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Research Article
Effect of Drainage Water Mixed with Untreated Sewage Water on Susceptibility of *Vicia fabato* Infection by *Botrytis fabae*

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Abstract

Background and Objectives: Farmers in many big cities have been using sewage polluted water for irrigation of crops. That increase the heavy metal contents of soils that affect on plant growth parameters and which makes the plant weak against any infection. The study was conducted to study the impact of the irrigation by drainage water mixed with untreated sewage water on the susceptibility of Giza 3 cultivar of *Vicia faba* to infection by *Botrytis fabae* pathogen in Egypt. **Materials and Methods:** Pathogen was isolated from infected *Vicia fabe* cultivars in Egypt. The drainage water mixed with untreated sewage water samples were collected from study area. Giza 3 was irrigated with drainage water mixed with untreated sewage water and infection with *Botrytis fabae* under greenhouse. Disease severity, enzyme activities, level of genes expression, percentage of total number of abnormalities cell, electrolytic leakage, growth parameters and mitotic index were determined. **Results:** The disease severity, enzyme activities (POD and PPO), level of POD and PPO gene expression, percentage of total number of abnormalities cell, electrolytic leakage (EC) and photosynthetic rate were increased. While photosynthesis pigments (chlorophyll a, b and the carotene), the fresh, dry weight of shoot and root and mitotic index were decreased. **Conclusion:** The irrigation by drainage water mixed with untreated sewage water increase the susceptibility of *Vicia faba* (Giza 3) cultivar to infection by *Botrytis fabae*.

Key words: Drainage water, sewage water, susceptibility, gene expression, abnormalities cell and *Botrytis fabae*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Industrial developments in the last years and the increased use of chemical pollutants and toxicants, such as metals, biocides, pesticides, chemicals, industrial effluents and Anthropogenic activities resulting from modern methods of agriculture led to increase the concentrations of chemical pollutant in the biosphere, which ultimately reach into aquatic environments and become responsible for the degradation of aquatic ecosystem[1,2]. The effect of chemical varies based on the quantities, toxicological and genotoxic potentials and at the same time the character of recipient water bodies (flowing or stagnant, sedimentation rate, temperature, salinity etc.). Many of these pollutants are non-biodegradable and can accumulate in the aquatic environment and in turn results biomagnifications in aquatic organisms and as well as in the consumer of aquatic products like humans. Thus they are harmful for the health of both human and other animals[3,4].

There are not many studies about the effect of heavy metals on pathogenicity of plant pathogens (bacteria, fungi and virus) but the heavy metals affect on plant growth parameters (photosynthesis pigments, the fresh and dry weight of shoot and root and mitotic index were decreased) which makes the plant weak against any infection and decreased the defenses against the pathogen that effect on plant production. The growth and yield characters of rice crop were not improved as a result of irrigation with treated wastewater[5]. The Increasing of soil contamination by heavy metal cadmium decreased the values of growth characteristics (root length, shoot length, root fresh mass, root dry mass, shoot fresh mass and shoot dry mass) and leaf water potential, whereas, activities of antioxidant enzymes and proline content increased with the increasing concentration of Cd[6]. The chlorophyll content was decreased when plants were irrigated with 100% raw wastewater, while enhanced at diluted wastewater due to high nutrients uptake[7]. The treatments with wastewater decreased chlorophyll content index in tomato cultivars[8].

Faba bean (*Vicia faba* L.) is one of the major crops grown in Egypt and many countries. Its recorded history in Egypt[9] at 1580. *Vicia faba* contain a high nutritive value in both energy and protein contents, it is a primary source of protein[10]. *Vicia faba* diseased by *Botrytis fabae* that is economic important disease and reduce faba bean production[11]. There are multiple sources of soil contamination with heavy metals such as industrial wastes, agriculture fertilizers and roadways. Crops grown in soils contaminated with heavy metals that are beside industrial sites suffer because of their uptake of heavy metals[12]. This available part of the heavy metals cause ecological, toxicological and health problems due to the possible penetration into most environmental segments, including food chains[13].

Many farmer in Egypt have been using the drainage water mixed with untreated sewage water for crops irrigation such as Tolompat parseqe village in Beheira Governorate Egypt. Farmers in many big cities have been using sewage contaminated water for irrigation of crops[14,15]. Wastewater irrigation is known to have a significant contribution to the heavy metal contents of soils[16,17].

This practice of loading heavy metals often leads to degradation of soil and contamination of food chain mainly through the vegetables grown on such soils[18]. The soils irrigated with sewage contaminated water led to decrease the production of crops. Heavy metals persist in the soil and can be adsorbed on soil particles or leached into groundwater. These metals, like other environmental stressors, also induce increased antioxidant enzyme activities in plants[19]. They are regarded as cumulative toxins which through biomagnifications in plants eventually affect human health[20]. It is a well-documented fact that serious health problems can develop as a result of excessive accumulation of dietary heavy metals such as Cd, Cr and Pb in the human body[21].

The use of untreated sewage sludge, industrial waste water or improper use of phosphate fertilizers in agriculture are progressively increasing the level of cadmium in the soil, which increases the accumulation of cadmium in crop tissues. Which not only poses threat to plant survival, but also induces several toxic responses and raises the danger of food adulteration in crop plant. The Cd competes with the uptake of other essential minerals and causes desiccation stress[22]. When taken up in the cellular environment it binds with membranes and enzymes interfering with their functions and stability[23]. Defense mechanisms are generated in plant species to resist of cadmium induced toxicity and to recover the subsequent damage[24,25] eliciting their genotype based biochemical responses. However, the resistance response relies on the interaction of genotype with a dose of toxicity to show comparative resistance.

Vicia faba is consider as a bioindicator for pollution and one of the most commonly used plant materials for cytological, radiobiological, soil toxicity particularly for cytotoxicity, genotoxicity and physiological studies[26]. In addition to its many favorable properties as a test material[27], *V. faba*, offer a wide range of possibilities of cytogenetic analyses. *Vicia faba* has been commonly used to study the effects produced by physical and chemical mutagens, having

the frequency of aberrations as an efficient indicator of mutagenic response[28]. *Vicia faba* has been recommended by the International Program on Chemical Safety (IPCS) to determine the root tip meristem chromosomal aberration assay for screening of chemicals for clastogenicity[29]. Also the effectiveness of *V. faba* chromosomal aberration assay has been used in assessing water quality conditions *in situ*[30]. Many farmers in Egypt irrigate crops with drainage water mixed with untreated sewage water, that may cause plant weakness, making them more susceptible to plant diseases. So the aim of this study determined the effect of irrigation by drainage water mixed with untreated sewage water on the susceptibility of Giza 3 cultivar to infection by *Botrytis fabae* through determination of Disease severity, enzyme activities, level of genes expression, percentage of total number of abnormalities cell, electrolytic leakage, growth parameters and mitotic index.

