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Modulation of cytotoxic effects of wastewater on barely seedlings by *Aloe vera* extract

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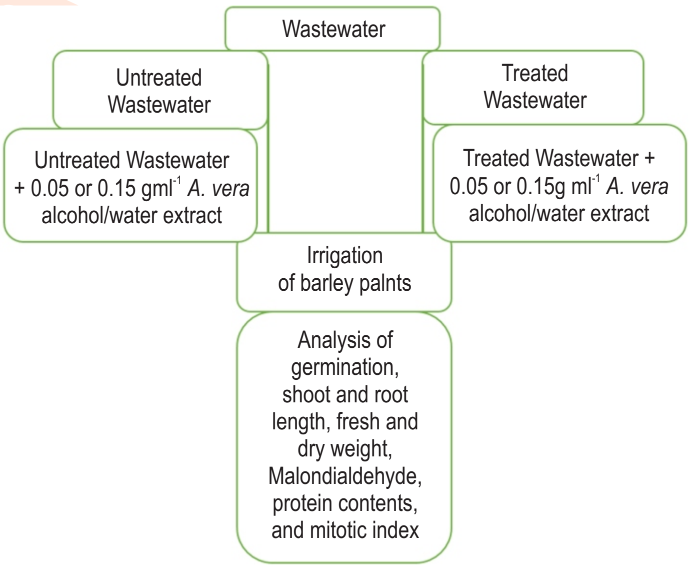
Abstract

Aim: This study was conducted to assess the mitigation effect of *A. vera* leaf ethanolic and water extracts on plant growth, MDA and protein content, and mitotic index of barley seedlings irrigated with sewage water.

Methodology: The experiment was conducted in a green house in Al-Ahsa, Saudi Arabia, in plastic bags containing air-dried loam soil and compost (1:1). Wastewater samples were collected from Al-Ahsa Sewage Treatment Plant/Al-Ahsa/Saudi Arabia. Seeds of barley were exposed to the following treatments for three weeks: 100% treated wastewater, treated wastewater mixed with: 0.05 g ml^{-1} *A. vera* alcoholic extract (Alc Ext) or 0.05 g ml^{-1} *A. vera* water extract (Wat Ext), 0.15 g ml^{-1} Alc Ext or 0.15 g ml^{-1} Wat Ext. Similar test doses were prepared for untreated wastewater. A separate set of seeds was prepared using distilled water as a control. Germination rate, fresh and dry weight, protein content, MDA content and mitotic index were determined.



Results: Untreated wastewater caused significant reduction in fresh weight while both treated and untreated wastewater showed insignificant decrease in dry weight of barley. No significant change in MDA contents were observed in all treatments, except for addition of 0.15 *A. vera* water extract to untreated wastewater. Protein content showed an increase in both treated wastewater (insignificant) and untreated wastewater (significant). Addition of *A. vera* extracts increased accumulation of protein in both shoot and root. *A. vera* alcoholic extract promoted significant increase in both root and shoot protein of treated wastewater, but only shoot protein of untreated wastewater. A significant reduction in mitotic index was induced by wastewater, while addition of *A. vera* extracts stimulated significant increase in mitotic index.

Interpretation: *A. vera* possess reactive oxygen species scavenging ability and enzyme conservation activity in addition to stimulatory effects on cell proliferation and cell-cycle process that add a modulation credit on protein content and mitotic index of barley.

Key words: *Aloe vera*, Cytotoxicity, Mitotic index, Wastewater

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Introduction

Rapid growth in municipal, agricultural and industrial activities has tremendously increased the demand for water. About 70% of the world freshwater is being used for agriculture purposes (Norton-Brandao *et al.,* 2013). Use of treated wastewater and desalinated water are the most probable alternatives of acceptable quality for water supply in agriculture (Antonio and Rico, 2019). Wastewater released from various industries and treatment plants is increasing rapidly in cities of developing countries, which is used for irrigation by farmers raising the risks on human health and environment pollution (Scott *et al.,* 2004). City sewage effluent and industrial wastewater may cause variable environmental problems including lower water quality for multipurpose usage, persistence of harmful residues in the environment, bioaccumulation and expected toxicity because of heavy metal accumulation (Al-Musharafi *et al.,* 2013). It is relevant to report that about 80% of untreated wastewater in developing countries flows directly into the environment, and 50% of these nations use untreated wastewater daily for various purposes, including agriculture (UNESCO, 2003).

