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Ameliorative effect of Salicylic Acid on the Growth of Lettuce (*Lactuca sativa* L.) Seedling under Cadmium Stress

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ABSTRACT

Worldwide, Lettuce (*Lactuca sativa* L.) is regarded as the most widely farmed vegetable. In Iraq, sewage water is used to irrigate lettuce that leading to health risks for consumers, particularly due to heavy metal contaminants, such as cadmium. This study was conducted as a factorial experiment in Taq-Taq city, Erbil, Iraq during the winter season 2021 to investigate the effects of soaking lettuce seeds in cadmium (Cd) (0, 10, 20, or 30mM) and salicylic acid (SA) (0, 2, 4, 6, or 8mM) on the germination and some seedling growth properties of this plant. Results show that soaking the seeds in distilled water led to a decrease in the percent of seeds germination compared to that soaked in Cd solutions, while exist of SA at moderate concentrations increase the germination percent. Most of the seedling growth characteristics exhibited a considerable increase with soaking seeds in Cd solution. SA in high concentration reduced significantly seeds germination and most of the seedling shoot and root characteristics, except the root length, which grew significantly. Most of the responses of Cd and SA were non-significant regarding chlorophyll a, b, and total carotenoids. This study concluded that soaking lettuce seeds in Cd up to 30mM and SA up to 4mM act as a stimulate hermetic effects on germination and subsequence growth of lettuce seedling, these findings confirm the increase growth and yield of lettuce irrigated by wastewater (including Cd) in Iraq and Iraqi Kurdistan region in spite of the increased health risks.

Index Terms: Cadmium Stress, Lettuce Seedling, Photosynthesis Pigments, Salicylic Acid, Seed Soaking

1. INTRODUCTION

Lettuce (*Lactuca sativa* L.), due to its high nutritional value, it is ranking among the most renowned and economically significant leafy vegetable crops globally [1]. It is also a crucial winter vegetable crop in Iraq [2]. Lettuce belongs to the Asteraceae family, and it propagated by seeds [3]. This plant is cultivated for its edible rosette leaves. In Iraq, 26507 tons are produced annually on 3317 hectares of farmed land [4]. The majority of lettuce is grown in the central part of Iraq, where seeds are sown in late summer to produce a crop in the winter. In the northern region, the growing season commences in late autumn, yielding a crop in the spring [5]. In Iraq, the majority of farmers utilize sewage water for the irrigation of leafy vegetables, particularly lettuce, resulting in the contamination of these crops and posing a health risk to consumer communities. In the Erbil governorate of Iraq, approximately 225 hectares are irrigated using non-pre-treated sewage water for the cultivation of various uncooked vegetables, which are consumed by nearly two million people as a significant component of their diet [6].

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A study by Hassoon [7] indicated that lettuce samples from Baghdad central exhibited significant cadmium consumption levels, measuring 310 μg/day for imported lettuce and 372 μg/day for locally sourced lettuce.

Iraq and the Iraqi Kurdistan region are a place for many crises and continual wars, in addition to many political, security, and incorrect practices problems, which led to make this region a fertile region for conducting studied regarding soil pollution. Hence, more information and studies are required to be implemented in this region. Erbil governorate, the most populous city in the Kurdistan region, experiences numerous pollution sources that contribute to significant soil contamination, particularly with cadmium (Cd) [8]. Notable sources include cement plants, phosphate fertilizers, urban traffic, waste incinerators, heating systems, metalworking industries, and power plants, which are recognized globally for their Cd emissions [9]. Approximately 660 metric tons of cadmium are utilized annually in soils through the application of phosphate fertilizers [10]. Cadmium is classified as a non-essential element for plants and is not recognized to provide any benefits to them. It exhibits significant toxicity to crops [11]. The National Food Safety Standard establishes a limit of 0.2 mg/kg FW for cadmium in leafy vegetables.

