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Molasses-based waste water irrigation: a friend or foe for carrot (*Daucus carota* L.) growth, yield and nutritional quality

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Abstract
Management of molasses-based wastewater generated in yeast and sugar industries is a major environmental concern due to its high chemical oxygen demand and other recalcitrant substances. Several strategies have been used to reduce the inland discharge of wastewater but the results are not satisfactory due to high operating cost. However, reuse of molasses-based wastewater irrigation in agriculture has been a major interest nowadays to reduce the freshwater consumption. Thus, it is crucial to monitor the impacts of molasses-based waste water irrigation on growth, metabolism, yield and nutritional quality of crops for safer consumer's health. In present study, carrot seeds of a local cultivar (T-29) were germinated on filter paper in Petri dishes under controlled conditions. The germinated seeds were then transplanted into pots and irrigated with three different treatments normal water (T0), diluted molasses-based wastewater (T1), and untreated molasses-based wastewater (T2), in six replicates. Results revealed that carrot irrigated with untreated molasses-based waste water had exhibited significant reductions in growth, yield, physiology, metabolism, and nutritional contents. Additionally, accumulation of Cd and Pb contents in carrot roots irrigated with untreated molasses-based waste water exceed the permissible limits suggested by WHO and their consumption may cause health risks. While, diluted molasses-based waste water irrigation positively enhanced the growth, yield of carrot plants without affecting the nutritional quality. This strategy is cost effective, appeared as most appropriate alternative mean to reduce the freshwater consumption in water deficit regions of the world.

Keywords Carrot cultivation, Waste water remediation, Water scarcity

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Introduction

Water is often considered an abundant natural resource but only 3% of it is available for consumption with the remaining 97% trapped in the icecaps and snow covers [1]. Therefore fresh water is facing scarcity issue worldwide, posing a significant threat to life sustainability [2]. Approximately, 2/3rd of the world population (~4 billion people) faces severe water scarcity for at least one month each year and 2 billion are living in inadequate water supply in various countries [3]. Pakistan is one of those countries which experience irregular patterns of annual rainfall (<100 mm) leading to water shortage in the soil and water bodies [4]. Southern Punjab is a dry arid region of Pakistan and Dera Ghazi (DG) Khan is a very deprived Division and lies in the hyper-arid zone of South Punjab [5]. DG Khan is experiencing high water scarcity in response of extreme droughts [6, 7] and contamination of available freshwater resources due to improper release of industrial effluents such as cement, sugar, detergent, fertilizer, pesticides, and other chemical production units [8]. Among several agro-industries such as sugar mills use a lot of freshwater and release half of it as effluent [9]. About 1500 to 2000 L freshwater is needed to crush one ton of sugarcane and >1000 L of this water [10] and 2.5 to 4% of molasses is released as waste but these wastes of this agro-industry are less harmful than other [11]. Molasses contains 35% of sucrose, 9% of fructose, 7% of glucose, 4% of non-reducing sugars, 3% of reducing sugars, 4.5% of various nitrogenous compounds, 5% of non-nitrogenous acids, and 12% of ash in 20% of water along with various other metal elements [12]. Thus it has been used as raw material in chemical industry for production of ethanol, bakery yeast, acetic acid, micronutrient, and cattle feed etc. or may be used for crop irrigation after recycling in the developed countries [10].

Waste water irrigation not only minimizes the pressure on freshwater resources, also provide economical support to farmers. Wastewater is an enriched source of organic matter but also contain high amount of various hazardous elements like Cd, Pb, Ni, Cr, Zn, Mn, and Hg etc. which adversely affect the crop yield [13]. On the other hand, recycled waste water irrigation serves as fertilizer and facilitates the microorganism to improve the soil and crop health [14]. It has been reported that ~20 million hectares of cultivated soil across the globe is being irrigated with treated and untreated waste water. Unfortunately, farmers are irrigating crops with untreated waste water in the developing countries like Pakistan resulting in the accumulation of various toxic elements in the soil and plants [15].

In Pakistan, farmers use untreated molasses-based waste water for crop irrigation to avoid the economic pressure of freshwater irrigation, and fertilizers. This poses a significant risk to human and ecosystem health, and these issues are escalating rapidly [16–18]. A previous study reported that Pb is one of the most persist pollutant due to its high retention time (150 to 1500 years) and enters in the food chain from different routes like water, soil, air [19, 20]. Cd is water soluble metal and easily absorbed by plant roots and translocated to arial parts [21–23]. These toxic metal elements interfere with the nutrient uptake ability, inhibit the seed germination, delay the plant growth with deformed morphology by altering various enzymatic activities, disrupted photosynthesis and respiration, increased plasmolysis and ROS production, membrane disintegration etc [24, 25]. Thus, use of untreated waste water for irrigation in agriculture serves as a severe risk for cereals, fruits and vegetable crops [14].

Vegetables accumulate toxic elements in edible and non-edible parts more easily as compared to fruits and cereal crops [26]. Leafy and root vegetables accumulate toxic elements in mesophyll and epidermal tissues of leaves and endodermal tissues of roots. Previous studies reported the accumulation of Fe, Cu, Zn, Cd, and Pb in different leafy and root vegetables including spinach, lettuce, cabbage, and turnip, radish, and sweet potato [27, 28]. Minhas et al., [29] also reported that tuberous root vegetables accumulate higher concentration of toxic metals than leafy vegetables in edible parts. Carrot is an apiaceous tuberous root vegetable and most economical source of various essential minerals, vitamins, pigments, and antioxidants [30] but can accumulates various toxic elements in its tuberous taproot [31, 32]. It is 3rd most consumed vegetable after potato and tomato in raw salads, juices, and various processed forms in Pakistan. Consumption of carrot is increasing due to its nutritious value and existing production quantity cannot fulfill the market demand [33]. Unfortunately, the average carrot yield in Pakistan is low (17.5 tons per hectare) due to various abiotic stresses including salinity, heat, and drought [34]. Farmers irrigate carrot field with molasses based untreated waste water in DG Khan Division to enhance the growth and crop yield with reduced cost of fertilizer and freshwater irrigation (informal discussion with local farmers). It has been reported in a previous study that carrot accumulates several toxic metals such as cadmium in root in response of waste water irrigations which can pose health risks in consumers [35]. Additionally, waste water irrigation resulted in excessive sodium accumulation in carrot root that affects the taste and texture as well [36]. Furthermore, some pharmaceutical compounds such as lamotrigine and carbamazepine have also been detected in carrot roots irrigated with waste water [37, 38]. Long term exposure of these pharmaceutical compounds can cause the cognitive problems (sedation, dizziness, and mood changes), skin allergies, and hematological diseases [39, 40]. Therefore, present study was aimed to evaluate the impacts of molasses-based waste water irrigation treatments on growth, biomass production and nutritional quality of local carrot cultivar (T-29).

Materials and methods

Experimental location and material collection

This experiment was conducted in a hyper-arid zone (District Dera Ghazi Khan) of South Punjab, Pakistan. Climatic conditions of this region remain dry round the year with long harsh summer, cool short winter and temperature varies from 47°F to 114°F. A Pot experiment was conducted to evaluate the impact of molasses-based waste water irrigation on carrot growth, metabolism and total yield, and nutritional quality. The seeds of carrot cultivar (T-29) were taken from Green Plant Seed Company, Multan. Normal tap water was used as positive control treatment (T_0) and molasses-based waste water (T_1, and T_2) collected from untreated drainage of Fatima Sugar Mill, was used for crop irrigation (Table 1). Soil (up to a depth of 20 cm) was collected from agricultural fields and the surface litter was scrapped by autoclaving.

Physico-chemical analysis of soil and water samples

The soil samples were air dried and subjected to acid digestion in solution of HNO_3, H_2SO_4, and $HClO_4$ (5:1:1 v/v/v) for 8 h at 80 °C [41]. The translucent material was filtered using Millipore nitocellulose membrane filter with a pore size of 0.45-µm and diluted up to 50 mL with distilled water. Total dissolved solids (TDs), pH, and electrical conductivity (EC) were measured for digested soil samples, and molasses-based wastewater and normal water samples using a pH meter (914 pH meter) and electric conductivity meter (Conductometer Metrohm AG). Mineral nutrient such as potassium (K), sodium (Na), calcium (Ca^{2+}), megnessium (Mg^{2+}) contents were measured using a flame photometer (CORNING M410), whereas phosphorus (P) content was measured spectrophotometerically at wavelength of 700 nm following method described by Bolland and Allen [42].

Moreover, nitrogen concentration was measured calorimetrically following method reported by Golterman [43]. Briefly, 20 mL of test solution was mixed with 1.0 mL of sodium salicylate, Nessler's reagent solution and incubated for 30 min and measured at wavelength of 420 nm spectrophotometrically. Sulphur (S), cadmium (Cd), iron (Fe), copper (Cu), zinc (Zn), and lead (Pb) were measured using atomic absorption spectrophotometer (PerkinElmer 3100) [44]. Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) were estimated by titrimetric analysis following the method described by Delzer and McKenzie, [45].