MATERIALS AND METHODS

Water sources: Tolompat Parseqe village, El Beheira Governorate, Egypt. The agriculture area of these village about 20,000 ha of different crops (cotton, wheat, rice, faba bean, clover, corn and vegetables). These crops are irrigated by drainage water mixed with untreated sewage water.

The experiments were replicated in two seasons. The drainage water mixed with untreated sewage water samples were collected from different sites in Tolompat Parseqe village.

Samples water analysis: During 2 winter seasons 2017 and 2018, the samples of drainage water mixed with untreated sewage water were collected in each season in different sites from Tolompat parseqe. The hypothesis governing the campaign is to provide good spatial coverage in the studied area. The mixed water and wastewater samples were collected using grab sampler at a depth of 10 cm below the surface. Tight-capped high quality polyethylene bottles were used for sample storage. Before use, the bottles were washed by distilled deionized water. The water samples brought to Pesticide Residue Analysis and Toxicity Laboratory (PRATL), Agriculture Faculty, Damanhour University to analysis.

Greenhouse experiments: The greenhouse experiment was conducted at Damanhour agriculture faculty. The *Vicia fabae* cultivar (Giza 3) was irrigated with different sources of water. The treatments were: Drainage water mixed with untreated sewage water+*Botrytis fabae* (DS+BF),drainage water mixed with untreated sewage water alone (DS), tap water+*Botrytis fabae* (T+BF) and tap water as control (T).

Isolation, purification and identification of *Botrytis fabae* isolate: Isolates were obtained from infected *Vicia fabe* cultivars and isolated on potato dextrose agar (PDA) medium[31]. To identify the pathogen isolate, the cultures were tentatively identified as *B. fabae* based on standard mycological parameters according to Subramanian[32] and maintained on PDA slants at 4°C for further study.

Inoculation with *B. faba*: *Vicia faba* plants (40 days old) were sprayed with spore suspension (2.5×10^5 spore mL^{-1}) of isolate. Three pots were used as replicates for each treatment. All pots were kept in the green house for 48 h at 20°C under high relative humidity. The inoculated plants were examined for chocolate spot disease and the data were recorded after 7 days post inoculation using the devised scale of Hanounik[33].

Assessment of growth parameters: Growth parameters were determined to achieve the objectives of this experiment after 47 days after seedling or 7 days after inoculation[34] such as:

- Shoot fresh and dry weight (g)
- Root fresh and dry weight (g)

Determination of photosynthetic pigments: Chlorophyll a, b and β-carotene were determined according to Wintermans and de Mots[35] as follows equations were used:

$$\text{Chl a (mg L}^{-1}) = 9.784\ E.662 - 0.99\ E.644$$

$$\text{Chl b (mg L}^{-1}) = 21.426\ E.644 - 4.65\ E.662$$

$$\text{Carotene (mg L}^{-1}) = 4.695\ E.440 - 0.268\ (\text{Chl a} - \text{Chl b})$$

where, E is the optical density at the wavelength indicated.

Electrolyte leakage: Relative electrolyte leakage (REC) or the membrane integrity index was calculated as a percentage of:

$$REC = \frac{EC1}{EC2}\times 100$$

where, EC1 and EC2 are the electrolyte conductivities measured before and after boiling, respectively[36].

Photosynthesis rate: Photosynthetic rate was measured as described by Akhkha[37] on the fully expanded fifth intact *Vicia faba* leaves of 47days old plants, using an LI-6400 XT Infra-Red Gas Analyser (IRGA), which was supplied by LICOR Inc. (Lincoln, NE, USA). Light intensities used were 0, 50, 150, 500, 750, 1000 and 1500 µmol quanta m^{-2} sec^{-1}.

Determination of mitosis index: Seeds were soaked in water for 12 h for each water source and then transferred to pots half-filled with sand. Three pots were used for each water source and 3 seeds were sown in each pot. The irrigation was continued till primary and secondary roots were appeared. division roots of 1-2 cm long for each examined plant were cut from treated and controlled and fixed in a freshly prepared carnoy fixation (3:1 v/v) (absolute ethyl alcohol:glacial acetic acid) for 24 h and preserved in 70% ethyl alcohol. Root tips squashes were made using 2% acetocarmine stain. Five slides were prepared per each treatment. The following parameters were determined: Mitotic index (MI) and chromosomal aberrations[38]:

$$\text{Mitotic index (MI)} = \frac{\text{TDC}}{\text{TC}} \times 100$$

$$\text{Total percentage of abnormal cells } (T_{Abn}) = \frac{TC_{Abn}}{\text{TDC}} \times 100$$

where, TDC is total dividing cells and TC is total dividing and non-dividing cells.

Determination of enzyme activates in *Vicia faba* against *Botrytis faba*

Estimation of peroxidase (POD) Activity: Approximately 1 g of leaf sample was homogenized in 2 mL of 0.1 M sodium phosphate buffer (pH 6.5) at 4°C. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C (Universal 32R, Hettich Zentrifugen model D-78532, Germany). The supernatant was used for POD enzyme activity. The reaction mixture was of 1.5 mL of pyrogallol (0.05 M), 0.5 mL of enzyme and 0.5 mL of H_2O_2 (1%). The reaction mixture was incubated at 28±2°C. The absorbance of the mixture was recorded every 20 sec interval for 3 min at 420 nm (Jenway UV/VIS spectrophotometer, Model 6305, Bibby Scientific Limited, Staffordshire, UK). Boiled enzyme preparation served as control[39]. The peroxidase activity was expressed as change in the absorbance of the reaction mixture min^{-1} g^{-1} of fresh tissue[40].

Estimation of polyphenol oxidase (PPO) activity: Leaf sample (1 g) was homogenized in 2 mL of 0.1 M sodium phosphate buffer (pH 6.5) using a pre-chilled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C (Universal 32R, Hettich Zentrifugen model D-78532, Germany) and supernatant was used as crude enzyme. Polyphenol oxidase activity was measured by mixing 1.5 mL of the phosphate buffer, 200 µL of the crude enzyme and 200 µL of catechol 0.01 M[41]. The reaction mixture was incubated at room temperature for 2 min and the absorbance was recorded at 495 nm (Jenway UV/VIS spectrophotometer, Model 6305, Bibby Scientific Limited, Staffordshire, UK). The changes in absorbance were recorded every 30 sec interval for 2 min and the activity was expressed as change in absorbance min^{-1} g^{-1} of fresh tissue[42].

Quantification of POD and PPO gene expression using real-time PCR: Total RNA was extracted from plant tissue using the GStract™ RNA Isolation kit II (Guanidinium Thiocyanate Method) (SA-40005, Maxim Biotech, Inc., USA) according to the manufacture's procedure.

