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Foliar application of salicylic acid improved morpho-anatomical features of potato by irrigating with wastewater

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Abstract

Background This study aimed to evaluate the suitability of using drain water as a source of irrigation and its effects along with salicylic acid on morphological, anatomical, physico-chemical as well as yield attributes of potato. For this study, potato tubers were grown in pots and irrigated with different concentrations of drain water. Salicylic acid treatments *vis*. 0, 0.5 and 1.0 mM were applied foliarly. Pre- and post-harvest analysis was carried out to determine different attributes of soil, water and plants after 60 days.

Results The growth of potato plant was increased as the concentration of SA increased through increasing shoot length, fresh/dry weight and tuber number/plant. In this research work, plant respond to overcome metal stresses by up regulating antioxidant defense system such as, peroxidase, catalase and superoxide dismutase) by application of highest treatment of SA when irrigated with 6% drain water. Plants accumulated the highest concentrations of Cd, Cr, and Pb in the leaves when treated with 1 mM of SA, compared to other plant parts. It was observed that photosynthetic pigment enhanced in 6% drain water treated plants when applied with 1mM SA as compared to control. An increase in epidermis and cortical cell thickness, as well as stomatal closure, was observed, helping to maintain water loss under stress conditions.

Conclusions According to these results, it can be suggested that SA is potent signaling molecule can play an essential role in maintaining potato growth when irrigated with drain water containing heavy metals through stimulating metal up take and up regulation of antioxidant enzymes.

Keywords Drain water, Metal stress, Irrigation water, Salicylic acid, *Solanum tuberosum*

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Introduction

As global industrialization and technological advancements continue, the release of pollutants has increased exponentially. The effects of the polluting substances on the neurological system of humans can have notable effects on population mental health [1]. Numerous pollutants, including inorganic ions, organic pollutants, organometallic compounds, radioactive isotopes, gaseous pollutants, heavy metals, and nanoparticles, have severely harmed the ecosystem. Anthropogenic activity has resulted in a rise in heavy metal pollution [2]. In addition to polluting the soil, heavy metals have an impact on the production and quality of food. They may accumulate in plant tissues [3] and have an impact on yield and are toxic to plants even at extremely low concentrations [4].

In Pakistan, there is a scarcity of freshwater, much of which is unsuitable for agricultural use or drinking in several regions [5]. Moreover, delivery efficacy of canal water to field is declining in Pakistan, which has enhanced dependence on groundwater and wastewater [6]. Generally, groundwater contains high sodium, calcium, total dissolved salts, free ions and in some cases high concentrations of toxic substances such as heavy metals [7, 8]. Several reports delineated high quantities of arsenic and other toxic metals in groundwater of country [9, 10].

Potatoes are now primary food and economic sources in emerging nations. It can be regarded as a key food crop for ensuring the nutritional security of global populations [11]. With a total production of 3,660 thousand tons, the province of Punjab produces the most potatoes, followed by Khyber Pakhtunkhwa with 143 thousand tons, Baluchistan with 22,000 tons, and Sindh with 6,000 tons. Punjab's primary potato-producing areas include Okara, Sahiwal, Kasur, Pakpattan and Chiniot [12]. To our knowledge, potatoes do not thrive well in soils heavily infested with pollutants and are considered a sensitive crop due to loss in tuber yield worldwide.

The use of antioxidants and biostimulants to improve crops under various stresses is well evidenced in the literature [13]. Evidence points out that salicylic acid having a unique and specific regulatory function under various stresses [14]. This signaling molecule improves the biochemical properties, resistance to abiotic stress and ultimately increase the growth and yield. It also helps in up regulation of several enzymatic and non-enzymatic antioxidants [15]. Although, studies regarding this positive role of salicylic acid are numerous in literature but with reference to cross talk with drain water having several toxic heavy metals are scanty. Thus, the present investigation aimed to evaluate the suitability of using drain water as a source of irrigation and its effects along with SA on growth, morphological, anatomical, physico-chemical and biochemical parameters as well as yield and quality of commercially important potato cultivar grown in Pakistan. Utilizing sewage or drainage water for irrigation of major crops like potato could reduce the need for freshwater in agriculture [16].

Materials and methods

Procurement of plant material, wastewater, and potting mix

Potato tubers (*cv. cardinal*) were procured from the Potato Seed Center, University of the Education Lahore, Pakistan. Wastewater was collected from the drain (containing sewage and domestic wastewater) running outside the University of Education, College Road Township, Lahore. For the potting mix, sieved garden soil was collected from the Botanical Garden of Department of Botany, University of Education, Lahore Pakistan.

Experimental design

Potato tubers of uniform size having equal nodes were sown in a plastic pot having potting mix (soils and organic matter 1:1) and irrigated with tap water whenever required for 15 days. Subsequently, the plants were irrigated with drain water at four different concentrations (0, 2, 4, and 6%), each with three replicates. Salicylic acid (Dejung Korea) was sprayed foliar in three treatments (0, 0.5 and 1 mM) manually. The experiment was set up in a Complete Randomized Block Design in wire house at Botanical Garden. After 60 days of treatment of Drain water and salicylic acid, the plants were harvested to study the various above and belowground morpho-anatomical characteristics of different parts of potato plant along with various physiological parameters. The data for pre- and post-harvest physicochemical analysis of garden, contaminated soil and drain water was also recorded.

Physicochemical analysis of garden soil and drain water

Different parameters of soil and drain water were carried out before and after experiment. The solutions of soil and drain water were made for analyzing pH by using a multiparameter (HI 2211 China). EC meters were used to record the readings (ETL-01). The organic matter content of different samples of soil was studied by using the methods of [17]. We measured the soil moisture content according to [18].

$$Moisture\ Content\ (MC) = \frac{(weight\ of\ moist\ soil\ (M) - weight\ of\ dry\ soil\ (D))}{weight\ of\ dry\ soil\ (D)} \times 100$$

The total solids were determined in various soil extract samples by using the method of [19]. According to this procedure, total dissolve, and suspended solids (TSS) were calculated by following formula:

$$TSS\ (mg\ L^{-1}) = \frac{(final\ weight - intial\ weight)}{sample\ volume} \times 1000$$

We measured the concentrations of calcium (Ca) and magnesium (Mg) ions using the hardness method described by Howari et al. (2005). The titration method of [20] was used to determine the total alkalinity, carbonates, and bicarbonates. The total alkalinity in the drain water was calculated by using the formula:

$$Total\ Alkalinity = \frac{Volume\ of\ sulphuric\ acid}{Volume\ of\ sample\ taken\ (ml)} \times 1000$$

Chemical and Biochemical oxygen demand in the wastewater samples was also determined using the method employed by [20]. The BOD and COD were calculated using the formula:

$$BOD\ \left(mg\ L^{-1}\right) = (D1 - D2) - (S) \times Vs/P$$

$$COD\ \left(mg\ L^{-1}\right) = (a - b) \times C \times 8000\ /\ sample\ volume\ (ml)$$

Morphological parameters of potato

The plants were harvested, and tubers were taken out of the pots to investigate the various morphological characteristics, such as shoot fresh weight, length, number, dry weight, leaf length, leaf width, leaf area, and leaf number. The number of tubers and their fresh weight were also recorded at the conclusion of experiment.

Physiological and biochemical parameters

Various physiological parameters, including chlorophyll a and b, carotenoids, and the ratio of chlorophyll a to b, were studied. Estimates of the protein, hydrogen peroxide and antioxidants (peroxidase and superoxide dismutase) were also analyzed in potato plant. Chlorophyll content was estimated by the method of [21, 22]. The hydrogen peroxide was measured by [23]. The protocol of [24] was used to determine the protein content whereas activity of peroxidases was estimated by following the procedure of Sakharov & Ardila (1999) [25]. Catalase activity was measured by using Bears and Sizer (1974) [26] method and superoxide dismutase by following Maral et al. (1977) [27].

Heavy metals analysis in leaves and tubers of solanum tuberosum L.

Sodium and potassium levels of samples were measured by using flame photometer (ETL-04 China). Heavy metals were determined in wastewater, soil, and plant samples by using Atomic Absorption Spectrophotometer (Perkin Elmer AA5000) before and after treatment. The samples were digested according to the methods of [28]. The heavy metal content in leaves was determined by following the method of [29]; similarly, the heavy metals in tubers were examined using the technique of [30].

Anatomical features

For anatomical studies, samples were inserted into the potato cylinder (2 cm piece) and a segment of the plant's root and leaves were cut in 2 cm [31]. Toluidine Blue stain was used, and the thin sections were cut then sliced and put on a slide. The anatomical characteristics of the root area of parenchyma cell (m^2), epidermal thickness (m), cortical cell thickness (m), pith area (m^2), and leaves epidermis (stomata density, stomatal cell area (m^2) were investigated.

Statistical analysis

One-way ANOVA and Duncan's Multiple Range Test (DMRT) were applied in all parameters to compare the means of all replicates at the significant threshold of $p \leq 0.05$. SPSS software (21.0.0) was used to statistically analyze the data.

Results

Pre harvest analysis of soil

The experiment was carried out by four different concentrations of drain water (0, 2, 4 and 6%) with three treatments of salicylic acid (0, 0.5 and 1 mM) followed by three replicates. Three heavy metal were traces i.e. Cadmium (Cd), chromium (Cr), and lead (Pb), at the start of experiment for the control soil samples. It was noted that the soil had a pH of 6.95. The electrical conductivity of the control soil was 107 dS cm^{-1}, the alkalinity in the soil was 15.05 mg L^{-1}. The bicarbonates were 15.05 mg L^{-1}, while the carbonates were found to be absent. The calcium ion in the soil was 40.1 mg kg^{-1}, the Mg ions were 40.1 mg kg^{-1}; the K ions were recorded to be 131.56 mg kg^{-1} while the amount of Na ions was 75 mg kg^{-1}. Cd, Cr and Pb were also studied; however, these metals were not detected in the control samples. The organic matter in the soil was 2.08%, while the moisture content was 6.31%. The data indicated that all values are below the permissible limits as indicated in the given Table 1.