Table 1 Detail of different irrigation treatments for carrot cultivation

Treatment	Different water proportion
T_0(Control)	100% Normal water
T_1(Diluted)	50% Normal water + 50% molasses-based waste water
T_2(Untreated)	100% molasses-based waste water

Experimental details

Carrot seeds were germinated on filter paper in petri dishes (twenty seeds each) under control conditions in complete darkness for 10 days at room temperature. Petri dishes were arranged in complete randomized design and six replicates were used for each treatment. Number of germinated seeds was counted and transferred to pots filled with soil and pots were divided into three groups arranged in complete randomized design with six replicates for each treatment. Seedlings were irrigated with normal tap water (T_0: control), diluted molasses-based waste water (T_1), and untreated molasses-based waste water (T_2) (Table 1) and seedling emergence rate was observed for next 7 days. After fifteen of seedling emergence, hand thinning was done to uproot the seedlings and only one healthy plant was kept in each pot of six replicates of each treatment for next 100 to 110 days. After that, carrot plants along with tap roots were harvested, washed, and dried with blotting paper and used for data recording. Total of 49 parameters in terms morphology, physiology and biochemistry were studied to evaluate effects of molasses-based irrigations on carrot.

Growth parameters

Growth parameters in terms of seed germination count, seedling emergence, leaf and root length, number of leaves, root weight and diameter, fresh plant biomass, and plant dry biomass were measured to evaluate the growth and yield of carrot plants.

Germinated seed on each Petri dish were counted very carefully after 10 days of incubation [32]. Emergence of seedling was recorded for each treatment and replicate after 15 days of sowing. After harvest, total of six from each treatment (one plant from all six replicates) were used for growth, yield and nutritional quality measurements. Leaf length and root length was measured using a measuring tape at maturity expressed in centimeters (cm). Number of leaves per plant was recorded at maturity when further growth was stopped. Plant fresh and dry weight and root weight was measured using digital weight balance for each treatment and replicate. Root diameter was measured in mm^2 by using Vernier Caliper for each replicate [46].

Biochemical parameters

Biochemical parameters were determined in terms of osmoprotectants (sugar, protein, and amino acid contents) while activity of stress responsive enzymes such as phenulalanine ammonialyase (PAL), superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), nitrate reductase (NAR), and nitrite reductase (NIR) was measured in fresh leaf tissues.

Osmoprotectants

Total of 0.5 g of fresh leaf tissue were macerated in 10 mL of C_2H_5OH (80%) and were incubated in water bathe at 80 °C for one hour. Leaf extract was mixed with 18% solution of phenol (0.5:1.0 mL respectively) in test tubes and were seated at room temperature. After one hour of incubation, 2.5 cc of sulphuric acid was mixed and absorbance was measured spectrophotometrically at 490 nm wavelength [47]. The reducing and non-reducing sugars, free amino acids and soluble protein were also determined on each sample by using method reported by Alabran et al. [48].

Activity of PAL

A homogenized extract of fresh leaf tissue (0.5 g) was prepared using liquid nitrogen and 50 mmol of Tris-HCl buffer (pH 8.8) and 0.5 mmol of EDTA. A mixture of leaf extract (0.5 mL), 20 mmol L-phenylalanine, with 50 mmol Tris-HCl buffer (pH 8.8) was incubated at 30 °C followed by adding 0.5 mL of 10% trichloroacetic acid to stop the reaction. Absorbance was measured at wavelength of 290 nm after 30 min and enzyme activity was explained in U µmol g^{-1} fw [49].

Activity of SOD

A reaction mixture of enzyme extract (0.5 mL) was prepared with 130 mM methionine, 1mM EDTA, 0.75 mM NBT, 0.02 mM riboflavin, and 50 mM phosphate buffer (pH 7) to determine the concentration of SOD. Absorbance of reaction mixture and blank was measured after 7 min exposure of fluorescent light at wavelength of 560 nm spectrophotometrically. Activity of SOD was calculated using Lambert–Beer law (A ¼ εLC; Where A is the absorbance, ε is the extinction coefficient, L the length of wall, and C the concentration of enzymes) [50].

Activity of CAT

Catalase activity was determined using a method reported by Cakmak and Marschner, [51]. 0.2 ml of enzyme extract was mixed with 25 mM buffer phosphate (pH: 7.0), and 10 mM hydrogen peroxide. Absorbance was measured at wavelength of 240 nm due to hydrogen peroxide extinction and enzyme activity was observed according to the degradation of H_2O_2 min^{-1} mg^{-1} of protein at 25°C and expressed in terms of U µmol g^{-1} of fw.

Activity of POX

To estimate the activity of POX, a reaction mixture containing 5.0 mM of 4-methylcatechol, 5.0 mM of hydrogen peroxide, and 500 µL of enzyme extract and sodium phosphate buffer (pH 7.0) was prepared at room temperature by making total volume of 3.0 mL. Absorbance was measured at wavelength of 420 nm spectrophotometrically and enzyme activity could be defined as 0.001 unit changes in absorbance min^{-1} in controlled condition and expressed in terms of U µmol g^{-1} of fw [52].

Activity of NAR and NIR

The activity of NAR activity was evaluated by using method reported by Kim & Seo [53]. For this, a reaction mixture was prepared by mixing 150 µL of enzyme extract with 850 µL of solution containing 40 mM of $NaNO_3$, 80 mM of Na_2HPO_4, 20 mM of NaH_2PO_4, 0.2 mM of NADH, 1% of sulphanilamide. Reaction mixture was incubated for 120 min and absorbance was measured at wavelength of 540 nm. Specific activity of nitrate reductase was calculated by using formula [change of nitrate concentration (µM)] x [extracted volume (mL)]/[fresh weight (g)]/[reaction time (h)] and expressed in terms of µmol g^{-1} h^{-1} of fw.

Physical parameters of juice

Physical parameters fresh carrot juice were determined in terms of juice volume, juice viscosity, juice torque, juice pH, total soluble solids, dietary fiber per 100 g of carrot. Fresh carrot roots were washed peeled off and head and tail were removed by knife. The peeled carrot roots were sliced into pieces, weighed and grinded in juicer and pulp residue was dried 60℃ for 24 h and various physical parameters were recorded. The juice volume was measured using volumetric flask in cm^3 per 100 g of carrot. The pH and viscosity of juice was measured using digital pH meter (PYE Unicam), and viscometer (Visco QC 100) using the method described in previous study [54]. Total soluble solids were determined in terms of brix (which describes the sweetness of juice) using refractometer by the method of AOAC official methodologies reported in a previous study [55]. Dietary fiber in terms of non-starchy polysaccharide were measured using enzymatic gravimetric method (AOAC) described by McCleary [56].

Proximate analysis

The proximate analysis of carrot juice was determined in terms of moisture, ash, protein, fiber, fat, and carbohydrates contents by following the protocol of the Association of Analytical Chemists (AOAC 2000) described by [57].

Moisture content

The AOAC (oven drying method) No. 934.01 was used to determine moisture content. The samples were kept overnight in an oven at 105°C and the moisture content

(%) was calculated by utilizing the following formula; [(weight loss on drying (g)/weight of sample (g)) x100].

Ash content

The crude ash content was calculated by following the AOAC official method No. 942.05. Each sample (2 g: W1) was placed in a crucible and subjected to burning under an oxidizing flame (15–20 min) or until no additional fumes were emitted. Subsequently, the sample was transferred to a muffle furnace and heated to 500 °C for a duration of three hours. After cooling in a desiccator, its weight (W2) was measured. Then, percentage of ash was determined by utilizing the following formula: [(1- (W1-W2)/weight of sample (g)) x100].

Protein content

The crude protein content was determined by AOAC official method No. 954.01. Two grams of the grounded sample was taken into a micro-kjeldahl digestion flask and 5 g of digestion mixture (100 g of K_2SO_4 + 10 g of $CuSO_4$ + 5 g of $FeSO_4$) was added along with 40 mL of concentrated sulfuric acid and kept on hot plate until solution became clear. The solution was cooled down and transferred to a volumetric flask to make dilution up to 1 L. A 10 mL aliquot of this dilution was boiled in a micro-kjeldahl distillation apparatus along with 10 mL of 40% sodium hydroxide (NaOH) solution. The liberated ammonia was condensed and collected into a beaker containing 2% of boric acid solution. Condensed ammonia (50 mL) was mixed with 2–3 drops of indicator (0.1% of BCG and 0.1% of methyl red in 95% alcohol) and mixture was titrated against 0.01 M of HCl with appearance of light pink color at the end. The crude protein (%) was calculated by following the formula: (NxTx0.0014Wx100x6.25).

Fiber content

The crude fiber content was determined by following the AOAC official method No. 962.09. Two gram of the sample was boiled taken in a 250 mL beaker along with 100 mL of 1.25% NaOH for 30 min and filtered through ordinary cloth using a suction pump. The filtrate residue on the cloth was first washed with distilled water, then with acetone and mixed with 100 mL of 1.25% sulfuric acid. The acid-treated washed residue was dried in an oven at 105°C for a few hours till the weight became constant (W1). Then sample was ignited in a muffle furnace at 550–600°C for 2 h and weighed (W2). The following formula was used to compute the percentage of fiber content: [(1- (W1-W2)/weight of sample (g)) x100].

Fat content

The fat content was extracted in a Soxhlet extractor by boiling in diethyl ether at 55°C and then ether was evaporated to measure the fat content using formula [(weight of ether extract (g)/weight of sample (g)) x100] (AOAC official method No. 945.16).

Carbohydrate contents

The carbohydrate content was estimated by subtracting the total mass from the total mass of moisture, ash, fat, and crude protein and expressed as a percentage.

