ANTIOXIDANT ACTIVITY AND SECONDARY METABOLITES IN SELECTED VEGETABLES IRRIGATED WITH SEWAGE WATER

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Abstract. The study was conducted to investigate the impact of various concentration of domestic waste water on growth, physiological, and biochemical characteristics of tomato, okra and pumpkin. Sewage water was applied of 50% and 100% on vegetables grown under field conditions. Irrigation with tubewell water was considered as control. At maturity data for growth attributes was recorded. Maximum fresh weight of root, shoot and fruit was in tomato at 50% sewage water treatment followed by control. Dry weight also exhibited a considerable increase at 50% sewage water application. However, a decline in chlorophyll a, b and carotenoid contents was observed at 100% sewage water. Antioxidants and secondary metabolites increased and their maximum values were recorded at 100% sewage treatment in all the three vegetables.
Keywords: *domestic effluents, contamination, flavonoid, growth, MDA*

Introduction

Shortage of fresh water resources is a serious issue, due to which resource poor countries are using marginal quality water for irrigation purposes. Waste water may contain some nutrients useful for agriculture, however, its continuous application increases toxic metals in plants and soil (Rattan et al., 2005). Sewage water also contains industrial wastes and toxic metals, polluting soil and food chain (Khan et al., 2008). In Pakistan inadequacy of irrigation water comes up with the integrative use of ground water and industrial and sewage effluents for agriculture (Khan et al., 2013). Major cities of Pakistan produce sewage 116,590 million gallons/day irrigating 32,000 ha of land (FAO, 2002; Musa et al., 2013).

Tomato (*Solanum lycopersicum* L.), okra (*Abelmoschus esculentus* (L.) Moench) and pumpkin (*Cucurbita pepo* L.) are a vital part of human diet, because they are important

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source of nutrients like proteins, fiber, vitamins, iron and calcium (Ullah et al., 2009). Waste-water irrigation results in elevated metal uptake by most of the vegetables grown particularly in peri-urban areas thus metals become a vital part of food chain (Farooq et al., 2008). Waste water irrigation may cause growth stage dependent sensitivity in vegetables (Baksh, 2005). It has been estimated that approximately 1/10th of the global population is considered to eat food from plants irrigated with wastewater (Kouser and Samie, 2009).

Consequently, it is imperative to look deep into the prospects of sewage water irrigation for managing this nutrient rich resource. On the other hand, unveiling specific relationship between fertigation with waste-water and a crop is also important for appropriate application (Kumar et al., 2010). Therefore, the present study was executed to evaluate the role of waste-water fertigation for vegetable growth, development and sustainable production. Additionally, incorporation of the present knowledge into fertigation systems will likely to be a promising strategy for optimizing crop productivity in irrigation scarce areas.

Materials and methods

A field experiment was performed in Pakpattan district, Punjab, Pakistan to evaluate the effect of domestic effluents on morpho-physiological and biochemical attributes in tomato (*Solanum lycopersicum* L.), okra (*Abelmoschus esculentus* (L.) Moench) and pumpkin (*Cucurbita pepo* L.).

Field preparation

The field was prepared by dividing the main plot into three sub plots of 3x3 m^2 and digging the soil upto 1 foot. The three sub plots were separated by polythene sheet, filled with clay and sand in 1:1 ratio. The experimental design was split plot. The treatments applied were: control (tube well water), 50% (sewage water blended with tube well water) and 100 % sewage water.

Seeds were collected from AARI (Ayub Agricultural Research Institute) Faisalabad, Pakistan and sown in the appropriate growing season.

Water analysis

Physico-chemical analysis of waste water (*Table 1*) indicated that the EC values recorded were higher than the suitable limit for most of the crops (EPA, 1991). The pH was slightly alkaline (United State Salinity Laboratory staff, 1954). Analysis of waste water indicated the presence of toxic metals were more than the permissible limits. Cadmium was much higher than the recommended value. Similarly Cu, Pb, and Zn were in higher concentrations.

