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تأثير مياه المجارى على الأيض والمحصول فى نبات الفول البلدى

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الملخص العربى

أجرى هذا البحث تحت ظروف الصوبة فى تجربة أصص على الفول البلدى لدراسة تأثير مياه المجارى على النمو والعلاقات المائية والصبغات النباتية والتركيب البيوكيميائى والمحصول ، وتم إجراؤها بقسم النبات الزراعى بكلية الزراعة جامعة المنوفية خلال موسم ٢٠٠٨/٢٠٠٩ ، وتم تخفيف مياه المجارى بمياه الصنبور لتُعطى نسبة ٢٥ ، ٥٠ ، ٧٥% وتم الرى بها وقد تم أخذ عينة عشوائية من كل معاملة بعد ٤٥ يوماً من الزراعة . وقد أظهرت النتائج زيادة فى كل صفات النمو ، البروتين الكلى ، النشاط الإنزيمى ، البرولين والمحصول . وقد زاد تركيز كل من الزنك ، المنجنيز ، والكادميوم والرصاص بكثرة فى جميع أجزاء النبات وكذلك قلت صبغات البناء الضوئى ومعدل فقد الماء ومحتوى الماء المرتبط بعد الرى بمياه المجارى .

EFFECT OF SEWAGE WATER ON GROWTH, METABOLISM AND YIELD OF FABA BEAN PLANTS

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ABSTRACT: *Two pot experiments were conducted under greenhouse conditions to study the effect of sewage water on growth analysis, water relations, plant pigments, certain biochemical composition and yield of faba bean plants. This experiment was performed in the Department of Agricultural Botany, Faculty of Agriculture, Minoufiya University, during successive season of 2008/2009. The sewage water was diluted with tap water to give three rates of 25, 50 and 75% used in irrigation. Plant sample was successively taken at random from every treatment starting 45 DAS. The results showed that sewage water increased all growth characters, total protein, total carbohydrates, activity of enzymes and proline as well as yield. The concentration of Zn, Mn, Cd and Pb were increased greatly in the different faba bean organs in response to sewage water application. Photosynthetic pigments, rate of water loss, relative water content were decreased by of sewage water Irrigation.*

Key words: *Sewage water, Faba bean, Growth, Enzymes activity, Heavy metals, Yield.*

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important Leguminous crops of high nutritive value in the world as well as in Egypt. In Egypt, the total area devoted to faba bean cultivation annually is about 198,000 feddan with production about 301.8 (1000 ton) with an average yield of 3.41 (ton / ha) in 2006 – 2007 season which covers only 67% of Egyptian consumption (Malr, 2007).

Water insufficiency is one of the most critical problems that confront the world particularly in the arid and semi arid regions. Re-use of domestic sewage effluents outlines the most substantial and self-controllable resource so far despite many obstacles and limitations to be retrieved. Pollution with ions of heavy metals results from excessive and / or unplanned uses of sewage water. In small quantities, certain heavy metals are nutritionally essential for a healthy life e.g., Fe, Cu, Mg and Zn. Increases loading of heavy metals ions in water and soil produce increases in mutation or cell death in plant and increases in human health hazards as the heavy metals enter and concentrated in the food chain through uptake by plants and ingestion by animals (Sancherz *et al.,* 1999).

Therefore the objectives of this study to evaluate the effect of sewage water on growth, some physiological and biochemical compositions, as well as yield of faba bean crop.

MATERIALS AND METHODS

This investigation was performed at the Experimental Farm of the Faculty of Agriculture, Minoufiya University at Shibin El-Kom, Egypt, during the successive season, 2008/2009. The seeds of Faba bean (*Vicia faba* L.) cv. Giza 843 used in this investigation were obtained from Crop Research Institute, Agricu1ture Research Center, Giza, Egypt. The polyethylene pots of 40 cm inner diameter and a depth of 30 cm were used and filled with 15 kg of clay loamy soil, and placed in an open air under a wired net cage. The physical and chemical of the soil used are shown in Table (1). The seeds were sown on November 20th 2008/2009. After 21 days from sowing, seedlings were thinned to 5 plants per pot.