2.9. Estimation of nutrients and heavy metals.

Concentrations of nutrients (Ca, Na, P, Mg, K) and heavy metals (Fe, Cu, Zn, Cd, Pb) in carrot juice were estimated by digesting of nitric acid (HNO_3) in microwave digestion system followed by Inductively Coupled Plasma-Optical Emission [58].

Estimation of vitamins

Vitamin A, B (riboflavin, thiamine, niacin), C contents in juice of carrot juice were measured using atomic absorption spectrophotometer [59]. About 5.0 mL of fresh carrot juice was treated with 50 mL of 1 N H_2SO_4 for 30 min and 3 drops of ammonia solution were mixed followed by filtration. Filtrate (10 mL) was mixed with 5 mL of 50% trichloroacetic acid (for vitamin A, and carotene), and 5 mL of potassium cyanide (for vitamin B-complex) and absorbance was measured at wavelength of 620 nm, 436 nm, 360 nm, 510 nm and 470 nm for vitamin A, carotenes, thiamine, riboflavin and niacin respectively [60]. Carotene was determined utilizing the methodology drawn by Valaden and Mummery [61], following its extraction in the mixture of petroleum ether and acetone. Vitamin C content was determined by the method of Dicholophenol indophenols, AOAC official method No. 985.33 reported by Hussain et al. [62].

Statistical analysis

Collected data was statistically analyzed using analysis of variance followed by LSD significant test using package "Agricolae" and lmer test in R. 3.4.4 (R Core Team, 2018). Violin plots and bar plots were drawn using package "ggplot2" in R.studio. Correlation, principal component analysis (PCA), and heat maps were also drawn using packages "corrr", "FactoMineR", and "pheatmap" respectively for better visualization of data.

Results

Physico-chemical analysis of water and soil samples

The present study reported that the physico-chemical characteristics of soil and normal water samples were below the permissible level (WHO standards), while above in molasses-based waste water samples collected from drainage of sugar industry Waste water contained an excessive quantity of TDS and higher value of pH, EC, BOD, and COD, and considerably high amount of cations, anions, macro and micronutrients, and heavy metals (Table 2).

Table 2 Physico-chemical characteristics of normal water and molasses-based waste water used for irrigation of carrot farming

Characters	Normal water	Soil	Molasses-based waste water
pH	7.7 ± 0.7	7.9 ± 0.23	8.2 ± 0.11
EC (dS m^{-1})	3.71 ± 0.65	4.7 ± 0.13	13.2 ± 0.09
TDS (ppm)	2261 ± 1.45	2373.5 ± 0.03	7345.7 ± 0.04
COD (mg L^{-1})	4.98 ± 0.61	4.5 ± 0.06	435.9 ± 0.61
BOD (mg L^{-1})	2.10 ± 0.45	2.6 ± 0.45	211.1 ± 0.45
Cations (meq L^{-1})			
Ca^{2+}	7.12 ± 1.23	4.04 ± 0.01	5.17 ± 0.21
Mg^{2+}	4.28 ± 1.42	3.02 ± 0.01	4.18 ± 0.14
K^{+}	1.56 ± 0.98	0.41 ± 0.01	5.73 ± 0.1
Na^{+}	22.6 ± 0.32	42.3 ± 0.46	78.6 ± 0.91
Anions (meq L^{-1})			
Cl^{-}	24.1 ± 1.7	34.4 ± 0.2	75 ± 0.4
CO_3^{2-}	0.03 ± 0.004	1.21 ± 0.13	0.95 ± 1.3
HCO_3^{-}	0.22 ± 0.32	0.87 ± 0.15	0.91 ± 1.7
SO_4^{2-}	8.74 ± 0.12	7.74 ± 0.18	13.41 ± 0.22
Macro and micro nutrients (mg L^{-1})			
P	6.4 ± 0.13	6.22 ± 0.01	9.47 ± 0.11
N	7.23 ± 0.33	7.84 ± 0.25	12.92 ± 0.22
S	7.3 ± 0.12	12.7 ± 0.11	24.3 ± 0.76
Fe	0.18 ± 0.25	0.63 ± 0.12	3.39 ± 0.34
Zn	0.01 ± 0.01	0.21 ± 0.01	6.02 ± 0.01
Cu	0.04 ± 0.02	0.01 ± 0.01	6.01 ± 0.01
Cd	0.03 ± 0.01	0.01 ± 0.01	9.01 ± 0.01
Pb	0.02 ± 0.01	0.01 ± 0.01	9.01 ± 0.01

Impact of irrigation treatments on growth and yield contributing traits of carrot cultivar (T-29)

It was observed that molasses-based waste water irrigation treatments (T_1 and T_2) showed significant effects on seed germination ($P<0.01$ each), seedling emergence ($P<0.001$each), leaf length ($P<0.01$each), number of leaves per plant ($P<0.001$, 0.01 respectively), plant fresh biomass ($P<0.001$ each), plant dry biomass ($P<0.01$each) with reference to normal water irrigation (T_0) (Supplementary Fig. S1). On comparison with normal water irrigation (T_0), seeds treated with diluted molasses-based waste water (T_1) showed 13.1%, and 66.4% increase in seed germination, seedling emergence while 20.4% and 16.9% decrease in seed germination, seedling emergence was observed in seeds treated with untreated molasses-based waste water (T_2) respectively (Fig. 1a and b). Later on, highest leaf length (57.8 cm) was recorded for plants irrigated with diluted molasses-based waste water (T_1) followed by plants irrigated with T_2 and T_0 i.e. 31.4 cm, and 25.6 cm respectively (Fig. 1c). The same pattern of growth in terms of number of leaves per plant was observed in plants irrigated with T_0 (10 leaves), T_1 (26 leaves), and T_2 (9 leaves) (Fig. 1d). Additionally, highest values of plant fresh and dry biomass were measured for plants irrigated with diluted molasses-based waste water (T_1) whereas a significant decrease was observed in plants irrigated with untreated molasses-based waste water (T_2) in comparison to normal water irrigation (Fig. 1e and f).

Furthermore, irrigation treatment (T1) of diluted molasses-based waste water significantly ($P<0.001$ each) affected the yield contributing traits (root length, root diameter, and root weight) of carrot cultivar T-29 (Fig. S1) and >90% increase in all these traits was recorded as compared to normal water irrigation. On the other hand, a significant decrease of 10.1%, 53.2%, and 17.3% in root length, root diameter, and root weight of plants irrigated with untreated molasses-based waste water was recorded as compared to normal water irrigation (T_0) respectively (Fig. 1g and h, and 1i).

Impact of irrigation treatments on osmoprotactants and activity of stress responsive enzymes in leave of carrot cultivar (T-29)

Analysis of variance exhibited that molasses-based irrigation treatments (T_1 and T_2) had non-significant effects on osmoprotectants in terms of total soluble sugars, reducing sugars and non-reducing sugars. While, effect of T_2 (untreated molasses-based waste water) was significant ($P<0.01$) on total soluble proteins and total free amino acids as compared to normal water irrigation i.e. T_0 (Supplementary Fig. S2). Total soluble sugar and reducing sugar contents were highest in plants treated with irrigation treatment T_2 followed by T_1 which were statistically at par with T_0 (Fig. 2a, and 2b). On the other hand, non-reducing sugars, total soluble proteins, and free amino acids were maximum in plants irrigated with T_2, followed by T_1 compared to T_0 (Fig. 2c and d, and 2e).

Analysis of variance showed non-significant variation ($P>0.05$) for all stress responsive enzymes including POX, PAL, SOD, CAT, NAR, and NIR activities among irrigation treatments (Supplementary Fig. S3). Mean comparison analysis exhibited that activity of all stress responsive enzymes was relatively higher in plants treated with untreated molasses-based waste water (T_2) followed by plants irrigated with diluted molasses-based waste water (T_1) and normal water (T_0) (Fig. 3a and f). These results suggest that molasses-based waste water irrigation posed negligible oxidative damage in carrot plants.

Impact of irrigation treatments on physical characteristics of carrot juice

Results revealed that effect of irrigation treatment T_1 (diluted molasses-based waste water) was non-significant on volume, torque, pH, and dietary fibers in carrot juice as compared to normal water irrigation (Supplementary Fig. S4), and mean values for these parameters was