Plants readily absorb cadmium (Cd) and accumulate it in various parts of plants [12]. This accumulation can adversely affect crops by inducing oxidative stress, characterized by increased reactive oxygen species, decreased activity of antioxidant enzymes, damaged cell membranes, and reduced biomass, ultimately compromising the quality of certain crops [13]. To mitigate the negative impacts of cadmium stress on plants, various strategies have been employed, including direct methods, such as mycosis fungoid, genetically modified plants, and grafting, which address heavy metal contamination issues [14], phytoremediation [15], and salicylic acid (SA) application [16]. SA is safe for human health [17]. It endogenous plant growth regulator that has a phenolic nature. It regulates various biochemical and physiological processes in plants, including seed germination, plant growth, flower induction, nutrient uptake, membrane permeability, transport, plant-water relations, stomatal conduction, photosynthesis, and enzyme activities [18]. Recent studies indicate that the application of SA significantly enhances plant adaptation to various stress factors, including heavy metals. SA functions as a natural signalling molecule, promoting tolerance to abiotic stress across different plant species [14], [19], including protection against cadmium toxicity [16].

Cadmium stress in plants can be reduced through various methods, including the reduction of Cd uptake and its accumulation in plant tissues. Reactive oxygen species (ROS) scavenging enhances the antioxidant defense system [20], [21] and improves photosynthetic capacity [22], [23].

Due to a lack of studies about the relationship between Cd and SA application on lettuce seedling, and as an attempt to decrease the health risk of heavy metals especially Cd in Iraq and Iraqi Kurdistan, this study was conducted as a complement to our previous study [24], where present study is regarding the seedling only, where it aims to use high concentrations of cadmium and SA to determine the tolerance of lettuce seeds and seedlings to these concentrations to avoid them in subsequent studies and to avoid cultivate lettuce in area polluted by high Cd concentrations, as well as to examine the capacity of SA to decrease the effects of cadmium on this lettuce seedling.

2. MATERIALS AND METHODS

2.1. Plant Materials, Cultivation, and Treatments

A factorial tray experiment was conducted in Taq Taq district (35.8857°N, 44.5932°E), Erbil, Kurdistan Region, Iraq, during the winter season 2021. Lettuce seeds (*L. sativa* L.) variety Romaine, produced by NADER company, Netherlands, were planted on the 1st of October in 105 cell plastic planting trays on peat-moss with 6.3 pH. Each experimental unit consists of 100 cells (Fig. 1). The average of the meteorological data during the study period from the 1st of October to the 15th of November ranged 22.10°C for temperature, 38.5 for relative humidity, and 1.95 mm for precipitation.

The experiment comprised two factors: The first involved soaking lettuce seeds in four concentrations of cadmium chloride ($CdCl_2.H_2O$) at 0, 10, 20, or 30 mM, designated as Cd0, Cd10, Cd20, and Cd30, respectively. The second factor involved soaking the seeds in five concentrations of SA (2-Hydroxybenzoic acid) at 0, 2, 4, 6, or 8 mM, referred to as SA0, SA2, SA4, SA6, and SA8, respectively, with distilled water serving as the control treatment. Seeds were immersed in the treatment solutions for 24 h.

Because of the average percentage of germination for the treatment (SA8) was only 7.62% (Table 1) and zero for most replications, we dropped this treatment, including their interactions, for the subsequent characteristics, including shoot, and root characteristics and photosynthetic pigments.

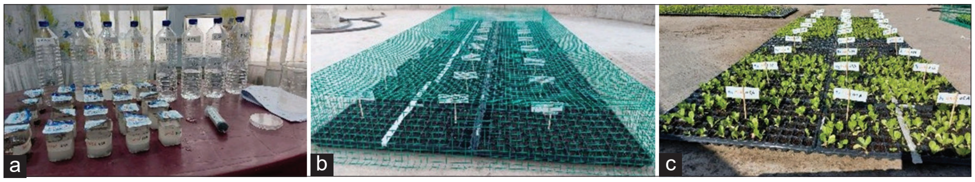

Fig. 1. The stages of (a) seeds soaking in different treatments solutions, (b) cultivated trays cells (c) emerged seedling.

TABLE 1: The effect of seed soaking in Cd, SA and their interactions on the var. Romaine germination of *Lactuca sativa* L

Concentration of SA (mM)	Concentration of Cd (mM)				Average of SA
	Cd0	Cd10	Cd20	Cd30	
SA0	49.07c*	82.86a	84.60a	83.50a	75.01a
SA2	61.45b	85.89a	77.45a	77.54a	75.58a
SA4	61.46b	83.17a	83.56a	87.97a	79.04a
SA6	10.75de	57.62bc	52.01bc	75.85a	49.06b
SA8	0.33e	2.68e	18.10d	9.37de	7.62c
Average of Cd	36.61b	62.45a	63.14a	66.85a	

*According to Duncan's Multiple Range test, means that have the same letters on columns and rows are not significantly different at $P \leq 0.05$.