The treatments were arranged in a complete randomized block design with 5 replicates. The normal practices for growing faba bean recommended for the region were followed. After one month from planting the sewage water was irrigated: The sewage water was obtained from the Sewage Water Station at Shibin El-Kom. The chemical characteristics of sewage water are illustrated in Table (2). The sewage water was diluted with tap water to give concentrations of 25, 50 and 75% used in irrigation. Tap water was used as a control. Every treatment was applied 4 times, the first application was after one month after sowing and then at 15 days intervals.

Table (1). The physical and chemical characteristics of the soil used.

Properties	Values	Properties	Values
Physical analyses:		Soluble 100g mg/100 g(mg /100 g soil): ions (mg /100 g soil):	
Sand %	17	Mg^{+2} + Ca	0.25
Silt %	35	Na^{+}	1.12
Clay %	48	K^{+}	0.33
Chemical analysis analysis:		Cl^{-}	0.80
pH	7.8	SO^{-2}_{4}	0.95
O.M	1.2	Total N %	0.12
Ec mmhos / cm	1.5	HCO^{-}_{3}	0.95

Table (2). Chemical characteristics of the sewage water used in the experiment.

Variable	Sewage water	Variable	Sewage water
E.C (1-10) sewage/water mhos/cm	2.6	K meq/L	2.30
pH (1-10) sewage/water mhos/cm	7.3	Fe µg/g	250
Total carbonate %	2.6	Mn µg/g	4.1
Total nitrogen %	4.00	Zn µg/g	3.9
Organic matter %	4.9	Pb µg/g	3.01
Water extractable:		Cd µg/g	< 0.02
P µg/g	36.9	K meq/L	2.30

The plants sample from each treatment was taken after 45 DAS, to determine the following data:

2.1. Growth analysis: Plant height (cm), Number of branches / plant, dry weights (gm) for stems and leaves and leaf area cm^2 / plant, dry weight of whole plant (g). Plant materials were dried in an electric oven at 70°C for 72 hours then used it for chemical analysis.

2.2. Photosynthetic pigments: Chlorophyll (a), (b) and carotenoids were determined from the middle fresh leaves using spectrophotometric method as described by Wettstein (1957)

2.3. Water Relations: Total Water Content (TWC%), leaf water deficit, (LWD%), relative water content (RWC%) and rate of water loss (RWL) were measured flowering the methods of Barrs and Weatherley (1962)

2.4. Carbohydrate concentration: was determined chlorometrically using the phenol sulfuric acid method as described by Dubois *et al.* (1956).

2.5. Enzymes activity: peroxidase activity and phenoloxidase activity were measured in fresh leaves using the method described by Fehrman and Dimond (1967)

2.6. Proline concentration: Total concentration of proline in leaves (mg / g Dr.wt.) was etermined according to Bates *et al.* (1973)

2.7. The total crud protein: The total crud protein was calculated by, multiplying the nitrogen percentage by 6.25 for faba bean, according to Osborn and Vooget (1978).

2.8. Nitrogen concentration of plant roots, stems and leaves was determined in the fine powder of dry matter using micro-kje1dahl method as described by Ling (1963).

2.9. Heavy metals: Some heavy metals such as Pb, Cd, Mn and Zn were determined in plant organs by using Parken Elemer (2830) atomic absorption spectrophotometer, the maximum absorbance was obtained by adjusting the cathode wave length (Stewart, 1973).

2.10 Yield and its components: At the harvest time the following data were recorded. Number of flowers / plant, Set percentage, Shedding percentage Seed yield / plant (gm) and Straw yield / plant (gm). Lead, cadmium zinc and manganese concentrations of faba bean seeds were estimated.

Statistical analysis:

Data were statistically analysed according to Gomez and Gomez (1984) with the help of COSTAT (1985) Computer program for statistics least significant differences test.