Heavy metals occur naturally in small amounts, but are potent environmental contaminants of soil. These heavy metal contaminants are released in the environment as a result of urban and/or industrial activities such as paints, gasoline, coal ash, explosives, shotgun bullets, agricultural practices that include the use of agrochemical products such as pesticides and herbicides, and disposal of municipal sewage sludge, beside atmospheric deposition (Li *et al.,* 2019). In plants, accumulation of heavy metals is known to affect various morphological, physiological, biochemical and developmental processes and induces production of reactive oxygen species. Balkhair and Ashraf (2016) reported the accumulation of heavy metals in edible parts of okra as follows: Cr>Zn>Ni>Cd>Mn>Pb>Cu>Fe. Sharma *et al.* (2007) reported the presence of heavy metals in different parts of the plant. Reactive oxygen species inactivate enzymes, damage DNA, harm protein oxidation and lipid peroxidation (Aslam *et al.,* 2014; Janku *et al.,* 2019). However, about 20 million hectares of the world's agriculture in 50 countries is irrigated with wastewater (Drechsel and Evans, 2010), a situation that necessitates the need for diagnostic analysis of quantitative, qualitative and socio-economic factors associated with wastewater as a resource of agricultural irrigation (Baawain *et al.,* 2020).

Unplanned use of untreated wastewater for irrigation of food crops was denoted as a reason for endemic and epidemic diseases spread among consumers (Blumenthal and Peasey, 2002; Iwu and Okoh, 2019). World Health Organization and the United Nations Children's Fund evaluated the reuse of wastewater in large cities to vary between 90% in Northern America, 66% in Europe and 35% in Asia (Scott *et al*., 2004). Fodder crops, which require continual irrigation is particularly well suitable to wastewater irrigation, due to its tolerance to high salinity levels in urban wastewater (Scott *et al.,* 2004). Treatment of plants with mint herbs reported to provide reactive oxygen species scavenging, antioxidant metal chelating, resist inflammation and mutation in addition to enhancement of DNA repair (Sreeranjini and Siril, 2011). Appreciated stress mitigation was obtained by Gingko extracts on *Vicia faba* root length and weight. *V. faba* seeds treated with untreated wastewater and gingko extract scored higher root length and weight and low lipid peroxidation compared to samples exposed to untreated wastewater without gingko extracts (Cavusoglu *et al.,* 2010). Turkmen *et al.* (2009) showed that addition of royal jelly to wastewater highly ameliorated the scores of lipid peroxidation and mitotic index compared to wastewater alone.

A. vera is used as a folk medicine and is known as a silent healer due to medicinal properties in wound and burn healing as anti-inflammatory and immunomodulatory agent (Choi and Chung, 2003), possess inhibitory effect on mutations (Ogunjobi *et al.,* 2007) and anticancer activity (Langmead *et al.,* 2004). Similarly, extract of *A. arborescens* exhibited a scavenging ability on hydroxyl radicals and reduced alterations in enzyme activity (Sato, 1990). Heavy metals ionic compounds have ionization ability in aqueous media with complex and expensive remediation process (Sarode *et al.,* 2019), but barley possess a considerable capacity for osmotic adjustment and ability to grow in salty and water deficit soils (Fayez and Bazaid, 2014), oxidative stress tolerance and ability to detain K^+ deficiency (Maksimovic *et al.,* 2013). The present study was designed to accommodate *A. vera* toxicity mitigation capacity accompanied by barley adaptation ability to evaluate the use of wastewater for irrigation of barley.

Materials and Methods

Extraction of *A. vera* leaves: Healthy fleshy leaves of *A. vera* (80 g) were cut into small pieces and grinded to slurry using mortar and pestle. To obtain ethanolic extract, one part of slurry was mixed with 95% ethanol, fitted on a rotary shaker (100-120 rpm) for 72 hr at room temperature, centrifuged at 3000 rpm for 10min, filtered, evaporated at 60°C to a volume of 12.5 ml, diluted to 1 liter by distilled water and stored at 4°C until further use. *A. vera* water extract was prepared by the same procedure. One millilitre contained 0.05g of *A. vera* extract. Concentrations of wastewater + *A. vera* extract were prepared by mixing either 1 or 3 ml of each extract (alcoholic or water extract) in 1 liter of treated or untreated wastewater.

