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Mitigating the Effect of Cadmium Toxicity on Growth and Lipid Composition of Sunflower by Foliar Application of Salicylic Acid

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ABSTRACT

Industrial emissions and human activities are the primary cause of heavy metals pollution in agricultural soils. An experiment was conducted for the investigation of healing effects of SA in sunflower growth and fatty acid composition on Cd exposure. This experiment was conducted according to factorial design. Sunflower plants were subjected to five different concentrations of Cd and subsequently treated with three treatments of salicylic acid via foliar spraying. Seven days after the application of final treatments of SA, plants of sunflower were collected and their roots, leaves and stem were separated, and growth parameters were measured. A Soxhlet system was used for the extraction of leaf oil, and fatty acid composition was analyzed by using gas chromatography. Statistical analyses revealed that growth parameters (length of roots and stems, number of leaves, and leaves fresh weight) were reduced when Cd concentration was increased, while application of SA enhanced these parameters as compared to controlled plants. The most prominent reduction in these parameters occurred at 0 µmol of SA and 200 µmol Cd. Cd exposure led to alterations in fatty acid composition, which resulted in an increase in saturated fatty acids and a reduction in the content of unsaturated fatty acids in sunflower leaves, whereas these effects were ameliorated on the foliar application of SA. Specifically, SA notably decreased the percentage of palmitic acid and increase linoleic acid. These findings suggest that by preserving membrane integrity through lipid esters, SA could serve as a potential growth regulator offering enhanced plant resistance to Cd stress and protection against oxidative stress induced by Cd.

Keywords: Foliar Application, Sunflower, Salicylic Acid, Cadmium, Heavy Metals

1. Introduction

Rapid expansion of industries in recent years, has led to a huge rise in heavy metal contamination in agricultural soils. Industrial emissions and human activities are the primary cause of this pollution. A highly toxic metallic element cadmium, which is resistant to biodegradation can damage the physiological processes of living organisms (Yang *et al.,* 2019). The buildup of bioaccumulation of cadmium in agricultural soil present a significant threat to agricultural products and public health via food chain (Uddin *et al.,* 2021). Biochemical and physiological processes of plants are disrupted on cadmium exposure, which lead to adverse effects on development and growth of plants (Benavides *et al.,* 2005). In plants activities of nitrite reductase and nitrate reductase are hindered by cadmium ions, as a result ability of roots to absorb nitrate is reduced (Li *et al.,* 2010).

Presently, cadmium poses a significant threat to agricultural soils (Zou *et al.,* 2021), contaminating water sources, farmland, and various ecosystems (Wang *et al.,* 2019), Additionally, even at low concentrations of cadmium toxicity poses risks to plants (Adrees *et al.,* 2020). The uptake of cadmium by roots from the soil usually results in initial root damage, leading to changes in cell membrane

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permeability and hindering the element's transport across the membrane (Riaz *et al.,* 2021). Which lead to diminish the ability of plant roots to uptake essential mineral nutrients (Dong *et al.,* 2006). Cadmium toxicity can elevate the production of reactive oxygen species (ROS), as a result cellular components of plants are damaged due to oxidative stress induced by ROS. This damage includes lipid peroxidation of membranes, resulting in significant harm to cellular lipids, proteins, or DNA (Sinnis *et al.,* 2010). Plants upregulates the activities of antioxidant enzyme such as catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) in response to excess ROS induced by cadmium stress (Xia *et al.,* 2023). However, under prolonged exposure to cadmium stress antioxidant capacity of enzymes decreases as a result photosynthesis of plants also decreases, thereby affecting their growth, development, and yield formation (Chu *et al.,* 2018).

Sunflower is an important oilseed crop worldwide (Forleo *et al.,* 2018), which boasts high levels of polyunsaturated fatty acids, including 64.35% linoleic and 19.81% oleic fatty acids. Additionally, sunflower seeds contain18% mineral elements, 65.42% lipids, and 33.85% proteins (Agnihotri *et al.,* 2019). Sunflower can accumulate 3000 mg/kg of heavy metals in plants so it can be considered as an affective source of phytoremediation of soils contaminated with heavy metals (Petraru *et al.,* 2021). This natural aptitude of sunflower phytoremediation is a cost efficient and ecofriendly source for sustainable agriculture (Rezania *et al.,* 2016).