Reverse transcription-polymerase chain reaction (RT-PCR) of mRNA: First-strand cDNA was synthesized in 25 µL reaction mixture contained 2.5 µL (5x) buffer with $MgCl_2$, 2.5 µL (2.5 mM) dNTPs, 1 µL (10 pmol) primer oligo (dT), 2.5 µL RNA (2 mg mL^{-1}) and 0.5 unit reverse transcriptase enzyme. The PCR amplification was performed in a thermal cycler programmed at 42°C for 1 h, 72°C for 10 min (enzyme inactivation) and the product was stored at 4°C until used.

POD and PPO gene expression using RT-qPCR: Samples were analyzed using the Fermentas kit (Sigma Egypt, Cairo) (Peng *et al.*[43]). Each reaction mixture had 12.5 µL of 2x Quantitech SYBR® Green RT Mix, 1 µL of 25 pm $µL^{-1}$ forward primer (Table 1), 1 µL of 25 pm $µL^{-1}$ reverse primer,1 µL of the cDNA (50 ng) and 9.5 µL of RNase free water for a total of 25 µL. Samples were mixed by spinning before loading in the Rotor's wells. The real time PCR program was as the following: Initial denaturation at 95°C for 10 min, 40 cycles of 95°C for 15 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec. Data acquisition performed during the extension step. This reaction was performed using Rotor-Gene- 6000-system (QIAGEN, USA).

Gene expression data analysis: The reaction was performed using a Rotor-Gene 6000 (Qiagen, ABI System, USA). Relative quantification of gene expression was performed[44] (ΔC q = C q-reference gene, ΔΔ C q = C q-control and ΔΔ C q expression = 2(-ΔΔCq). The expression levels of the target genes were normalized relative to 18S rRNA gene and relative expression of untreated control plants were set as T.

Table 1: Sequence of primers used in the real-time PCR

Primers	Primer sequence 5→3'
Peroxidase (F)	GCTTTGTCAGGGGTTGTGAT
Peroxidase (R)	TGCATCTCTAGCAACCAACG
Catalase (F)	AGGAGGCGGATCTAGCCTTA
Catalase (R)	TGTCAAGAAAGGGGTGTCGT
18S (F)	GTGCATGGCCGTTCTTAGTTG
18S (R)	CAGGCTGAGGTCTCGTTCGT

Statistical analysis: Data collected from *in vitro* and pot experiments were analyzed by two-way analysis of variance (treatments and times) using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Treatment means were separated by Duncan's multiple range test at 5% probability. Data of enzymes activity and gene expression were analyzed with GraphPad PRISM version 7 (GraphPad Software Inc.,California, USA). Tukey's HSD test was used to determine the significant differences between means at a probability level of $p \leq 0.05$.

RESULTS

The results in Table 2 showed the effect of irrigation by drainage water mixed with untreated sewage water on susceptibility of Giza 3 cultivar to infection by *Botrytis fabae*. The disease severity (PDS) was increased in irrigation by drainage water mixed with untreated sewage water+*Botrytis fabae* (DS+BF) compared the tap water+*Botrytis fabae* (T+BF)

The effect of irrigation by drainage water mixed with untreated sewage water on plant growth recorded in Table 3. Fresh weight and dry weight were decreasing after irrigation by drainage water mixed with untreated sewage water as compared irrigation by tap water as a control. The irrigation with DS+BF was the lowest decreased, followed by DS alone. While the T+BF was significantly increased compared with irrigation by DS+BF compared with the control (T).

The irrigation by drainage water mixed with untreated sewage water was decreased chlorophyll contents (chl a, b and carotene) that was observed in treatment with irrigation by DS+BF and irrigation by DS alone compared with control (T). While the T+BF was significant increased compared with the irrigation by DS+BF (Table 4).

Electrolyte leakage of Giza 3 cultivar showed significant increase by irrigation with drainage water mixed with untreated sewage water (Table 5). The higher degree of membrane injury caused by irrigation with DS+BF, followed by DS alone. While tap water (T) was the lowest in electrolyte leakage (27.800). There a significant increase between DS+BF and T+BF. The maximum photosynthesis rate (254.936 µmol CO_2 m^{-2} sec^{-1}) was observed in irrigation by DS+BF, followed by T+BF (209.948 µmol CO_2 m^{-2} sec^{-1}). The lowest of photosynthesis rate was in tap water T (89.908 µmol CO_2 m^{-2} sec^{-1}). While the irrigation by DS alone was 131.626 µmol CO_2 m^2 sec^{-1} (Table 5). There a significant increase in DS+BF compared to T+BF.

The analysis of water showed that the different concentrations of some heavy metals in irrigation water were detected such as Mn, Fe, Cu, Zn, Cd and Pb in the 2 seasons, while the concentrations was different from the first season to the second season (Table 6). The levels of the heavy metals in irrigation water were a high concentrations, specially Cu and Cd levels were a higher than the FAO's allowable limits. The GC-MS analysis of water showed many of compounds such as saponin (Table 7).

Table 2: Percent disease intensity of Giza 3 that irrigation by drainage water mixed with untreated sewage water after 7 days of inoculation by BF

Treatments	Disease intensity (%)
DS+BF	52.25±0.17
T+BF	35.56±1.23
DS	0.00
T	0.00

DS+BF: Drainage water mixed with untreated sewage water+*Botrytis fabae*, DS: Drainage water mixed with untreated sewage water alone, T+BF: Tap water+*Botrytis fabae* and tap water as control (T)

Table 3: Effect of irrigation by drainage water mixed with untreated sewage water on fresh weight and dry weight of Giza 3 to infection by *Botrytis fabae*

	Shoot weight plant (g)		Root weight plant (g)	
Treatments	Fresh	Dry	Fresh	Dry
T	11.47^{a}	1.01^{a}	5.26^{a}	0.59^{a}
DS+BF	8.27^{d}	0.55^{c}	4.20^{c}	0.22^{c}
T+BF	10.16^{b}	0.79^{b}	4.36^{b}	0.43^{b}
DS	9.20^{c}	0.69^{b}	3.51^{d}	0.34^{b}
$LSD_{0.05}$	0.92	0.12	0.84	0.10

*Data are average of 3 replicates, means followed by the same letter(s) are not significantly different at $p \leq 0.05$, DS+BF: Drainage water mixed with untreated sewage water+*Botrytis fabae*, DS: Drainage water mixed with untreated sewage water alone, T+BF: Tap water+*Botrytis fabae* and tap water as control (T)

Table 4: Effect of irrigation by drainage water mixed with untreated sewage water on photosynthesis pigments of Giza 3 cultivar to infection by *Botrytis faba*

Treatments	Chl a	Chl b	Carotene
T	0.89^{a}	0.63^{a}	0.42^{a}
DS+BF	0.72^{c}	0.51^{d}	0.31^{c}
T+BF	0.80^{b}	0.59^{b}	0.35^{b}
DS	0.75^{b}	0.55^{c}	0.37^{b}
$LSD_{0.05}$	0.05	0.03	0.03