RESULTS AND DISCUSSION

3.1. Growth analysis:

Data presented in Table (3) showed that all sewage water treatments significantly increased all growth parameters of bean plants. This effect may be. attributed to the high concentration of organic matter, macro and micronutrients in sewage water and beneficial nutrients enhanced the metabolic activities and hence the vegetative growth These finding are in agreement with those reports by reports Al-Fredan (2008) in bean, Sakr *et al.* (2008) and Singh and Agrawal (2010) in rice plants. The lowest concentration of sewage water more effective than the highest concentration in increasing bean stem and leaves dry weight after 45 DAS, resulting in water stress which decreasing cell division, cell enlargement and the intensity of photosynthesis on soybean. Similar results were obtained by Gaballah and Gomaa (2004) on faba bean. Moreover, Al-Fredan (2008) and El-Okkiah, Samira (2010) in faba bean, who indicated that waste water effluent significantly increased the stem and root lengths and growth.

Table (3). Effect of sewage water on growth analysis of faba bean leaves after 45 DAS on season (2008/2009).

Characters / SW level	Plant height (cm)	Branch No. / plant	Leaf area (cm^2 / plant)	Dry weight (g / plant)		
				Stem	Leaves	Whole
Control	48.3	1.2	121.7	1.6	1.3	2.9
SW 25%	55. 7	2.7	182.6	2.3	2.0	4.3
SW 50%	61. 7	1.3	166.0	1.9	1.4	3.3
SW 75%	63.3	1.3	109.2	1.4	1.1	2.5
L.S.D at 5%	3.1	0.7	15.5	0.5	0.5	2.5

3.2 Photosynthetic pigment:

Application of sewage water negatively affected synthesis of photosynthetic pigments (Table 4). The sewage water application had the same effect on chlorophyll (b) and total chlorophyll. Chlorophyll (b) and total chlorophyll were decreased by using the all levels of sewage water. Concerning carotenoids content, it can be observed from the same table that, the high level of sewage water (75%) treatments caused a reduction in carotenoids concentration.

The sewage water at high level of 75% reduced this aspects at 45 DAS of faba bean leaves chlorophyll (a + b) caused in general a significant decrease in this respect. This decrease in total chlorophyll content may be attributed to elevated levels of heavy metal accumulation in bean plants, which inhibited the chlorophyll formation (Table 4). The decrease of photosynthetic pigments especially chlorophyll a because of the high level of sewage water may be attributed to the high uptake and accumulation of heavy metals consequently their harmful effects on the photosynthesis. Similar results were obtained by who found that, when high level of sludge were applied, some soybean seedlings became chlorotic and died (Krupa and Baszynski, 1995).

3.3. Water relations:

Data presented in Table (5) showed that, there was an increase in total water content (%) of leaves. Data presented in Table (5) show that application of sewage water increased total water content (%) and leaf water deficit. Meanwhile, the relative water content and rate of water loss were decreased. This decrease might be explained on the fact that, heavy metals especially Cd decrease the water uptake by root in different plant species. These results were in agreement with those obtained by Haroun *et al.* (2003). Singh and Agrawal (2009) reported that, the transpiration rate in lady's finger plants decreased with increasing sewage water concentration when compared with those grown in un amended soil. The decrease in the transpiration rate in faba bean plants may be due to the harmful effect of the applied sewage water on decreasing the stomatal conductance. Additionally, this effect may also be attributed to increasing the heavy metals concentration in faba bean plants.

3.4. Protein and carbohydrate concentration:

Data presented in Table (6) showed that application sewage water was increased total carbohydrates as well as proline in leaves. Mazen *et al.* (2010) found that, the total carbohydrate contents of sewage sludge treated test plants were positively affected. The sewage water application on carbohydrate metabolism depended on the plant species and the plant organ. In faba bean, the levels of soluble and insoluble fractions of carbohydrate increased in the sludge-supplemented soil and sewage water irrigation. Bearing in mind that leaf total soluble sugars amounts depended primarily on photosynthesis, the percentage of sugar in *Phyllanthus amarus* plants decreased by Cd (Rai *et al.,* 2005). El-Maghraby and Gomaa

(1992) reported that, sewage water application increased total soluble sugars, polysaccharide and total carbohydrates content were higher in sewage water irrigated plants compared with control. The increased content of carbohydrates in sewage water irrigated plants may be due to the presence of some mineral ions e.g. Mn that stimulate the two photosystems Mn^{+2} is required for PSII (O_2 evolving system) and there is also a direct interaction between copper and ferredoxin on the reducing site of PSI. Cu^{++} stimulate the rate of overall electron transfer from water to NADP (Marschner, 1998). Protein synthesis is essential for normal cell proliferation, differentiation and growth. A variety of environmental factors have been reported to influence the synthesis of plant protein (William, 1989). In this connection Mazen (2003) reported that, the cultivation of plants (Eruca, Daucus, Lactuca and Spinacia) on sewage sludge amended soil was generally enhanced the total proteins. In Zea mays, total protein also was increased (Mazen, 1995).