Salicylic acid, a very important compound for bolstering resilience and can promote plant development. Under difficult soil conditions it can enhance crop productivity (Asma & Ashraf, 2023). Moreover, in plant defense mechanisms against various stresses, it also serves as a signaling molecule (Liu *et al.,* 2022). SA serves as a precursor for nitrogen metabolism and growth regulators, it also plays a key role in regulating photosynthesis (Nazar *et al.,* 2015). Furthermore, SA helps plants in improving membrane stability, antioxidant defense, and the production of osmoprotectants (Ogunsiji *et al.,* 2023). SA can also stimulate gene expression related to antioxidant defense mechanisms and ROS scavenging, to detoxify ROS (Agnihotri *et al.,* 2018). As cadmium toxicity becomes a prominent contaminant posing threats to environmental and agriculture sustainability worldwide, it is mandatory to unravel the changes in plant growth and percentage of lipid content in response to Cd exposure. Sunflower which is known as a vital oilseed crop can withstand to harsh conditions but is not much resistant to Cd toxicity. The underlying regulatory mechanisms that enable sunflower to withstand Cd toxicity remain incompletely understood. Therefore, this experiment was conducted aiming to analyze growth and lipid content under Cd toxicity and alleviate this toxicity by foliar application of SA.

2. Materials and Methods

2.1. Experimental Design

Homogeneous seeds of sunflower were obtained from the Agricultural Research Center in Faisalabad, Pakistan. The seeds were sterilized for 15 minutes in a 1% NaClO solution, then seeds were washed with distilled water. In each pot six seeds were cultivated, and after germination, at seedling stage were thinned to four uniform seedlings in each pot. Irrigation of these pots were done by distilled water. The plants were kept in a greenhouse with temperatures ranging from 24.5ºC to 33.5ºC. At the two-leaf stage, five different concentrations (0, 50, 100, 150, and 200 µM) of $CdCl_2$ were applied on their respective labelled plants. Seven days after the application of Cd treatments, foliar application of three different concentrations (0, 250, and 500 µM) of salicylic acid (SA) was done on their respective labelled plants. Each treatment was replicated three times.

2.2. Plant Growth Analysis

The plants were harvested after one week of application of the final salicylic acid treatment, their leaves, roots and stems were separated. The lengths of the roots and stems were measured, number of leaves per plant was counted. The fresh weight of the leaves for both treated and control plants was recorded and expressed in grams per plant.

2.3. Oil Extraction

Leaves were dried in an oven at 40°C for four hours to minimize moisture percentage up to 5%. The dried leaves were then crushed into a fine powder with a mortar. One gram of the grounded leaf tissues was used to extract oil with petroleum ether for 6 hours following the AOCS method (AOCS, 1993). At 40°C under reduced pressure, a rotary evaporator was used to evaporate solvent from the extracted oil.

2.4. Analysis of Fatty Acids

According to Marquard (1987) methods, the extracted oil from the test sample using a hexane/methanol (3:2, v/v) mixture was changed into fatty acid-methyl esters. A gas chromatograph equipped with flame ionizing detector and a fused silica capillary column was used to analyze these methyl esters (0.1 µl). The conditions of gas chromatograph operations were: for initial 1 minute, oven temperature was set to 120°C, then at a rate of 6°C/min increase, temperature was increased to 250°C and were held for 15 minutes at 250°C. A carrier gas Helium was used, flow rate and split ratio of gas were 40 ml/min, 1/20 ml/min respectively. The peaks of fatty acid methyl esters were identified by comparing the relative retention times. By using a computing integrator, the contents of acids were quantified on percentage basis.

2.5 Statistical Analysis

Statistical analysis was performed using factorial ANOVA with Statistix 8.1 software. While mean values of each treatment was compared using LSD test at $P \leq 0.05$ to check the significance/ non significance differences among all treatments.