Seed germination: Seeds of *Hordeum vulagre* were obtained from a local market and wastewater was collected from Al-Ahsa Sewage Treatment Plant/Al-Ahsa/Saudi Arabia. Seeds were soaked overnight in separate beakers containing: 100% treated wastewater or treated wastewater mixed with; 0.05 g ml^{-1} *A. vera* alcoholic extract (0.05 g ml^{-1} Alc Ext), 0.05 g ml^{-1} *A. vera* water extract (0.05 g ml^{-1} Wat Ext), 0.15 g ml^{-1} *A. vera* alcoholic extract (0.15 g ml^{-1} Alc Ext) or 0.15 g ml^{-1} *A. vera* water extract (0.15 g ml^{-1} Alc Ext). Similar treatment doses were prepared for untreated wastewater. The control treatment consisted of distil water. A part of soaked seeds were transferred into Petri dishes wetted with suitable treatment doses and left to germinate for estimation of seed germination percentage and cytological analyses. Three replicates of seeds in a completely randomized design were transferred into plastic bags containing 1kg of air-dried loam soil and compost (1:1) in a plastic greenhouse. After 3 weeks, the seedlings were harvested for determining the shoot length, root length, fresh weight, dry weight, malondialdehyde and protein contents.

Estimation of protein: Protein content of barely seedlings roots and leaves was estimated by following the standard method of Ohnishi and Barr (1978).

Lipid peroxidation: Lipid peroxidation of barely seedlings roots and leaves was estimated by the method of Peever and Higgins (1989) and expressed in terms of malondialdehyde content.

Mitotic index: Root tips (1.0-1.5 cm long) of all treatments (treated or untreated wastewater without or mixed with 0.05g ml^{-1} Alc Ext, 0.05g ml^{-1} Wat Ext, 0.15g ml^{-1} Alc Ext or 0.15g ml^{-1} Wat Ext) and the control were fixed in ethyl alcohol: acetic acid mixture. Root tips were heated for 10 min in 1N HCl at 60°C, incubated in a mixture of 2% cellulase and pectinase for 2 hr, transfered to a clean slide and two drops of aceto-orcein were added. After 30-60 min, the specimens were squashed and slides were examined under a microscope (Olympus, Japan). Five root tips and 1000 cells were examined for each treatment. The mitotic index was calculated according to Ozmen and Summer (2004).

Statistical analysis: One-way analysis of variance (ANOVA) was applied to compare the results of tested treatments using post-hoc, Dunnett Multiple Comparison Test. Statistical analysis was performed using the SPSS version 16 and significance levels of $P< 0.05$, $P< 0.01$ and $P< 0.001$ were applied.

Results and Discussion

The present study evaluated the effect of treated and untreated wastewater alone or mixed with 0.05 g ml^{-1} or 0.15 g ml^{-1} of *A. vera* extracts on cell proliferation and growth of *H. vulgare*. Germination of *H. vulgare* seeds in distilled water, *i.e.,* control treatment was 82.0%, while in treated and untreated wastewater was 74.0 and 85.0%, respectively (Table 1). Treated wastewater slightly inhibited seed germination, (74%) while untreated wastewater slightly induced germination (84%) compared to the control (82%). The result is partially in agreement with Zeid and Abou El Ghate (2007) who attributed the increase of germination rate to sewage stimulatory effects on amylases and proteases enzymes.

Addition of 0.05 g ml^{-1} of *A. vera* alcoholic extract improved the germination rate to 78 and 90%, while *A. vera* water extract reduced germination rate to 69% and 83% in treated and untreated wastewater, respectively. Addition of 0.15 g ml^{-1} of *A. vera* alcoholic extract increased germination rate to 80% in the case of treated wastewater, but reduced the germination rate to 78% in untreated wastewater, while 0.15g ml^{-1} of *A. vera* water extract increased germination rate to 82 and 89% for treated and untreated wastewater, respectively. It is clear that the addition of 0.05g ml^{-1} of *A. vera* alcoholic extract and 0.15 g ml^{-1} of *A.* water extract to untreated wastewater showed 90% and 89% germination rate of seeds.

Shoot length of barley seedlings irrigated with distilled water, *i.e.,* control was 8.8cm, which was similar to plants irrigated with treated wastewater. Seedlings irrigated with untreated wastewater showed a considerable increase in shoot length to 13.27cm, but addition of *A. vera* extracts reduced the shoot length, however, they were higher than the control seedlings (Table 1). Addition of *A. vera* extracts to treated wastewater significantly increased the shoot length of *H. vulgare* seedlings,

Table 1: Seed germination rate, shoot and root length, and fresh and dry weight of barley seedlings irrigated with untreated or treated wastewater without or mixed with different concentrations of *A. vera* extracts.