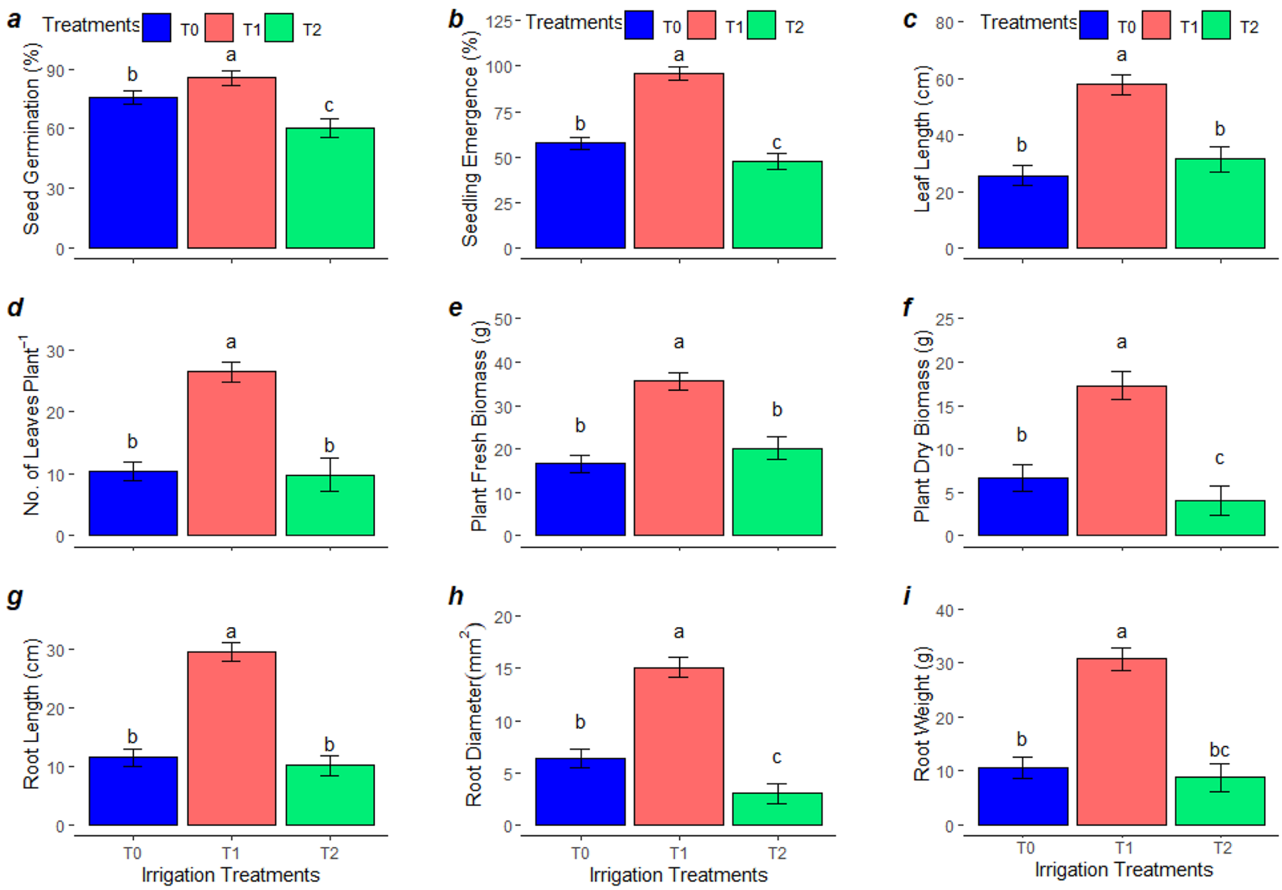

Fig. 1 Classified filled barplots **(a)** seed germination (%), **(b)**, seedling emergence (%), **(c)** leaf length (cm), **(d)** number of leaves per plant, **(e)** plant fresh biomass, **(f)** plant dry biomass, **(g)** root length (cm), **(h)** root diameter (mm^2), and **(i)** root weight **(g)** carrot cultivar T-29. The three irrigation treatments from T_0 to T_2 represented by different color filled violins. One-way ANOVA calculated significant differences, followed by LSD test based lettering are also presented, where same letter did not vary significantly. Each barplot is presenting mean value and error bar represents standard error of six replicates

statistically at par with T_0 (Fig. 4). On the other hand, plant irrigated with untreated molasses-based waste water (T_2) showed significant variation for all physical parameters of juice except pH compared to normal water irrigation (Fig. S4). Mean comparison showed highest values of juice volume (82.7 cm^3), viscosity (38%), torque (16%), and total soluble solids (88%) for plants irrigated with untreated molasses-based waste water (Fig. 4a, b and c, and 4e), while values of pH and dietary fiber content (19%) were higher in plant irrigated with normal water (Fig. 4d and f).

Impact of irrigation treatments on proximate, nutrient and vitamin content of carrot juice

Statistical analysis exhibited that effect of irrigation treatments was non-significant on crude protein, crude fat, crude carbohydrate, crude fiber, and total ash contents of carrot juice except moisture content (Fig. 5). Same pattern of non-significant variation was observed for heavy metal contents (Fe, Cu, Zn, Cd and Pb) between molasses-based waste water irrigation treatments (T_1 and T_2) compared to normal water irrigation (T_0). On the other hand, molasses-based waste water irrigation treatments showed significant variation for mineral nutrients (Ca, Na, P, Mg, and K) in juice with respect to normal water irrigation (Supplementary Fig. S5). Additionally, non-significant variation was observed among irrigation treatment for carotenes, vitamin B-complex (thiamin, riboflavin, niacin), vitamin C except vitamin A content of carrot juice (Supplementary Fig. S6). Mean comparison analysis of these chemical contents of juice showed highest values of proximate, mineral nutrients, and vitamin contents in juice extracted from carrot roots irrigated with normal water (T_0) followed by diluted molasses-based waste water (T_1) and minimal in irrigated with untreated molasses-based waste water (T_2). Whereas, heavy metal content was relatively higher in juice obtained from carrot plants irrigated with untreated molasses-based waste water (T_2) followed by diluted molasses-based waste water (T_1) and normal water irrigation (T_0). Additionally, it was observed that values of heavy metals in all juice samples were below the WHO's

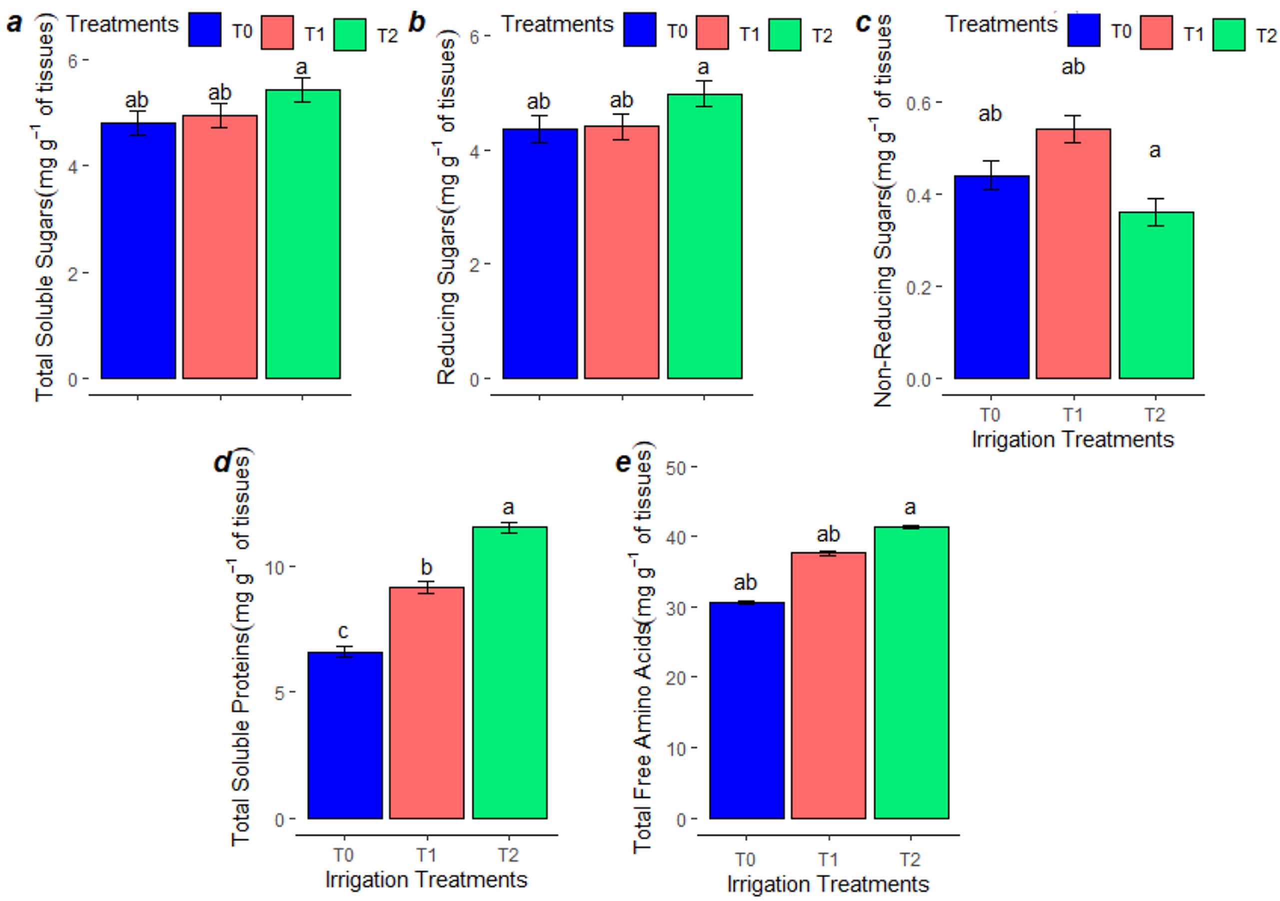

Fig. 2 Classified filled barplots **(a)** total soluble sugars, **(b)** reducing sugars, **(c)** non-reducing sugars, **(d)** total soluble proteins, and **(e)** total free amino acids in leaves of carrot cultivar T-29. The three irrigation treatments from T_0 to T_2 represented by different color filled violins. One-way ANOVA calculated significant differences, followed by LSD test based lettering are also presented, where same letter did not vary significantly. Each barplot is presenting mean value and error bar represents standard error of six replicates

permissible limits except that cadmium and lead contents exceeded the permissible limits in juice obtained from plants irrigated with treatment T_2: untreated molasses-based waste water which may cause health issues in human (Table 3).