Table (4). Effect of sewage water on photosynthetic pigment concentrations (mg/g DW) of faba bean leaves at 45 DAS on season (2008/2009).

Characters / Sw level	Chlo (a)	Chl (b)	Total Chl (a + b)	Carotenoids
Control	2.73	1.14	3.87	1.39
SW 25%	1.05	0.86	1.91	0.83
SW 50%	1.37	0.74	2.11	0.96
SW 75%	1.13	0.43	1.56	0.51
LSD at 5%	0.28	0.39	1.19	0.20

Table (5). Effect of sewage water on water relations of faba bean leaves at 45 DAS on season (2008/2009).

Characters / (SW) level	Total water content (%)	Leaf water def. (%)	Relative water content (R.W.C) (%)	Rate of water loss (R.W.L) (mg / gm / h)
Control	83.10	41.23	58.77	4.35
SW 25%	86.15	41.74	58.26	4.00
SW 50%	85.46	45.08	54.92	3.68
SW 75%	84.25	46.42	53.67	2.40
LSD at 5%	4.20	2.85	3.26	0.82

Table (6). Effect of sewage water on protein, carbohydrate, proline and enzymes activity in faba bean leaves at 45 DAS on season (2008/2009).

Characters / (SW) level	Protein %	Total Carbohydrate	Proline mg / g D.W	Enzymes activity	
				Phenoloxidase OD / g FW	Peroxidase OD / g FW
Control	29.79	231.53	0.19	3.79	7.09
SW 25%	35.83	233.10	0.25	3.42	7.70
SW 50%	39.58	238.36	0.35	2.56	6.92
SW 75%	39.79	240.11	0.47	2.49	6.90
LSD at 5%	4.86	1.33	0.09	1.15	0.25

3.5. Enzyme activity:

Data in Table (6) revealed that, peroxidase and phenoloxidase activities were significantly decreased in faba bean leaves by sewage water application. In this respect, Mengel and Kirkby (2001) reported that, heavy metal pollution alter the metabolic processes inside cells through their effect on the enzymatic system.

3.6. Proline concentration:

Proline concentration of sewage water treated and untreated leaves of faba bean plant are shown in Table (6). All levels of sewage water caused significant increase in proline concentration This increase may be due to the toxic heavy metals in the production of proline (Shah and Dubey, 1998 b; Mehta and Gaur, 1999 and Verma, 1999). The accumulation of proline in stressed plants is associated with the reduced damage to the membranes and proteins (Verma, 1999). Proline synthesis has been implicated in the alleviation of cytoplasmic acidosis and may maintain NADP / NADPH ratios at values compatible with metabolism (Hare and Cress, 1997). Rapid catabolism of proline upon relief of stress also may provide reducing equivalents that support mitochondrial oxidative phosphorylation and the generation of ATP for recovery from stress-induced damage (Hare and Cress, 1997). Free proline has been proposed to act as an osmoprotectant (Taylor, 1996), a protein stabilizer (Shah and Dubey, 1998), an inhibitor of lipid peroxidation (Mehta and Gaur, 1999), a hydroxyl radical scavenger (Smirnoff and Cumbes, 1989), and a singlet oxygen scavenger (Alia *et al.,* 2001).