A. vera extract (g ml^{-1})	Wastewater	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
Control (DW)	0.0	82.0±6.0	8.80±0.71	15.13±1.51	0.74±0.13	0.06±0.03
0.00 Aloe extract	UWW	85.0±1.0	13.27±1.52	15.20±0.62	0.41±0.04*	0.04±0.02
	TWW	74.0±2.0	8.60±1.17	17.97±1.84	0.57±0.05	0.04±0.02
0.05 Alc Ext	UWW	90.0±0.0	10.83±1.81	13.63±1.88	0.43±0.02*	0.04±0.02
	TWW	78.0±6.0	7.33±0.54	14.23±0.53	0.44±0.05*	0.03±0.02*
0.05 Wat Ext	UWW	83.0±7.0	10.10±2.58	14.83±0.20	0.44±0.11*	0.05±0.02
	TWW	69.0±5.0	17.23±5.84	15.70±0.88	0.51±0.04	0.04±0.02
0.15 Alc Ext	UWW	78.0±6.0	10.50±1.06	13.50±0.53	0.50±0.14	0.05±0.01
	TWW	80.0±18.0	12.20±4.40	15.47±1.31	0.56±0.04	0.06±0.03
0.15 Wat Ext	UWW	89.0±9.0	8.93±1.70	11.47±0.36	0.41±0.06*	0.04±0.01
	TWW	82.0±6.0	10.67±2.48	16.00±0.65	0.49±0.05	0.04±0.02

*Statistically significant at $p<0.05$, when compared with control. WW: Wastewater; UWW: Untreated wastewater; TWW: Treated wastewater; DW: Distilled Water; Alc Ext: *A. vera* alcoholic extract and Wat Ext: *A. vera* water extract.

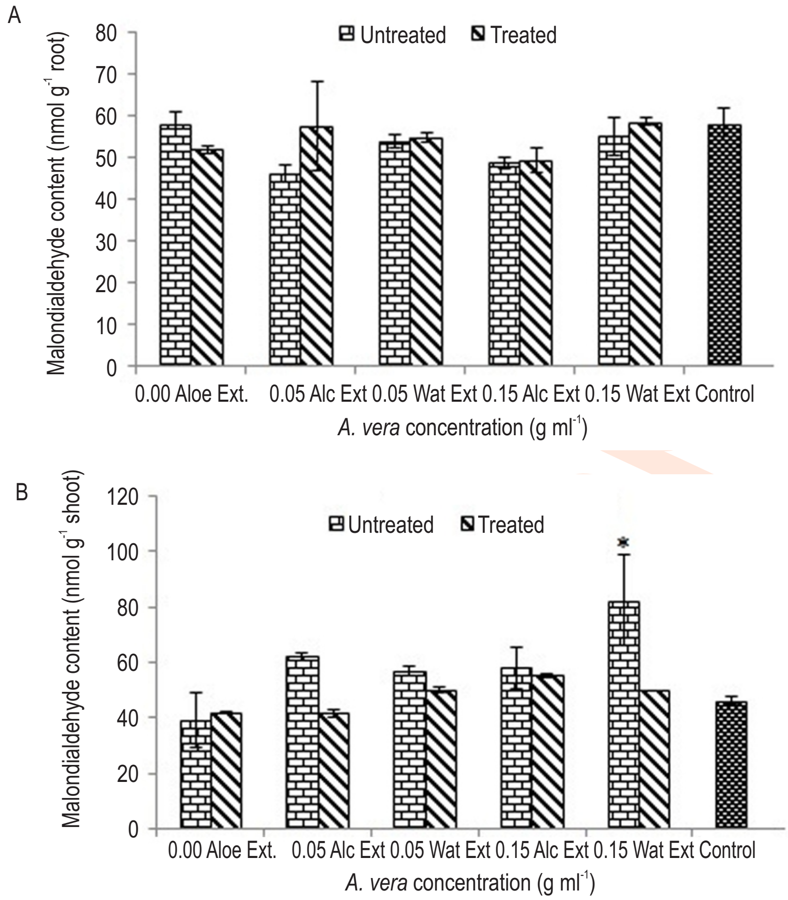

Fig. 1: Malondialdehyde concentration in root (A) and shoot (B) of barley plants irrigated with untreated/treated wastewater without or mixed with *A. vera* extracts.