Table 1. *Physico-chemical characteristics of waste-water used for irrigation*

Parameters	Unit	Waste water	Recommended values
pH		7.3	6.0- 8.5
EC	$mScm^{-1}$	3.73	3
Carbonate	$meqL^{-1}$	2.4	
Bicarbonate	$meqL^{-1}$	5.1	
Heavy metals/ Ions	$mgkg^{-1}$	-	

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Zn	-	3.145	≤ 2.0
P	-	13.78	
Cd	-	0.625	< 0.01
K	-	115	
Fe	-	0.519	≤ 5.0
Pb	-	7.432	< 5.0
Cu	-	1.11	< 0.2

Physico-chemical characteristics of the soil

The pH of soil was 7.3, slightly alkaline and was in safe limit with reference to standard limit of 8.5 (*Table 2*). EC value of soil was 2.1 mS/cm, which was in the safe limit according to the standard permissible limit (Ilaco, 1985; MAAF, 1988; CCME, 2007; WHO, 2007). In soil, heavy metals were also in the safe limit.

Table 2. *Physico-chemical properties of the soil*

Parameters	Unit	Soil	Safe limits
Texture		Sandy loam	
pH		7.3	≤ 8.5
EC	$mScm^{-1}$	1.9	2-4
Heavy metals/ Ions	$mgKg^{-1}$	-	
Zn	-	3.01	300
K	-	1400	-
Fe	-	2.35	-
P	-	40.1	300
Cd	-	0.21	3
Cu	-	17	140
P	-	41.1	

Fresh and dry weight

The fresh weight and dry weight was measured in grams for shoot, root and fruit by using electrical balance. For dry weight samples were kept in an oven at 70 ºC for 72 hours.

Pigment analysis

The fresh leaves (0.5 g) were homogenized in chilled acetone (80%). Centrifuge (3000 rpm/10 mints) at 4 ºC. Separate the supernatant and measure absorbance at 663,645 and 480 nm respectively with spectrophotometer. Chlorophyll contents were determined by the method of Arnon (1949) and carotenoid contents were calculated as described by Kirk and Allen (1965).

Antioxidant enzymes

Fresh leaves (0.5 g) of tomato, okra and pumpkin were ground in 8 ml of 50 m*M* phosphate buffer pH (7.8). The homogenate was centrifuged at 15000 x g for 20 min at 4 ºC. The supernatant was used for the assay of enzymes activity.

Catalase (CAT)

The activity was assayed in a 3 ml reaction solution containing phosphate buffer (50 m*M* ,7.0 pH), H_2O_2 (5.9 m*M*) and 0.1 ml of enzyme extract as described by Chance and Maehly (1955). A decline in activity of catalase enzyme due to H_2O_2 consumption was measured at 240nm absorbance after every 20 sec. A one unit catalase activity defined as a change in absorbance of 0.01 unit min^{-1}.

Peroxidase (POD)

The activity was determined as the peroxidation of H_2O_2 and guaiacol as an electron donor (Chance and Maehly, 1955). Add phosphate buffer (50 m*M*, pH 5), 20 m*M* of guaiacol, H_2O_2 (40 m*M*) and 0.1 mL enzyme extract in a reaction solution. Formation of tetra-guaiacol resulted in an increase in the absorbance at 470 nm measured after every 20 sec. One enzyme unit was the amount responsible for an increase in OD value of 0.01/ 1 min. The activity was expressed as unit min^{-1} g^{-1} fresh weight basis.

Superoxide dismutase (SOD)

It was determined by the method of Giannopolitis and Ries (1977) by measuring the inhibition rate of nitroblue-tetrazolium (NBT) reduction by xanthine oxidase acting as hydrogen peroxide generating agent. The absorbance was measured at 560 nm by using a UV-visible (IRMECO U2020) spectrophotometer. One unit activity reflected 50% photochemical inhibition of NBT.

Ascorbate peroxidase (APX)

The activity was monitored by a decrease in ascorbic acid absorbance at 290 nm (extinction coefficient 2.8 mM cm-1) in 1 ml reaction mixture containing phosphate buffer (50 m*M*, pH 7.6), 0.1 m*M* Na-EDTA, 12 m*M* H_2O_2, 0.25 mM ascorbic acid as described by Cakmak, (1994).