Solutions of the cadmium ($CdCl_2.H_2O$) with molecular weight = 201.33 g/mole was used to prepare the concentrations 10, 20, and 30 mM/L by dissolving 2.013, 4.026 and 6.039 g of $CdCl_2.H_2O$ in one liter distilled water, whereas for preparing the SA solutions, extra pure SA (2-Hydroxybenzoic acid) with molecular weight = 138.12 g/mole was used to prepare the solutions 2, 4, 6, and 8 mM/L by dissolving 0.276, 0.552, 0.828, and 1.104 g of SA in one liter of distilled water.

2.2. Studied Characteristics

Seed germination (G) where taken on 21th of October after no more seeds were germinated by the equation (1), which was described by Ahmadloo *et al.* [25].

$$G(\%) = \frac{L}{S} \times 100 \quad (1)$$

Where G (%) = Germination percentage, L = Number of planted seed, and S = Total number of emerged seedlings.

The following characteristics were studied on the seedling 25 days after seeds emerging were complete; seedling leaf area (cm^2) by taking 30 known area discs from the leaves, then dried and weighted. Seedling leaf area was calculated by the equation (2), which was described by AbuEl-Zahaba *et al.* [26]:

$$\text{Leaf erea} = \frac{\text{Leaves dry weight} \times \text{Known discs area}}{\text{Discs dry weight}} \quad (2)$$

The length of the seedling shoot and primary root (cm) was measured using a standard metric tape measure, along with the fresh and dry weight, and the percentage of shoot and root dry weight, as outlined in Tudela *et al.* [27]. Chlorophyll a (Chl.a), chlorophyll b (Chl.b), and total carotenoids (Car.) were quantified according to the method described by Lichtenthaler and Wellburn [28], utilizing a 50 ml 80% acetone to 1 g sample ratio.

2.3. Experimental Design and Statistical Analysis

This study conducted a factorial experiment utilizing a completely randomized design with three replications. Data were analyzed using analysis of variance, with Duncan's multiple range tests applied at a 5% probability level for comparison of experimental means, utilizing the SAS program [29].

3. RESULTS AND DISCUSSIONS

The percent of seeds germination affected significantly by each of Cd, SA concentrations and their interactions as shown in Table 1, where soaking seeds in Cd solutions regardless to their concentrations increased significantly seeds germination compared to the tap water, where higher germination percent (66.85%) records in Cd30, whereas, the lowest percent recorded in the control treatment (36.61%). There were non-significant differences between low SA concentrations and soaking in tap water in their effect on this property, where SA4 records higher seed germination (79.04%), whereas the concentration rises to 6 and 8 mM, germination percent considerably decreased to 49.06 and

7.62%, respectively. The interactions of Cd10, Cd20, and Cd30 with SA0, SA2, and SA4 mM significantly enhanced seed germination relative to all other interactions, with the exception of the interaction between Cd30 and SA6 treatment. From the results of the percent of germination, it is clear that the highest concentration of SA (8 mM) led to a significant decrease in the germination percent to a very low percent, reached to zero in some experimental units, so we exclude this treatment from the experiment for the shoot, root, and photosynthesis pigments properties.

Characteristics of the seedling vegetative growth (Table 2) that demonstrated by the leaves number, seedling length, seedling dry and fresh weight were rose considerably with maximize Cd concentration especially the high concentration 30 mM which records highest values 7.03 leaves per seedling, 5.06 cm, 0.209 g, and 1.09 g, respectively, whereas, the control records the lowest values 5.67 leaves/seedling, 3.96 cm, 0.62 g, and 0.128 g, respectively. In contrast, Cd30 treatment resulted in the lowest percentage of dry matter at 19.26%, compared to 21.50% for the Cd20 treatment, which indicated the smallest seedling leaf area at 12.78 cm². This was significantly lower than all other treatments, with the control group recording the highest value at 20.06 cm². The study results demonstrated that the application of a high concentration of SA (6mM) led to a result that was more adverse than the control treatment. Highest of seedling leaves (6.62), leaf area (19.98 cm²), dry weight (0.180 g), fresh weight (0.84 g), dry matter percent (21.50%), where recorded between SA2 and SA4 treatments, while the lowest values were recorded for the control and SA6 treatments. SA had non-significant effects on seedling length. Combinations of 10 and 20 mM of Cd with 2 and 4 mM of SA increased most of the seedling vegetative growth, including leaves number, seedling length, and seedling fresh and dry weight.

