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Effect of wastewater irrigation on anatomical structure of faba bean plant (*Vicia faba* L.)

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Abstract

Two pot experiments were carried out at the Experimental Farm of the Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University, Assiut governorate, Egypt, during two successive seasons 2019/2020 and 2020/2021. To study the effect of wastewater concentrations compared with the control on anatomical structure of faba bean variety's (Giza 716 and Nubaria 3). The experiments were arranged in random whole sectors in which two levels of wastewater were diluted with Nile water at (50 and 100% ww) in addition to control. Data recorded that anatomical characteristic *i.e*. (Ø of root, cortex thickness, Ø of V.C, Ø pith, Ø of meta xylem) geared the highest in Giza 716 var. values compared with Nubaria 3. Added to all characteristic as affected the concentration of wastewater irrigation used. The highest values were cleared with Ø of root and thickness of cortex while Ø of V. C was the lowest in both varieties especially with control plant (Giza 716) at the same Time the anatomical characters of Nubaria 3 var. were better by using 50% ww irrigation compared with the control plants. Data recorded that the anatomical characteristics of the stems were affected by all wastewater concentrations, 100% ww concentration recorded that decrease in Ø of stem, epidermal thickness, and cortex for both cultivars. While the 50% ww concentration showed superiority in the thickness of the cortex, especially with the Giza 716 var., compared to the control. At the same there was a significant decrease in the number of V.B, the length of the xylem arch and the Ø of the pith in both cultivars compared to the control. Data recorded that the anatomical characteristics of the leaves were affected by all the concentrations of wastewater used, where the concentration of 100% ww showing decrease in the thickness of the upper and lower epidermis and the mesophyll tissue (palisade and spongy tissue), as well as the diameter of the midrib and length of xylem arch in both types compared to the control. Generally, the Data obtained in this study indicated that uses of wastewater irrigating improved the anatomical characters faba bean plant, especially with Nobaria 3 var., can be considered as resistance wastewater irrigation under the Assiut conditions, Egypt.

Keywords: faba bean, wastewater, anatomical structure.

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1. Introduction

The faba bean is an important temperate zone grain legume and is used for food and feed worldwide. For food, it is used more commonly in Asia, and Africa .in Europe it is mainly used as animal feed Beside it considers the source for human and animals' food, faba bean splay important role in crop rotation and soil improvement since it can fix a relatively large amount of nitrogen (60-250 kg/ha). According to statics in 2010, the biggest producer of faba beans was China with 1.4 million tons (Mt) followed by Ethiopia (0.6 Mt) and France (0.48 Mt). Total world production of faba bean in 2010 was about 4 Mt from about 2.5 million hectares with an average yield of about 1.6 ton/ha (Faostat, 2012). The use of municipal wastewater in agriculture is a centuries-old practice that is receiving renewed attention with the increasing scarcity of freshwater resources in many arid and semi-arid regions of the world. The use of wastewater for irrigation is a reality, not a matter of choice, not only in arid and semi-arid regions, but also in more humid areas where seasonal water shortage occurs (Huibers and van Lier, 2005). Even in areas where wastewater is not the sole water source for agricultural irrigation, farmers still prefer using sewage for irrigation by reason of its crop nutrient value, which reduces expenditure on chemical fertilizers (Lazarova and Asano, 2005). The high concentrations of salts may adversely affect physiological processes and growth of wastewater-irrigated plants, especially salt-sensitive crops (Bañón *et al*., 2011). The net effect on growth may be a reduction in crop yields and potential loss of income to farmers. Irrigation water pollution affects the overall shape of the plant where the root morphology appears at 0% to 100% concentration of effluent. Root branching was more intense at higher waste concentration. In the control condition, the root color was lighter; the surface was smoother, while at higher concentrations of litter, the roots appeared darker and had a rougher skin texture. Vidya and Mariani (2020) showed that all sewage water treatment showed decrease root anatomical traits such as root diameter, central cylinder diameter, and cortex thickness, whether these are parenchymatous tissues in the root cortex and/or xylem vessels, thereby resulting in shrinkage of root diameter. It is clear from same data that application of sewage water at 100% gave the highest reduction in thickness of cortex tissue, and diameter of vascular cylinder (El-Okkiah, 2015). This research aims to study the effect of irrigation rates with different sewage water on anatomical structure of (Giza716 and Nubaria3) cultivars of *Vicia faba*.