3.7. Heavy metals concentration in plant organs:

Heavy metals by sewage water application, seems to be one of the most limited factors affecting the potential value of sewage water. So, the harmful effect of these heavy metals on plant growth, photosynthesis, enzyme activity and other physiological processes, then which reflected on the final yield, is in compact with their concentration in the used sates. Therefore, some of heavy metals e.g. Pb, Cd, Mn and Zn were determined in different organs of faba bean plants treated with sewage water and data presented in Table (7). The application with sewage water at all levels significantly increased the Pb, Cd, Zn and Mn concentration of bean roots and stems. The increase of heavy metals concentration can be attributed to the high uptake of heavy metals by plants and then accumulated in root compared to shoots or translocated to seeds (Abd El-Hady, 1996).The accumulation of Cd in roots resulted from sewage water levels was more announced than those in the other organs. (Zeid and Abou El-Ghate, 2007).

El-Maghraby and Gomaa (1992) found that, sewage water application increased heavy metals in soil. Horak *et al.* (1998) Cd concentration tended to increase in the sewage sludge treatment soils. It could be related to the higher levels of Cd in sewage sludge treated soils. These concentration were quite below the toxicity levels 5 – 30 mg. kg^{-1} according to Alloway and Ayers (1997). The Pb concentrations ranged from 7 – 11 mg . kg^{-1} which were far below the critical level of toxicity. Berton *et al.* (1989) found that, the sewage water application increased Zn in maize plants using higher sludge doses. Binder *et al.* (2002), Hogan *et al.* 2001) and Xiong *et al.* (2004) reported that, higher concentration of Cd and Zn in rice plant by applied sewage sludge. The heavy metals were highest in roots followed by leaf expect Mn which was higher in stem than in leaf. The increase of heavy metals concentration in different organs of faba bean resulting from sewage water can be attributed to the high uptake of heavy metals by plants and then accumulated in roots compared to shoots or translocated to seeds (Table 7). The recommended levels are for Cd (0.05 – 0.20 ppm) and Pb (0.5 – 1.0 ppm), while the toxic levels of these elements are 5 – 30 ppm and 10 – 100 ppm, respectively (Davis *et al.,* 1978). The concentration of Cd, Pb, Mn and Zn in the edible parts of the test plants treated by sewage water (Table7) were generally near the normal range and less than the toxic levels as mentioned before. Zeid and Abou El-Ghate (2007) stated that accumulation of Zn, Mn, Cd and Pb in plants irrigated with sewage water was also observed.

Table (7). Effect of sewage water on heavy metals concentration (ppm) in faba bean organs at 45 DAS on season (2008/2009).

Characters / Sewage level	Pb	Cd	Mn	Zn
	Roots			
Control	9.3	2.5	13.7	38.0
SW 25%	10.2	2.9	14.1	39.4
SW 50%	11.1	3.7	14.6	39.9
SW 75%	11.8	4.6	14.9	40.3
Mean	11.0	3.7	14.5	39.9
	Stem			
Control	7.01	1.3	9.66	31.11
SW 25%	9.9	3.1	11.1	34.7
SW 50%	10.3	3.3	11.6	36.2
SW 75%	10.7	3.6	11.9	36..38
Mean	10.3	3.3	11.5	35.8
	Leaf			
Control	7.21	1.22	10.53	28.36
SW 25%	10.2	2.77	12.1	31.25
SW 50%	10.88	3.17	12.68	31.97
SW 75%	10.95	3.87	12.97	32.44
Mean	10.7	3.3	12.6	31.9

3.8. Yield and its components:

Data presented in Table (8) show that application of sewage water at 50% recorded the highest flowers No. / plant, set percentage, seed yield / plant and weight of 100 seeds in faba bean plants compared to the control and other levels of sewage water. These results are in harmony with those obtained by Zeid and Abou El-Ghate (2007). The beneficial effects of sewage water application may be due to the greater capacity of sewage to supply nutrients to the plant and to improve soil properties (Heckman *et al.,* 1986). These nutrients may activate the hydrolytic enzymes during germination, which in turn increase the amount of hydrolyzates e.g., glucose and amino acids, which required for growth of embryo axes (Zeid and Shedeed, 2006). Moreover, Staniforth and Smith (1991) mentioned that, the increased crop yield as a result of increasing rate of sewage application to the available nutrients supplied particularly nitrogen, which is reflected in the increased total nitrogen control of the crop. These results are in harmony with those obtained by Mostafa (1994), Zeid and Abou El-Ghate (2007) and El-Okkiah Samira (2010). Moreover, seed yield / plant, straw yield / plant and weight of 100 seeds recorded highly significant weights at SW_2 levels compared to the untreated plants. Seed yield / plant and set percentage were increased, meanwhile shedding percentage was decreased.