Multivariate analysis of collected data

The results indicated that all growth traits except seed germination (that showed no correlation) and yield traits, biochemical contents in leaves except non-reducing sugars (that is negatively correlated with almost all traits) and physical parameters of juice except pH of juice (negatively correlated), total soluble sugars and crude fibers are positively correlated with their own and each other's traits. However, All minerals and vitamin content and almost all biochemical contents in carrot juice showed negative correlation with most of the parameters except biochemical, mineral and vitamins contents in carrot juice showed positive correlation with pH of the juice, dietary fibers, vitamin A, moisture contents, crude proteins, crude carbohydrates, crud fibers, total ash contents, Ca, Na, P, Mg and K, similarly, metal and vitamin contents in carrot juice showed positive correlation with their own contents and metal contents with total soluble solids and crude fats (Fig. 6).

The PCA showed the distribution of irrigation treatments on carrot growth, metabolism, nutrition quality and yield (Fig. 7). Cumulative variance was 100% i.e. the first factor explained 89.6% and second 10.4%. A great difference among the irrigation treatments was evident in carrot cultivation. The maximum coordinate on PCA Biplot was untreated molasses-based wastewater. PCA further showed most of the growth traits are positively correlated with almost all enzymes, vitamins and some biochemical contents and have no correlation with crude proteins, carbohydrates, Ca, K and pH of juice while negatively correlated with non-reducing sugars, seed germination and some other metals and growth parameters. Likewise, enzymes are positively correlated with each other but have negative correlation with some mineral and metals. Vitamins are also positively correlated with each other's but have negative correlation with various

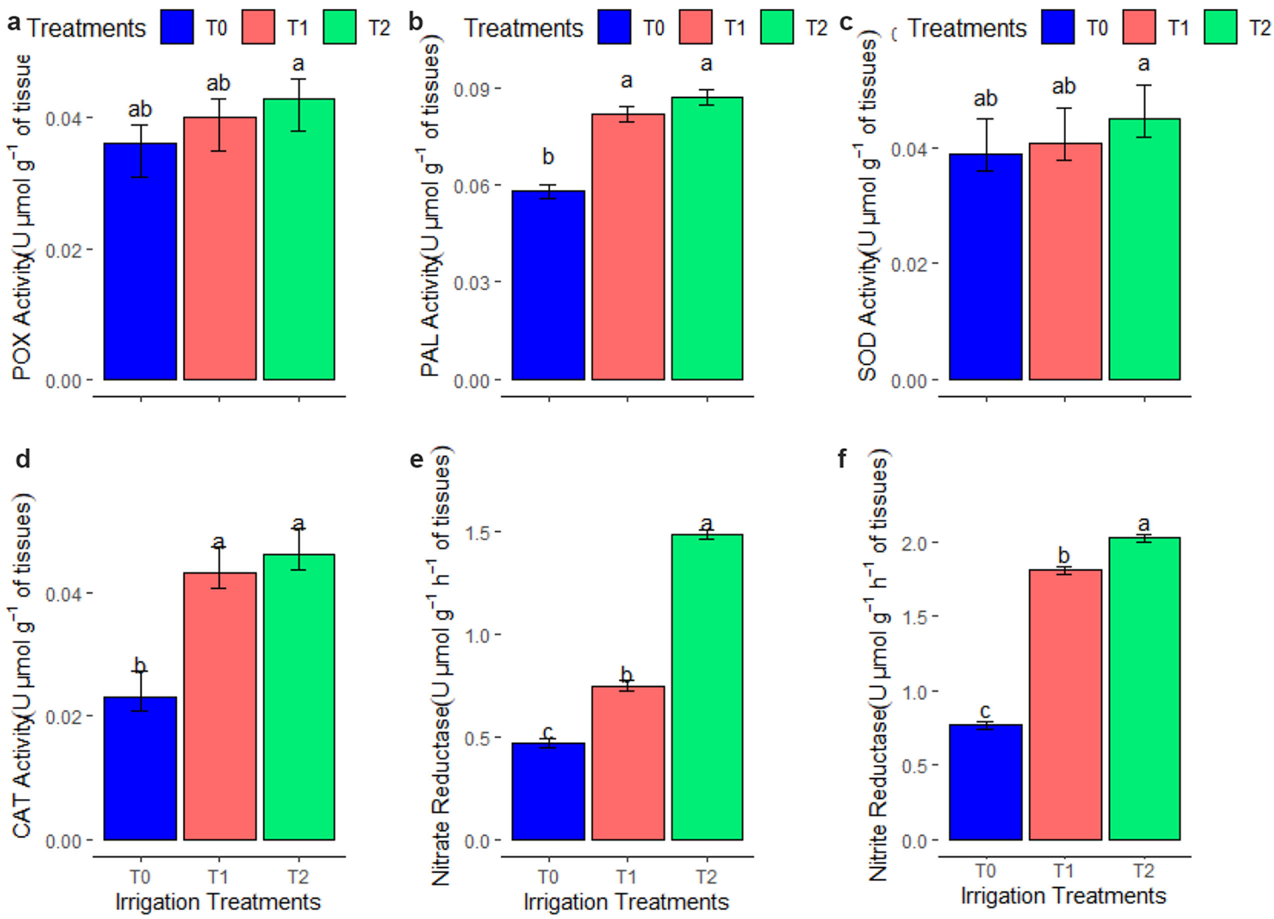

Fig. 3 Classified filled barplots of activity of stress responsive enzymes **(a)** POX, **(b)** PAL, **(c)** SOD, **(d)** CAT, **(e)** nitrate reductase, **(f)** nitrite reductase in leaves of carrot cultivar T-29. The three irrigation treatments from T_0 to T_2 represented by different color filled violins. One-way ANOVA calculated significant differences, followed by LSD test based lettering are also presented, where same letter did not vary significantly. Each barplot is presenting mean value and error bar represents standard error of six replicates

minerals. Additionally, significant variation was observed among the studied parameters (response variable) of carrot plants irrigated with different irrigation treatments in cluster-based heatmap. Total of 49 response variables were classified in three main groups with number of subgroups according to the extent of variation and similarity between them (Fig. 8).

Discussion

Global water scarcity and wastewater use

Freshwater scarcity is a pressing issue exacerbated by several factors such as climate change, population explosion, and unsustainable management practices [63]. Currently, 40% of the global population (∼700 million people) is experiencing the water scarcity [64]. The world population is growing steadily and is projected to touch 9.8 billion by 2050 which will exert extreme pressure on available resources including food and water [65]. The increasing pressure on freshwater resources demands wise use of available fresh water resources [66]. Thus agriculture sector needs to reduce its share of freshwater use and look for alternative sources. Developed nations are using recycled wastewater for their crops irrigation for centuries but it has been estimated 10% of global population is consuming plant food irrigated with raw, partially treated or untreated wastewater in ∼50 countries [4, 67]. Additionally, long term waste water irrigations can flood the soil with persistent toxic elements, salts which ultimately lower the crop yield and also accumulated in edible parts [68]. In Pakistan 26% of national vegetable production is irrigated with municipal wastewaters generated by domestic and or industrial sector despite the associated risks of soil and crops contamination with heavy metals and pathogenic microbes [69].

Impact of molasses-based waste water on carrot growth

Present study was designed to evaluate the impacts of molasses-based waste water of a sugar mill on carrot cultivation because sugar mills use a lot of fresh water for processing and release half of the water as effluent which