Malondialdehyde (MDA)

The estimation was done by using the method of Camak and Horst (1991). Ground fresh leaves (1g) in 20ml tri-chloroacetic acid (0.1%) and centrifuge at 12000g for 10 min. Take 1 ml of the supernatant and added 4 ml of 20 % TCA comprising 0.5% thiobarbituric acid and then it was heated for 30 min., at 95°C in a water bath and then immediately cooled on ice. After centrifuge for 10 min., at 12000 g, the absorbance of the supernatant was read at 532 and 600 nm. The contents of MDA were calculated using extinction coefficient of 155/ (mM/cm) with the help of formula:

$$\text{MDA level (nmol)} = \Delta\,(\text{A 532nm} - \text{A 600nm})/1.56\times10^{5} \qquad \text{(Eq.1)}$$

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Total leaf phenolics content

To the 5 ml Folin-Ciocalteu reagent (formerly diluted with water 1:10 v/v) along with 4 ml (75 g/L) of Na_2CO_3, added the plant extract was added. The tubes were vortexed for 15 sec and allowed them to stay for 30 min at 40 °C for developing color. The absorbance read at 765 nm using Folin-Ciocalteu method (Wolfe et al., 2003). The absorbance was expressed as mg g^{-1} tannic acid and the amount worked out by using the equation based on the following calibration curve:

$y = 0.1216x$, $r^2 = 0.9365$, x was the absorbance, y the tannic acid equivalent (mg/g)

Total flavonoid contents

The contents were determined with the help of spectrophotometer by using the method of Park et al. (2008). Total flavonoid contents were expressed as mg of rutin equivalents per gram of dried fraction.

Statistical analysis

The results were statistically analyzed by using the software program Statistix 8.1 at $P \leq 0.05$. Means and standard errors were assessed on Microsoft Excel -2007 Version and the significance of means was tested at 5% probability level using Least Significant Difference test.

Results

Sewage water applied in 100% concentration reduced plant fresh weight in tomato (*Solanum lycopersicum* L.), okra (*Abelmoschus esculentus* (L.) Moench) and pumpkin (*Cucurbita pepo* L.) (*Fig. 1*). Application of 50% concentration of sewage water resulted in an increase in fresh weight in shoot of okra and pumpkin by 36% and 16%. In tomato 50 and 100% sewage reduced shoot fresh weight from control (773 g) to 630 and 352 g respectively. However, 50 % domestic sewage water increased fresh weight in tomato, okra and pumpkin (*Fig. 1*). Irrigation with 100% sewage water decreased root fresh weight by 4%, 8% and 43% in pumpkin, tomato and okra, respectively. Fruit fresh weight decreased in the order as 50% sewage water >control >100% sewage water.

Polluted water significantly affected shoot dry weight of all vegetables (*Table 3.*). A decrease in dry weight at 100% concentration of sewage water was observed in tomato, okra and pumpkin by 30%, 20% and 13%, respectively. At 100% sewage water dry weight of fruit was 99 g in tomato and 42g in okra. Different concentrations of sewage water also affected root dry weight (*Fig. 1*). An increase of 20, 28 and 60% in root dry weight was observed in tomato, okra and pumpkin, respectively as compared to that in control. In general tomato plants had the highest root dry weight 25g at 50% sewage water and pumpkin roots had minimum 5g at 100% sewage water. In okra and pumpkin, dry weight of fruit increased at 50% concentration of sewage water over control (*Fig. 1*). Dry weight of fruit in tomato, okra and pumpkin decreased by 38, 17 and 32% respectively at 100% sewage water application. Overall, maximum dry weight of fruit was in pumpkin (6 g) at 50 % sewage water application.

A decline in chlorophyll contents was observed at 50 and 100 % application of domestic sewage water in all the vegetables (*Fig. 1*). Reduction in tomato, okra and

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pumpkin at 50% sewage water was 12%, 30% and 32% respectively and at 100% sewage water it was 40%, 56% and 21% as compared to control.