3. Results

3.1. Plant Growth

With the increase in Cd concentrations, the number of leaves and their fresh weight were gradually decreased. In contrast, plants which received foliar application of SA showed a slight increase in number of leaves and weight for all Cd concentrations as compared to their controlled values. Cd exposure also significantly reduced the lengths of stems and roots. However, 500 µmol SA enhanced stem and root elongation in both Cd-exposed and non-exposed plants (Tang *et al.,* 2023; Nawaz *et al.,* 2024).

3.1.1 Number of Leaves

Figure 1 shows the increase in Cd concentrations, the number of leaves were gradually decreased. Lowest number of leaves (13.95) were found at 200 µM cadmium concentration. But plants which received foliar application of SA showed a slight increase in number of leaves for all Cd concentrations as compared to their values at 0 µM SA concentrations. At 200 µM Cd concentration and 250 µM SA concentrations sunflower showed a 3% increase and at 500 µM SA concentrations showed a 14% increase in number of leaves as compared to 200 µM Cd concentrations without the foliar application of SA.

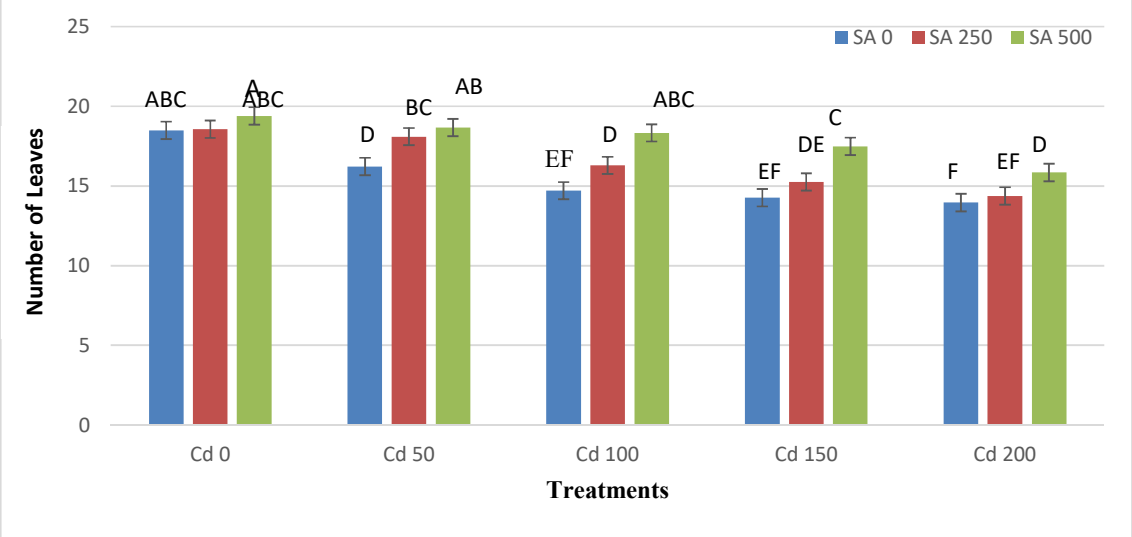

Fig. 1: Graphical representation of LSD test for number of leaves at Cd and SA treatments

3.1.2. Leaves Fresh Weight

The plants were harvested after one week of application of the final salicylic acid treatment, their leaves, roots and stems were separated. The fresh weight of the leaves for both treated and control plants

was recorded and expressed in grams per plant. Cd toxicity significantly affected on leaves fresh weight. Without foliar application of SA 68% leaves fresh weight was decreased as compared to their controlled (Figure 2). However, when SA was applied, sunflower plants showed increase in leaves fresh weight for all Cd concentrations as compared to their values at 0 µM SA concentrations. At 200 µM Cd concentration and 500 µM SA concentrations showed a 57% increase in leaves fresh weight as compared to 200 µM Cd concentrations without the foliar application of SA.