Contrary to expectations, it is found from the results that Cd in low concentrations reduced the inhibitory effect of high concentration of SA on the growth of lettuce seedling in most growth characteristics, this confirm what Carvalho *et al.* [30] have reported that Cd stimulate hormetic effects on plants, where exposure to Cd improves the performance of some plant species, despite of accumulation of Cd in their roots and shoots, where protective strategies have been developed at these plants to neutralize the side effects of Cd toxicity or mechanisms that service Cd as beneficial element.

TABLE 2: The effect of seed soaking in Cd, SA, and their interactions on some vegetative shoot growth parameters of *Lactuca sativa* L. var. Romaine seedling

Treatments	Leaf number	Leaf area (cm²)	Length (cm)	Fresh weight (g)	Dry weight (g)	Dry matter (%)
Cd concentration (mM)						
Cd0	5.67c*	20.06a	3.96b	0.62c	0.128c	20.22 ab
Cd10	6.20b	18.89a	4.20b	0.67bc	0.139bc	20.74ab
Cd20	6.38b	12.78b	4.09b	0.69b	0.149b	21.50a
Cd30	7.03a	18.89a	5.06a	1.09a	0.209a	19.26b
SA concentration (mM)						
SA0	6.13b	16.23b	4.23a	0.73b	0.155b	21.22a
SA2	6.62a	19.64a	4.31a	0.83a	0.180a	22.01a
SA4	6.52a	19.98a	4.46a	0.84a	0.168ab	21.66ab
SA6	6.02b	14.29b	4.30a	0.67c	0.122c	18.26b
Interactions between Cd and SA						
Cd0SA0	5.40i	18.64bc	4.12bcd	0.59g	0.123de	21.11abcd
Cd0SA2	5.80ghi	18.26bc	4.32bc	0.73efg	0.157cd	21.56abcd
Cd0SA4	6.00fgh	30.75a	4.04bcd	0.73efg	0.150cde	20.62abcd
Cd0SA6	5.47hi	12.59de	3.34d	0.45h	0.080f	17.48d
Cd10SA0	6.27defg	17.34bcd	4.14bcd	0.77ef	0.157cd	20.34abcd
Cd10SA2	6.67cd	26.77a	3.93cd	0.68fg	0.157cd	23.08ab
Cd10SA4	6.07efg	14.98cde	4.41bc	0.64fg	0.133de	20.74abcd
Cd10SA6	5.80ghi	14.51cde	4.33bc	0.59g	0.110ef	18.80cd
Cd20SA0	5.93ghi	13.16de	4.20bcd	0.66fg	0.157cd	23.50a
Cd20SA2	6.73bcd	14.28cde	4.04bcd	0.83de	0.190bc	22.91abc
Cd20SA4	6.60cde	11.96e	3.92cd	0.64fg	0.130de	20.43abcd
Cd20SA6	6.27defg	11.71e	4.17bcd	0.63g	0.120de	19.18bcd
Cd30SA0	6.93abc	15.78cde	4.46bc	0.92cd	0.183bc	19.91abcd
Cd30SA2	7.27ab	19.23bc	4.93ab	1.07b	0.217b	20.41abcd
Cd30SA4	7.40a	22.22b	5.49a	1.36a	0.260a	19.17bcd
Cd30SA6	6.53cdef	18.35bc	5.35a	1.01bc	0.177c	17.55d

*According to Duncan's Multiple Range test, means that have the same letters on columns are not significantly different at $P \leq 0.05$.