2. Materials and methods

Two pot experiments were carried out at the experimental farm of the Department of Agricultural Botany, Faculty of Agriculture, Al-Azhar University, Assiut governorate, Egypt, during two seasons of 2019/2020 and 2020/2021 to study the effect of wastewater treated at (25, 50, 75 and 100% TWW) compared with the control treatment on anatomical structure of (Giza716 and Nubaria3) cultivars of (*Vicia faba* L.) of the bean plant at 45

DAS from sowing. Seeds of bean (*Vicia faba* cv. Giza 716 and Nubaria 3) were obtained from the Food Legumes Research Department of Sakha Agricultural Research Station (SARS), North Delta, Egypt. Faba bean seeds were sowed on 26th October for two seasons in pots of 30 cm in diameter which filled with 6 kg with clay, then seeds were sowed (2-3 seeds/pot). After emergences of seedlings, were thinned to three plants per pot, this experiment included 5 treatments, each treatment was about 3 replications (4 pots for each replicate). The pots were arranged in a randomized completely blocks design (CRBD). Cultivation and all cultural practices except irrigation *i.e.*, weeding, fertilization and pest control and so on were performed according to the recommendations as usual. The plants were irrigated with concentrations at (25, 50, 75 and 100% ww) of wastewater over the course of the growth period. The experiment was arranged in a randomized completely blocks design arrangement as follow:

1. Irrigation with freshwater (control) Nile water (100).
2. Irrigation with a mixture of 75% Nile water and 25% wastewater treated (3:1).
3. Irrigation with a mixture of 50% Nile water and 50% wastewater treated (1:1).
4. Irrigation with a mixture of 25% Nile water and 75% wastewater treated (1:3).
5. Irrigation with undiluted wastewater treated (100% TWW).

2.1 Sampling and collecting data

Anatomical characters and it was as follows specimens of faba bean root, stem and terminal leaflet for all treatments were collected. The root samples were taken 0.5 cm from the root tip and the 5th apical internode of the main stem and its corresponding leaf of treated plants and those of the control at 45 DAS during the second season only (2020/2021). All samples were killed and fixed for 48 h in FAA (10 ml formalin; 5 ml glacial acetic acid; 50 ml ethyl alcohol and 35 ml water). The dehydrated samples were infiltrated and embedded in paraffin (50-52°C m. p.). The Embedded samples were sectioned on a rotary microtome at a thickness of 5-7 µm. Sections were mounted on slides and deparaffinized. Staining was accomplished with safranine and light green, cleared in xylene and mounted in Canada balsam (Geriach, 1977). Slides were microscopically examined, measurements and counts were taken and averages of 9 readings of 3 slides were calculated. The sections were computerized morphometrical analysis, the morphometrical analysis was done by Research Microscope type Axiostar plus made by Zeiss transmitted light bright field examinations upgrade able to professional digital image analysis system (Carl Zeiss Axiovision Product Suite DVD 30).

2.1.1 Transverse section of the root of Vicia faba

1. Root diameter (Ø of root) (µ).
2. Epidermis thickness (µ).
3. Cortex thickness (µ).

4. Diameter Vascular Cylinder (Ø of V. C) (µ).
5. Diameter of pith (Ø of Pith) (µ).
6. Diameter of Meta xylem vessels (Ø of M X (µ)

2.1.2 Transverse section of the stems of Vicia faba

1. Stem diameter (Ø= 1/2 diameter stem) (µ).
2. Epidermis thickness (µ).
3. Cortex thickness (µ).
4. Length of vascular bundle (µ).
5. Diameter of pith (µ).
6. Number of vascular bundle (Number of V. B) (µ).

2.1.3 Transverse section of leaflets of Vicia faba

1. Upper epidermis thickness (µ).
2. Lower epidermis thickness (µ).
3. Spongy tissue thickness (µ).
4. Palisade tissue thickness (µ).
5. Length of xylem arch (µm).
6. Thickness of leaf midrib (µ).