particularly 0.05g ml^{-1} of *A. vera* water extract, which increased the shoot length to 17.23cm (196%); almost double as compared to control seedlings. However, the addition of 0.05g ml^{-1} *A. vera* alcohol extract to treated wastewater reduced shoot length to lower than that of control seedlings, while 0.15g ml^{-1} *A. vera* alcohol extract produced better shoot length than the control group but lower than a similar dose of water extract. Root length of barely seedlings irrigated with treated wastewater showed slight increase (19.0%) as compared to the control seedlings, while the root length of seedlings irrigated with untreated wastewater was similar to control. The root length of barely seedlings was reduced when irrigated with *A. vera* extracts mixed with untreated wastewater, and the highest reduction in root length was observed with *A. vera* water extract (between 2.0 to 24.6% compared to the control). Generally, no significant difference was noted in root length of seedlings treated with treated wastewater mixed with *A.* extracts (Table 1).

It was reported that salt water decreases root and shoot length, fresh and dry weight of barley and the effect is usually more notable in roots (Khosravinejad *et al.,* 2009). The present study showed that shoot and root length were slightly increased, which indicates the suitability of treated or untreated wastewater for barley growth. This result was similar to Zeid and Abou El Ghate (2007) who observed a considerable increase in root length, fresh and dry mass of bean seedlings. Addition of *A. vera* extract showed slight stimulation of shoot growth and mild inhibition of root growth. Alawsy *et al.* (2018) reported stimulant effect of wastewater on crop growth, which was attributed to microelements present in wastewater. Stimulatory effects of wastewater encouraged farmers to save fresh water and reduce the use of fertilizers (Lima *et al.* 2021).

Fresh weight of *H. vulgare* seedlings exhibited a significant decrease of 44.6% when irrigated with untreated wastewater and insignificant decrease of 23.0% when irrigated with treated wastewater as compared to the control seedlings (Table 1). Fresh weight of seedlings treated with treated wastewater mixed with *A. vera* extracts decreased ranging between 23.3 to 40.5% ($p<0.05$) and the later was reported when plants were irrigated with treated wastewater mixed with 0.05g ml^{-1} of *A. vera* alcoholic extract. Fresh weight of seedlings maintained

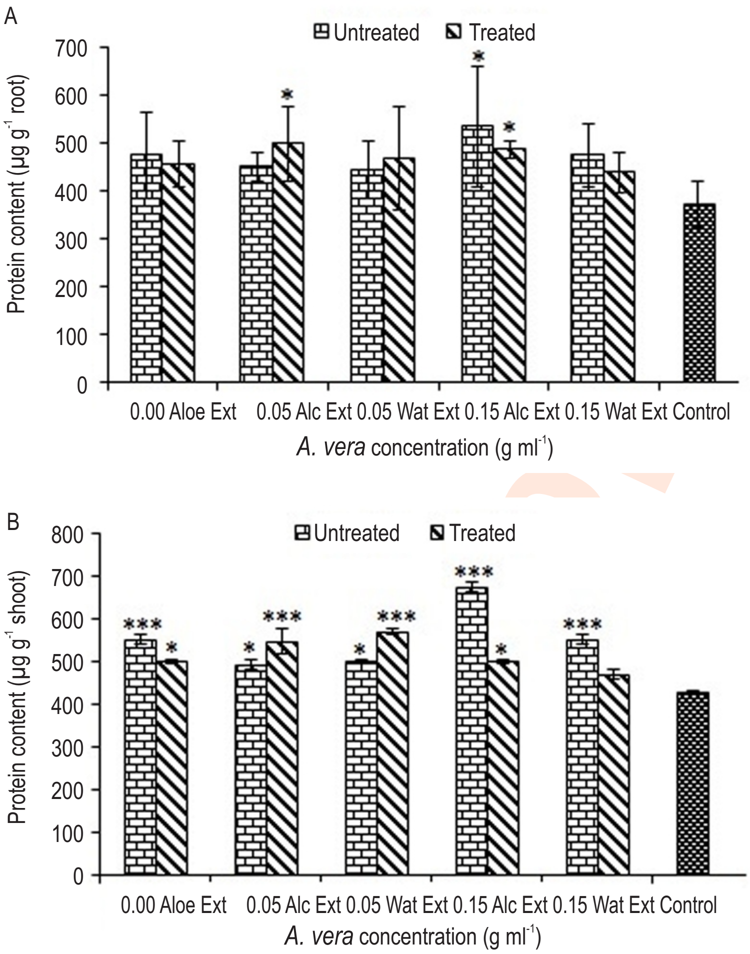

Fig. 2: Protein contents in root (A) and shoot (B) of barley plants irrigated with untreated/treated wastewater without or mixed with *A. vera* extracts.