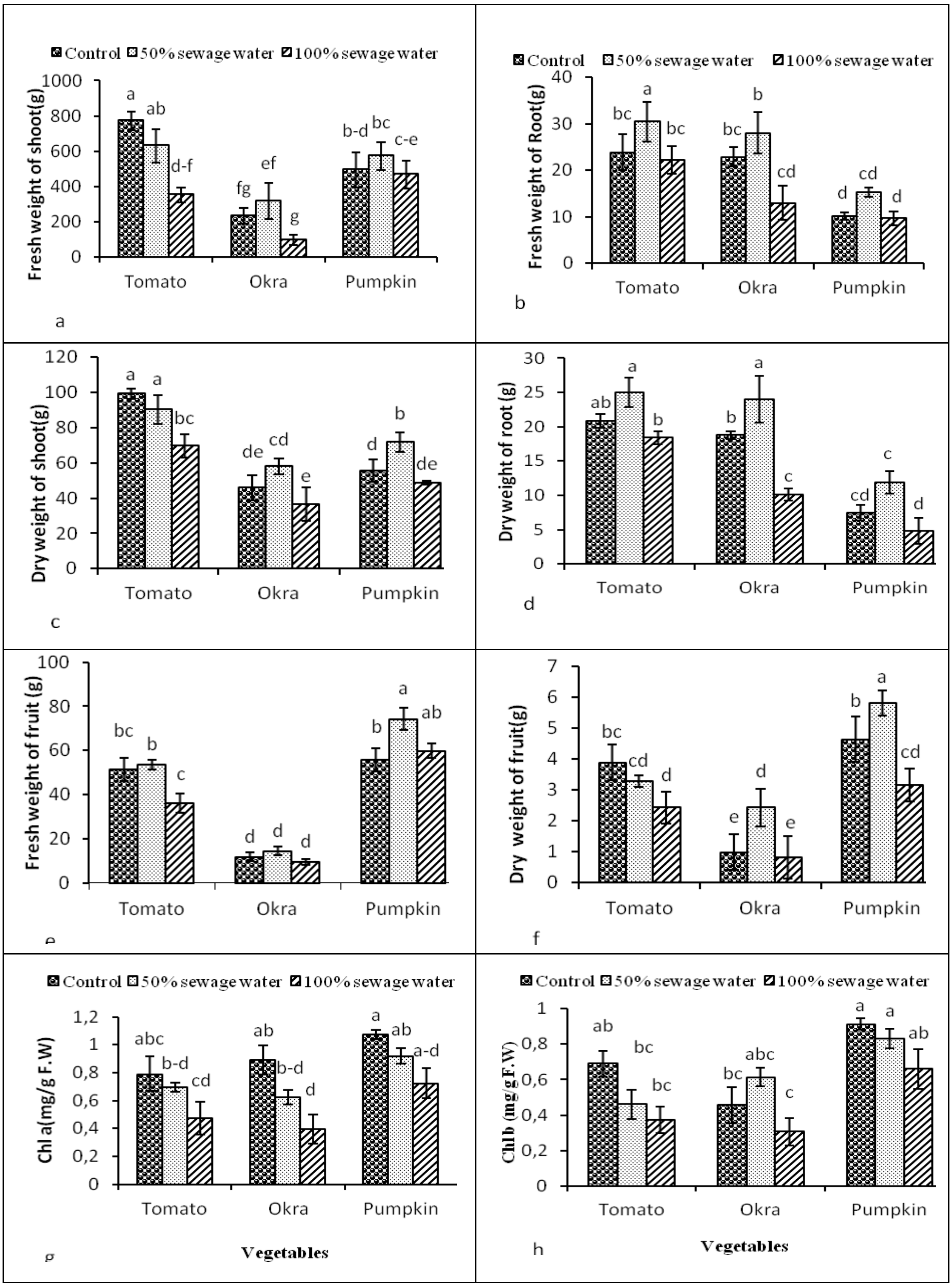

***Figure1.** Effect of domestic sewage water on morpho-physiological attributes of tomato, okra and pumpkin. Bars with different letters in each group show significant difference at $p < 0.05$*