Pre harvest analysis of drain water

The pH of the drain water was determined in four different concentrations of., (0, 2, 4 and 6%). The lowest pH value (6.87) was found in control, while the highest pH was 8.1 (6% DW). The EC of drain water was found to be maximum (555 µS cm^{-1}) in 6% while the lowest value found in control 491 µS cm^{-1}. As the concentration of drain water increased, the amount of the total dissolved solids was also increased. The TDS was minimum (225 mg L^{-1}) in control while it was maximum in 6% DW (260 mg L^{-1}). The highest BOD value was 62.56 mg L^{-1} in 6% drain water while the lowest value (45.11 mg L^{-1}) was found in 0% DW. The COD of the drain water was found to be minimum in control (72.56 mg L^{-1}) as compared to 6% drain water having the highest value (132.44

Table 1 Analysis of different physio-chemical parameters of different concentrations of soil before experimentation

Parameter	Control soil	Permissible limits (WHO 2011)
pH	6.95 ± 0.19	7–8.5
EC (dS cm^{-1})	107 ± 1	1400
Alkalinity (mg L^{-1})	15.05 ± 0.01	-
Carbonate (mg L^{-1})	N.D	-
Bicarbonate (mg L^{-1})	15.05 ± 0.01	-
Calcium (mg kg^{-1})	40.1 ± 0.01	100
Magnesium (mg kg^{-1})	40.1 ± 0.01	150
Potassium (mg kg^{-1})	131.56 ± 0.04	-
Sodium (mg kg^{-1})	75 ± 0.2	200
Lead (mg kg^{-1})	N.D	0.1
Cadmium (mg kg^{-1})	N.D	0.03
Chromium (mg kg^{-1})	N.D	0.1
Organic matter content (%)	2.08 ± 0.01	-
Moisture (%)	6.31 ± 0.02	-

± = Standard deviation

N.D = Not detected

mg L^{-1}). The sulphate ion in 0% (control) drain water was the lowest with a value of 56 mg L^{-1}. In 2% drain water, sulphate ions reached their highest value of 61 mg L^{-1} and in 6% DW (61 mg L$^{-1)}$ and 8% DW (71 mg L^{-1}), respectively. The chloride content was highest in 4% DW (53.11 mg L^{-1}). In 0% drain water, 41 mg L^{-1} of chloride ions were found while in highest concentrations of DW have maximum chloride ions (59.11 mg L^{-1}). The alkalinity level was noted and lowest amounts for alkalinity was found in the control sample (43 mg L^{-1}). The value of total alkalinity in 6% drain water was 71.94 mg L^{-1}. The concentration of drain water was examined for the presence of carbonates, which was found to be absent. The lowest concentration of bicarbonates was detected in the DW in control (43 mg L^{-1}), while the highest concentration was found at 4% (63.65 mg L^{-1}) and 6% DW (76.94 mg L^{-1}). The concentration of Ca was 24.06 mg L^{-1} in 0% drain water and 44.11 mg L^{-1} in 6% drain water as depicted in the Table. Mg concentrations ranged from 36.39 mg L^{-1} in 0% drain water, 30.06 mg L^{-1} in 2% DW, 36.04 mg L^{-1} in 4% DW and 62.08 mg L^{-1} in 6% drain water. Potassium ions in 0% drain water were detected with value of 5 mg L^{-1} while highest potassium ions were found in 4 and 6% DW i.e., 9 mg L^{-1} and 11 mg L^{-1} respectively. The maximum concentration of Na was found in 6% drain water (81 mg L^{-1}) while the lowest value was found in control (24 mg L^{-1}). Among heavy metals, the concentration of cadmium in 6% drain water was the highest (0.030 mg L^{-1}) as compared to 2, 4 6% DW. The amount of chromium was observed to be 0.036 mg L^{-1} in 4% DW and 0.049 mg L^{-1} in 6% DW whereas no chromium is detected in control and 2% drain water concentrations. The lead content was 0.016 mg L^{-1} in 6% drain water, which was the highest concentration. But Pb was not found in the lowest concentration of drain water as shown in Table 2.

Table 2 Analysis of different physio-chemical parameters of different concentrations of drain water before experimentation

Parameter	(Tap water) 0% DW	2% DW	4% DW	6% DW	Permissible limits WHO
pH	6.87^{c} ± 0.1	7.7^{c} ± 0.01	7.9^{b} ± 0.06	8.1^{a} ± 0.04	6.5–8.5
EC (µS cm^{-1})	491^{c} ± 1	522^{b} ± 1	528^{b} ± 1	555^{a} ± 1	≤ 1000
TSS (mg L^{-1})	62^{c} ± 0.1	76 ^{b}±0.01	81^{c} ± 0.06	90^{a} ± 0.04	150
TDS (mg L^{-1})	225^{c} ± 0.01	245^{b} ± 0.1	250^{c} ± 0.01	260^{a} ± 0.01	≤ 500
Chloride ions (mg L^{-1})	41^{d} ± 1	53.11^{c} ± 1	57.67^{b} ± 1	59.11^{a} ± 1	≤ 250
COD (mg L^{-1})	72.56^{d} ± 1	88.77^{c} ± 1	105.22^{b} ± 1	132.44^{a} ± 1	150
BOD (mg L^{-1})	45.11^{d} ± 1	52.33^{a} ± 1	57.67^{b} ± 1	62.56^{a} ± 1	80
Sulphate ion (mg L^{-1})	56^{d} ± 1	61^{c} ± 1	65^{b} ± 1	71^{a} ± 1	≤ 100
Alkalinity (mg L^{-1})	43^{a} ± 1	55.5^{c} ± 0.1	66.65^{bc} ± 0.01	71.94^{b} ± 0.01	50–200
Carbonate (mg L^{-1})	N.D	N.D	N.D	N.D	-
Bicarbonate (mg L^{-1})	43^{a} ± 1	51.5^{c} ± 0.1	63.65^{b} ± 0.01	76.94^{b} ± 0.01	-
Calcium (mg L^{-1})	24.06^{b} ± 0.01	30.03^{d} ± 0.01	36.04^{c} ± 0.01	44.11^{a} ± 0.01	≤ 200
Magnesium (mg L^{-1})	36.39^{d} ± 0.01	42.03^{c} ± 0.01	52.13^{b} ± 0.01	62.08^{a} ± 0.01	≤ 250
Potassium (mg L^{-1})	5^{c} ± 0.01	7^{b} ± 0.01	9^{ab} ± 0.01	11^{a} ± 0.01	≤ 50
Sodium (mg L^{-1})	24 ^{c}±0.2	76 ^{c}±0.2	79 ^{b}±0.2	81 ^{a}±0.2	≤ 200
Lead (mg L^{-1})	N.D	N.D	N.D	0.016 ± 0.1	0.5
Cadmium (mg L^{-1})	N.D	N.D	0.013^{b} ± 0.2	0.030^{a} ± 0.1	0.1
Chromium (mg L^{-1})	N.D	N.D	0.036^{a} ± 0.01	0.049^{a} ± 0.01	1.0

± = Standard deviation

SA = salicylic acid

N.D = Not detected

The values are mean of three replicates. The alphabets show level of significance at $p \leq 0.05$ based on DMRT using ANOVA

Morphological attributes of potato plants in leaves

The potato plants showed maximum growth in terms of fresh weight (78.2 g) with highest concentration (6%) of drain water under highest treatment of salicylic acid 1 mM. It was observed that the growth of plants was reduced (37.3 g) when irrigated with 0% DW concentration followed by control treatment (0 mM SA). An increase in dry weight of the plants was observed with highest treatment of SA (1 mM) as compared to the control plants (1.31 g). As the concentration of drain water and salicylic acid increased it also increased the dry weight of the shoot. The shoot length of the potato plant showed significant results against the salicylic acid treatments and drain water concentration (Fig. 1A-H). The maximum shoot length was observed in plants along with replicated when 1 mM SA was applied. The shoot length was increased from 36.1, 44.5 and 74.7 cm in (0, 0.5 and 1mM SA in 0% DW) to 39.4, 51.2. The number of shoots was 11.33 in (0% DW and 0 mL SA), 14 in 2% DW and 15 in 6% DW concentration. When salicylic acid was applied as a treatment to the plants of potato, the no. of shoots increased i.e., 20 in 0.5 mM and 42.6 in 1 mM SA. The no. of shoots was further increased from 20 to 42.6 in highest treatment of SA in 4% DW as compared to the control treatment. However, the highest no. of shoots (80) was observed in highest concentration of DW under highest treatment as compared to the control 0 mM SA, where the no. of shoots was reduced to be 11.33 (Fig. 2A & B). The length of the leaves was measured in all DW concentrations under all treatment of salicylic acid, and it was noticed that as the plants were irrigated with different drain water concentrations, the leaf increases in size gradually. The leaves looked healthier (25.3 cm) as compared to the control plants (3.53 cm). Similar response was observed in leaf width of potato plants as well. The width of the potato leaves was recorded in the form of table in all treatments of salicylic acid and concentrations of drain water. It was observed that leaf width was reduced in control concentration (19.6 cm) with 1 mM SA whereas it was increased by increasing concentration of DW and SA treatment for 2% DW (23.1 cm), 4% DW (43.1 cm) and 6% DW (49.43 cm) respectively. For the measurement of the area of the leaves, length and width of the leaf was recorded initially. The maximum area of leaves (61.3) was observed in 6% drain water concentration as compared to the 4% (58.83) and 2% DW (49.5) as compared to the control i.e., 0% DW. The number of leaves increased gradually as the treatment of SA and the concentrations was increased in potato plants. As the maximum growth of leaves (101) was observed in highest concentration of drain water as compared to the control.