a significant decrease in all treatments when irrigated with untreated wastewater mixed with *A. vera* extracts ($p<0.05$), except for treatment with 0.15 g ml^{-1} of *A. vera* alcoholic extract that showed insignificant decrease in fresh weight. Similarly, dry weight of *H. vulgare* seedlings exhibited similar pattern of decline as fresh weight, except for seedlings treated with treated wastewater mixed with 0.15g ml^{-1} of *A. vera* alcoholic extract, which showed a slight increase to a level similar to control seedlings (Table 1). Therefore, it seems that 0.15 g ml^{-1} *A. vera* alcoholic extract eliminated the effect of wastewater on barley growth. It is important to note that these variations were insignificant, except for plants irrigated with treated wastewater mixed with 0.05 g ml^{-1} *A. vera* alcoholic extract that their dry weight reduced by 44.1% ($p<0.05$) as compared to control.

This reduction in plant fresh and dry weight may be due to salinity present in wastewater (Mohammadi *et al.,* 2018). Salinity enhances the production of reactive oxygen species (Arora *et al.,* 2019) that causes high cellular damage, degradation of proteins and enzymes and production of high lipid peroxidation (Zhou *et al.,* 2017) that consequently inhibits plant growth. Hag El Amin *et al.* (2020) reported a significant reduction in root and shoot lengths as well as fresh and dry weight at low 1.8 dsm^{-1} and high 7.2 dsm^{-1} salinity concentrations. The reduction in fresh and dry weights may also be attributed to heavy metals, as they are known to inhibit physiological and biochemical functions like photosynthesis, respiration and metabolism (Ashfaque *et al.,* 2016). Badr *et al.* (2020) ranked heavy metals in wastewater in the order: Zn>Cu>Pb>Cr>Ni>As>Cd>Hg>Co.

Accumulation of Pb in plants affects various morphological, physiological, biochemical, and developmental processes that affect transpiration, photosynthetic rate, chlorophyll content and plant growth (Kopittke, 2007). Cd is another major pollutant in wastewater and known to induce genotoxicity in plants due to its direct impact on DNA structure and function (Aslam *et al.,* 2014). Environmental stress generates reactive oxygen species, which causes major cellular damage in the form of lipid peroxidation (Zhou *et al.,* 2017). Untreated wastewater showed no change in malondialdehyde content in

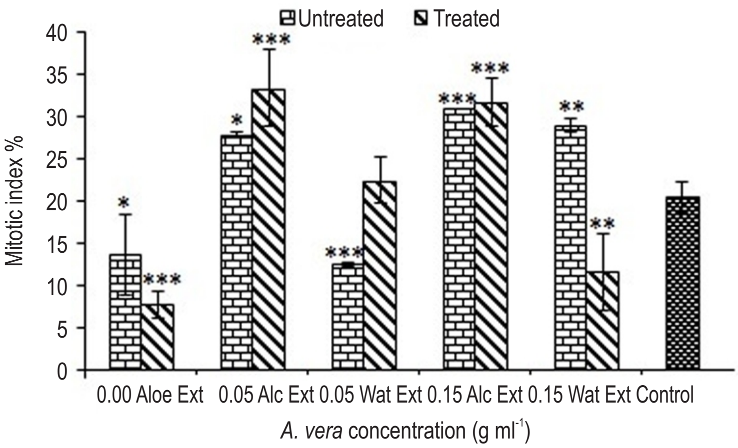

Fig 3: Mitotic index of barley plants irrigated with treated/untreated wastewater without or mixed with *A. vera* alcoholic/water extract.

roots of *H. vulagare* as compared to control samples, however, addition of *A. vera* extracts further decreased the malondialdehyde concentration in roots as compared to seedlings treated with untreated wastewater or control samples. Treated wastewater slightly decreased (10.5%) the malondialdehyde content in roots (Fig. 1. A), but when treated wastewater was mixed with A. vera extracts, the malondialdehyde content slightly increased, though less than the concentration in the control samples. Therefore, it is possible to say that the *A. vera* extracts mitigated the toxic effects of treated wastewater, but induced the effects of untreated wastewater on malondialdehyde concentration in barley roots.