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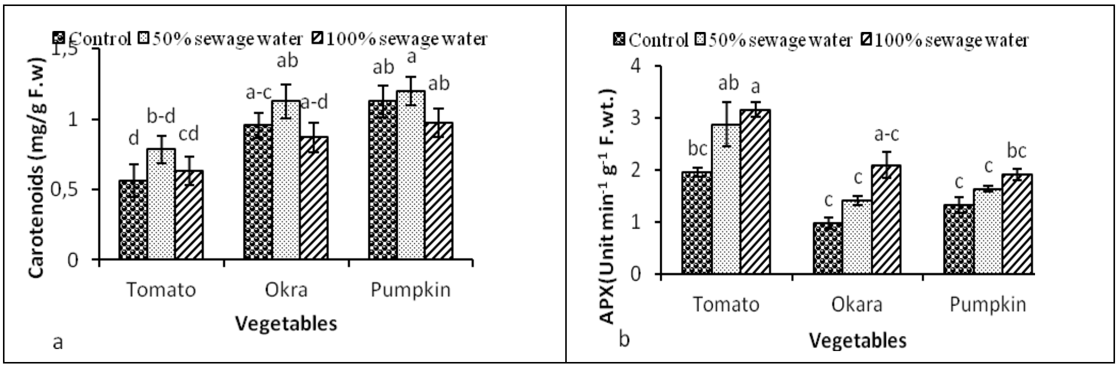
Reduction in chlorophyll *b* contents was 0.6 to 0.4 and 0.9 to 0.8 mg/g respectively at 50% waste-water treatment in tomato and pumpkin (*Fig. 1*). However, an increase in chlorophyll *b* contents from 0.4 to 0.6 mg/g was observed in okra at 50% waste-water treatment. At 100% irrigation of polluted water reduction in chlorophyll *b* was 0.6 to 0.37, 0.4 to 0.31 and 0.9 to 0.66 mgg^{-1} respectively in tomato, okra and pumpkin. An increase in the carotenoid contents at 50 and 100 % concentration of domestic sewage water was observed in all three vegetables. At 50% irrigation of polluted water, the carotenoid contents increased from 0.5 to 0.7, 0.9 to 1.1 and 1.12 to 1.2 mg ml^{-1} in tomato, okra and pumpkin, respectively.

Sewage water irrigation with 50 and 100% increased the ascorbate peroxidase activity in all three vegetables. Generally the APX activity at 100% sewage water in tomato was 3.15 (min^{-1} g^{-1} F. wt.) and 1.908 (min^{-1} g^{-1} F. wt.) in okra. A significant increase in the SOD activity 247 to 276 (min^{-1} g^{-1} F. wt.) was observed in tomato with irrigation of sewage water. At 100% sewage water the SOD activity was the highest in tomato 276(min^{-1} g^{-1} F. wt.) and the least in okra 176 (min^{-1} g^{-1} F. wt.).

Peroxidase activity increased with the irrigation of different concentrations of sewage water in all the vegetables (*Fig. 2*). Sewage water at 50% irrigation increased the POD activity in pumpkin, tomato, okra and from 57 to 68, 91 to 108 and 52 to 75 (min-1 g-1 F. wt.) respectively. The highest POD activity at 50% irrigation with domestic sewage water was 108 (min-1 g-1 F. wt.) in tomato and the least was in okra 68 (min-1 g-1 F. wt.). Irrigation with 50% domestic sewage water increased the catalase activity from182 to 199 and 69 to 101 (min^{-1} g^{-1} F. wt.) in tomato and pumpkin, respectively. An increase in MDA content was observed in pumpkin 43% and 49%, tomato 69%, 117% and okra 76%, 111% over control under 50% and 100%. The order of increase in flavonoid contents was 100% sewage water > 50 % sewage water >control. Irrigation of 50% and 100% sewage water increased the phenolic content in okra 27%, 33%, tomato 56%, 91% and pumpkin 46% and 96 % over control (*Fig. 2*). Overall, the highest concentration of phenolics was found in tomato followed by okra and pumpkin.