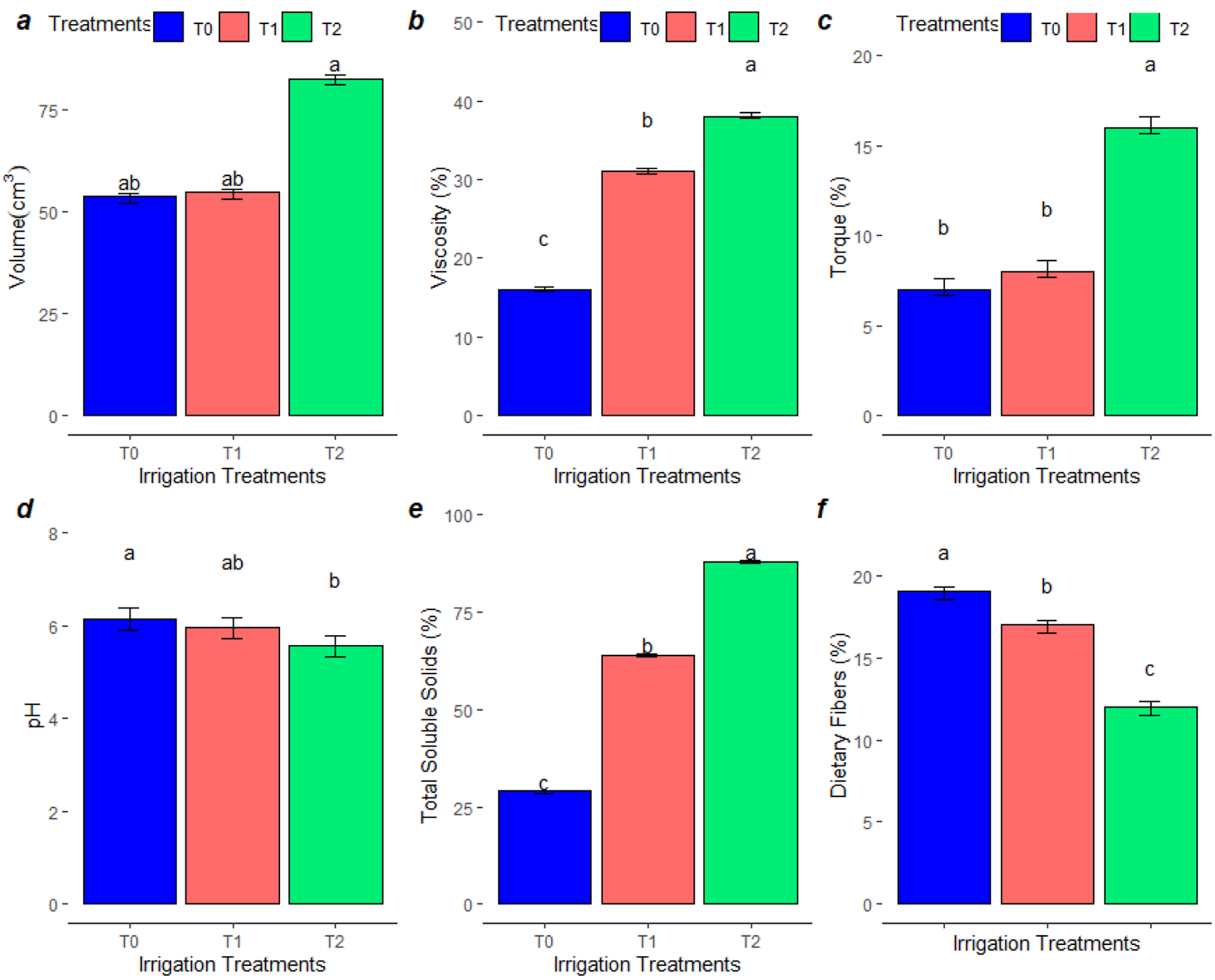

Fig. 4 Classified filled barplots of physical parameters of carrot juice **(a)** volume, **(b)** viscosity, **(c)** torque, **(d)** pH, **(e)** TSS: total soluble solids, **(f)** dietary fibers. The three irrigation treatments from T_0 to T_2 represented by different color filled violins. One-way ANOVA calculated significant differences, followed by LSD test based lettering are also presented, where same letter did not vary significantly. Each barplot is presenting mean value and error bar represents standard error of six replicates

is used for crop irrigation in the arid area, receive low annual rainfall [10, 70]. Molasses-based waste water of sugar mill was found to be a rich source of various important cations, anions, mineral nutrients, organic matter along with accumulation of toxic heavy metals such as Fe, Zn, Cu, Pb, and Cd (Table 1). These toxic traces accumulated in the effluent during processing and purification of sugarcane and beet root juices in response of covalent collisions of colliding particles [71]. It was assumed in this study that presence of high concentration of organic matter and dissolved substances will enhance the crop growth. While, high pH, EC, BOD, COD values will not only suppress the biological activities of microorganisms [72], toxic metal elements will accumulate in edible carrot roots and negatively affect the nutritional quality [73]. Previous studies also reported the accumulation of various heavy metals (Fe, Zn, Cu, Cr, Cd, Pb) in the edible parts of the different vegetables irrigated with municipal waste water [74–78]. Thus, water analysis of present study and findings of previous studies suggest that untreated molasses-based waste water is not appropriate for irrigation whereas, diluted waste water with some proportion of freshwater could be a potential source of plant nutrients and can be used for irrigation purposes [29, 79, 80]. It was observed in present study that growth and yield of carrot plants was significantly increased when carrots are irrigated with diluted molasses-based waste water as compared to normal water irrigation (Fig. 1). Enhanced growth of carrot crop in response of diluted molasses-based waste water irrigation could be attributed to presence of mineral nutrients, and organic matter contents [81]. Similar findings were reported by Saharan et al. [82], and Rael, [83] in spinach and barley. Previous studies showed that molasses waste of sugar

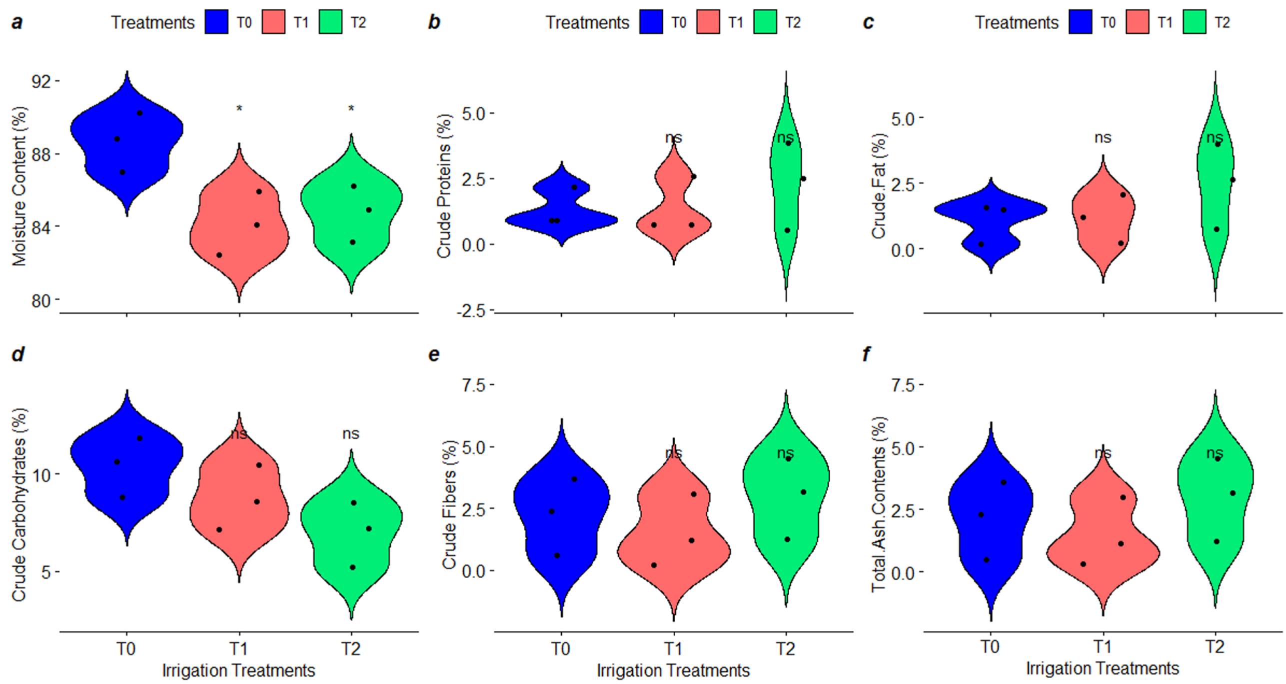

Fig. 5 Classified filled violin plots **(a)** moisture content, **(b)** crude proteins, **(c)** crude fat, **(d)** crude carbohydrates, **(e)** crude fibers, **(f)** total ash contents in leaves of carrot cultivar T-29. The three irrigation treatments from T_0 to T_2 represented by different color filled violins. Each violin is delimited 25th and 75th percentile and mean of 5 to 7 seedlings from six replicates represented by dots. One way ANOVA calculated significant differences among the treatment represented with *** ($P<0.0001$), ** ($P<0.001$), * ($P<0.05$), and ns: non-significant ($P>0.05$) notations

Table 3 Mean comparison analysis for proximate composition, vitamins, and minerals in carrot roots treated with different irrigation treatments

Treatments	**Moisture (%)**	**Crude protein (%)**	**Crude fat (%)**	**Carbohydrates (%)**	**Crude fiber (%)**	**Total ash (%)**
T_0	88.8 ± 1.7 a	0.9 ± 0.23 a	0.2 ± 0.11 a	10.6 ± 1.21 a	2.4 ± 0.2 a	2.3 ± 0.21 a
T_1	84 ± 1.65 b	0.7 ± 0.13 a	0.2 ± 0.09 a	8.6 ± 1.35 ab	1.2 ± 0.25 b	1.1 ± 0.16 b
T_2	83 ± 1.45 b	0.5 ± 0.03 a	0.7 ± 0.04 a	5.2 ± 0.27 b	1.2 ± 0.11 b	1.2 ± 0.06 b
Treatments	**Carotene (mg 100 mL^{-1})**	**Thiamine (mg 100 mL^{-1})**	**Riboflavin (mg 100 mL^{-1})**	**Niacin (mg 100 mL^{-1})**	**Vitamin C (mg 100 mL^{-1})**	**Vitamin A (mg 100 mL^{-1})**
T_0	5.3 ± 1.23 a	0.04 ± 0.01 a	0.02 ± 0.0 a	0.2 ± 0.1 a	4.2 ± 0.23 a	89.8 ± 1.7 a
T_1	3.1 ± 1.42 b	0.002 ± 0.01b	0.008 ± 0.0 b	0.03 ± 0.0 b	2.4 ± 1.34 b	75.1 ± 1.65 b
T_2	2.56 ± 0.98 c	0.002 ± 0.00 b	0.003 ± 0.1 b	0.002 ± 0.0 b	2.1 ± 1.45 b	67.3 ± 1.45 b
Treatments	**Ca (mg 100 mL^{-1})**	**Na (mg 100 mL^{-1})**	**P (mg 100 mL^{-1})**	**Mg (mg 100 mL^{-1})**	**K (mg 100 mL^{-1})**	
T_0	34 ± 1.7 a	40 ± 0.21a	25 ± 0.14a	9.2 ± 1.0a	42.2 ± 1.2 a	
T_1	27 ± 0.4 b	32 ± 0.13b	16 ± 1.23b	4.4 ± 0.21b	35.1 ± 0.3 b	
T_2	22 ± 0.32c	27 ± 0.15c	12 ± 1.47c	3.21 ± 0.27b	26.1 ± 0.12 c	
Treatments	**Fe (mg L^{-1})**	**Cu (mg L^{-1})**	**Zn (mg L^{-1})**	**Cd (mg L^{-1})**	**Pb (mg L^{-1})**	
T_0	0.001 ± 0.0 b	0.001 ± 0.0 b	0.001 ± 0.0 b	0.001 ± 0.0 b	0.001 ± 0.0 b	
T_1	0.04 ± 0.12 a	0.87 ± 0.11 a	1.53 ± 0.76 a	0.014 ± 0.12 a	0.005 ± 1.22 ab	
T_2	0.078 ± 0.25 a	0.93 ± 0.12 a	1.69 ± 0.34 a	2.96 ± 0.03 a	2.41 ± 0.23 a	
WHO Limits	**0.3**	**2.0**	**3.0**	**0.03**	**0.01**	