3.9. Protein and total carbohydrates in seeds:

Data in Table (9) demonstrate that, all sewage water levels significantly increased both total carbohydrates and protein percentage in faba bean seeds compared to the control plants. These results are in agreement with those obtained by Mazen (1995) and El-Okkiah, Samira (2010). The increased content of protein and carbohydrates by sewage water irrigated plants may be due to the presence of some mineral ions e.g., Mn and Cu that stimulate the two photo systems. Mn^{+2} is required for PSII CO_2 evolving system and there is also a direct interaction between copper and ferredoxin on the reducing site of PSI C (Marschner and 1998).

3.10. Heavy metals content in faba bean seeds:

Data in Tables (10) showed that, there was a remarkable gradual significant increase in faba bean seeds from heavy metals with increasing sewage water concentrations. This increasing in heavy metals content may be attributed to increasing of heavy metals content in sewage water. According to Kabata-Pendias and Pendias (1992); such results agreed with those obtained by Abd El-Naim and El-Houseini (2002) who showed that sewage water caused increase in the edible parts contents of heavy metals of corn and sunflower. Moreover, Abdel-Sabour and Rabie (2003) revealed that irrigation with different waste water significantly increased the concentration of heavy metals in vegetable plants especially the leafy species.

Table (8). Effect of sewage water on the yield of faba been plants at the harvest stage on season (2008/ 2009).

Characters / SS level	Flowers No. / plant	Set %	Shedding %	Seed yield / plant	Strow yield (g/plant)
Control	38.0	22.8	77.2	17.0	23.8
SW 25%	40.7	26.2	73.8	35.7	49.9
SW 50%	52.0	23.7	76.3	49.3	69.1
SW 75%	58.0	6.0	76.4	45.3	63.5
L.S.D at 5%	4.5	2.5	0.9	10.8	15.1

Table (9). Effect of sewage water on the total protein and total carbohydrates percentages in faba been seeds on season (2008/2009).

Characters / SW level	Total protein%	Total carbohydrate%
Control	34.9	30.6
SW 25%	34.5	32.5
SW 50%	44.3	34.8
SW 75%	38.9	33.5
LSD at 5%	1.6	1.4

Table (10). Effect of sewage water on heavy metals (ppm) in faba bean seeds on season (2008/2009).

Characters / (SW) level	Micro elements (ppm)			
	Pb	Cd	Mn	Zn
Control	0.04	0.20	10.5	15.11
SW 25%	0.06	0.35	12.6	16.01
SW 50%	0.17	0.38	13.8	17.01
SW 75%	0.20	0.42	14.2	19.00
LSD at 5%	0.04	0.85	0.95	2.00

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تأثير مياه المجارى على الأيض والمحصول فى نبات الفول البلدى

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الملخص العربى

أجرى هذا البحث تحت ظروف الصوبة فى تجربة أصص على الفول البلدى لدراسة تأثير مياه المجارى على النمو والعلاقات المائية والصبغات النباتية والتركيب البيوكيميائى والمحصول ، وتم إجراؤها بقسم النبات الزراعى بكلية الزراعة جامعة المنوفية خلال موسم ٢٠٠٨/٢٠٠٩ ، وتم تخفيف مياه المجارى بمياه الصنبور لتُعطى نسبة ٢٥ ، ٥٠ ، ٧٥% وتم الرى بها وقد تم أخذ عينة عشوائية من كل معاملة بعد ٤٥ يوماً من الزراعة . وقد أظهرت النتائج زيادة فى كل صفات النمو ، البروتين الكلى ، النشاط الإنزيمى ، البرولين والمحصول . وقد زاد تركيز كل من الزنك ، المنجنيز ، والكادميوم والرصاص بكثرة فى جميع أجزاء النبات وكذلك قلت صبغات البناء الضوئى ومعدل فقد الماء ومحتوى الماء المرتبط بعد الرى بمياه المجارى .