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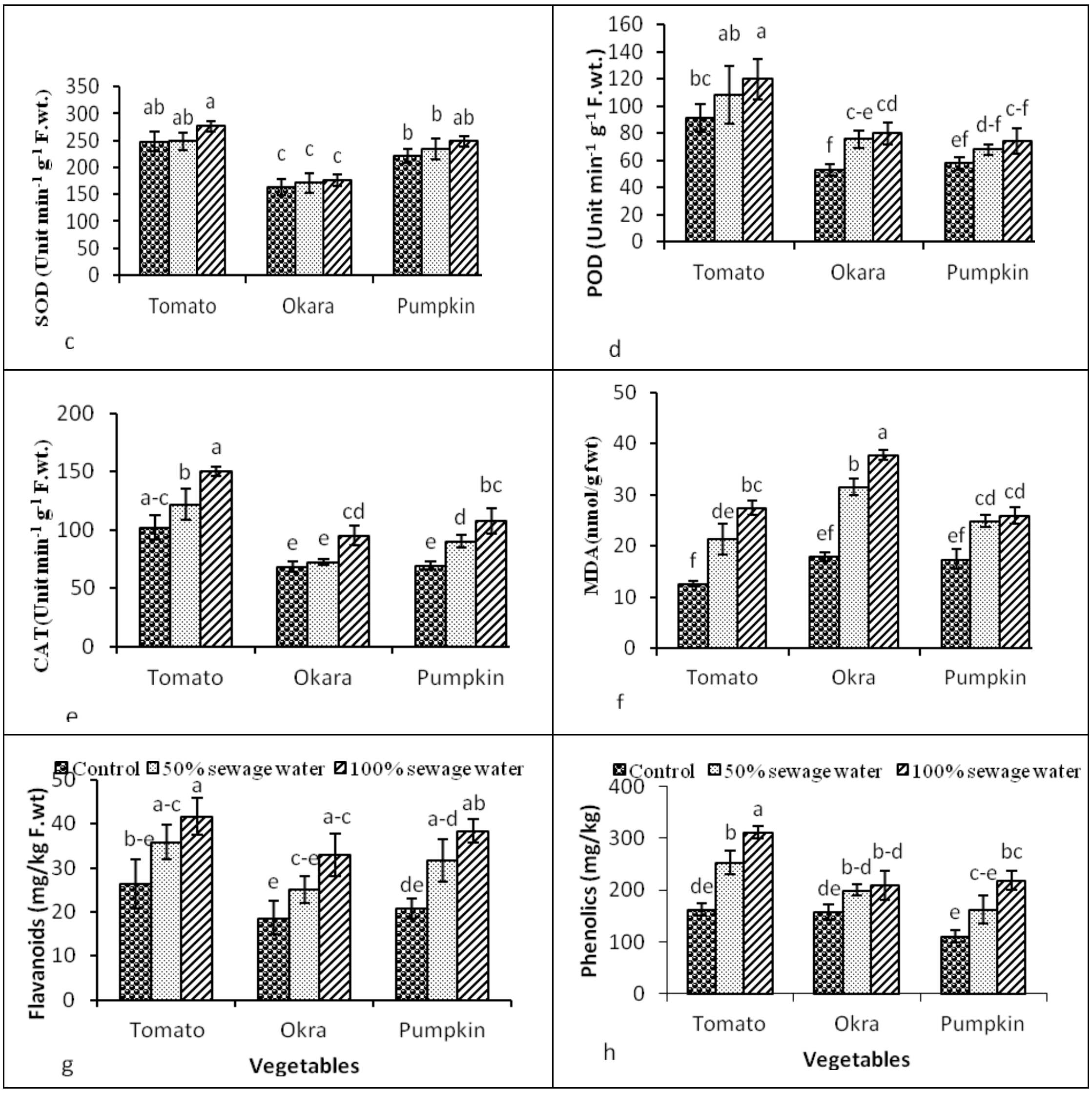

Figure 2. *Effect of domestic sewage water irrigation on antioxidant activities of tomato, okra and pumpkin irrigated with sewage water. Bars with different letters in each group show significant difference at $p < 0.05$.*

Discussion

Application of 100 % sewage water reduced the fresh and dry weights of tomato (*Solanum lycopersicum* L.), okra (*Abelmoschus esculentus* (L.) Moench) and pumpkin (*Cucurbita pepo* L.). Similar, decrease in biomass was observed with the application of 20 and 30 % polluted water in tomato plants (Saeed and Ahmed, 2009). A decrease in fresh biomass of shoot under sewage application was also investigated in wheat (Kakar et al., 2010). Irrigating *Leucaena leucocephala* with polluted water reduced growth and plant height (Hassan and Ali, 2013). Toxic metals may accumulate in the foliage parts of the plant, disturbing the physiological and biochemical activities in plant that ultimately reducing the growth (Sing and Agrwal, 2007). Physico-chemical analysis of waste water (*Table 2*) indicated that the EC value may cause increase in the soil salinity which may cause reduction of plant growth (Iqbal et al., 2013).

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A reduction in chlorophyll contents was recorded in tomato, okra and pumpkin. Chlorophyll *b* exhibited more decline than chlorophyll *a*. Similar, reductions in chlorophyll contents were exhibited by *Beta vulgaris* subjected to sewage water irrigation (Sing and Agrawal, 2010). According to Marwari and Khan (2012) irrigation of tomato plants with 20 and 30 % sewage water badly affected the chlorophyll contents, indicating that the decrease may be due to reduction of chlorophyll biosynthesis under stress (Bamniya et al., 2010).