TABLE 3: The effect of seed soaking in cadmium chloride (Cd), salicylic acid (SA) and their interactions on some root growth parameters of *Lactuca sativa* L. var. Romaine seedling

Treatments	Length (cm)	Fresh weight (g)	Dry weight (g)	Dry matter (%)
Cd concentration (mM)				
Cd0	9.05c	0.360b	0.075a	20.769a
Cd10	11.09a	0.381b	0.077a	20.235a
Cd20	10.14b	0.360b	0.073a	20.060a
Cd30	10.69ab	0.502a	0.083a	16.577b
SA concentration (mM)				
SA0	9.12b	0.413a	0.080a	19.600a
SA2	9.87b	0.439a	0.087a	19.895a
SA4	11.12a	0.428a	0.080a	19.427a
SA6	10.86a	0.323b	0.060b	18.719a
Interactions between Cd and SA				
Cd0SA0	8.25e	0.343def	0.073abc	21.477ab
Cd0SA2	8.49e	0.420bcde	0.093a	22.210a
Cd0SA4	10.11bcde	0.440bcd	0.087ab	19.793abcd
Cd0SA6	9.34cde	0.236g	0.047d	19.597abcd
Cd10SA0	9.29cde	0.460bc	0.087ab	18.773abcd
Cd10SA2	10.73bcd	0.380cdef	0.077ab	20.230abc
Cd10SA4	12.99a	0.346def	0.077ab	22.113a
Cd10SA6	11.35abc	0.337ef	0.067bcd	19.823abcd
Cd20SA0	9.83bcde	0.363cdef	0.073abc	20.220abc
Cd20SA2	9.58bcde	0.453bc	0.093a	20.587abc
Cd20SA4	10.06bcde	0.333ef	0.070bc	21.077ab
Cd20SA6	11.08abcd	0.290fg	0.053cd	18.357bcd
Cd30SA0	9.11de	0.483b	0.087ab	17.930bcde
Cd30SA2	10.67bcd	0.503ab	0.083ab	16.553de
Cd30SA4	11.29abc	0.590a	0.087ab	14.723e
Cd30SA6	11.67ab	0.430bcde	0.073abc	17.100cde

*According to Duncan's Multiple Range test, means that have the same letters on columns are not significantly different at $P\leq 0.05$.

In comparison to the control treatment, seeds soaked in Cd regardless concentrations increased seedling root length significantly (Table 3), which means that the Cd consider as a stress by the embryo so increasing the root length [31], this result was disagree with Bautista *et al.*, [32] who found that 25, 35 and 50 μM/L of Cd interfered significantly with root elongation compared to the control treatment. The highest concentration of Cd increased the root fresh weight significantly to (0.502 g) compared to other treatments, while an inverse respond demonstrated in root dry matter to the higher Cd concentration (30 mM), which reached 16.577% compared to other treatments, where the control records 20.769%. There were non-significant differences between Cd concentrations on roots dry weight.

Similar to Cd, SA at concentrations of 4 and 6 mM significantly increased root length to 11.12 cm and 10.86 cm, respectively, compared to the control and SA2 treatments. This finding matches the results of Talabany and Albarzinji [24], who reported that SA up to 2 mM enhanced root length, as well as fresh and dry weight. A high concentration of SA (6 mM) significantly reduced both the fresh and dry weight of roots to 0.323 g and 0.060 g, respectively, in comparison to all other treatments. The percentage of dry matter content was not significantly affected by SA treatment (Table 3).

The interaction treatment Cd10SA4 produced the tallest root at 12.99 cm, significantly surpassing all other treatments, with the exception of the treatments involving Cd 10 mM with SA at 4 and 6 mM. Soaking seeds in tap water and 2mM of SA recorded the lowest root length 8.25 and 8. 49 cm, respectively (Table 3). The interactions of Cd30 with SA2 and SA4 resulted in the highest root fresh weights of 0.503 g and 0.590 g, respectively, which were significantly greater than those observed in all other treatments. In contrast, the interaction treatment Cd0SA6 recorded the lowest value at 0.236 g compared to all other treatments except the treatment Cd20SA6 (Table 3). Each of the interaction treatments Cd0SA2 and Cd20SA2 records the highest root dry weight (0.093 g) significantly compared only to the interactions Cd10SA6 and Cd20SA6. Higher root dry matter percent noted for the interactions Cd0SA2 (22.210%) and Cd10SA4 (22.113%), significantly compared to the interaction treatment Cd20SA6 and the interaction of Cd30 with all SA concentrations, where the interaction Cd30SA4 records the lowest value (14.723%).