Data are average of 3 replicates, means followed by the same letter(s) are not significantly different at $p \leq 0.05$, DS+BF: Drainage water mixed with untreated sewage water+*Botrytis fabae*, DS: Drainage water mixed with untreated sewage water alone, T+BF: Tap water+*Botrytis fabae* and tap water as control (T)

Table 5: Effect of irrigation by drainage water mixed with untreated sewage water on electrolytic leakage (EC) and photosynthetic rate of Giza 3 cultivar to infection by *Botrytis fabae*

Treatments	EC (µS CO_2 m^{-2})	CO_2 (µmol m^{-2} sec^{-1})
T	27.80^{d}	89.90^{d}
DS	31.36^{b}	131.62^{c}
T+BF	29.13^{c}	209.94^{b}
DS+BF	32.83^{a}	254.93^{a}
LSD	1.24	8.17

Data are average of 3 replicates, means followed by the same letter(s) are not significantly different at $p \leq 0.05$, DS+BF: Drainage water mixed with untreated sewage water+*Botrytis fabae*, DS: Drainage water mixed with untreated sewage water alone, T+BF: Tap water+*Botrytis fabae* and tap water as control (T)

Mitotic index measurements were given in Table 8. Mitotic index value of Giza 3 was irrigated by drainage water mixed with untreated sewage water was decreased (16.61%) compared to tap water (20%). The percentage of total abnormalities is given in Table 8. The highest value was scored in the plants that irrigated by drainage water mixed with untreated sewage water (27.50%) compared to tap water (9.38%).

Types and percentages of mitotic abnormalities were listed in Table 8. In the present study irrigated by drainage water mixed with untreated sewage water induced different types of abnormalities (Fig. 1) such as stickiness, breaks, bridges, lagging chromosomes, micronuclei, multipolar cell, C-metaphase and polyploidy. The highest value of stickiness was recorded in plants which irrigated by drainage water mixed with sewage water (8.33%), followed by bridge (4.17%), while the micronuclei, lagging chromosome and polyploidy recorded the lowest value (1.67%) compared the control (T).

Antioxidant enzymes peroxidase (POD) and polyphenol oxidase (PPO) showed an enhanced increase in their activities in Giza 3 cultivar was irrigated by drainage water mixed with untreated sewage water as compared with the control (Table 9). Giza 3 was irrigated by drainage water mixed with untreated sewage water were significantly increased the enzyme activities in all treatments. The maximum activity POD and PPO enzyme were observed in DS+BF, followed by DS alone compared with control (T). The irrigation by DS+BF and DS were significant increase of enzyme activities compared with T+BF.

Giza 3 was irrigated by drainage water mixed with untreated sewage water increased the gene expression in all treatments (Fig. 2). The maximum level POD and PPO gene expression were observed in DS+BF, followed by DS alone compared with control (T).

Table 6: Concentration (mg L^{-1}) of some heavy metals in drainage water mixed with untreated sewage water samples in tolompat parseqe

	Samples type (mg L^{-1})					
Seasons	Mn	Fe	Cu	Zn	Cd	Pb
1	0.180	0.586	0.235	0.130	0.056	0.042
2	0.192	0.577	0.221	0.153	0.047	0.038

Table 7: GC-MS analysis of irrigation with drainage water mixed with untreated sewage water at the two seasons

		Season (Rt)	
Chemicals	Class	1	2
1-(2-Acetoxyethyl)-3,6-diazahomoadamantan-9-one oxime	Azolines	5.65	4.32
11,16-Bis(acetyloxy)-3,20-dioxopregn-4-en-21-yl acetate	Prostaglandin	6.41	6.23
Dodecamethylcyclohexasiloxane	Cyclosiloxane	7.13	6.89
2-(9-Borabicyclo[3.3.1]non-9-yloxy)-3-([2-(9-borabicyclo[3.3.1] phenyl non-9-yloxy)ethyl]sulfanyl)propyl ether	Organoborane	7.28	8.34
((5LPregnane- 3,20 L diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	Steroids	8.04	8.12
2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	Alkaloid	8.21	7.43
3-Hydroxyspirost-8-en-11-one	Sterol	10.91	10.65
Phthalic acid, isobutyl octadecyl ester	Phthalate (PVC plasticizer)	13.30	14.32
1,2-Benzenedicarboxylic acid, dibutyl ester	Phthalate	14.32	14.71
Decamethylcyclopentasiloxane	Saponin cyclic siloxane	5.37	4.77
Hexadecamethyl-cyclooctasiloxane	Saponin cyclosiloxane (shampoo, antifoaming agent)	11.10	10.76
1-Hexadecanol, 2-methyl-	Saponin, fatty alcohol (skin creams and lotion)	26.23	27.01
2,3-Bis[(trimethylsilyl)oxy]propyl (9E,12E,15E)-9,12,15-octadecatrienoate	Triglycerides (oleic acid)	18.18	18.12
6,9,12,15-Docosatetraenoic acid, methyl ester	Triglycerides (linolenic acid)	19.83	20.32
Cyclopropaneoctanoic acid, 2-octyl-, methyl ester	Triacylglycerols	22.08	22.13
16-Octadecenoic acid, methyl ester	Triglycerides (fatty acid)	26.61	23.45
9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	Triglycerides	32.19	35.07
Olean-12-ene-3,15,16,21,22,28-hexol, (3β,15α,16α,21β,22α)-	Triglycerides	35.05	32.21

Table 8: Percentage of mitotic index, total abnormal cells and types of mitotic abnormalities induced by irrigation by drainage water mixed with untreated sewage water and tap water

			Types and percentage of mitotic abnormalities							
Water sources	Mitotic index	Total number of abnormal cell	Stickiness	Break	Bridge	Lagging chromosome	Micronuclei	Multi-polar cell	C-metaphase	Polyploidy
Tap water	20.00	9.38	4.37	2.18	1.87	0.00	0.31	0.62	0.00	0.00
Drainage mixed sewage	16.61	27.50	8.33	3.75	4.17	1.67	1.67	2.50	3.33	1.67

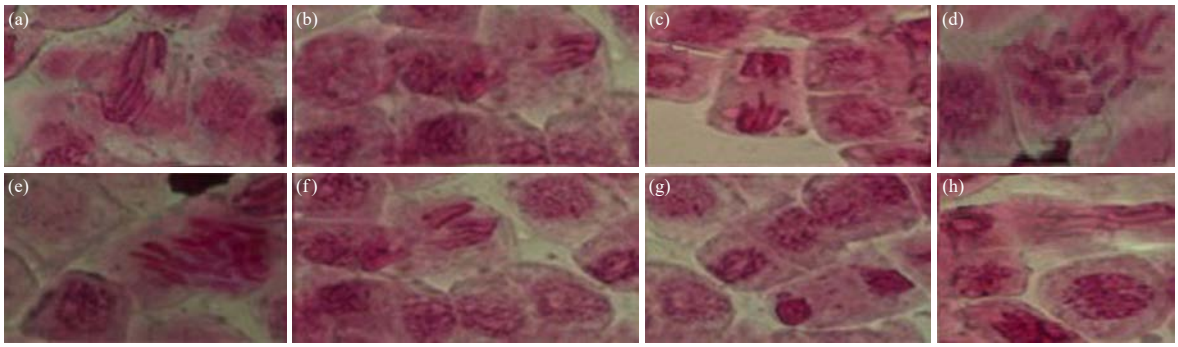

Fig. 1(a-h): Types of abnormalities induced in different stages of mitosis in *V. faba* root tip cells irrigated with drainage water mixed with untreated sewage water, (a) Chromosome bridge, (b) Chromosome breaks in anaphase, (c) Lagging chromosome in anaphase, (d) Polyploidy, (e) C-metaphase, (f) Multipolar cell, (g) Micronuclei in anaphase and (h) Stickiness in prophase