2.1.4 Statistical analysis of the data

Data were subjected to statistical analysis using the two-way analysis of variance (ANOVA) [Costat statistical program, 1990] and separated by the least significant difference (LSD) test and the probability level $P < 0.05$ was considered statistically significant by Duncan (1955).

3. Results and Discussion

3.1 Anatomical structure of faba bean

3.1.1 Roots

The data in Table (1) and Figure (1) showed that the root was affected by all concentrations of wastewater irrigation (50% and 100%), The concentration wastewater (100%) was more effective as It showed in increase Ø of root and increase in cortex thicken in both cultivars, decrease in vascular cylinder Ø in both cultivars and the best concentration was in Giza control cultivar, while in (Nubaria 3) cultivar the concentration was (50%) better compared to control, increase of Ø The pith in both cultivars, a decrease in the Ø of the meta xylem vessel in both cultivars compared to the control. Anatomical characteristics measurements of faba bean root were affected by all concentration of sewage water irrigation, sewage water at 100% gave the lowest values of diameter thickness of cortex tissue, diameter of xylem vessel, and these decreases were significant compared with the control in faba bean (El-Okkiah, 2015). Anatomical characteristics in root and stem were decreased in Giza957 compared with Reina mora under irrigation with dilued sea water at 1:5 A slight increase in diameter of stem and root were observed the irrigation dilued sea water at 1-7 in both varieties in faba bean (El-Emary, 2010). A low Tr was accompanied by a low conductive rate in the narrow vessels due to salt stress. Similarly, the reduction in xylem vessels of roots and leaves of both cultivars was followed by a significant reduction in Tr, which can be explained by the high salinity of the wastewater in lucerne (*Medicago sativa*) (Nja *et al*. 2018). The high concentration of Cd (*i.e*., 250µM) for just 24 h of treatment results in mitosis disorder in the roots in pea (Lee *et al*., 2010). Increasing addition of Cd in soils enhanced Cd concentration in plants, resulting in decreased plant growth and cell ultra-structural changes in both roots and leaves (Bola *et al*., 2003). The vascular system This may be due to a decrease in the elasticity of cell walls of the root Heavy metal-induced reduction in the cell size includes all root tissue, whether these are parenchymatous tissues in the root cortex and/or xylem vessels, thereby resulting in a shrinkage of root diameter in *sorghum bicolor* (L.) (Kasim, 2006). A significant decrease in xylem vessels, in particular, meta xylem vessels may significantly limit the movement of water and mineral nutrients from root to aerial parts of the plant. A number of studies reported that reduction in root growth may be due to a decrease in cell division that led to increase the thickness of cell wall, and or a disorder in the activity and contents of phytohormones like auxin in the roots exposed to heavy metals (Schilcher *et al*. 2005; Sharma and Dietz, 2006; Stohs *et al*. 2000). Also reported a reduction as a result of heavy metal stress in root anatomical traits such as root diameter, central cylinder diameter, and cortex thickness, cross section area of root and cross section area of central cylinder in maize (*Zea mays* L.). Gowayed and Almaghrabi (2013) showed that the cortex thickness difference between treatments is not significant, but the appearance of the cortex looked different. The greater the concentration of waste the more damaged the cells that form the root cortex. Cortex cells in control plants are polygonal, meanwhile in treated plants (75-100%) the shape of the cells is elongated or tend to be irregularly arranged in marigold (*Tagetes erecta* L.) (Vidya and Maryani, 2020).

3.1.2 Stem

The data in Table (2) and Figure (2) showed that the stem was affected by all concentrations of wastewater irrigation (50% and 100%), The concentration waste water (100%) was more effective as it showed in a decrease in all studied traits in the stem, Ø of stem, epidermal thicken, cortex thicken, length of xylem arch, Ø of pith and number of V. b, as a result of irrigation with high concentrations of wastewater compared to the control. A number of studies reported that the decrease in stem elongation in the presence of cadmium is due to a decrease in photosynthesis.