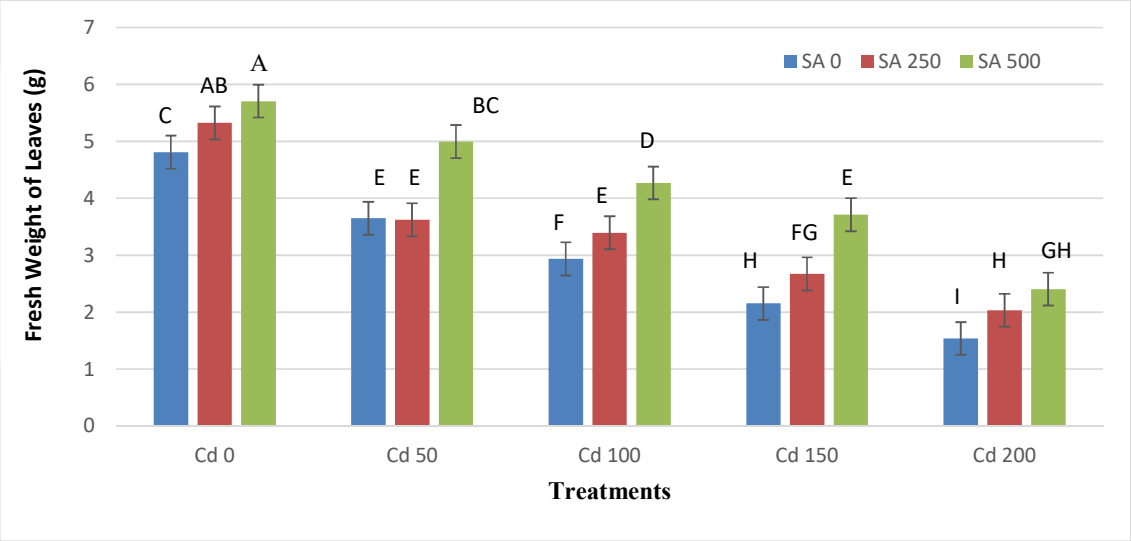

Fig. 2: Graphical Representation of LSD Test for Leaves Fresh Weight at Cd and SA Treatments

3.1.3 Stem and Root Length

The plants were harvested after one week of application of the final salicylic acid treatment, their leaves, roots and stems were separated. Stem and root length were measured with the help of measuring tape. Results showed that Cd significantly affected on stem and root length. With increasing Cd concentrations stem and root length were gradually decreased as shown in Figure 3 and Figure 4. At 200 µM Cd concentration and 500 µM SA concentrations showed a 9% increase in stem length and 60% increase in root length as compared to 200 µM Cd concentrations without the foliar application of SA.