Generally, most of the Cd and SA effects on photosynthetic pigments were non-significant. Chl. a increased significantly in Cd10 and Cd30 to 0.310 and 0.321 mg/g fresh weight compared to the Cd20 treatment only, Cd30 increased Chl.b to 0.280 mg/g compared to Cd20 (0.232 mg/g) treatment only, which gave the lowest total carotenoids (0.148 mg/g). About the effect of SA, there were only a decrease in each of Chl.a and b where 2mM SA was used (0.269 and 0.223 mg/g), respectively, as compared to all other treatments. Increasing the SA to 6mM increased significantly total carotenoids to 0.184 mg/g compared to all other treatments.

Each of the interactions Cd0SA6 and Cd10SA0 records the highest Chl.a (0.377 and 0.383 mg/g) significantly compared to all other treatments except the interactions Cd10SA4, Cd20SA6, Cd30SA0, Cd30SA4, and Cd30SA6 treatments. Regarding Chl.b same interaction treatments were superior, where the treatment Cd30SA6 recorded higher value significantly compared to other treatments except Cd0SA6, Cd10SA0, Cd10SA4, Cd20SA6, Cd30SA0, and Cd30SA4 treatments. Total carotenoids increased significantly in each of the interactions Cd0SA6 and Cd10SA0 (0.217 and

0.207 mg/g) significantly compared to all other treatments except the interaction Cd30SA6.

Significant increase in seeds germination in Cd treatments may be due to the hormetic effects of Cd, where Carvalho *et al.* [30] have reported that Cd stimulates hormetic effects on plants, where exposure to Cd improves the performance of some plant species. This result was not agree with Bautista *et al.* [32] who reported that the percent of lettuce seeds germination decreased significantly 64%, 82.5%, and 92.5% by 25, 35, and 50 μM/L of Cd, respectively, compared to the control treatment.

Lower concentrations of SA (2 and 4 mM) were more beneficial than higher concentrations (6 and 8 mM). Bankole *et al.* [33] reported that a foliar treatment of 1 mM SA resulted in an 8% increase in lettuce height and a 53.16% increase in plant biomass. In addition, a foliar treatment of 3 mM stimulated a 13% growth increase compared to the control plants. The application of SA also slightly decreased the effects of water potential in lettuce, which typically leads to significant reductions in growth and yield during water stress conditions. The findings of Hassoon [7] revealed that cadmium concentrations in local lettuce from Baghdad, Iraq, were measured at 1.93 mg/kg dry matter, exceeding the normal range of 0.05–1 mg/kg. The enrichment of soil with cadmium results in increased concentrations due to two interrelated factors: Its relative mobility and its affinity for association with organic matter [34].

The notable reduction in plant leaf area due to elevated SA concentration (Table 2) aligns with observations by Talabany and Albarzinji [24], where increasing SA concentration to 2 mM decreased lettuce leaf area and the results of Youssef *et al.* [35] who demonstrated beneficial effects of foliar application of SA up to 0.5 mM on the vegetative growth parameters of lettuce leaf area.

Our findings contrast with those of Zhao *et al.* [36], who reported a decrease in net growth of plants with increasing cadmium concentration. This discrepancy may be attributed to the lower Cd concentration utilized in the present study. However, our results align with Zhao's observations regarding the reduction in shoot and root dry matter at a Cd concentration of 30 mM. This decrease may be linked to an increase in seedling fresh weight in both shoot and root, resulting from an imbalance of certain plant growth hormones, particularly auxins [37], or a disruption in the balance of essential minerals for plant growth [38].