Table (1): Root anatomical structure of the faba bean plant (Giza 716 and Nubaria 3) was affected by irrigation with concentrations of wastewater after 45 days (2020/2021) season.

Treatment	Ø of root (mm)		Ø of V.C (mm)		Cortex thickness (mm)		Ø of pith (mm)		Ø of xylem big vessels (mm)	
	Giza 716	Nubaria 3	Giza 716	Nubaria 3	Giza 716	Nubaria 3	Giza 716	Nubaria 3	Giza 716	Nubaria 3
Control	3.235	3.798	0.592	0.566	0.476	0.694	0.459	0.580	0.108	0.102
50%	2.949	3.467	0.333	0.830	0.580	0.828	0.478	0.302	0.052	0.062
100%	3.525	4.215	0.246	0.560	0.761	1.083	0.480	0.594	0.030	0.048
LSD	0.168	0.202	0.020	0.086	0.047	0.061	0.017	0.033	0.012	0.014

Ø of root = diameter of root. Ø of V.C. = diameter of vascular cylinder. Ø of pith = diameter of pith.

Figure (1): Transverse section of roots faba bean plant (Giza 716 and Nubaria 3) as affected by wastewater irrigation (45 days), xy = xylem tissue, ph = phloem tissue, pi = pith. Par =1 mm.

Table (2): Stem anatomical structure of the faba bean plant (Giza 716 and Nubaria 3) was affected by irrigation with concentrations of wastewater after 45 days (2020/2021) season.

Treatment	Ø of Stem (mm)		Thickens of epidermal cell (mm)		Thickness of cortex layer (mm)		Length of xylem arch (mm)		Ø of pith (mm)		No. of V.B	
	Giza 716	Nubaria 3	Giza 716	Nubaria 3	Giza 716	Nubaria 3	Giza 716	Nubaria 3	Giza 716	Nubaria 3	Giza 716	Nubaria 3
Control	3.907	2.61	0.086	0.085	0.117	0.130	0.263	0.14	2.099	2.05	27	26
50%	2.413	2.103	0.070	0.067	0.287	0.085	0.129	0.097	1.710	1.796	26	24
100%	2.363	1.823	0.06	0.058	0.059	0.060	0.105	0.067	1.575	1.43	23	23
LSD	0.14	0.17	0.49	0.01	0.04	0.04	0.04	0.02	0.06	0.16	1.15	3.44

Ø of stem = diameter of stem. Ø of pith = diameter of pith. No. of V.B = number of vascular bundles.

Similar observation of having anatomical changes only in roots and stems in *B. juncea* plants exposed to higher concentration of Cd had been reported (Sridhara *et al*., 2005). Anatomical characteristics measurements of faba bean stem were affected by all concentration of sewage water irrigation, sewage water at 100% gave the lowest values of diameter thickness of cortex tissue, diameter of xylem vessel, and these decreases were significant compared with the control in faba bean (El-Okkiah, 2015).