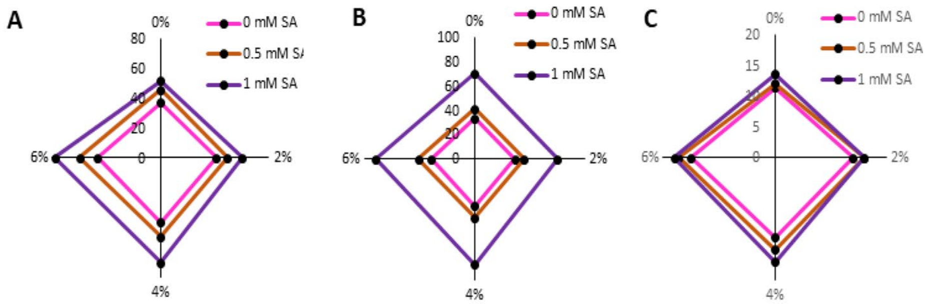
Morphological attributes of potato tubers

Among tuber parameters, the number of tubers was counted to be highest (18.67) in 1 mM salicylic acid treatment when irrigated with 6% drain water concentration. In 2% drain water concentration, the no. of tubers was 8 (0 mM SA), 15 (0.5 mM SA) and 16 in 1 mM SA. The number of tubers was also increased in 4% DW as recorded to be 17 in 1 mM, 16 in 0.5 mM and only 10 in 1 mM SA. The maximum weight of the tuber was recorded 144.7 g in 6% DW, 143.7 g in 4% DW, 132.45 g in 2% DW, and 120.36.7 g in 0% DW under 1 mM SA treatment as compared to the control treatment (89.59 g). The data was also recorded for yield of potato tuber. The maximum yield (916.05 g) was observed in 1 mM SA when irrigated with 6% drain water as compared to all other treatments (Fig. 3A-C).

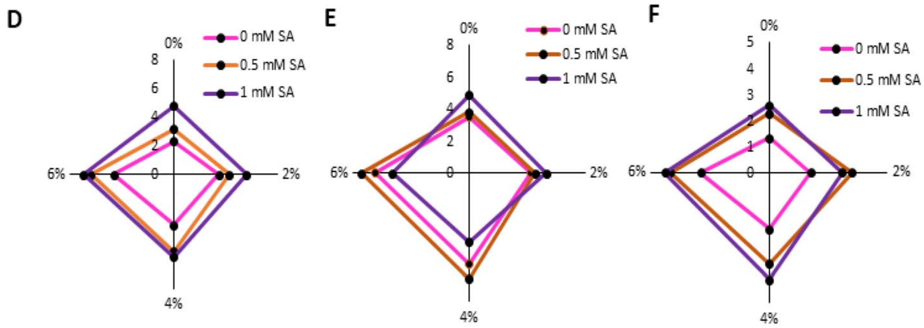
Biochemical and physiological attributes of potato plants

Various physiological attributes in the plants of potato were measured like H_2O_2, protein, peroxidases, Chlorophyll (a, b, a & b, a/b) and carotenoids. When the plants treated with 0% DW in the presence of 0 mM SA, the amount of H_2O_2 in the potato plant was minimum (17.57 g mol^{-1}), and highest in 6% DW in the presence of 1 mM SA (59.86 g mol^{-1}). Hydrogen peroxide provides defense system to plants by actively regulate the plant growth under stress condition. The nutritional need of the plant was measured in terms of amount of protein that is also increased in the highest treatment of salicylic acid after irrigation with 6% drain water i.e., 0.89 mg 100 g^{-1} as compared to the control plants (0.044 mg 100 g^{-1}). Peroxidases were found to produce the reactive oxygen species in plants, the level of POD was observed to be lowest in 0% DW (0.733 U m L^{-1} of enzyme) and the highest peroxidase level (2.633U mL^{-1} of enzyme) was recorded in 6% DW as compared to 2 and 4% DW concentrations. Catalase and superoxide dismutase activity was also increased significantly by applying salicylic acid to wastewater (6%) treated plants as presented in Table 3. Catalase and superoxide dismutase also showed an increasing trend by increasing concentration of wastewater.

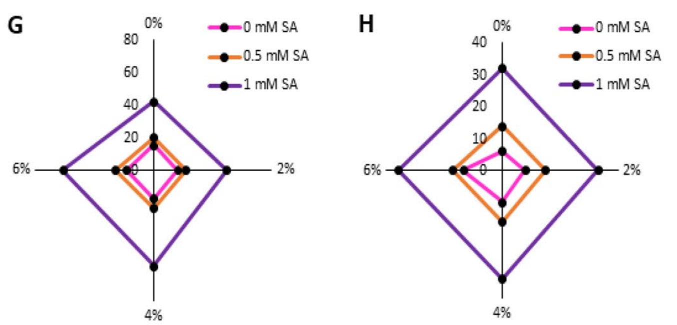
The highest concentration of chlorophyll a (1.93 mg g^{-1} FW) was found in leaves treated with 1 mM SA and 6% DW. Low levels of chlorophyll a (0.013 mg g^{-1} FW) were found in 0 mM SA and 0% drain water. The leaves treated with 1 mM SA and 6% DW have the highest chlorophyll b content (0.198 mg g^{-1} FW) while the plants treated with lower treatment (0% DW and 0 mM SA) have value (0.019 mg g^{-1}) lower than the highest treatment. The ratio of chlorophyll a and b in potato leaf tissue was measured and the maximum value (4.01) was observed in 1 mM SA when the plants were irrigated with the highest concentration of drain water (6%). The lowest chlorophyll



Shoot fresh weight (g)					Shoot length (cm)					No. of shoots				
	0%	2%	4%	6%		0%	2%	4%	6%		0%	2%	4%	6%
0 mM SA	C	b	b	a	0 mM SA	c	b	b	a	0 mM SA	c	ab	bc	a
0.5 mM SA	C	bc	b	a	0.5 mM SA	d	c	b	a	0.5 mM SA	d	c	b	a
1 mM SA	D	C	b	a	1 mM SA	d	c	b	a	1 mM SA	d	c	b	a



Shoot dry weight (g)					Leaf length (cm)					Leaf width (cm)				
	0%	2%	4%	6%		0%	2%	4%	6%		0%	2%	4%	6%
0 mM SA	d	c	b	a	0 mM SA	a	a	a	a	0 mM SA	b	b	a	b
0.5 mM SA	c	b	a	a	0.5 mM SA	c	bc	b	a	0.5 mM SA	c	bc	b	a
1 mM SA	b	a	a	a	1 mM SA	b	a	a	a	1 mM SA	b	a	a	a



Leaf area					No. of leaves				
	0%	2%	4%	6%		0%	2%	4%	6%
0 mM SA	a	a	a	a	0 mM SA	c	b	a	c
0.5 mM SA	c	bc	b	a	0.5 mM SA	c	b	a	b
1 mM SA	b	a	a	a	1 mM SA	c	b	a	b

Fig. 1 (**A-H**): Study of different morphological parameters of potato plants irrigated with different concentrations of drain water and salicylic acid after 60 days