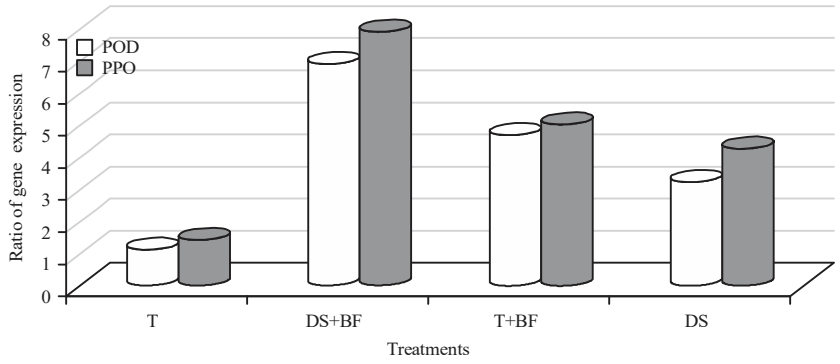

Fig. 2: Effect of irrigation by drainage water mixed with untreated sewage water on POD and PPO gene expression of Giza 3 cultivar

BF+DS: Drainage water mixed with untreated sewage, T+BF: Tap water+*Botrytis fabae*, DS+BF: Drainage water mixed with untreated sewage+*Botrytis fabae*, T: Tap water, Each value represents Mean±SE (n = 3)

Table 9: Effect of irrigation by drainage water mixed with untreated sewage water on POD and PPO enzyme activates of Giza 3 cultivar to infection by BF

Treatments	POD	PPO
T	0.52^{d}	0.67^{c}
DS+BF	0.98^{a}	1.37^{a}
T+BF	0.68^{c}	1.11^{b}
DS	0.73^{b}	0.95^{b}
$LSD_{0.05}$	0.13	0.24

Data are average of 3 replicates, means followed by the same letter(s) are not significantly different at p<0.05

DISCUSSION

This research study the impact of the irrigation with drainage water mixed with untreated sewage water on the susceptibility of *Vicia faba* cultivar to infection by *Botrytis fabae*. There is not many studies about the effect of heavy metals on susceptibility of the plant to diseased by plant pathogens. The results showed that impact of the irrigation with drainage water mixed with untreated sewage water increase the susceptibility of crops to infection by *Botrytis fabae*.

The analysis of irrigation water in this study showed that the heavy metals (Mn, Fe, Cu, Zn, Cd and Pb) were found in high concentrations in the two seasons, specially Cu and Cd levels were a higher than the FAO's allowable limits. Some studies that reported the allowable limits of the levels of some heavy metals in irrigation water are available elsewhere[45,46]. The GC-MS analysis of water showed many of compounds such as saponin. High levels of these metals could be attributed to the contribution from anthropogenic sources. Industrial and/or domestic activities may be the source of contribution by Cu in the environment of the study area.

The pathogenicity was increased in irrigation by drainage water mixed with untreated sewage water (52.25%) compared the tap water (35.56%). But it's also effected on plant growth (photosynthesis pigments, the fresh and dry weight of shoot and root and mitotic index) were decreased which makes the plant weak against any infection and decreased the defenses against the pathogen that effect on plant production.

In this study the fresh weight and dry weight were decreasing after irrigation by drainage water mixed with untreated sewage water as compared irrigation by tap water as a control. While the tap water+BF(T+BF) was significant increased compared the irrigation by drainage water mixed with untreated sewage water+BF(DS+BF). The decrease in some growth parameters under some treatments in some genotypes carried out by Alghobar and Suresha[5], who reported that growth and yield characters of rice crop were not improved as a result of irrigation with treated waste water. Increasing contamination by heavy metal cadmium in soil decreased the values of growth characteristics (root length, shoot length, root fresh mass, root dry mass, shoot fresh mass and shoot dry mass) and leaf water potential, whereas, activities of antioxidant enzymes and proline content increased with the increasing concentration of Cd[6].

Chlorophyll content measured as chlorophyll content index is another limiting factor of photosynthesis, the present study found that this parameter was reduced under irrigation with drainage water mixed with untreated sewage water compared the irrigation by tap water. While tap water+BF (T+BF) was significant decreased compared with the irrigation by drainage water mixed with untreated sewage water+BF (DS+BF). This was in line with the study carried out by El-Nahhal *et al.*[7], who observed that chlorophyll content was decreased when plants were irrigated with 100% raw wastewater, while enhanced at diluted wastewater due to high nutrients uptake. The treatments with wastewater decreased chlorophyll content index in tomato cultivars[8].

The present study confirmed that the effect of irrigation with drainage water mixed with untreated sewage water on the rate of photosynthesis increased compared the irrigation by tap water and there was significant increase in drainage water mixed with untreated sewage water+BF (DS+BF) compared to tap water+BF (T+BF). These results agree with the findings of Tak *et al.*[47], who reported that wastewater irrigation of chickpea plants increased the rate of photosynthesis and such increase was reflected in the growth parameters and wastewater irrigation of tomato[8]. Singh and Agrawal[48] also reported an increase in the rate of photosynthesis when plants were irrigated with wastewater compared to ground water irrigated ones.

It was observed that electrolyte leakage was a significant increase of Giza 3 cultivar showed significant increase by irrigation with drainage water mixed with untreated sewage water compared tap water, while we observed a significant increase between drainage water mixed with untreated sewage water+BF(DS+BF) and tap water+BF(T+BF). The effects of certain heavy metals such as copper, iron and cadmium can increase the electrolyte leakage[49]. There was a significant increase in electrolyte leakage (EL%) of broad bean leaves with increasing the duration of chilling stress revealing the disturbance of plasma membrane integrity and that might be attributed to generation of ROS[50,51].