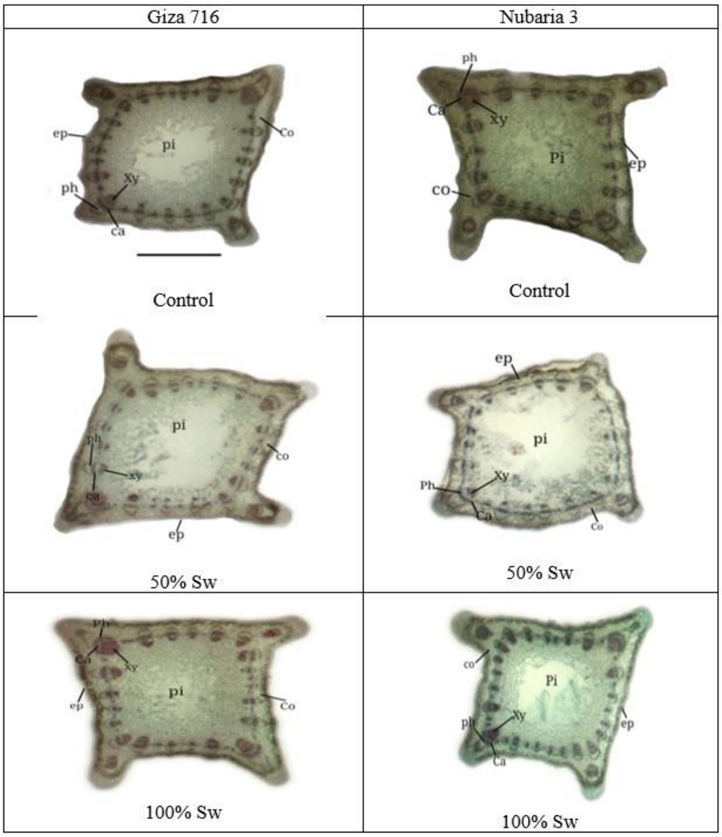

Figure (2): Transverse section of stems faba bean plant (Giza 716 and Nubaria 3) as affected by wastewater irrigation (45 days). ep = epidermis, co = cortex, vc = vascular cylinder, xy = xylem tissue, ph= phloem tissue, pi = pith. Par =1 mm.

Heavy metals have been reported to cause anatomical changes in leaves and stems in *Brassica napus* L. plants (Wan *et al*., 2011) in *Matricaria Chamomilla* plants (Farzadfar and Zarinkamar, 2012). revealed that There was significant alteration in anatomical feature of stem of cow pea seeding imposed to various level of drought. Transverse section of stem V. unguiculata showed decrease in thickness of upper epidermal layer at 0.1g Cd and 0.2g Cd compared to control. The thickness of cortex layers of stem was reduced by different concentration level. The thickness of hypodermal layer and pith area of stem were decreased by 0.1g Cd, 0.2g Cd treated plants. The pith cell diameter increased significantly in stem of Cadmium treated seedling as compared to control. There was significant change observed in the xylem vessels diameter of the stem of cow pea plant (Lekshmi and Jayadev, 2017). In the root anatomy of C. mori folium Ramat. The higher concentration of wastewater of batik staining decreased the thickness of epidermal tissue, met xylem cell size and phloem cell size, increased stele diameter especially at 75% and 100 % (age of 1 and 3 weeks), increased cortical tissue thickness especially at concentrations of 25%, 50%, and 100% (age of 1 week), and decreased cortical tissue thickness at 3 weeks after treatment in marigold (*Tagetes erecta* L.) (Vidya and Maryani, 2020).

3.1.3 leaves

The data in Table (3) and Figure (3) showed that a decrease in the anatomical parameters of the leaves when using high concentrations (50% and 100%) of wastewater to irrigate the faba bean plant in all studied traits, the upper epidermis thicken , lower epidermis thicken, Ø midrib, the length of xylem arch , as well as the palisade tissue and spongy tissue as a result of irrigation with high concentrations of wastewater compared to the control.