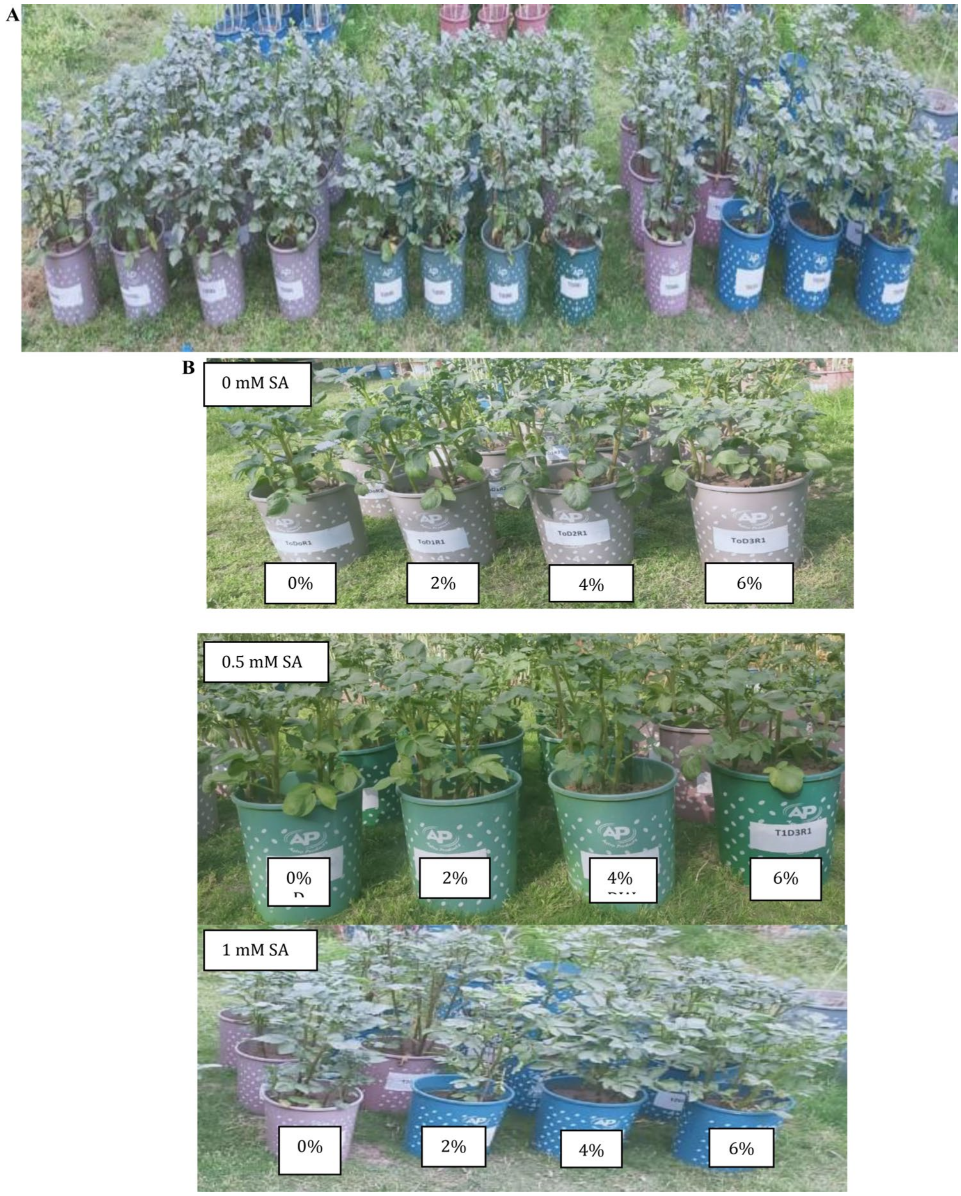


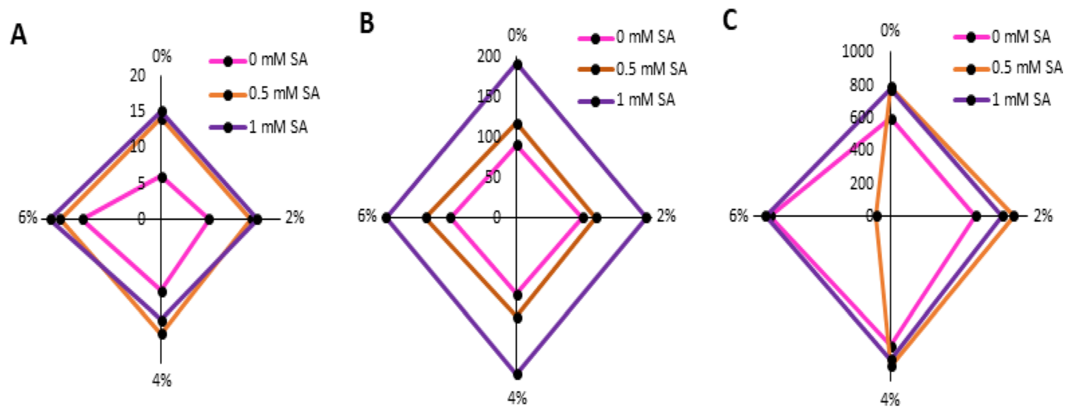
Fig. 2 **A** Study of various morphological attributes in leaves of *Solanum tuberosum* L. grown under various treatments of salicylic acid after 60 days of experiment. **B** Comparison of various treatments of salicylic acid in plants of *Solanum tuberosum* L. irrigated with different concentration of drain water at the time of harvest

ratio (0.68) was observed in case of control treatment and control DW concentration. Low levels of carotenoids (1.15 mg g^{-1} FW) were found in leaves treated with 0 mM salicylic acid and irrigated with 0% drain water. The highest concentration of carotenoids (3.55 mg g^{-1} FW) was observed in maximum treatment with highest DW concentration (Fig. 4A-D).

Estimation of heavy metals in the leaves and tubers of potato

The potato tubers were also analyzed against heavy metals (Pb, Cr and Cd) presented graphically (Fig. 5A-C). The concentration of all metals was below the permissible limits as described by WHO. When plants were treated with 1 mM SA and 6% drain water, the lead (Pb) was found to be maximum (0.011 mg Kg^{-1}) and in 0 mM SA and 0% drain water, the lead was not detected. The highest concentration of chromium (0.009 mg Kg^{-1}) was recorded in 1 mM SA irrigated with 6% drain water as compared to the other concentrations. Similar results were recorded for cadmium 0.017 mg kg^{-1}. No metal was detected in tubers under control concentration and control treatment.

The data for the estimation of heavy metals like Lead (Pb), Chromium (Cr) and Cadmium (Cd) in the leaves and tubers was recorded after harvesting of plants (60



No. of tubers					Tuber wieight (g)					Tuber yield (g)				
	0%	2%	4%	6%		0%	2%	4%	6%		0%	2%	4%	6%
0 mM SA	c	bc	b	a	0 mM SA	c	ab	b	a	0 mM SA	b	b	a	a
0.5 mM SA	b	ab	ab	a	0.5 mM SA	c	c	b	a	0.5 mM SA	c	b	a	d
1 mM SA	b	ab	ab	a	1 mM SA	d	c	b	a	1 mM SA	d	c	b	a

Fig. 3 (**A-C**): Study of various parameters of potato tubers irrigated with different concentrations of drain water and Salicylic acid after 60 days of experiment

Table 3 Study of various physiological attributes of potato plants irrigated with different concentrations of drain water and salicylic acid after 60 days of experiment

Parameter	Concentration of DW	Treatment 0 mM SA	0.5 mM SA	1 mM SA
H_2O_2 (g mol^{-1})	0%	$17.57^a \pm 0.4$	$17.69^d \pm 0.5$	$18.64^d \pm 0.2$
	2%	$20.70^b \pm 0.4$	$20.79^c \pm 0.2$	$21.71^c \pm 1.8$
	4%	$21.98^b \pm 0.2$	$21.99^b \pm 0.4$	$22.58^b \pm 0.5$
	6%	$22.52^c \pm 0.5$	$23.55^a \pm 0.1$	$24.86^a \pm 0.04$
Protein (mgg^{-1})	0%	$0.044^a \pm 0.03$	$1.321^d \pm 0.02$	$1.664^d \pm 0.07$
	2%	$0.94^b \pm 0.03$	$1.343^c \pm 0.02$	$2.695^c \pm 0.04$
	4%	$1.158^c \pm 0.01$	$2.414^b \pm 0.06$	$2.753^b \pm 0.06$
	6%	$2.181^d \pm 0.01$	$2.450^c \pm 0.02$	$2.893^a \pm 0.03$
POD (U mL^{-1} of enzyme)	0%	$0.733^a \pm 0.09$	$1.202^d \pm 0.01$	$2.244^d \pm 0.02$
	2%	$0.754^a \pm 0.02$	$1.237^c \pm 0.02$	$2.551^c \pm 0.01$
	4%	$0.774^a \pm 0.05$	$1.263^b \pm 0.01$	$2.653^a \pm 0.01$
	6%	$0.815^a \pm 0.01$	$1.334^a \pm 0.01$	$2.633^b \pm 0.02$
CAT (U mL^{-1} of enzyme)	0%	$0.55^a \pm 0.02$	$1.67^d \pm 0.07$	$4.21^d \pm 0.01$
	2%	$0.124^a \pm 0.04$	$1.675^c \pm 0.06$	$3.44^c \pm 0.02$
	4%	$0.233^a \pm 0.01$	$1.866^b \pm 0.04$	$4.21^a \pm 0.02$
	6%	$0.354^a \pm 0.02$	$2.342^a \pm 0.07$	$5.231^b \pm 0.03$
SOD (Unit mg^{-1} of protein)	0%	$1.234^a \pm 0.02$	$2.542^d \pm 0.03$	$2.789^d \pm 0.02$
	2%	$2.671^a \pm 0.03$	$3.764^c \pm 0.01$	$5.678^c \pm 0.06$
	4%	$2.113^a \pm 0.01$	$4.421^b \pm 0.03$	$6.123^a \pm 0.04$
	6%	$3.251^a \pm 0.02$	$5.227^a \pm 0.04$	$7.892^b \pm 0.01$

DW = Drain Water

SA = Salicylic Acid

± = Standard deviation

The data representing mean of three replicates. Different alphabets show level of significance at $p \leq 0.05$ based on DMRT using ANOVA

days) as shown graphically in Fig. 5 (D-F). No metals uptake was observed in 0% drain water concentration in both leaves and tubers. In leaves, the highest metal uptake was observed for Cd in the highest treatment of salicylic acid i.e., 0.23 mg kg^{-1}. Whereas the amount of Pb was 0.19 mg kg^{-1} and the concentration of Cr was 0.17 mg kg^{-1} in 1mM SA. In 2% DW, the concentration of Pb was 0.09 mg kg^{-1} in control, 0.11 mg kg^{-1} in 0.5 mM and 0.15 mg kg^{-1} in 1mM SA. The amount of cadmium was 0.11, 0.14 and 0.15 mg kg^{-1} found in 0, 0.5 and 1 mM SA treatment.

