ANATOMICAL STUDIES ON CANOLA PLANTS (*Brassica napus* L.) IRRIGATED WITH SEWAGE WATER

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ABSTRACT

Pollution of irrigation water was increased in the recent years by different environmental pollutants i. e., sewage. Seeds of canola (*Brassica napus* L.) were sown on 28 November 2005 in lyzimeters, that were filled clay soil. Canola plants were irrigated with 100% and 50% sewage water. Untreated plants (control) were irrigated with Nile water. Anatomical differences of root, stem and leaf of canola plants irrigated with sewage water at the both two levels and the untreated plants (control) were studied.

It was found that, irrigation with sewage water reduced the development of vascular tissues in root and stem of canola plants, while increased it in the leaves compared with the control. The highest level of sewage water increased root diameter, while the two levels of sewage water decreased the diameter of stem. Scattered groups of cortex cells which had thicker walls were appeared in canola root sections of plants irrigated with 100% sewage water. These transformed cells seems to be sclereid cells (sclerification). Other anatomical parameters of canola plants as affected by sewage water irrigation are presented in detail. Generally, these may be explain the effect of irrigation with sewage water on plant growth and yield characters.

Key words: *Brassica napus* L., sewage water, sclerification.

INTRODUCTION

Competition for limited water resources is increasing among agricultural interests. When wastewater are discharged into water bodies (rivers, sea, lakes, stream and drains), they can be a serious source of pollution because of their high biological oxygen demand (BOD5), chemical oxygen demand (COD), suspended solids, high concentration of nutrients, toxic elements and pathogens (cited by Abou El-khair *et al.*, 2005). In some areas, fresh water (Nile water) is polluted by the sewage effluent which are damped into the agricultural drainage system. Sewage water contains many essential nutrients for plant growth and its application may reduce fertilizer application rates (Neilsen *et al.*, 1989). Potential disadvantages of using reclaimed wastewater include accumulation of phytotoxic levels of heavy metals, Pb, Mn, Cd, Ni and Cu, (Omran *et al*., 1988, Zein and El-Kady, 1997 and Zein, *et. al*., 2002), and high salinity (Basiouny, 1982). In north Delta region, soil irrigated by drainage water polluted by waste water effluent of some factories were higher in heavy metals content than the normal soil (Zein, *et. al*., 1998). The content of heavy metals in soil, water and plants become of increasing interest due to their impact on polluting natural resources and public health.

Canola (*Brassica napus* L.) belong to family Brassicaceae. It is grown in more than 120 countries around the world, which a traditional source of oil (about 40%) and protein (about 25%) and other compositions. Grown sparingly for young leaves used as potherb; more generally grown forage for livestock feed, and as source of rapeseed oil. Canola is the Canadian Canola Association Trademark, which refer to rapeseed with less than 2% of Erucic acid in the oil and less than 30 micromoles of aliphatic glucosinolate in the oil free meal (Severo, 1993). Rape oil used in food industry. Also, the seed powdered with salt is said to be a folk remedy for cancer. Rape oil is used in massage and oil path, believed to strengthen the skin and keep it cool and healthy.

To the authors knowledge it appear that such anatomical study has not been previously investigated. Therefore, the objective of this study was to evaluate the effect of irrigation with municipal wastewater (sewage) on anatomical structure of canola plants.

MATERIALS AND METHODS

A lyzimeter trials were carried out at the Experimental farm of Agric. Res. Station, Sakha, at Kafr El-Sheikh Governorate, Egypt, during 2005/2006 season. Seeds of canola were sown on 28 November 2005 in lyzimeters (100 x 70 x 90 cm), that were filled with clay soil. Canola plants were irrigated with 100% and 50% sewage water (from Drain No. 7), when required with equal water volume to field capacity. Untreated plants (control) were irrigated with Nile water (from Meetyased canal). Heavy metals of Nile and drainage water (sewage water) used for irrigation are shown in Table (1), according Zein, *et. al*., (2002). Climatological normals for Sakha Station through the growth season Table (2).

Table (1): Heavy metals content of irrigation water qualities.

