Stem weight and total biomass: High doses of detergent (T1 and T2) reduced stem weight and total biomass (Tab. 3). T1 produced lower stem weight compared to T2. Total biomass had a positive and significant correlation with most traits except leaf to stem ratio (Tab. 4). This negative correlation is due to that plant under high doses of contaminated water (T1 and T2) had higher leaf to stem ratio, but produced lower total biomass.

High doses of detergent (T1 and T2) reduced plant height, leaf number per plant, stem weight and total biomass, but increase leaf to stem ratio. Many studies observed reduction in plant height due to environmental stresses (Kang *et al*., 2000). This decrease in plant height may be

Tab. 3. Effect of contaminated water by different doses of detergent powder on sunflower traits

x Treatments	Plant height (cm)	Leaf number per plant	Stem weight (mg/plant)	Leaf weight (mg/plant)	Leaf to stem ratio	Total biomass (mg/plant)
T1	14.8 b y	4.0 b	18.07 c	43.40 bc	2.41 a	61.47 c
T2	16.7 b	4.3 b	30.60 b	34.20 c	1.11 b	64.80 c
T3	26.6 a	6.1 a	58.20 a	47.93 ab	0.82 cd	106.13 ab
T4	26.3 a	6.2 a	61.13 a	50.93 ab	0.84 cd	112.07 a
T5	27.2 a	6.0 a	55.20 a	37.40 c	0.68 d	92.60 b
T6	27.6 a	6.2 a	62.80 a	54.00 a	0.86 c	116.80 a
T7	26.0 a	6.2 a	60.07 a	49.80 ab	0.83 cd	109.87 ab
T8	25.2 a	6.4 a	53.73 a	50.33 ab	0.94 c	104.07 ab

x T1, T2, T3, T4, T5, T6, T7 and T8 are different doses of contaminated water by detergent powder (T1=20, T2=2, T3=0.2, T4=0.02, T5=0.002, T6=0.0002, T7=0.00002 and T8=0 g/L respectively). y Means followed by the same letter within each column are not significantly different at $p<0.05$ as determined by Duncan's Multiple Range Test

Tab. 4. Pearson's correlation coefficients among studied traits in sunflower under different doses of contaminated water by detergent powder

	Plant height	Leaf number per plant	Stem weight	Leaf weight	Leaf to stem ratio	Total biomass
Plant height	1.000	0.970**	0.980**	0.566	-0.834*	0.936**
Leaf number per plant	0.970**	1.000	0.967**	0.653	-0.802*	0.954**
Stem weight	0.980**	0. 967**	1.000	0.612	-0.868**	0.966**
Leaf weight	0.566	0.653	0.612	1.000	-0.196	0.796*
Leaf to stem ratio	-0.834*	-0.802*	-0.868**	-0.196	1.000	-0.729*
Total biomass	0.936**	0.954**	0.966**	0.796*	-0.729*	1.000

*.Correlation is significant at the 0.05 level. **.Correlation is significant at the 0.01 level

attributed to intelligent response of plant to prevent shoot transpiration (Karam *et al*., 2003), reduction of cell size and internodes length and accumulation of abscisic acid (Sharp, 1996).

These data show that by increasing detergent dose, the leaf became thicker. Pace and Benicasa (2010) reported similar results. Save *et al.* (1993) reported that water stress resulted in decreasing cell size and increasing solute concentration so specific leaf weight increases under high level of detergent stress. Elevated salinity reduced water uptake by plant, thereby inhibits root elongation (Rahimi *et al*., 2006). Presence of elements such as Na in contaminated water can inhibit activities of some enzymes and result in unbalanced nutrient uptake by the seedlings (Al-Taisan, 2010). Ultimately, regarding total biomass as the main growth parameter, lower biomass accumulation under higher doses of contaminated water by detergent powder can be attributed to three stresses; salinity stress, water stress and heavy metal stress (Jadia and Fulekar, 2008; Sharifzadeh *et al*., 2006).

Conclusions

Cloth detergent powder in high concentration (20 and 2 g/L) reduced seed germination, seedling vigor, plant height, leaf number per plant, total biomass and stem weight in sunflower probably by means of high osmotic potential, oxidative stress, salinity stress and heavy metal stress. Germination stage was more sensitive to detergent than the next stage (early growth stage). However in lower doses (<2 g/L), the adverse effects were not observed, study of sunflower oil content under contaminated water can be useful at the next studies.

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