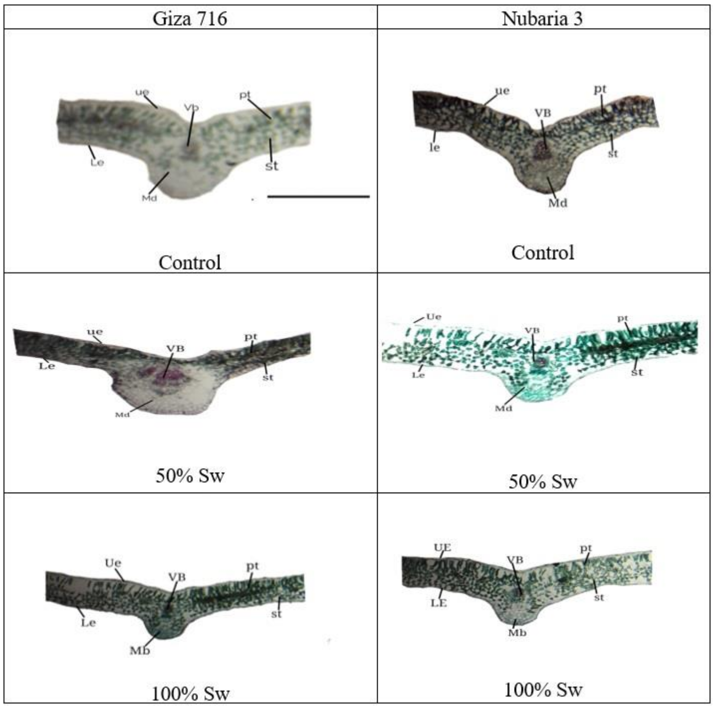

Figure (3): Transverse section of leaves faba bean plant (Giza 716 and Nubaria 3) as affected by wastewater irrigation (45 days). Ue = Upper epidermis, Le = Lower epidermis, pt = Plastid tissue, st = Spongy tissue, Md = Midrib. Par =500 µm.

Table (3): Leaves anatomical structure of the faba bean plant (Giza 716 and Nubaria3) was affected by irrigation with concentrations of wastewater after 45 days (2020/2021) season.

Treatment	Ø of U. E (μm)		Ø of L. E (μm)		Thickness spongy tissue (μm)		Thickness palisade tissue (μm)		Ø of Mi (μm)		Length of xylem arch (μm)	
	Giza 716	Nubaria 3	Giza 716	Nubaria 3	Giza 716	Nubaria 3	Giza 716	Nubaria 3	Giza 716	Nubaria 3	Giza 716	Nubaria 3
Control	10.56	17.27	10.13	22.87	104.61	167.96	64.70	90.78	299.72	415.72	60.54	98.5
50%	8.44	16.18	8.76	20.24	91.26	133.52	53.7	77.97	268.97	290.53	52.93	70.95
100%	7.73	13.22	6.81	15.73	86.07	103.72	44.94	61.50	238.27	258.34	46.23	50.72
LSD	1.41	3.23	0.84	2.21	5.90	8.42	2.81	3.24	32.72	76.29	3.02	5.06

Ø of L. M = Diameter of midrib. Ø of U. E = Upper epidermis. Ø of L. E = Lower epidermis.

Ali *et al*. (2009) reported that foliar application with zinc registered favorable anatomical changes in leaf due to the effect of micronutrients on broad bean plants (*Vicia faba* L.) Anatomical characteristics in leaf were decreased in Giza957 compared with Reina more under irrigation with diluted sea water at (1:5) specially the leaf structure and diameter of vessels in faba bean (El-Emary, 2010). Abbatial epidermis of faba bean leaves showed slightly elongated cells irregular in shape with reduced size at the higher concentrations of sewage water treatment in faba bean (El-Okkiah, 2015). It has been reported that, heavy metals may cause anatomical changes in leaves in *Brassica napus* L. plants (Wan *et al*., 2011) in *Matricaria Chamomilla* plants (Farzadfar and Zarinkamar, 2012). There was a decrease in thickness of leaves in the midrib veins along with a reduction in mesophyll width and vascular bundle diameter in response to wastewater irrigation. Reported that salt stress only decreased the cross-sectional area of leaves in the midrib and large veins, which agrees with results here. The reduction of xylem vessel area in the leaves' midrib veins can lead to the reduction of water flow to the leaves as an active sink for water. The reduction of water flow to the leaves via midrib and large veins under saline condition leads to disturbances in the transport of mineral nutrients along with loading and transport of carbohydrates in wheat. Hu *et al*. (2005) revealed that the leaf cross-sections appeared different between the controls and the wastewater-irrigate cultivars. In the major vein region, leaf thickness, vascular bundle diameter, xylem vessel area width, phloem vessel region width, and solid cell region width were significantly decreased with increasing wastewater concentration in both cultivars. Wastewater induced larger bubble cells in both cultivars compared to controls, with the largest bubble cells occurring at 25% and 50% in wheat (Hajihashemi *et al*, 2020). Generally, we recommend using wastewater to irrigate the bean plant in case of necessity, not more than 25% of wastewater to 75% natural water. Where this study showed that this concentration gives the best growth indicators *i.e.*, anatomical strictures especially with Nubaria 3 (with 50%) can be considered as resistance under Assiut conditions, Egypt.

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