Water sources	Heavy metals content (mg/L)					
	Pb	Mn	Zn	Cd	Ni	Cu
Nile	0.03	0.011	0.10	0.004	0.021	0.022
Drainage	0.73	0.27	0.18	0.030	3.470	0.060
RMC (mg/L)*	5.00	0.011	0.01	0.01	0.200	0.200

* Recommended Maximum concentration of trace elements in irrigation water mg/L according to Nat. Acad. of Sci. (1972).

Table (2): Monthly climatological normals for Sakha Station from November 2005 to January 2006.

Month	Mean of air temperature °C		Mean of relative humidity %		Solar Rad. Mega Joule/m^2
	Max	Min	7:30	13:30	
November	24.2	10.6	77.3	56	9.4
December	20.0	7.0	85.5	60	6.6
January	18.8	5.1	86.0	61	8.0

For preparing sections, the stem, root and leaf specimens were taken 45 days after sowing.. Stem and root pieces 4-5 mm in length were taken from 5th internode and about 2 cm from the tip of the main roots, respectively. Concerning the leaves pieces 5 mm in length were taken from fourth leaf (from the shoot apex) including the midrib. Specimens were killed and fixed in formalin alcohol acetic acid mixture (FAA, 1:18:1 v/v) and washed and dehydrated in alcohol series. The dehyderated specimens were infiltrated and embedded in paraffin (52-54 °C m. p.). The embedded specimens were sectioned on a rotary microtome at a thickness of 10 – 12 μ m. Sections were mounted on slides and deparaffinised. Staining was accomplished with safranine and light green, cleared in xylol and mounted in Canada balsam (Gerlach, 1977).

Measurement of transverse sections of stem of canola plants i. e. stem diameter, vascular bundle phloem and xylem tissues thickness, and diameter of xylem vessels. Measurement of transverse sections of root of studied canola plants i. e. root, vascular cylinder and vessel diameters and No. of vessels/bundle were measured. Measurement of transverse sections of leaves of studied canola i. e. leaf lamina, palisade, spongy tissues thickness and midrib vascular tissue thickness were recoded.

Data were subjected to statistical analysis of variance according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The root internal structure of canola plants is similar to other dicotyledons plants. It consists of the exodermis, ground tissue and the vascular system (Fig. 1). Ground tissue differentiated into cortex and pith. The radical vascular bundles arranged in complete protostele (haplostele) cylinder.

Measurement of canola root cross sections as affected by irrigation with sewage water concentrations (100 and 50% sewage water) was studied at the time of plant samples tacking. Data illustrated in Fig. (1) and recorded in Table (3) show that, soil irrigation with two different levels of sewage water caused a significant anatomical differences of the root. The highest values of root and vessel diameter and number of vessel/bundle were found by irrigation with 100% sewage water, while the longest diameter of vascular cylinder was obtained by control (irrigated with tap water). Scattered groups of cortex cells which had thicker walls and stained by safranin dye were appeared in canola root sections of plants irrigated with 100% sewage water. These cells seems to be sclereid cells.

A reduction in vascular cylinder diameter may be due to high salinity Basiouny, (1982) and Hussien, (2000). Sclerification of cortex parenchyma cells may be due to heavy metals accumulation. Jarvis *et al.*, (1976) studied the uptake of cadmium by the roots of some plants and its transport to shoots using solution culture. They found that, the cadmium concentration in the roots was always greater than in the shoots. The physiological reasons for scarification of parenchyma cells are not known, but many botanists thought the fact that stone cells often appear close to wound tissues suggests that they develop in response to some physiological disturbances (internal and external factors) Fahn, (1990). These transformed cells may reduce nutrients and water absorption and their transport, cited by Moore, *et. al.*, (1998).

The stem structure of canola plants as seen in transverse sections consists of the epidermis, ground tissue and vascular system (Fig. 2). Ground tissue differentiated into cortex and pith. The vascular collateral bundles arranged in complete cylinder (Siphonostele: ectophloic). Two types of collateral vascular bundles are present, i. e., large and small bundles. The large bundles are separated with few small one. Data illustrated in Fig. (2) and presented in Table (4) indicated that, significant difference of measurements of transverse stem sections of canola plants were recorded as affected by irrigation with two different sewage water concentrations compared with the control. Stem and vessel diameter, vascular bundle length and vascular tissues (phloem and xylem tissues) thickness were significantly decreased by irrigation with two levels of sewage water. The lowest values of anatomical stem parameters were found by irrigation with 100% sewage water.