Anatomical attributes of potato plants

Root anatomy

The roots were examined for various anatomical features like epidermal thickness, cortical cells thickness, pith area, area of vascular bundle and area of parenchyma cells Fig. 6(A-L). The roots were treated with various SA treatments like 0, 0.5 and 1mM. The results indicated that the highest epidermal thickness (36.60 μm) was observed in 6% DW whereas 0% DW and 0 mM SA showed the lowest epidermal thickness (7.33 μm). The results indicated that the highest epidermal thickness (36.60 μm) was observed in 6% DW whereas 0% DW and 0 mM SA showed the lowest epidermal thickness (7.33 μm). The cortical cell thickness was observed to be reduced in the 0% drain water (87 μm) and the highest cortical thickness was reported in the 6% drain water under 1 mM SA (219.68 μm) as compared to the other treatments. The maximum pith area was found in the plants when irrigated with the highest drain water concentration (117.45 μm) while the lowest pith area was

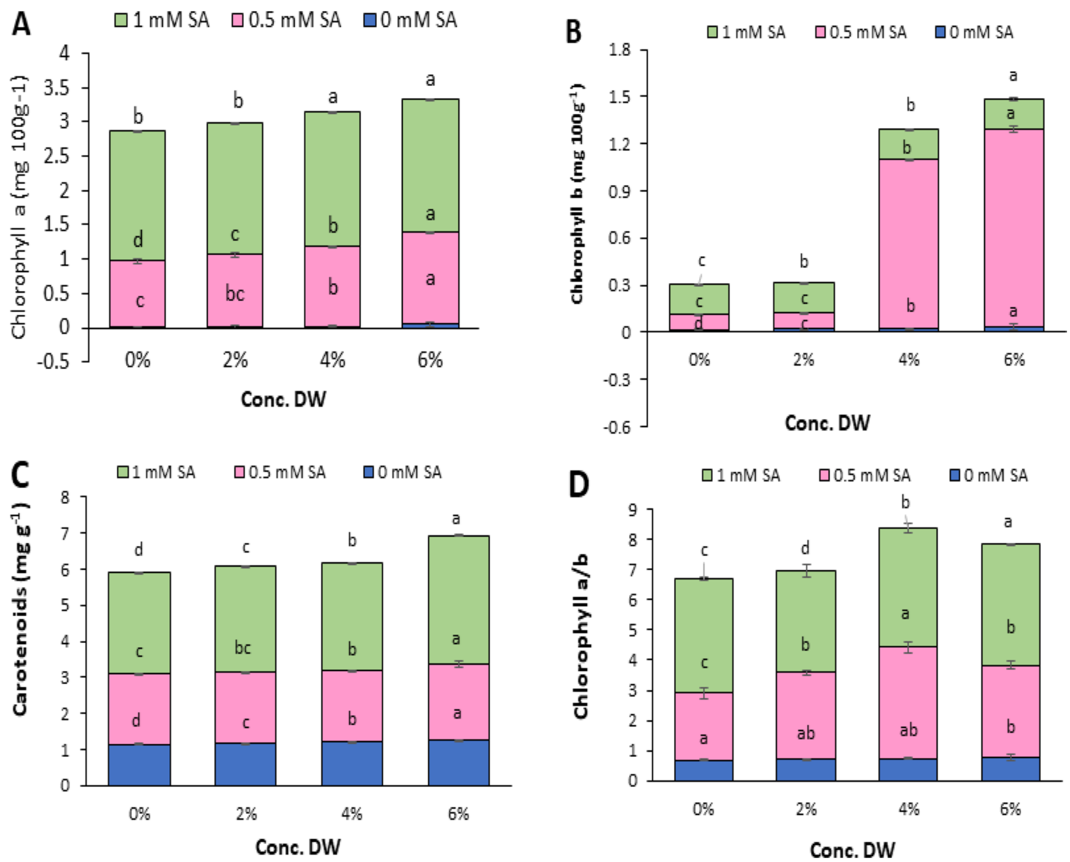


Fig. 4 (**A-D**): Determination of chlorophyll content in leaves of potato plants irrigated with different concentrations of drain water and Salicylic acid after 60 days of experiment

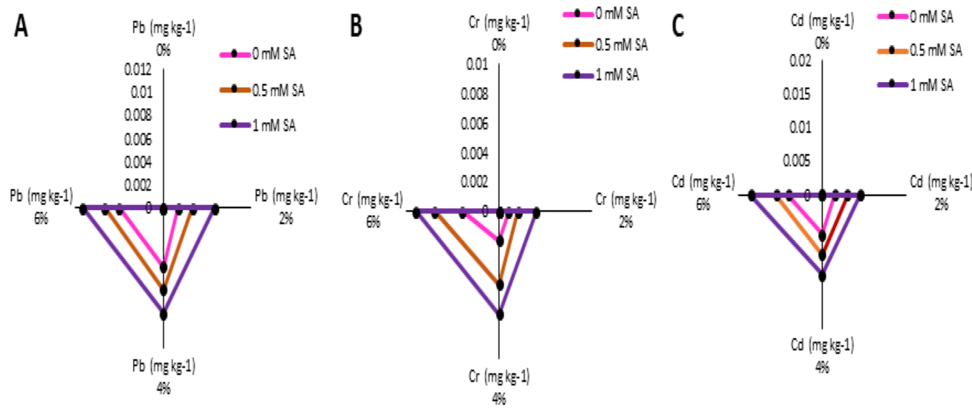
recorded in 0% drain water (57.88 µm) and the highest was (193.7 µm) in 1 mM SA. The minimum area of the vascular bundle was found in 0% DW (66.8 µm), 2% DW (69.47 µm), 4% (72.33 µm), and 6% (75.23 µm) in and 0 mM SA respectively. While maximum area of vascular bundles (161.55 µm) was found in maximum treatment (1 mM). The area of parenchyma cells was lowest in the root treated with 0% and 0 mM SA with value of 14595.13 µm, and highest in the root treated with 1 mM SA and 6% drain water (58875.63 µm) as presented graphically in Fig. 7(A-E).

Leaf anatomy of potato plants

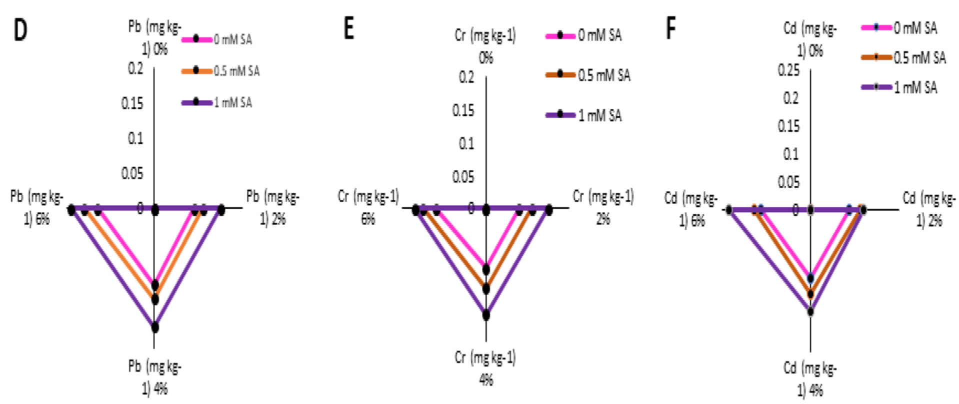
The hand section technique was used to observe the anatomical characters of potato leaves under various salicylic acid (SA) treatments and drain water concentrations, as illustrated in Fig. 8(A-L). The leaves irrigated with 0% DW showed the stomatal density of 21, 27 and 61 in 0, 0.5 and 1 mM SA treatment as shown graphically in When the plants irrigated with 2 and 4% DW, stomatal density was increased in all treatments of salicylic acid. The maximum stomatal area covered (1264173.1 µm^2) in the leaves when 1 mM SA was applied after every week having value of and the minimum stomatal cell area was recorded in the epidermis of potato leaves than all other drain water concentrations under control treatment i.e., 1,146,625 µm^2. Similar results were recorded with other concentrations. The results are statistically significant at $p \leq 0.05$ (Fig. 9A & B).

Analysis of soil after final harvest

After two months of experiment, the soil was examined for various parameters to compare the initial and final analysis as shown in Table 3. The lowest soil pH (6.75) was recorded in the 0% drain water with 0 mM SA treatment, and the highest (6.9) in the 1 mM SA treatment with 6% drain water. The EC was minimum (193.3 dS/cm) in 0% DW and 0 mM SA while maximum (282 dS/cm) in 6% DW under highest salicylic acid treatment. The percentage of organic matter was found to be highest in 6% DW under 1 mM SA treatment (8.97%), whereas the lowest percentage was found in the control treatment (3.42%). The maximum amount of moisture content (7.09%) was found in highest concentration of DW and SA as compared to the control treatment (1.54%). The level of soil alkalinity was 6.46 mg L^{-1} (0 mM SA), 5.07 mg L^{-1} (0.5 mM SA) and 8.34 mg L^{-1} (1 mM SA) in 0% DW. The samples showed highest alkalinity level (9.30



Tubers														
Pb (mg kg^{-1})					Cr (mg kg^{-1})					Cd (mg kg^{-1})				
	0%	2%	4%	6%		0%	2%	4%	6%		0%	2%	4%	6%
0 mM SA		c	b	a	0 mM SA		c	b	a	0 mM SA		b	b	a
0.5 mM SA		c	b	a	0.5 mM SA		c	b	a	0.5 mM SA		b	ab	a
1 mM SA		c	b	a	1 mM SA		b	ab	a	1 mM SA		a	a	a