Cytotoxicity defined as a decrease in the mitotic index and it considered as an acceptable measure of cytotoxicity for all living organism[52]. Mitotic index is considered a parameter that allows estimating the frequency of cellular division[53,54]. The mitotic index values showed a significant reduction values[55] in *Allium cepa* under effects of industrial wastewater. In present study, the mitotic index values showed decreased compared the control. The reduction in mitotic index by cytotoxic substances and polluted water may be due to the effect on microtubule configuration[55,56] or the blocking of the mitotic cycle during interphase[57] or the inhibition of DNA synthesis[58], which could be due to blocking of G1, there by suppressing DNA synthesis[59]. Blocking in G2 prevents the cell from entering V' H 96 p synthesis in the cell cycle[60], which leads to inhibit the formation of various metabolic events necessary for mitosis[61]. Moreover, the present study showed that many abnormalities such as stickiness, breaks, bridges, lagging chromosomes, micronuclei, multipolar cell, C-metaphase and polyploidy. In addition to the highest number of sticky chromosomes was recorded under the effect of irrigation by drainage water mixed with untreated sewage water . These results were in agreement with that obtained in *Vicia faba*[55] and *A. cepa* of wastewater[62]. Moreover, there was a negative correlation between mitotic index and mitotic abnormalities as previously recorded in *Allium cepa*[63]. All metals can exhibit toxicity at different levels. Heavy metals are toxic to all organisms when higher concentrations of them is present in the soil[64].

The cellular stress response is a ubiquitous defense mechanism when cells are treated with different chemicals and wastewater. The induction of the stress response leads to expression of a group of proteins referred to as stress proteins, which are thought to protect the cell[65]. Activities of peroxidase expression have been shown in several plant systems to be altered by stress chemicals and infection[66]. Under stress conditions, the enhanced peroxidase activity in the intercellular spaces can probably lead to reduction of cell

growth, stimulating cell wall stiffening. It has been observed that peroxidase induction is a general response of higher plants to the uptaking of toxic amounts of metals in roots and leaves of various species after application of toxic doses[67] of Zn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+}. Also different plants can give different responses against wastewater treatment in terms of peroxidase activities as in this study. In this study the antioxidant enzymes (peroxidase and polyphenol oxidase) showed an enhanced increase in their activities in giza 3 was irrigation with drainage water mixed with untreated sewage water as compared with the control, while the irrigation by drainage water mixed with untreated sewage water+BF (DS+BF) and drainage water mixed with untreated sewage water were significant increase of enzyme activates compared with tap water+BF (T+BF). The enzyme activities of catalase and peroxidase were increased in wastewater-irrigated maize seedlings[68]. The polyphenol oxidase activity (PPO) in pisum grown under various waste stresses showed a progressive increase in plants irrigated with wastewater in all sites as compared with the control. The antioxidant enzymes play a significant role in defense system against oxidative stress and considered to be as indicators of metal toxicity[69]. The POD and PPO gene expression were increased of under salt stress in all broad bean cultivars[39]. The resistance responses were investigated by El-Komy[70] during the interaction of *Botrytis fabae* with two faba bean cultivars expressing different levels of resistance against this pathogen. These results indicated that the induction of oxidant/antioxidant responses and the accumulation of PRPs are part of the faba bean defense mechanism against the necrotrophic fungus *B. fabae* with a different intensity and timing of induction, depending on the resistance levels.

CONCLUSION

Current findings on use the irrigation of crops with drainage water mixed with untreated sewage water increase the susceptibility to infection by plant pathogens. Water should be treated before irrigation in order to avoid increased pathogenicity, decreased productivity and reduced genetic toxicity affecting human health.

SIGNIFICANCE STATEMENT

This study discover the irrigation with drainage water mixed with untreated sewage water (polluted water) increase the susceptibility of crops to infection by plant pathogens, but also reconfirmed with pathological molecular analysis that can be beneficial for determinate the causes of increasing plant infection rates in developing countries that irrigate crops with drainage water mixed with untreated sewage water that led to decrease the crop productivity and thus increase toxicological and genotoxic, which affects health and increases the spread of human diseases. Most recent studies did not address the effect of irrigation with polluted water on crop sensitivity to pathogens, but studied with the effect on crop productivity and measures of plant growth. This study measures the impact on the rate of infection because the irrigation with this water makes the plant weak and low resistance to pathogens. This study is used to know the effect of this water on the genes and metabolic pathways in *Vicia faba*e plant such as salicylic pathway.

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REFERENCES

1. Wang, W.X., 2002. Interactions of trace metals and different marine food chains. Mar. Ecol. Prog. Ser., 243: 295-309.
2. Dautremepuits, C., S. Paris-Palacios, S. Betoulle and G. Vernet, 2004. Modulation in hepatic and head kidney parameters of carp (*Cyprinus carpio* L.) induced by copper and chitosan. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., 137: 325-333.
3. Kalay, M., O. Ay and M. Canil, 1999. Heavy metal concentrations in fish tissues from the Northeast Mediterranean sea. Bull. Environ. Contam. Toxicol., 63: 673-681.
4. Ashraf, W., 2005. Accumulation of heavy metals in kidney and heart tissues of *Epinephelus microdon* fish from the Arabian Gulf. Environ. Monit. Assess., 101: 311-316.
5. Alghobar, M.A. and S. Suresha, 2016. Effect of wastewater irrigation on growth and yield of rice crop and uptake and accumulation of nutrient and heavy metals in soil. Applied Ecol. Environ. Sci., 4: 53-60.
6. Irfan, M., A. Ahmad and S. Hayat, 2014. Effect of cadmium on the growth and antioxidant enzymes in two varieties of *Brassica juncea*. Saudi J. Biol. Sci., 21: 125-131.
7. El-Nahhal, Y., K. Tubail, M. Safi and J. Safi, 2013. Effect of treated waste water irrigation on plant growth and soil properties in Gaza Strip, Palestine. Am. J. Plant Sci., 4: 1736-1743.