industry has long been used to irrigate the crops including sugarcane, and rice resulted in remarkable economic benefits [84]. Additionally, present study also revealed a marked reduction in growth and yield was observed when plant was irrigated with untreated molasses-based waste water (Fig. 1). Use of untreated waste water induces the oxidative stress leads to the overproduction of ROS that may inhibit the growth and development of crops [85]. Overproduction of ROS triggers the lipid peroxidation, membrane disruption which ultimately affects

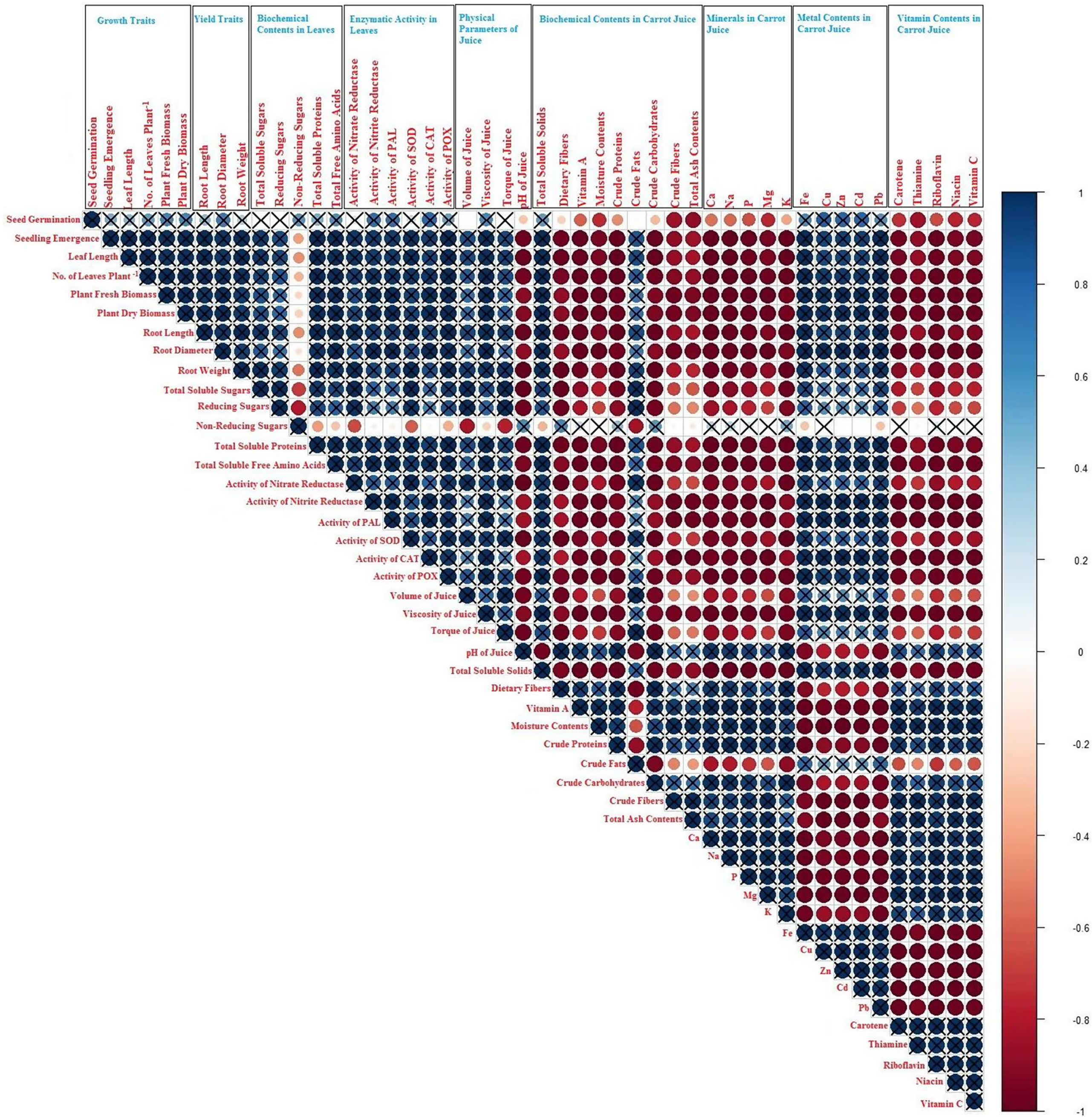

Fig. 6 Pearson's correlation coefficients among 49 pairs of response variables of carrot cultivar treated with different irrigation treatments

the osmotic and ionic homeostasis and severe damage to plant cells [86, 87]. However, plants have potential to avoid or tolerate these oxidative cellular damages by higher accumulation of antioxidants, stress responsive enzymes, and various osmoprotectants [88, 89].

Impact of molasses-based waste water irrigation on osmoprotectants and stress responsive enzyme activity in carrot plants

In present study, concentration of various osmoprotectans such as total soluble sugars, reducing and non-reducing sugars, total soluble proteins, and free amino acids were increased in plants exposed to untreated and diluted molasses-based waste water treatments as compared to normal water irrigation (Fig. 2). The extent of the increase in osmoprotectants could be linked with

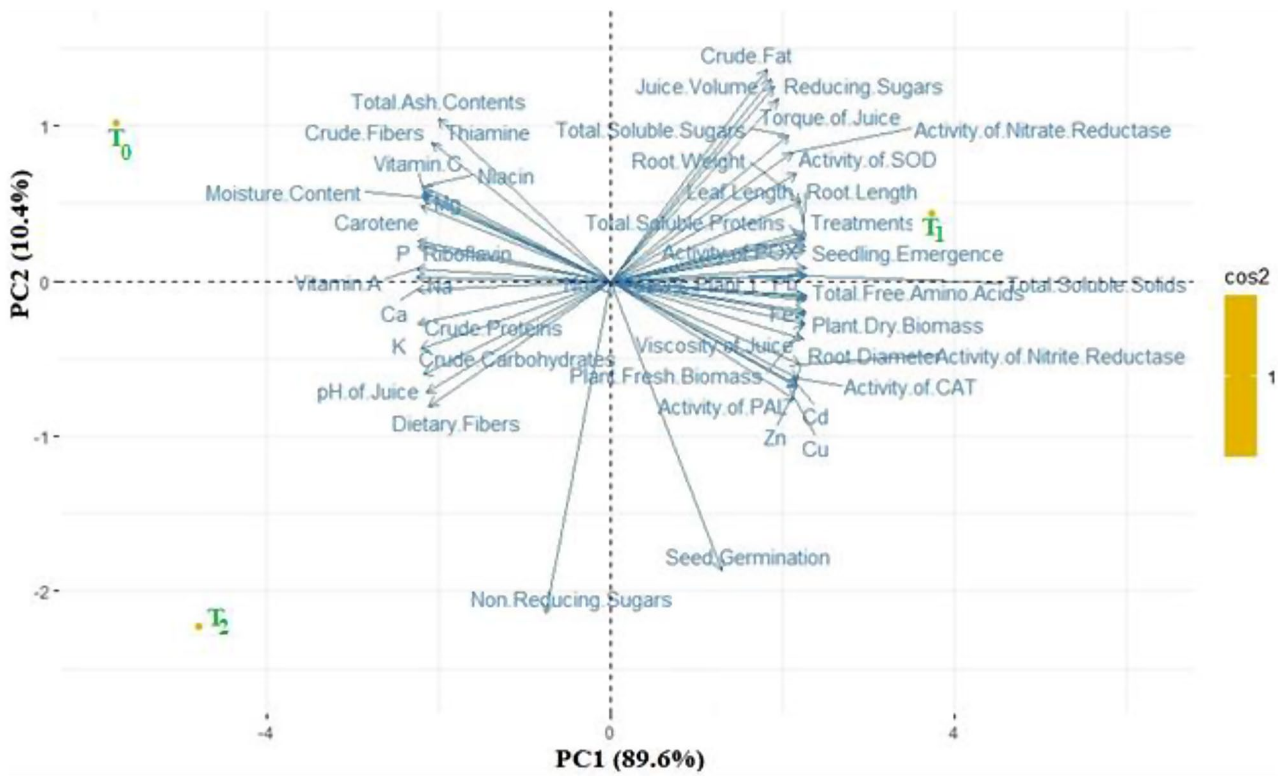

Fig. 7 Biplot of principal components analysis of 3 irrigation treatments and 49 response variables of carrot cultivar T-29

concentration of toxic metal elements in the water used for the irrigation of crop [90] and it is regarded as an important adaptive plant response to avoid the metal toxicity. It has been previously reported that accumulation of soluble sugars along with other osmolytes in response of stress conditions assists the osmotic adjustments and strengthen the plant metabolism by storage of sufficient reserves [91, 92]. Additionally, it is also believed that proline accumulation in plant tissues under toxic metal stress enhance the hydroxyl radicals scavenging along with osmotic adjustments and lipid stabilization [93]. These osmoprotectants can reduce the oxidative damages by chelating with toxic heavy metal elements in cytoplasm of the cell. Similar findings were also reported by [94]. Improved growth of carrot plants irrigated with diluted molasses-based waste water depicts the improved potential of plant to uptake nutrients which maintain the turgor potential, osmotic balance and membrane stability [95].