Leaves															
Pb (mg kg^{-1})						Cr (mg kg^{-1})					Cd (mg kg^{-1})				
	0%	2%	4%	6%			0%	2%	4%	6%		0%	2%	4%	6%
0 mM SA		c	b	a	0 mM SA			c	b	a	0 mM SA		b	b	a
0.5 mM SA		c	b	a	0.5 mM SA			c	b	a	0.5 mM SA		b	ab	a
1 mM SA		c	b	a	1 mM SA			b	ab	a	1 mM SA		a	a	a

Fig. 5 (**A-F**): Analysis of different heavy metals in tubers and leaves of potato plant irrigated with different concentrations of drain water and Salicylic acid after 60 days of experiment

mg L^{-1}) in 6% DW concentration. The highest concentration of Pb (0.26 mg kg^{-1}) was found in highest treatment, whereas the in control samples, it was not detected. The concentration of Pb in 2, and 4%DW was 0.23 and 0.24 mg kg^{-1} in 1mM SA. The uptake of Cr was highest 0.23 mg kg^{-1} in 6% DW as compared to the 0.19 mg kg^{-1} in 0% DW and 0mM SA treatment. But Cr was absent in all control samples. Similar results were recorded for Cadmium. When 1 mM salicylic acid was applied to the plants at 6% drain water, the amount of Na was highest 101.35 mg kg^{-1} as compared to the 2% (99.5 mg kg^{-1}) and 4% drain water concentration (101.06 mg kg^{-1}). The

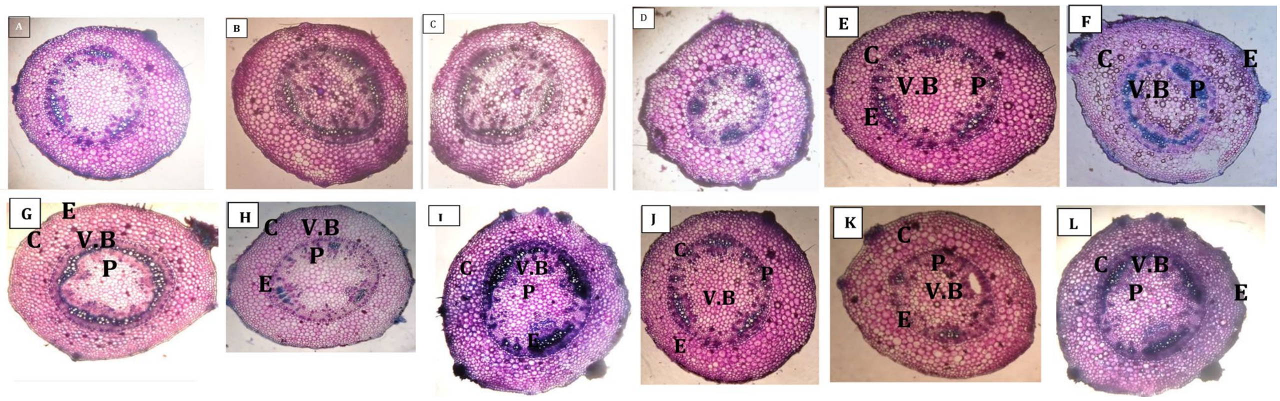


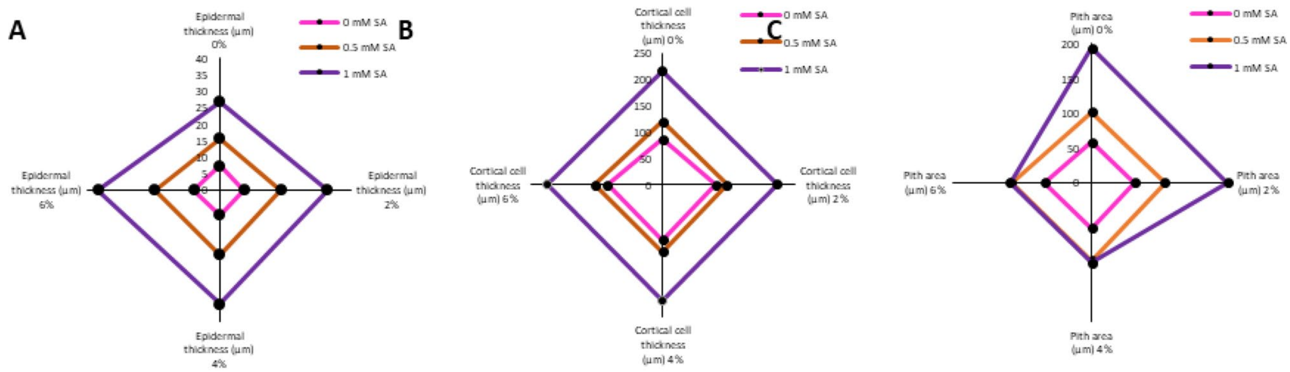
Fig. 6 (**A-D**): Root anatomy of potato plant (*Solanum tuberosum* L.) treated with 0.5 mM SA irrigated with different concentrations of drain water (A: 0% DW, B: 2% DW, 4% DW and 6% DW) (C: cortex, V.B: vascular bundles, P: pith and E: epidermis). (**F-H**): Root anatomy of potato plant (Solanum tuberosum L.) treated with 0.5 mM SA irrigated with different concentrations of drain water (A: 0% DW, B: 2 % DW, 4% DW and 6% DW) (C: cortex, V.B: vascular bundles, P: pith and E: epidermis). (**I-L**): Root anatomy of potato plant (*Solanum tuberosum* L.) treated with 1 mM SA irrigated with different concentrations of drain water (A: 0% DW, B: 2% DW, 4% DW and 6% DW) (C: cortex, V.B: vascular bundles, P: pith and E: epidermis)

control treatment showed 76 mg kg^{-1}, 90.17 mg $kg^{-1}$and 98.5 mg kg^{-1} in 0, 0.5 and 1mM SA. The concentration of potassium ions in post-harvest soil was 132.6 mg kg^{-1} in control plants (0% DW) than 143.8 mg kg^{-1} in 6% DW. The amount of calcium in 2% DW was 56.60 mg kg^{-1} in 0.5 mM SA, 72.16 mg kg^{-1} in 1 mM SA. The amount was further increased as the concentration of drain water increased i.e., 59.80 and 76.48 mg kg^{-1} in the similar treatments (0.5 and 1mM SA). Similarly, highest concentration of calcium was found in in 6% DW (78.76 mg kg^{-1}) in 1 mM SA. The maximum uptake of magnesium was found in the soil treated with 0, 0.5 and 1 mM SA in highest concentration of drain water as compared to the control soil samples (Table 4).

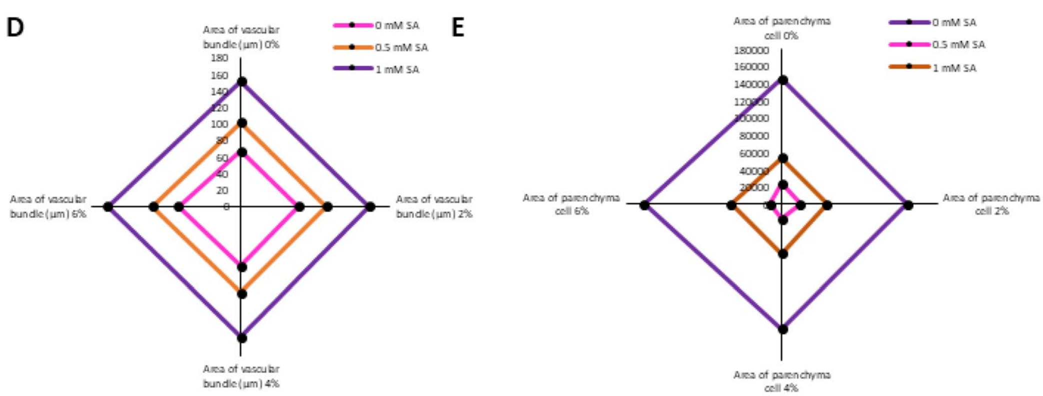
The research was carried out to determine various pollution parameters in the pre- and post-harvest soil. It was observed that there was a gradual increase in all parameters as the concentration of drain water was increased. Similar to findings in [32], the highest pH levels caused an increase in cation concentrations. The similar results were recorded by [33] who explain that the drain water is alkaline in nature. The electrical conductivity and total dissolved solids of drain water in 6% DW was found to be highest as compared to the control and other concentrations as well. The similar findings were reported [34] and [35] that the EC of the drain water increased due to the presence of different ions. High TDS levels affected the plant growth, and a decline growth of plants was attributed to high dissolved solids concentrations because an increase in salts reduces osmotic potential, resulting in less water availability to the plants [36]. The values for biological and chemical oxygen demand for various concentrations of drain water increased indicating the presence of high organic matter content in wastewater that is not only difficult to biodegrade rather it is more toxic as studied by [37, 38] Sulphate ions were found to be highest in drain water because it is mixed with many other liquid wastes which cause the increase in the absorption of sulphate ions as determined by [39]. Carbonates were found to be absent in all samples while bicarbonates were present. The highest values of bicarbonate content were found in highest concentration of DW as compared to the control as documented [40].

The highest moisture content was observed in the 6% drain water concentration, compared to the control. Similar results were described by [41]. The highest level of calcium was noted in 6% concentration DW in the soil. When calcium and magnesium levels in water exceed permissible limits, it negatively affects soil quality and plant growth [42]. Heavy metals such as Cr, Cd, and Pb were analyzed in soil samples irrigated with various concentrations of drain water. Effluents released by factories and industries cause an increase in chromium levels in wastewater [43]. Lead was in high concentration in the drain water may cause long-term health risks as reported by [44] However, all detected heavy metals were below the threshold levels .