The inhibiting effect on stem structure of sewage water treated canola plants may be due to its phototoxic heavy metals content and its inhibiting effect on both cell division and elongation, cited by Fouda and Arafa (2002). Differentiation of the vascular tissues is correlated to the rate of cambial activity, which is the results of many internal and external factors, cited by Fahn, (1990). Generally, the activity of vascular cambium is correlated to endogenous phytohormones content i. e., auxin, Moore, *et. al.*, (1998) and El-Nady (2003). Therefore, it can be deduced that, sewage water plays a direct or indirect role with auxin biosynthesis.

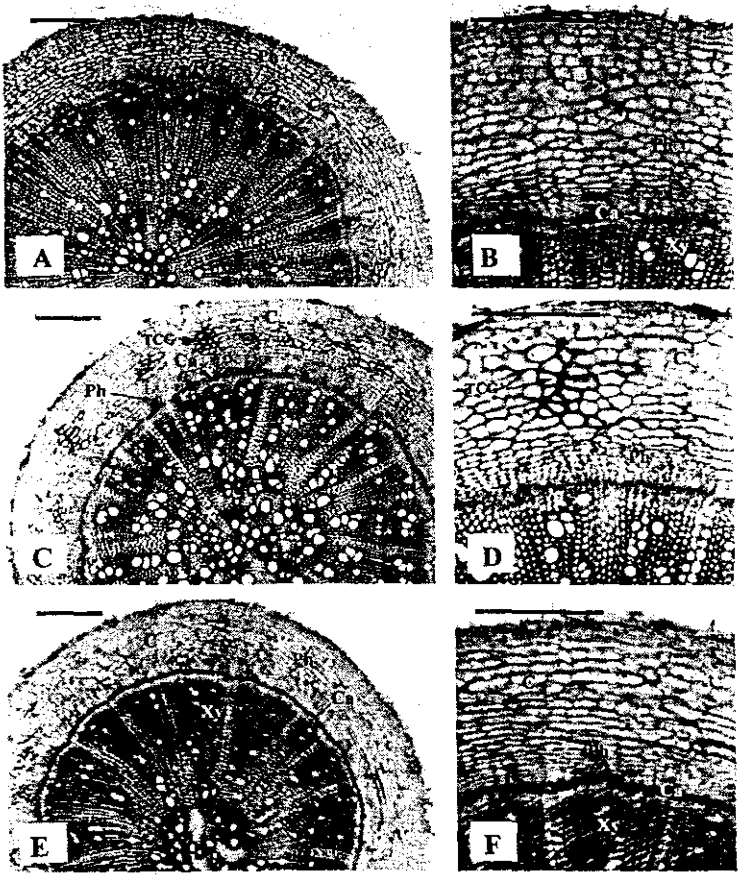

Fig. (1): Root cross sections of canola plants as affected by sewage soil irrigation (C: cortex, Ca: vascular cambium, Ph: phloem tissue, Xy: xylem tissue, TCG: transformed cell groups). (Pars = 400 µm)

A-B: control. C-D: 100% sewage water. E-F: 50% sewage water.

Table (3): Anatomical parameters of canola roots as affected by irrigation with sewage water.

Parameters / Treatments	Root diameter (mm)	Vascular cylinder diameter (µm)	Vessel diameter (µm)	No. of vessels/ bundle
Control	2.75 b	2325 a	42 b	13 b
Sewage water (100%)	3.00 a	2000 b	46 a	17 a
Sewage water (50%)	2.25 c	1550 c	38 c	9 c

Values having the same alphabetical latter within column are not significantly different ($P < 0.05$).