Malondialdehyde content in leaves showed a reverse trend as compared to roots (Fig 1. B). It reduced by 9.5% in the leaves of seedlings treated with treated wastewater or treated wastewater mixed with 0.05g ml^{-1} *A. vera* alcoholic extract, however, reduction (15.1%) was higher with untreated wastewater as compared to control seedlings. All other treatments with treated or untreated wastewater mixed with *A. vera* extracts recorded an increase in malondialdehyde content ranging between 7.6 and 36.0%. However, the highest and significant ($p<0.05$) increase in malondialdehyde content was noted in leaves of *H. vulgare* seedlings treated with untreated wastewater plus 0.15g ml^{-1} *A. vera* alcoholic extract compared with control. However, it is notable that *A. vera* extracts reduced the effects of untreated and treated wastewater on malondialdehyde content in barley leaves. The results revealed that *A. vera* extracts modulated malondialdehyde concentration in roots almost throughout all treatments while its modulation effect on shoots was in one treatment. These results may be explained by the direct contact between roots and wastewater mixed with *A. vera* extracts, but *A. vera* extracts may require traveling longer distances to reach the leaves and exert effect. This finding shows imperative need of foliar spray of *A. vera* extracts on leaves. Fayez and Bazaid (2014) applied KNO_3 to ameliorate oxidative stress in barley. They maintained considerable modulation through low malondialdehyde content and concluded that it is possible to cultivate barley in salt and water deficit soils without oxidative stress. Reddy *et al*. (2005) and Emamverdian *et al.* (2021) indicated that lowering of malondialdehyde consequently supported the plant tolerance and improved photosynthetic parameters and high yield. Similar results of improved shoot and root growth and higher dry material were achieved by lowering oxidative damage, which coincided with lowered malondialdehyde concentration (Abdelaziz *et al*., 2018).

Barley seedlings irrigated with treated and untreated wastewater showed increased root and shoot protein contents compared to control samples (Fig. 2. A,B). The increase in root protein contents was significant ($p<0.05$) in seedlings irrigated with treated wastewater mixed with 0.05g ml^{-1} or 0.15g ml^{-1} of *A. vera* alcoholic extracts (33.7 and 30.5% over the control samples, respectively) and seedlings irrigated with untreated wastewater mixed with 0.15g ml^{-1} *A. vera* alcoholic extract (43.3% over the control samples) (Fig. 2. A). Similar, but insignificant increase in root protein contents were induced by other treatments with *A. vera* extracts. The increase of root proteins induced by *A. vera* extracts coincided with a decrease in malondialdehyde content. In other words, treatment with *A. vera* extracts decreased the level of malondialdehyde, increased protein contents in roots, and hence encouraged normal growth of barley roots.

Barley seedlings irrigated with treated wastewater showed significant increase ($p<0.05$) in shoot protein contents (16.8% more than the control samples), while those irrigated with treated wastewater mixed with 0.05g ml^{-1} *A. vera* alcoholic extract or 0.05g ml^{-1} *A. vera* water extract showed highly significant increase in ($p<0.001$) shoot protein contents. At the same time, plants irrigated with untreated wastewater, untreated wastewater mixed with 0.15 g ml^{-1} *A. vera* alcoholic extract or 0.15 g ml^{-1} *A. vera* water extract exhibited highly significant increase ($p<0.001$) in shoot proteins exceeding the control values by 29.4%, 57.5 and

29.4%, respectively (Fig. 2. B). Highly significant ($p<0.001$) increase in shoot protein contents was also observed in plants irrigated with treated wastewater mixed with 0.05 g ml^{-1} of *A. vera* alcoholic or 0.05g ml^{-1} of *A. vera* water extract (exceeding the control by 28.0 and 33.6%, respectively). Saha *et al.* (2010) found higher protein concentration in wheat irrigated with sewage water. Abdel Latef and Sallam (2015) attributed accumulation of proteins and total free amino acids in root/shoot of maize to higher growth rate due to abundance of soluble organic and/or inorganic substances in sewage water. Almazov and Kholuyako (1990) and Baghel and Singh (1995) noted that the necessary products for protein biosynthesis such as CO_2, NO_3, PO_4, SO_4, NH_3, H_2S and CH_4 are provided by microbial activity that break down the organic matter in sewage. In addition, barley possess a considerable capacity for osmotic adjustment and the ability to grow in salty and water deficit soils (Fayez and Bazaid, 2014). It was also reported that barley possess tolerance to oxidative stress harmonized with higher potassium ion holding capacity (Maksimovic *et al.,* 2013).