Carotenoid contents increased in all vegetables due to sewage water irrigation and this increase was more pronounced at 100 % level of domestic sewage water irrigation. Increase of carotenoid contents is considered, a defense role in plants to alleviate metal stress (Sinha et al., 2007).

The antioxidants like SOD, APX, CAT and POD also showed an increment in activity (*Fig. 2*), responsible for scavenging of reactive oxygen species (ROS) (*Table 4*) (Noctor and Foyer, 1998). They play a defensive role against oxidative stress and indicators of metal uptake (Radotic et al., 2000). Similar results are observed in palak (*Beta vulgaris* var All Green) irrigated with different concentrations of sewage water (Singh and Agrawal, 2007). According to Ashraf (2009) antioxidants detoxify H_2O_2 (ROS) into H_2O (water) into O_2 (Sairam *et al*., 2005) and relieve the oxidative damage in plants caused by ROS. Total phenolics and flavonoids behave like antioxidants and also act in H_2O_2- scavenging system. Under multiple stresses, phenolic metabolism induction is observed in plants (Michalak, 2006). Flavonoids play an important role in plant-environment interactions under low concentrations (Fini et al., 2011). An increase in phenolic compounds was observed in *Albizia lebbek* under heavy metal stress (Tripathi and Tripathi, 1999). In the present work irrigation with waste-water increased phenolic contents and flavonoids with maximum values recorded at 100% concentration of sewage water (*Fig. 2*). Similarly, nickel and aluminum contents resulted increase in phenolic contents in wheat (Diáz et al., 2001) and maize (Winkel-Shirley, 2002). In biological systems, the presence of malondialdehyde as oxidation products is directly related to the peroxidation of unsaturated fatty acids constituting cellular membranes (Turton et al., 1997). Plants irrigated with waste-water exhibited higher MDA concentration, as compared to those irrigated with fresh water. Heavy metals cause peroxidation of lipid membranes due to the formation of ROS and free radicals, leading to enhanced permeability and oxidative stress to the plants (Nada et al., 2007; Zhang et al., 2007).

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Table 3. *Mean squares values from analysis of variance (ANOVA) of data for morpho-physiological attributes of tomato, okra and pumpkin treated with sewage water*

SOV	df	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Fruit fresh weight	Fruit dry weight	Chla	Chlb
Variety (V)	2	571588.54****	1160.066***	6207.34***	711.846***	10425.6***	36.933***	0.342*	0.512**
Treatment (T)	2	196299.68***	1087.189***	1869.59***	315.384***	573.6**	11.044**	0.564**	0.238*
Interaction (VxT)	4	54865.411*	345.412**	277.57*	24.379ns	189.3ns	2.199**	0.0139ns	0.048ns
Error	36	15577.445	75.983	110.71	12.515	163.2	0.595	0.086	0.0622
Total	44								

*, **, ***= Significant at 0.05, 0.01, 0.001 levels, ns= non-significant

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Table 4. *Mean squares values from analysis of variance (ANOVA) of data for antioxidants of tomato, okra and pumpkin treated with waste water.*

SOV	df	Carotenoid contents	APX	SOD	POD	CAT	MDA	Flavanoids	Phenolics
Variety (V)	2	0.781***	3.701***	3.701***	1354.182**	3228.694***	160.267***	185.258*	14129.317***
Treatment (T)	2	0.177ns	2.119***	2.119***	4466.678***	5272.703***	502.708***	559.065***	23921.799***
VxT	4	0.017ns	0.133ns	0.239ns	48.058ns	122.394ns	30.536*	4.584	1871.005ns
Error	36	0.071	0.174	0.879	160.809	75.297	7.800	49.047	1061.001
Total	44								

*, **, ***= Significant at 0.05, 0.01, 0.001 levels, ns= non-significant

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Conclusion

Irrigation with waste-water is a common technique for cultivation of crops including vegetables in third world countries. It enhanced the biomass of tomato, okra and pumpkin on 50% dilution. However, its use as a nutrient source depends on the type of a crop grown, fertility level of soil and nutrients concentration. Waste water should be diluted with fresh water in areas having shortage of irrigation water. But still it should be checked for its heavy metal contents to avoid risk on human and animal lives.

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