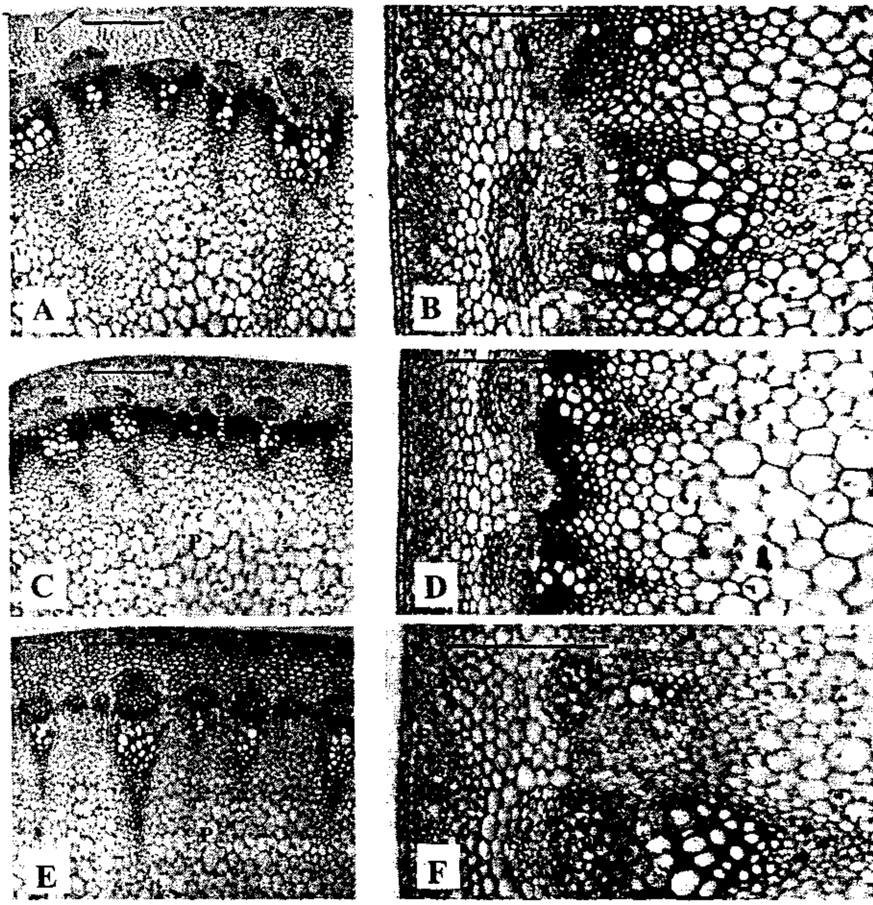

Fig. (2): Stem cross sections of canola plants as affected by sewage soil irrigation (E: epidermis, C: cortex, Ca: vascular cambium, P: pith, Ph: phloem tissue, Xy: xylem tissue), Pars = 500 µm.
A-B: control. C-D: 100% sewage water. E-F: 50% sewage water.

Table (4): Anatomical parameters of canola stems as affected by irrigation with sewage water.

parameters / Treatments	Stem diameter (mm)	Vascular bundle length (µm)	Phloem thickness (µm)	Xylem thickness (µm)	Vessel diameter (µm)
Control	7 a	600 a	150 a	390 a	45 a
Sewage water (100%)	4 c	500 c	100 c	250 c	30 c
Sewage water (50%)	5.5 b	530 b	120 b	350 b	37 b

Values having the same alphabetical latter within column are not significantly different ($P < 0.05$).

The leaf blade internal structure of canola plants is similar to other dicotyledons plants. It consists of upper and lower epidermis and mesophyll tissue, which differentiate into palisade and spongy parenchyma. Epidermis, one layer of completely arranged parenchymatous cells, which are flattend parallel to the leaf surface. Mesophyll tissue differentiates into palisade and spongy tissues. The palisade parenchyma cells are elongated and completely arranged. The spongy parenchymatous cells are lossely arranged with numerous large intercellular spaces.

Data illustrated in Fig. (3) and presented in Table (5) show that, canola plants irrigated with 50% sewage water gave the highest values of lamina, mesophyll tissues (palisade and spongy tissues) thickness. On the other hand, the highest values of phloem and xylem tissues of midrib vascular bundle were recorded by irrigation with 100% sewage water. Irrigation with 100% sewage water increased leaf blade thickness in the midrib region due to an increase in the parenchyma cells and their diameter. The size of the midvein vascular bundle was also increased as indicated by its dimension as well as xylem and phloem tissues thickness. Moreover, metaxylem vessels diameter seen to be longer.

The increasing of internal leaf structure may be due to essential nutrients, which occurred in sewage water, Neilsen *et al.*, (1989) and Zein and El-Kady (1997). These may be explain the effect of irrigation with sewage water on plant growth and yield characters. Zein and El-Kady (1997) found that, soybean yield characters were significantly decreased by irrigation with drainage water.