In the present study, *Solanum tuberosum* L. showed vigorous growth in terms of morphological attributes such as length of root, fresh and dry weight of root, root length, diameter and volume of root. Salicylic acid plays an important role in the increase of tuber weight, greater tuber yield and increase in the number of tubers. The similar results were observed by [45] who investigated that under the treatment of salicylic acid increased the biomass of the plants as it serves as a growth regulator and is involved in the plant development. The salicylic acid was helpful in regulating various the physiological responses in the potato plant. The results were in line with those reported by [4], who found that tuber yield, as well as the uptake of photosynthetic pigments, nitrogen, phosphorus, and potassium, increased as the



	Epidermal thickness (µm)					Cortical cell thickness (µm)					Pith area (µm)			
	0%	2%	4%	6%		0%	2%	4%	6%		0%	2%	4%	6%
0 mM SA	c	ab	ab	a	0 mM SA	d	c	b	a	0 mM SA	d	c	b	a
0.5 mM SA	c	b	a	a	0.5 mM SA	d	c	b	a	0.5 mM SA	d	c	b	a
1 mM SA	d	c	b	a	1 mM SA	d	c	b	a	1 mM SA	b	a	c	d



	Area of vascular bundle (µm)					Area of parenchyma cell			
	0%	2%	4%	6%		0%	2%	4%	6%
0 mM SA	d	c	b	a	0 mM SA	b	b	b	a
0.5 mM SA	d	c	b	a	0.5 mM SA	b	b	b	a
1 mM SA	d	c	b	a	1 mM SA	c	d	b	a

Fig. 7 (**A-E**): Study of various anatomical attributes of roots of potato plants irrigated with different concentrations of drain water and Salicylic acid after 60 days of experiment

concentration of salicylic acid foliar spray increased, due to its regulatory role in physiological processes.

In this research study, plant respond to overcome various environmental stresses by producing antioxidant defense system i.e., hydrogen peroxide, POD, catalase and superoxide dismutase etc. the potato plants showed a significant stimulation of all antioxidants by application of highest treatment of salicylic acid. The hydrogen peroxide is helpful in maintaining the defense system when the potato plant was treated with salicylic acid. These results conform the findings of [46]. The protein content in potato showed the positive effect on the plant growth by treating with salicylic acid. These results conform the findings of [35] that potato is composed of starch but it also contains trace amount of protein and alkaline salts as well. The plants treated with salicylic acid enhance the tuber yield by stimulating the antioxidant system in the plant. Increased consumption of potato tubers may increase antioxidant levels in blood and tissues and protect against oxidative stress, which is responsible for lipid, protein and enzyme damage. The activation of antioxidant level in the plant causes the increase in the growth and yield parameter of potato plant described by [47]. Our result exhibited that all antioxidants increased in the leaves of potato after salicylic acid application. All these parameters are comparable with the findings of

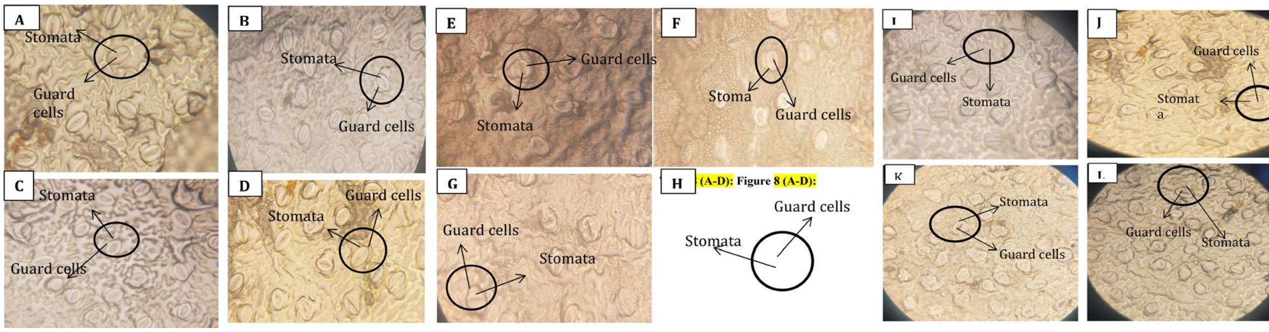
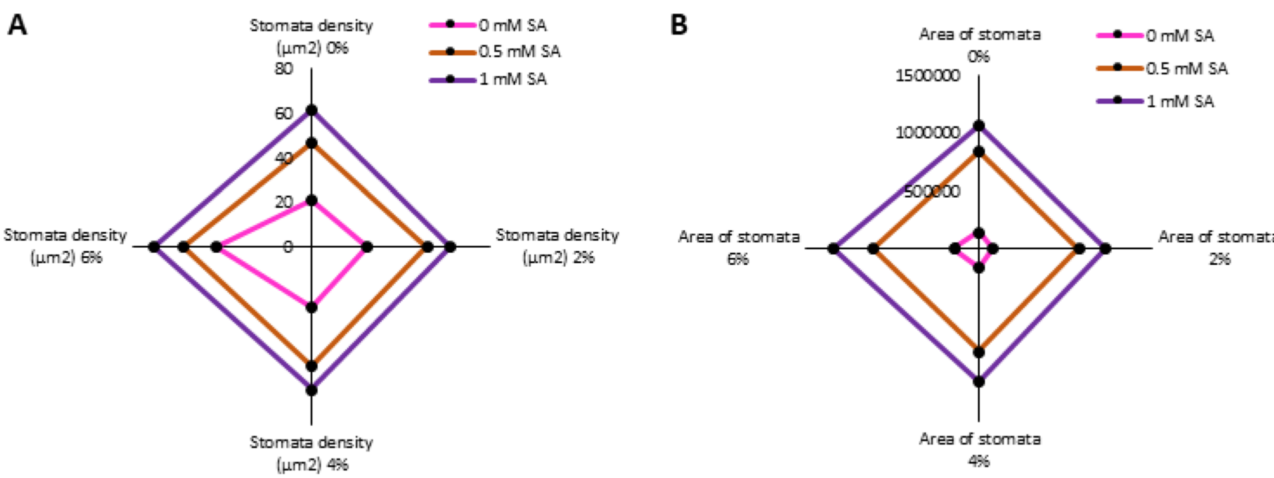


Fig. 8 (**A-D**): Leaf epidermal anatomy of the potato plant (*Solanum tuberosum* L.) treated with the 0 mM treatment SA irrigated with different concentrations of drain water (A: 0% DW, B: 2% DW, 4% DW and 6% DW). (**E-H**): Leaf epidermal anatomy of the potato plant (*Solanum tuberosum* L.) treated with 0.5 mM SA irrigated with different concentrations of drain water (A: 0% DW, B: 2% DW, 4% DW and 6% DW). (**I-L**): Leaf epidermal anatomy of the potato plant (*Solanum tuberosum* L.) treated with 1 mM SA irrigated with different concentrations of drain water (A: 0% DW, B: 2% DW, 4% DW and 6% DW)



Stomata density (µm²)					Area of stomata				
	0%	2%	4%	6%		0%	2%	4%	6%
0 mM SA	d	c	b	a	0 mM SA	c	d	b	a
0.5 mM SA	c	b	b	a	0.5 mM SA	c	b	d	a
1 mM SA	b	b	b	a	1 mM SA	d	c	b	a

Fig. 9 (**A-B**): Study of various anatomical attributes in leaves of potato plants irrigated with different concentrations of drain water and Salicylic acid after 60 days of experiment

[48]. The chlorophyll is a useful indicator for measuring potatoes nutritional status [49]. The chlorophyll content of potato indicated a high level of chlorophyll *a* and chlorophyll *b* in the leaves of potato under highest treatment in the current study [50]. The loss of chlorophyll is most likely due to the stress and chlorophyll content is reduced because the enzymes that works to produce chlorophyll loss their activity studied by [29].

The highest uptake of cadmium was observed in the leaves of *Solanum tuberosum* L. as compared to the other metals like Cr and Pb. Highest concentrations of heavy metals were reported in green vegetables irrigated with wastewater as described [51]. According to [52] the plant can promote heavy metal ion chemical transformations, which increase the potential toxicity of heavy metals but often improve their availability for plant uptake. Similar response of heavy metals was observed in the current study when plants of potato irrigated with the highest treatment of salicylic acid. These results conform the finding of [53] who explained that the Cd was found in maximum concentrations in the upper parts of the plants of *Cistus libanotis*. After chromium, maximum concentration of lead was the found in the leaves. The growth of plants does not affect much under Pb even in high concentrations of drain water. According to [16], maximum lead uptake was detrimental for the growth and yield of quinoa plants. The minute uptake of chromium was observed in the leaves of potato plants under highest

Table 4 Post harvest analysis of various physio-chemical parameters of soil irrigated with different concentrations of drain water