The beneficial effects of SA at low concentrations on shoot and root growth were consistent with the results of Shehata *et al.* [39], which reported that foliar application at 100-300 PPM resulted in enhanced shoot growth. Pre-treatment with SA before Cd application showed a significant increase in root dry weight, while SA exhibited a non-significant effect on root fresh weight. The combination of SA with Cd significantly decreased root fresh weight. Shehata *et al.* [39] reported similar findings, indicating that SA pretreatment significantly increased the fresh weight of roots under drought stress. In addition, Hou *et al.* [40] demonstrated that cadmium may inhibit growth by interfering with essential metabolic processes, including photosynthesis and the transport of photosynthetic products and nutrients. The decrease in the percent of dry matter in lettuce leaves under cadmium stress may result from disruption of photosynthesis due to the displacement of Cd^{2+} ions by Mg^{2+} ions in the chlorophyll molecule and Cd stress inhibited the synthesis

TABLE 4: The effect of seed soaking in cadmium chloride (Cd), salicylic acid (SA) and their interactions on some photosynthetic pigments of *Lactuca sativa* L. Var. Romaine seedling

Treatments	Chlorophyll a	Chlorophyll b	Total carotenoids
	mg/g Fresh weight		
Cd Concentration (mM)			
Cd0	0.306ab*	0.255ab	0.168a
Cd10	0.310a	0.262ab	0.167a
Cd20	0.274b	0.232b	0.148b
Cd30	0.321a	0.280a	0.165ab
SA Concentration (mM)			
SA0	0.306a	0.256a	0.163b
SA2	0.269b	0.223b	0.146b
SA4	0.303a	0.263a	0.157b
SA6	0.333a	0.286a	0.184a
Interactions between Cd and SA			
Cd0SA0	0.287bcde	0.243bcdefg	0.160cde
Cd0SA2	0.283bcde	0.250bcdefg	0.167bcd
Cd0SA4	0.277cde	0.237cdefg	0.130de
Cd0SA6	0.377a	0.290abcd	0.217a
Cd10SA0	0.383a	0.310ab	0.207ab
Cd10SA2	0.263de	0.217efg	0.137de
Cd10SA4	0.313abcd	0.270abcdef	0.170bcd
Cd10SA6	0.280bcde	0.250bcdefg	0.157cde
Cd20SA0	0.237e	0.203fg	0.123e
Cd20SA2	0.263de	0.197g	0.140de
Cd20SA4	0.273cde	0.243bcdefgh	0.153cde
Cd20SA6	0.323abcd	0.283abcde	0.173bcd
Cd30SA0	0.317abcd	0.267abcdef	0.160cde
Cd30SA2	0.267de	0.230defg	0.140de
Cd30SA4	0.347abc	0.303abc	0.173bcd
Cd30SA6	0.353ab	0.320a	0.190abc

*According to Duncan's Multiple Range test, means that have the same letters on columns are not significantly different at $P \leq 0.05$.

of photosynthetic pigments and photosynthesis-related proteins or subunits, eventually affected the fixation of CO2, as manifested by the decreased plant biomass [41], [42].

The results reported in Table 4 indicate that soaking seeds in cadmium (Cd), SA, or their combinations resulted in varied responses. The increase in chlorophyll a and b at high SA concentration supports the findings of Youssef *et al.* [35] who found that foliar application of SA up to 20 mM increased chlorophyll a and b significantly compared to the control and lower and higher SA concentrations. In addition, it aligns with the findings of Shehata *et al.* [39], which indicated an increase in Car. Due to foliar spray with SA [23]. The significant decreases in Chl. a, b, and total Car. With Cd20 application (Table 4) were reported by Matraszek *et al.* [6], who demonstrated that chlorophyll content significantly decreased with increasing concentrations of Cd in lettuce. This finding contrasts with the concentration of Cd30, which may be attributed to the enhancement of certain hormones and antioxidant defenses in cells. The decrease in chlorophyll levels in lettuce leaves under cadmium stress may result from the damage to chloroplast ultrastructure, the expression of important enzymes in the pathway of chlorophyll synthesis in leaves delayed, resulting in a reduction in chlorophyll content, degradation of carotenoids, and a reduction in carotenoids content [41], [43].

4. CONCLUSIONS AND RECOMMENDATIONS

This study concludes that soaking lettuce seeds in a solution of 30 mM of Cd and 2 or 4 mM of SA improve germination percent and subsequent vegetative growth of lettuce seedlings. Hence, low concentrations of Cd can estimate the growth of lettuce seedlings. Cd at 10 mM without SA increased photosynthetic pigments specially Chl.a and total carotenoids. SA concentration more than 4 mM is not recommended regarding seeds and seedling treatments. More studies are recommended about the effects of higher concentrations of Cd to reach the toxic concentration on seed germination and subsequent vegetative growth.

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