8. Akhkha, A., T. Boutraa and A.K. Al-Shoaibi, 2017. The influence of Al-Madinah Al-Munawwara treated and untreated domestic wastewater on growth and physiology of three tomato (*Lycopersicon esculentum* Mill.) genotypes. Pak. J. Bot., 49: 879-890.
9. Abdalla, M.M.F., 1979. The origin and evoluation of *Vicia faba*. Proceedings of the First Mediterranean Conference of Genetics, March 28-30, 1979, Cario, Egypt, pp: 713-746.
10. Nassib, A.M., S.A. Khalil and A.H.A. Hussein, 1991. Faba bean production and consumption in Egypt. Options Mediterr., 10: 127-131.
11. Awaad, H.A., A.H. Salem, A.M.A. Mohsen, M.M.M. Atia, E.E. Hassan, M.I. Amer and A.M. Moursi, 2005. Assessment of some genetic parameters for resistance to leaf miner, chocolate spot, rust and yield of faba bean in F_2 and F_4 generations. Egypt. J. Plant Breed., 9: 1-15.
12. Teklic, T., J.T. Hancock, M. Engler, N. Paradikovic and V. Cesar *et al*., 2008. Antioxidative responses in radish (*Raphanus sativus* L.) plants stressed by copper and lead in nutrient solution and soil. Acta Biol. Cracoviensia: Ser. Bot., 50: 79-86.
13. Kucharski, R., A. Sas-Nowosielska, E. Malkowski, J. Japenga, J.M. Kuperberg, M. Pogrzeba and J. Krzyzak, 2005. The use of indigenous plant species and calcium phosphate for the stabilization of highly metal-polluted sites in Southern Poland. Plant Soil, 273: 291-305.
14. Ghafoor, A., A. Rauf, M. Arif and W. Muzaffar, 1994. Chemical composition of effluents from different industries of the Faisalabad city. Pak. J. Agric. Sci., 31: 367-370.
15. Lone, M.I. and M. Rizwan, 1997. Evaluation of industrial effluents fore irrigation and their effect on soil chemical properties. Proceedings of the International Symposium on Advanced Materials, September 17-21, 1997, Islamabad, Pakistan, pp: 269-279.
16. Mapanda, F., E.N. Mangwayana, J. Nyamangara and K.E. Giller, 2005. The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. Agric. Ecosyst. Environ., 107: 151-165.
17. Nan, Z., J. Li, J. Zhang and G. Cheng, 2002. Cadmium and zinc interactions and their transfer in soil-crop system under actual field conditions. Sci. Total Environ., 285: 187-195.
18. Rattan, R.K., S.P. Dutta, S. Chandra and N. Saharaan, 2002. Heavy metals in environments-Indian scenario. Fertile News, 47: 21-40.
19. Iannelli, M.A., F. Pietrini, L. Fiore, L. Petrilli and A. Massacci, 2002. Antioxidant response to cadmium in *Phragmites australis* plants. Plant Physiol. Biochem., 40: 977-982.
20. Groten, J.P. and P.J. van Bladeren, 1994. Cadmium bioavailability and health risk in food. Trends Food Sci. Technol., 5: 50-55.
21. Oliver, M.A., 1997. Soil and human health: A review. Eur. J. Soil Sci., 48: 573-592.
22. Hernandez, L.E., R. Carpena-Ruiz and A. Garate, 1996. Alterations in the mineral nutrition of pea seedlings exposed to cadmium. J. Plant Nutr., 19: 1581-1598.
23. Karcz, W. and R. Kurtyka, 2007. Effect of cadmium on growth, proton extrusion and membrane potential in maize coleoptile segments. Biol. Plant., 51: 713-719.
24. Meharg, A.A., 1994. Integrated tolerance mechanisms: Constitutive and adaptive plant responses to elevated metal concentrations in the environment. Plant Cell Environ., 17: 989-993.
25. Mohamed, A.A., A. Castagna, A. Ranieri and L.S. di Toppi, 2012. Cadmium tolerance in *Brassica juncea* roots and shoots is affected by antioxidant status and phytochelatin biosynthesis. Plant Physiol. Biochem., 57: 15-22.
26. White, P.A. and L.D. Claxton, 2004. Mutagens in contaminated soil: A review. Mutat. Res., 567: 227-345.
27. Kihlman, B.A., 1971. Root Tips for Studying the Effects of Chemicals on Chromosomes. In: Chemical Mutagens, Volume 2: Principles and Methods for their Detection, Hollaender, A. (Ed.). Plenum Press, New York, USA., ISBN: 978-1-4615-8971-6, pp: 489-514.
28. Villalobo-Spietrini, R., A.R. Flores-Marquez and S. Gomez-Arroyo, 1994. Cytogenetic effects in *Vicia faba* of the polluted water from rivers of the Tlaxcala hydrological system, Mexico. Rev. Int. Contam. Ambient., 10: 83-87.
29. Kanaya, N., B.S. Gill, I.S. Grover, A. Murin, R. Osiecka, S.S. Sandhu and H.C. Andersson, 1994. *Vicia faba* chromosomal aberration assay. Mutat. Res./Fundam. Mol. Mech. Mutagen., 310: 231-247.
30. Grant, W.F., H.G. Lee, D.M. Logan and M.F. Salamone, 1992. The use of *Tradescantia* and *Vicia faba* bioassays for the in situ detection of mutagens in an aquatic environment. Mutation Res., 270: 53-64.
31. Adss, I.A.A., M.A. Abdel-Gayed, W. Botros and E.E. Hafez, 2017. Multilocus genetic techniques, RAPD-PCR and ISSR-PCR markers and polygalacturonase activity as tools for differentiation among *Alternaria solani* isolates on tomato fruits and relation to their pathogenicity in Egypt. Asian J. Plant Pathol., 11: 18-27.
32. Subramanian, C.V., 1971. Hyphomycetes: An Account of Indian Species, Except Cercosporae. Indian Council of Agricultural Research, New Delhi, India, Pages: 930.
33. Hanounik, S.B., 1986. Screening Techniques for Disease Resistance in Faba Bean. International Center for Agricultural Reesearch in the Dry Areas (ICARDA), Aleppo, Syria, Pages: 59.
34. El-Argawy, E. and I.A. Adss, 2016. Quantitative gene expression of peroxidase, polyphenoloxidase and catalase as molecular markers for resistance against *Ralstonia solanacearum*. Am. J. Mol. Biol., 6: 88-100.
35. Wintermans, J.F.G.M. and A.S. de Mots, 1965. Spectrophotometric characteristics of chlorophylls A and B and their phenophytins in ethanol. Biochim. Biophys. Acta (BBA)-Biophys. Incl. Photosynth., 109: 448-453.