Parameters	Concentration of DW	Treatments		
		0 mM SA	0.5 mM SA	1 mM SA
pH	0%	$6.75^{b} \pm 0.04$	$7.07^{b} \pm 0.05$	$7.23^{a} \pm 0.03$
	2%	$6.91^{a} \pm 0.03$	$7.10^{b} \pm 0.05$	$7.25^{b} \pm 0.01$
	4%	$6.97^{a} \pm 0.03$	$7.13^{ab} \pm 0.03$	$7.26^{c} \pm 0.03$
	6%	$6.98^{a} \pm 0.05$	$7.16^{a} \pm 0.03$	$7.27^{c} \pm 0.02$
EC (dS cm^{-1})	0%	$193.3^{c} \pm 0.1$	$216^{c} \pm 0.2$	$256^{a} \pm 0.1$
	2%	$313^{bc} \pm 0.1$	$220^{c} \pm 0.3$	$266^{a} \pm 0.1$
	4%	$331^{b} \pm 0.1$	$228^{b} \pm 0.3$	$272^{a} \pm 0.3$
	6%	$370^{a} \pm 0.1$	$258^{a} \pm 0.2$	$282^{a} \pm 0.4$
Organic matter (%)	0%	$3.42^{d} \pm 0.01$	$5.61^{d} \pm 0.02$	$8.29^{c} \pm 0.01$
	2%	$4.16^{c} \pm 0.01$	$6.38^{c} \pm 0.03$	$8.64^{bc} \pm 0.01$
	4%	$5.45^{b} \pm 0.01$	$8.29^{b} \pm 0.01$	$8.86^{b} \pm 0.01$
	6%	$6.56^{a} \pm 0.01$	$8.50^{a} \pm 0.1$	$8.97^{a} \pm 0.01$
Moisture content (%)	0%	$1.54^{c} \pm 0.01$	$4.79^{c} \pm 0.01$	$6.88^{c} \pm 0.01$
	2%	$1.93^{c} \pm 0.01$	$5.11^{bc} \pm 0.03$	$6.95^{c} \pm 0.02$
	4%	$2.55^{b} \pm 0.02$	$5.21^{b} \pm 0.01$	$6.98^{b} \pm 0.03$
	6%	$3.04^{a} \pm 0.02$	$5.47^{a} \pm 0.01$	$7.09^{a} \pm 0.01$
Alkalinity (mg L^{-1})	0%	$4.46^{d} \pm 0.02$	$5.07^{c} \pm 0.03$	$8.34^{d} \pm 0.03$
	2%	$4.70^{c} \pm 0.03$	$5.25^{c} \pm 0.03$	$8.37^{c} \pm 0.03$
	4%	$4.71^{b} \pm 0.1$	$5.69^{b} \pm 0.03$	$8.98^{b} \pm 0.03$
	6%	$4.21^{a} \pm 0.1$	$5.93^{a} \pm 0.03$	$8.30^{a} \pm 0.03$
Pb (mg kg^{-1})	0%	N.D	N.D	N.D
	2%	$0.12^{c} \pm 0.01$	$0.18^{b} \pm 0.01$	$0.23^{c} \pm 0.01$
	4%	$0.16^{b} \pm 0.01$	$0.20^{a} \pm 0.01$	$0.24^{b} \pm 0.01$
	6%	$0.20^{a} \pm 0.01$	$0.23^{a} \pm 0.01$	$0.26^{a} \pm 0.01$
Cd (mg kg^{-1})	0%	N.D	N.D	N.D
	2%	$0.23^{c} \pm 0.01$	$0.26^{c} \pm 0.01$	$0.29^{b} \pm 0.01$
	4%	$0.26^{b} \pm 0.01$	$0.27^{b} \pm 0.01$	$0.31^{a} \pm 0.01$
	6%	$0.28^{a} \pm 0.01$	$0.28^{a} \pm 0.01$	$0.32^{b} \pm 0.01$
Cr (mg kg^{-1})	0%	N.D	N.D	N.D
	2%	$0.15^{b} \pm 0.01$	$0.18^{b} \pm 0.01$	$0.21^{c} \pm 0.01$
	4%	$0.21^{a} \pm 0.01$	$0.19^{b} \pm 0.01$	$0.22^{b} \pm 0.01$
	6%	$0.23^{a} \pm 0.01$	$0.20^{a} \pm 0.01$	$0.23^{a} \pm 0.01$
Na (mg kg^{-1})	0%	$76^{b} \pm 0.1$	$90.17^{ab} \pm 0.01$	$98.5^{d} \pm 0.003$
	2%	$78.66^{b} \pm 0.05$	$91.64^{b} \pm 0.01$	$99.5^{c} \pm 0.04$
	4%	$82.01^{a} \pm 0.1$	$93.92^{b} \pm 0.02$	$101.6^{b} \pm 0.02$
	6%	$84.04^{a} \pm 0.03$	$95.39^{a} \pm 0.04$	$101.35^{a} \pm 0.06$
K (mg kg^{-1})	0%	$132.6^{d} \pm 0.2$	$145.8^{c} \pm 1$	$167.2^{c} \pm 0.09$
	2%	$138.4^{c} \pm 0.06$	$149.6^{b} \pm 0.6$	$168.6^{b} \pm 0.07$
	4%	$141.4^{b} \pm 0.09$	$152.5^{a} \pm 0.02$	$169.2^{b} \pm 0.01$
	6%	$143.8^{a} \pm 0.1$	$164.4^{a} \pm 0.08$	$173.1^{a} \pm 0.01$
Ca (mg kg^{-1})	0%	$40.33^{c} \pm 0.1$	$53.58^{d} \pm 0.03$	$67.49^{c} \pm 0.02$
	2%	$41.43^{c} \pm 0.1$	$56.60^{c} \pm 0.01$	$72.16^{c} \pm 0.01$
	4%	$44.07^{b} \pm 0.01$	$59.80^{b} \pm 0.01$	$76.48^{b} \pm 0.01$
	6%	$49.08^{a} \pm 0.02$	$65.72^{a} \pm 0.01$	$78.76^{a} \pm 0.01$
Mg (mg kg^{-1})	0%	$40.41^{d} \pm 0.1$	$60.54^{d} \pm 0.01$	$69.49^{d} \pm 0.01$
	2%	$47.83^{c} \pm 0.1$	$62.04^{c} \pm 0.01$	$71.08^{c} \pm 0.01$
	4%	$53.39^{b} \pm 0.01$	$64.03^{b} \pm 0.01$	$75.08^{b} \pm 0.01$
	6%	$58.09^{a} \pm 0.01$	$67.09^{a} \pm 0.01$	$79.82^{a} \pm 0.01$

± = Standard deviation

DW = Drain Water

SA = salicylic acid

The values are mean of three replicates. The alphabets show level of significance at $p \leq 0.05$ based on DMRT using ANOVA treatment. The levels of Cr in cabbage, carrot, green pepper, onion, and tomato demonstrated that the Cr levels in various vegetables might not constitute a health danger to consumers [43]. All metals estimated in the leaves of potato plants lies within the permissible limits as described [53].

In the present study it was recorded that the number of tubers increases with the increase in the concentration of drain water (6%) with highest treatment salicylic acid (1 mM). These results were in line with [53, 54] who recorded that potato tuber number per plant and total yield increase with adequate irrigation. In the present research work, the concentrations of Pb and Cd in the potato tubers was below than threshold value of food safety standards as reported [55]. The irrigation with wastewater would gradually increase Cr in soil but the quantity of metal in the tuber was below the permissible limits. Pb is present in crops including potatoes at very low concentrations due to its uptake from soils, and this may be due to a low rate of translocation of Pb to potato flesh [56]. Various anatomical parameters of root and leaves of potato were studied in this research study. The epidermis thickness of root of *Solanum tuberosum* L. was increased in the highest salicylic acid treatment irrigated with wastewater. [57] estimated that the epidermis protects plants from dehydration and ensures proper organ growth and development. According to [58], increasing epidermis thickness increases plant water efficiency. Cortex is a nutrient and water storage area. According to [59], reducing the thickness of plant stem cortical cells is helpful for growth by storing energy for existence in critical environment. In the recent study, the area of cortex was minimum in the least concentration of treatment, which may lead to the depletion of nutrients as conclude by [60, 61]. estimated the pith of the plant was maximum in the highest concentration of treatment that's why the cells can store more water that was used for the growth of the plant.

In the current study, the area of vascular bundle of the potato plant was maximum in the maximum treatment of salicylic acid (1 mM). According to [60, 62], the reduction in photosynthesis and plant growth is caused by the decrease in the area of vascular bundles which leads to decreases in water uptake. Ground parenchyma cell wall sub-layers were thicker than vascular parenchyma cells. Ground parenchyma cells had a thicker pith membrane than vascular parenchyma cells, but ground parenchyma cells diameter was smaller [24]. It was studied that the parenchyma cell area and stomatal density increased as the concentration of DW and SA increased. The similar results were described by [58] that in *Arabidopsis thaliana*, lower stomata density would minimize cuticular transpiration through closed stomata.

Post- harvest analysis of soil indicated that the maximum treatment of salicylic acid enhances the growth of *Solanum tuberosum* L. irrigated with various concentrations of drain water. The increase in the concentration of the pollution parameters indicates that the plants have the ability to uptake the metals from the soil to the aerial parts of plant and have the capacity to tolerate and survive in the stress conditions.

Conclusion

The study concluded that the best growth of potato plants was observed when irrigated with drain water and treated with 1 mM salicylic acid. Overall, maximum plant growth was observed with 6% drain water and 1 mM salicylic acid, outperforming the control across all measured soil and plant parameters. Highest concentration of drain water was beneficial for various parameters like morphological, physiological and anatomical characters enhancement. Regarding heavy metals, only minute amounts were detected in potato tubers, all of which were below threshold levels. Furthermore, growing potato plants with industrial wastewater can help address pollution problems as the availability of fresh water continues to decrease. Therefore, using drain water for irrigation, particularly when combined with salicylic acid treatment, can increase potato yields. Use of sewage water for irrigation may help to minimize the use of freshwater for agriculture. This practice can mitigate the harmful effects of contaminants found in drain water.

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Author contributions

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Data availability

All relevant raw data, will be freely available on request from corresponding author Anis Ali Shah.

Declarations

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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