According to the obtained results, it's obvious that irrigation with sewage water caused anatomical differences in the main canola plant organs (root, stem and leaf). Generally, this may be explain the effect of irrigation with sewage water on plant growth and yield characters.

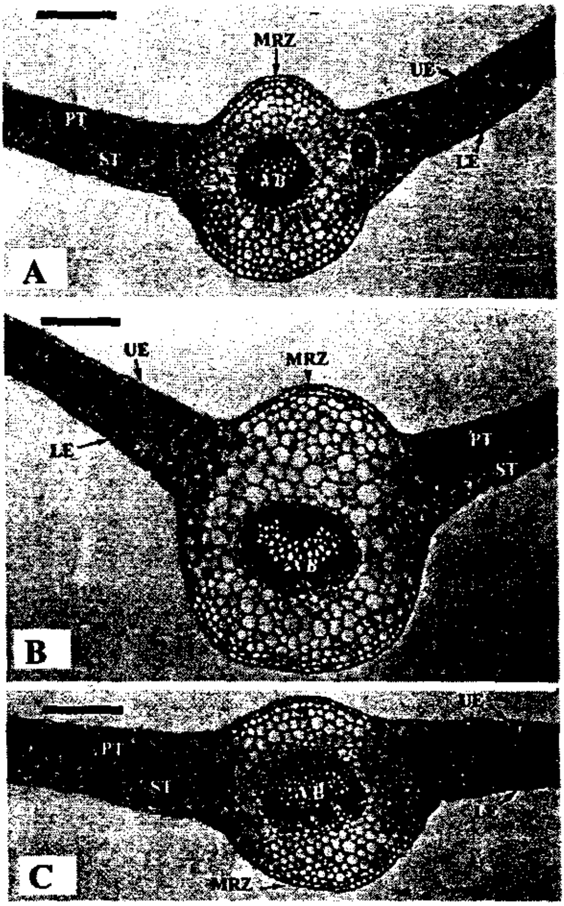

Fig. (3): Leaf cross sections of canola plants as affected by sewage soil irrigation (UE: upper epidermis, LE: lower epidermis, VB: vascular bundle, MRZ: midrib zone), Par= 250 µm.
A: control. B: 100% sewage water. C: 50% sewage water.

Table (5): Anatomical parameters of canola leaves as affected by irrigation with sewage water.

parameters / Treatments	Lamina thickness (µm)	Palisade t. thickness (µm)	Spongy t. thickness (µm)	Xylem t. thickness (µm)*	Phloem t. thickness (µm)*
Water	260 bc	108 b	110 b	110 c	100 b
Sewage water	265 b	125 a	110 b	175 a	105 a
Sewage water (50%)	280 a	125 a	135 a	150 b	100 b

Values having the same alphabetical latter within column are not significantly different ($P < 0.05$). * vascular tissues in midrib.

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الملخص العربي

دراسات تشريحية علي نباتات الكانولا تم ريها بمياه الصرف الصحي

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نظراً لزيادة تلوث مياه الري في السنوات الأخيرة بملوثات البيئة وخاصة بالصرف الصحي، أجري هذا البحث لدراسة تأثير الري بمستويين (٥٠ و ١٠٠ ٪) من مياه الصرف الصحي على نباتات الكانولا مقارنة بالكنترول (ماء النيل).فتمت زراعة بذور الكانولا في ٢٨ نوفمبر لسنة ٢٠٠٥م بأحواض أسمنتية مملوءة بتربة طينية، وقد أدت معاملتي الري بمياه الصرف الصحي إلى نقص في تكشف الأنسجة الرعائية فى كل من جذور وسيقان نبات الكانولا بينما زاد هذا في الأوراق. وقد أدي الـــري بمياه الصرف الصحي (١٠٠ ٪) إلى زيادة في قطر الجذور ونقص في قطر السيقان، كما أدي إلى ظهور مجموعات متفرقة من خلايا القشرة سميكة الجدر في الجذور، هي على الأرجح خلايا إسكلرنشيمية. كما وجدت اختلافات تشريحية أخري في نبات الكانولا نتيجة ريها بمستويات مختلفة من ماء الصرف الصحي ذكرت بالتفصيل. تساهم هذه النتائج في تفسير تأثير الري بمياه الصرف الصحي على صفات النمو والمحصول.