36. Shi, Q., Z. Bao, Z. Zhu, Q. Ying and Q. Qian, 2006. Effects of different treatments of salicylic acid on heat tolerance, chlorophyll fluorescence and antioxidant enzyme activity in seedlings of *Cucumis sativa* L. Plant Growth Regul., 48: 127-135.
37. Akhkha, A., T. Boutraa and A. Alhejely, 2011. The rates of photosynthesis, chlorophyll content, dark respiration, proline and abscicic acid (ABA) in wheat (*Triticum durum*) under water deficit conditions. Int. J. Agric. Biol., 13: 215-221.
38. Bhatta, P. and S.R. Sakya, 2008. Study of mitotic activity and chromosomal behaviour in root meristem of *Allium cepa* L. treated with magnesium sulphate. Ecoprint: Int. J. Ecol., 15: 83-88.
39. Adss, I.A., E. Abdel Raouf and M. Eldakrory, 2015. Effects of salt stress on gene expression and activities of POD and PPO in of broad bean (*Vicia faba* L.) cultivars. Alex. J. Agric. Res., 60: 65-75.
40. Hammerschmidt, R. and J. Kuc, 1982. Lignification as a mechanism for induced systemic resistance in cucumber. Physiol. Plant Pathol., 20: 61-71.
41. Nassar, A.M.K. and I.A.A. Adss, 2016. 2,4-dichlorophenoxy acetic acid, abscisic acid and hydrogen peroxide induced resistance-related components against potato early blight (*Alternaria solani*, Sorauer). Ann. Agric. Sci., 61: 15-23.
42. Mayer, A.M., F. Harel and R. Ben-Shaul, 1966. Assay of catechol oxidase a critical comparison of methods. Phytochemistry, 5: 783-789.
43. Peng, J.L., Z.L. Bao, H.Y. Ren, J.S. Wang and H.S. Dong, 2004. Expression of $Harpin_{Xoo}$ in transgenic tobacco induces pathogen defense in the absence of hypersensitive cell death. Phytopathology, 94: 1048-1055.
44. Livak, K.J. and T.D. Schmittgen, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta}C_T$ method. Methods, 25: 402-408.
45. Ayers, R.S. and D.W. Westcot, 1985. Water quality for agriculture. FAO Irrigation Drainage Paper No. 29, Food and Agriculture Organization of the United Nation, Rome, Italy.
46. Chapman, D., 1996. Water Quality Assessments: A Guide to the Use of Biota, Sediments and Water in Environmental Monitoring. 2nd Edn., E&FN Spon, London, UK., ISBN-13: 9780203476710, Pages: 648.
47. Tak, H.I., A. Inam and A. Inam, 2010. Effects of urban wastewater on the growth, photosynthesis and yield of chickpea under different levels of nitrogen. Urban Water J., 7: 187-195.
48. Singh, A. and M. Agrawal, 2010. Effects of municipal waste water irrigation on availability of heavy metals and morpho-physiological characteristics of *Beta vulgaris* L. J. Environ. Biol., 31: 727-736.
49. Benavides, M.P., S.M. Gallego and M.L. Tomaro, 2005. Cadmium toxicity in plants. Braz. J. Plant Physiol., 17: 21-34.
50. Taspinar, M.S., R. Dumlupinar, F. Demir, T. Cakmak and Y. Gulen, 2009. Determination of chilling temperature effects on nutrient elements composition and distribution in cole (*Brassica Oleracea* L. Cv. Acephala) using the WDXRF spectroscopic technique. Afr. J. Biotechnol., 8: 6569-6575.
51. Hassan, A., N. Saber and H.M. Abdulaziz, 2017. Changes of enzymatic antioxidants and minerals content of broad bean (*Vicia faba* L.) plants in response to chilling stress and reacclimation. Egypt. J. Bot., 57: 539-553.
52. Smaka-Kincl, V., P. Stegnar, M. Lovka and M.J. Toman, 1996. The evaluation of waste, surface and ground water quality using the *Allium* test procedure. Mutat. Res./Genet. Toxicol., 368: 171-179.
53. Leme, D.M. and M.A. Marin-Morales, 2009. *Allium cepa* test in environmental monitoring: A review on its application. Mutat. Res./Rev. Mutat. Res., 682: 71-81.
54. Marcano, L., I. Carruyo, A. Del Campo and X. Montiel, 2004. Cytotoxicity and mode of action of maleic hydrazide in root tips of *Allium cepa* L. Environ. Res., 94: 221-226.
55. Mattar, M.Z., R.H. Sammour, S.A. Haroun, A.S. Elkholy and M.L. Seada, 2014. Cytogenetic impact of different types of polluted water on *Vicia faba* L. at Kafr El-Sheikh governorate, Egypt. J. Am. Sci., 10: 100-107.
56. Armbruster, B.L., W.T. Molin and M.W. Bugg, 1991. Effects of the herbicide dithiopyr on cell division in wheat root tips. Pestic. Biochem. Physiol., 39: 110-120.
57. Mohandas, T. and W.F. Grant, 1972. Cytogenetic effects of 2,4-D and amitrole in relation to nuclear volume and DNA content in some higher plants. Can. J. Genet. Cytol., 14: 773-783.
58. Chand, S. and S.C. Roy, 1981. Effects of herbicide 2,4-dinitrophenol on mitosis, DNA, RNA and protein synthesis in *Nigella sativa* L. Biol. Plant., 23: 198-202.
59. Schneiderman, M.H., W.C. Dewey and D.P. Highfield, 1971. Inhibition of DNA synthesis in synchronized Chinese hamster cells treated in G1 with cycloheximide. Exp. Cell Res., 67: 147-155.
60. Kim, J.C. and E.L. Bendixen, 1987. Effects of haloxyfop and CGA-82725 on cell cycle and cell division of oat (*Avena sativa*) root tips. Weed Sci., 35: 769-774.
61. Rost, T.L. and S.L. Morrison, 1984. The comparative cell cycle and metabolic effects of chemical treatments on root tip meristems. II. Propham, chlorpropham and 2, 4-dinitrophenol. Cytologia, 49: 61-72.
62. Amin, A.W., 2002. Cytotoxicity testing of sewage water treatment using *Allium cepa* chromosome aberrations assay. Pak. J. Biol. Sci., 5: 184-188.

63. Ateeq, B., M. Abul Farah, M.N. Ali and W. Ahmad, 2002. Clastogenicity of pentachlorophenol, 2,4-D and butachlor evaluated by *Allium* root tip test. Mutat. Res./Genet. Toxicol. Environ. Mutagen., 514: 105-113.
64. Arnebrant, K., E. Baath and A. Nordgren, 1987. Copper tolerance of microfungi isolated from polluted and unpolluted forest soil. Mycologia, 79: 890-895.
65. Gabara, B., M. Sklodowska, A. Wyrwicka, S. Glinska and M. Gapinska, 2003. Changes in the ultrastructure of chloroplasts and mitochondria and antioxidant enzyme activity in *Lycopersicon esculentum* Mill. leaves sprayed with acid rain. Plant Sci., 164: 507-516.
66. Herbette, S., C. Lenne, D.T. de Labrouhe, J.R. Drevet and P. Roeckel-Drevet, 2003. Transcripts of sunflower antioxidant scavengers of the SOD and GPX families accumulate differentially in response to downy mildew infection, phytohormones, reactive oxygen species, nitric oxide, protein kinase and phosphatase inhibitors. Physiol. Plant., 119: 418-428.
67. Sinha, S., S. Singh and S. Mallick, 2008. Comparative growth response of two varieties of *Vigna radiata* L. (var. PDM 54 and var. NM 1) grown on different tannery sludge applications: Effects of treated wastewater and ground water used for irrigation. Environ. Geochem. Health, 30: 407-422.
68. Daud, M.K., S. Hassan, A. Azizullah, M. Jamil and N. Rehan *et al*., 2016. Physiological, biochemical and genotoxic effects of wastewater on maize seedlings. Pol. J. Environ. Stud., 25: 563-571.
69. Singh, R.P. and M. Agrawal, 2007. Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of *Beta vulgaris* plants. Chemosphere, 67: 2229-2240.
70. El-Komy, M.H., 2014. Comparative analysis of defense responses in chocolate spot-resistant and -susceptible faba bean (*Vicia faba*) cultivars following infection by the necrotrophic fungus *Botrytis fabae*. Plant Pathol. J., 30: 355-366.