In present study, a significant increase in activity of stress responsive enzymes including POX, PAL, SOD, CAT, NAR and NIR were observed in response to irrigation with molasses-based waste water as compared to normal water irrigation (Fig. 3). The extent of the increase in enzymatic activity could be linked with concentration of toxic metal elements in the waste water [90]. Increase in the activity of antioxidant stress responsive enzymes like CAT, POX, SOD confirms the stress level in plants as it has been observed in different plants in response to abiotic stress conditions and accumulation of toxic heavy metals [96]. Sahin et al. [97] also reported increased activity of enzymes in cabbage leaf tissues and soil irrigated with waste water as soil enzymes are also crucial for different reactions occurring in biogeochemical nutrient recycling and assist the pollutants detoxification into beneficial smaller elements boost the plant and microfauna [98].

Health risks and environmental concerns associated with molasses-based waste water irrigation

Presence of toxic metal elements in the molasses-based waste water cannot be ignored as it poses serious environmental and health concerns [98, 99]. Present study revealed toxic metal elements accumulation was observed in edible carrots and their fresh juice. Concentration of cadmium and lead was also exceeded in response of molasses-based waste water irrigation which may be linked with persistent chemical nature of these heavy metals. In contrast with these toxic metal elements, mineral nutrients, proximate contents, and vitamin contents were reduced in these samples (Table 3). Previous studies reported that heavy metals easily accumulated in vegetables than cereals and fruits [100–102] and lessen their nutritional value by reducing the magnitude

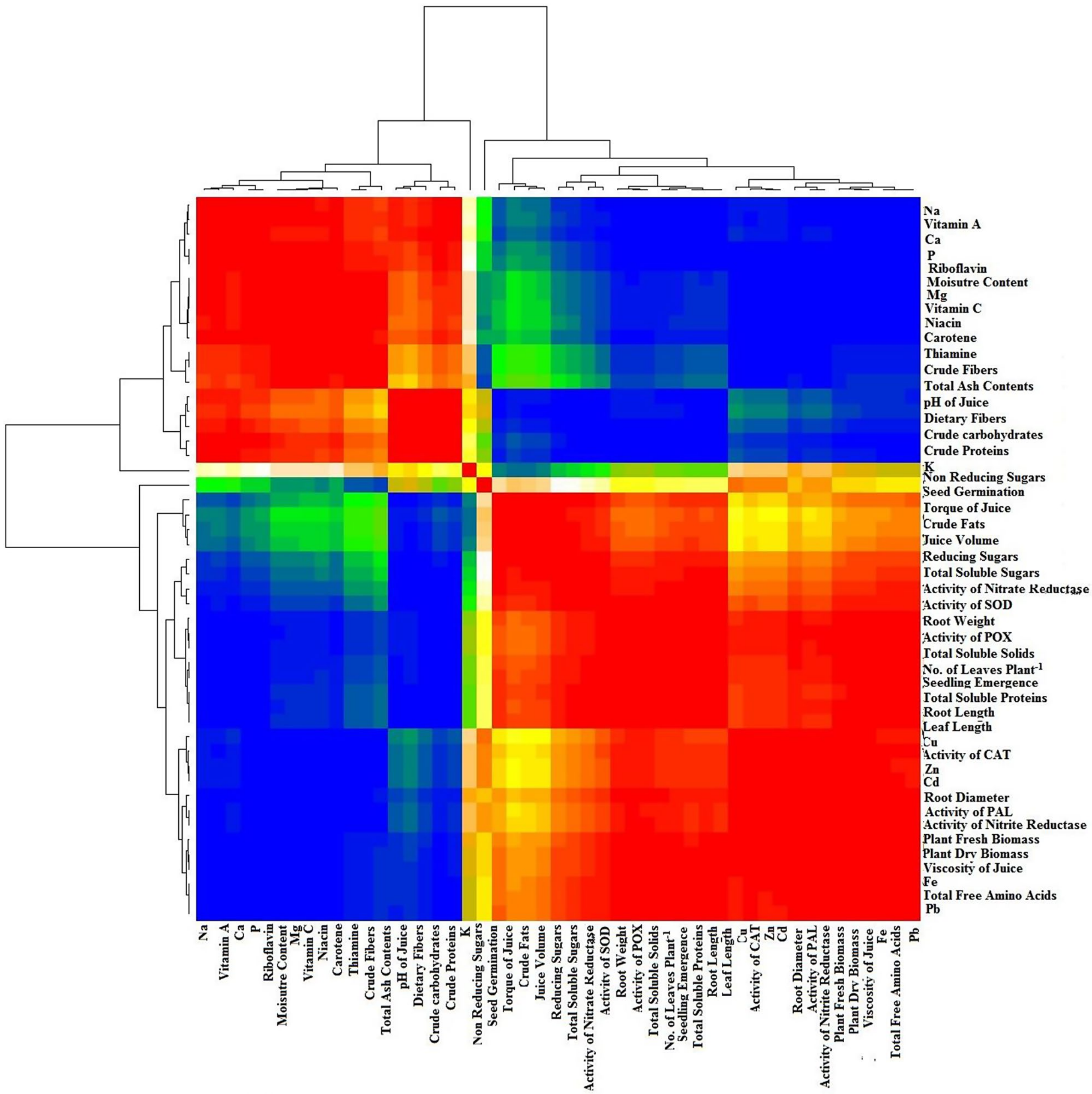

Fig. 8 Cluster based heatmap of three irrigation treatments and 49 response variables of carrot cultivar (T-29)

of antioxidants, pigments, and fibers etc. It could be explained as toxic heavy metals are absorbed by roots and their translocation to arial parts is slow [103]. Thus findings of present study and a previous study [31] confirms that carrot is not hyper-accumulator of toxic metal because carrot root is major site for the synthesis of chelating elements and accumulates high magnitude of toxic metals. Moreover, permissible limits of these toxic heavy metals reported by WHO's Standards for edible liquids were compared with the detected amounts in carrot root juice (Table 3). It was observed that concentration of Cd and Pb in carrot juice only exceeded the permissible limits in plants irrigated with untreated molasses-based waste water due to their persistent chemical nature. Similarly, hyper-accumulation of these two toxic metals in edible parts of vegetables irrigated with waste water [80]. The hyper-accumulation of metal contents in edible crops irrigated with different wastewaters exceeding the permissible limits set by WHO is a serious food safety concerns. Redox reactions are triggered by toxic heavy metals in the metabolism which generate the free radicals that damage the DNA and proteins [104]. Consumptions of such food crops contaminated with toxic metal elements may cause several foodborne illnesses including neurological disorders, renal damage, cardiovascular diseases, reproductive toxicity, skin lesions, and carcinomas in consumers [105]. Frequent and long term consumption of contaminated foods can cause severe morbidity and mortality as well as serious other environmental concerns that must be addressed carefully [106]. Thus, Present study also discourage the use of untreated waste water for irrigation purposes, however 50% dilution of molasses-based waste water is a safe, cost effective, and potential alternative of freshwater irrigation of vegetables including carrot.

Conclusion

Present study evaluated the impacts of molasses-based waste water irrigation on carrot growth, yield and nutritional quality. Results revealed that the effects of untreated molasses-based waste water exhibited significant reduction in growth, yield, physiology, metabolism, and nutritional contents of carrot. Additionally, Cd and Pb contents accumulation in carrot root of plants irrigated with untreated molasses-based waste water were found to exceed the permissible limits suggested by WHO and their consumption will cause health risks. While, diluted molasses-based waste water irrigation positively enhanced the growth, yield of carrot plants without affecting the nutritional quality. This strategy is cost effective, appeared as most appropriate alternative mean to reduce the freshwater consumption in water deficit regions of the world. This study suggest that policymakers should focus to develop guidelines for safe use of waste water irrigation and implement by raising awareness in farmers regarding risk associated with untreated waste water irrigation. The future research must be focused on developing strategies such as heavy metals remediation to avoid their accumulation in crops, and long term impact of diluted waste water irrigations on the soil health as well.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05527-1.

Supplementary Material 1

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Author contributions

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Data availability

The author confirms that all data generated or analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate

We all declare that manuscript reporting studies do not involve any human participants, human data, or human tissue. So, it is not applicable.

Consent for publication

Not applicable.

Study protocol must comply with relevant institutional, national, and international guidelines and legislation

Our experiment follows the relevant institutional, national, and international guidelines and legislation.

Competing interests

The authors declare no competing interests.

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