In this study, barley seedlings irrigated with wastewater mixed with *A. vera* alcoholic extract exhibited a significant protein accumulation in roots, while seedlings irrigated with wastewater mixed with *A. vera* alcoholic or water extract showed highly significant accumulation of protein concentrations in shoot. It was reported that protein accumulation increase resistance to mutations (Ogunjobi *et al.,* 2007), scavenges hydroxyl radicals and reduces alterations in enzyme activities (Sato, 1990).

Mitotic index is an important sensitive measure of tissue proliferation, growth, and cell cycle machinery (Cotelle *et al.,* 2015), and it is a reliable method to check the level of cytotoxicity (Fiskesjo, 1985; Fernandes, 2007). A decreased rate of mitotic index reveals the cytotoxicity level of pollutants (Smaka-Kinel *et al.,* 1996) and conversely an increase of mitotic index reveals the stimulation activity of pollutants.

In the present study, samples of barley seedlings irrigated with treated or untreated wastewater showed a significant decrease in mitotic index ($p<0.001$ and $p<0.05$, respectively). Mitotic index of seedlings irrigated with treated wastewater was 7.8% and for seedlings irrigated with untreated wastewater was 13.7%, while that of control seedlings was 20.5% of dividing cells. A significant decrease of mitotic index indicated the potent cytotoxicity of both treated and untreated wastewater. The reduction in mitotic index induced by untreated wastewater was 66.8% of the control value, while that induced by treated wastewater was almost destructive to cells division scoring only 38.0% of control value (Fig. 3).

A reduced rate of mitotic index reflects the cytotoxicity level; the decline of mitotic index lower than 50% of the control induces sub-lethal changes (Mesi *et al*., 2012). Rosculete *et al*. (2019) reported sub-lethal and lethal effects of untreated wastewater on plants. Thus, wastewater may interfere with the cell cycle causing inhibition of DNA synthesis (Yildiz *et al.,* 2009). Reduction of mitotic index may also result from a blockage of cell cycle in G_2 phase if the DNA replication was affected at S phase (Hlavova *et al.,* 2011), or due to DNA damage (Knapik and Ramsdorf, 2020). High concentration of heavy metals is known to induce high frequencies of chromosomal aberrations (Ivanova *et al.,* 2005).

Barely seedlings irrigated with treated wastewater mixed with 0.05g ml^{-1} or 0.15g ml^{-1} of *A. vera* alcoholic extract showed significantly increased mitotic index (33.4 and 31.7% ($p<0.001$), respectively). Compared to control, these mitotic index values exceeded control samples by 62.9%, 54.6%, respectively. Seedlings treated with untreated wastewater mixed with 0.05 g ml^{-1} or 0.15g ml^{-1} of *A. vera* alcoholic extract also showed significantly increased mitotic index (27.9 and 31.0%), which exceeded the control samples by 51.2% and 36.1%, respectively. As a result, it is possible to say, *A. vera* alcoholic/water extracts modulate the mitotic effects of wastewater. *A. vera* possess biochemical, morphological, and biomechanical effects (Oryan *et al.*, 2016).

A. vera was also reported to significantly stimulate cell proliferation, migration and viability (Teplicki *et al.,* 2018). It was found to cause remarkable changes in cell cycle, increases cell number and cell thickness (Moriyama *et al.,* 2016). Thus, either of these activities may be the cause of significant increase in mitotic index that was observed in this study.

In conclusion, the cytotoxicity of wastewater on barley seedlings was observed in the form of reduced growth, significantly reduced mitotic index and increased shoot protein contents. The addition of *A. vera* alcoholic/water extract modulated the cytotoxic effects of wastewater by a significant increase in mitotic index and a moderate decrease in malondialdehyde. Further studies are required for example to investigate the effects of foliar spray of *A. vera* extract.

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Add-on Information

Authors' contribution: O.A.H. El Amin: Resources, Methodology, Data analysis, Formal analysis, writing original draft; **R.I.H. Ibrahim:** Resources, Data analysis, Formal analysis, investigation, writing final draft.

Research content: The research content is original and has not been published elsewhere.

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Conflict of interest: The authors declare that there is no conflict of interest.

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