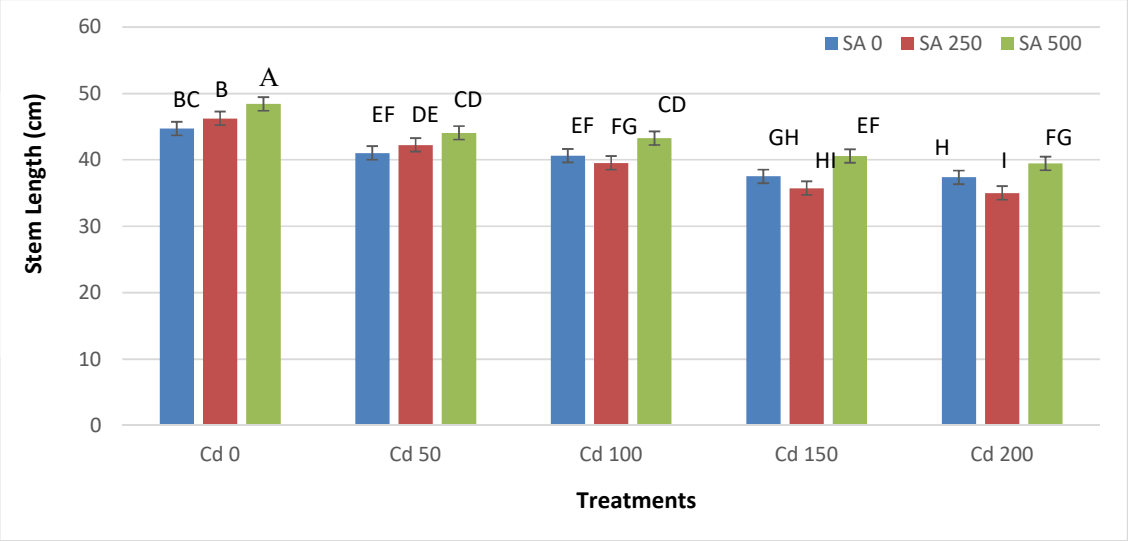

Fig. 3: Graphical Representation of LSD Test for Stem Length at Cd and SA Treatments

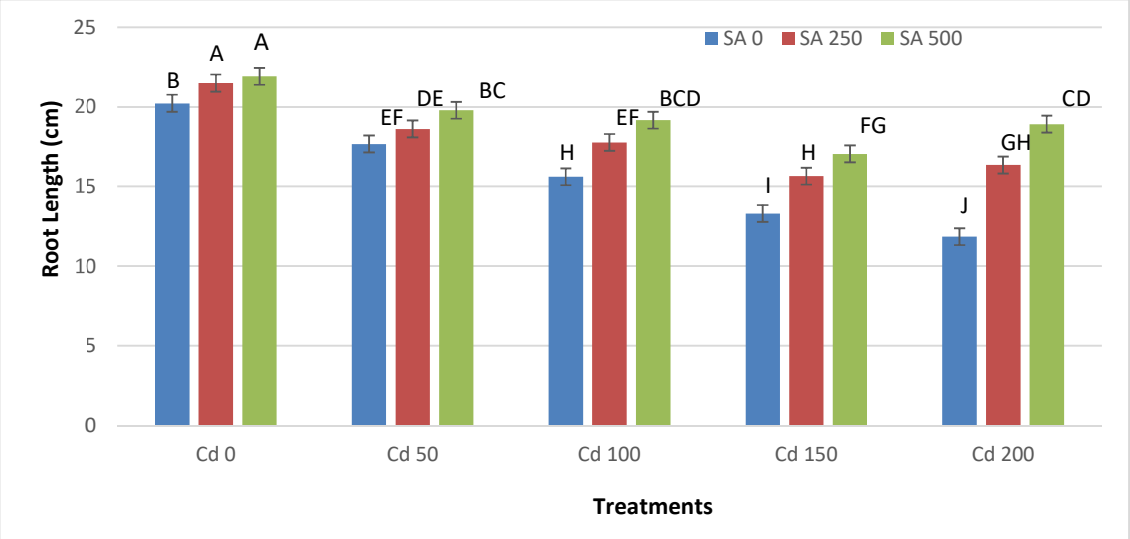

Fig. 4: Graphical Representation of LSD Test for Root Length at Cd and SA Treatments

3.2. Fatty Acids Composition

Fatty acids content in sunflower leaves showed prominent variations when exposed to cadmium toxicity. While foliar application of SA alleviated this oxidative stress caused by Cd toxicity. linoleic, oleic, stearic and palmitic acids are the primary fatty acids. Analysis of data showed that content of unsaturated fatty acids were reduced while content of saturated fatty acids were enhanced on cadmium exposure as compared to control treatment. However, the presence of an antioxidant like SA, whether or not Cd was present, content of unsaturated fatty acids were enhanced and that of saturated fatty acids content were reduced ((Popova *et al.*, 2008; Sakineh *et al.,* 2012).

3.2.1. Saturated Fatty Acids

The results in Figure 5 and Figure 6, expressed as percentages of saturated fatty acids, show that the key difference in fatty acid composition between control and Cd-contaminated sunflower leaves was an increase in saturated fatty acids, with palmitic acid increasing by 57.04% under 200 µmol Cd compared to control treatment. Specifically, palmitic acid (C16:0) increased by 1.6-fold. Treatment with 250 µmol SA, whether or not Cd was present, significantly decreased stearic acid (18.9%). Additionally, 500 µmol SA reduced palmitic acid (27.8%) in leaves with or without Cd treatment.

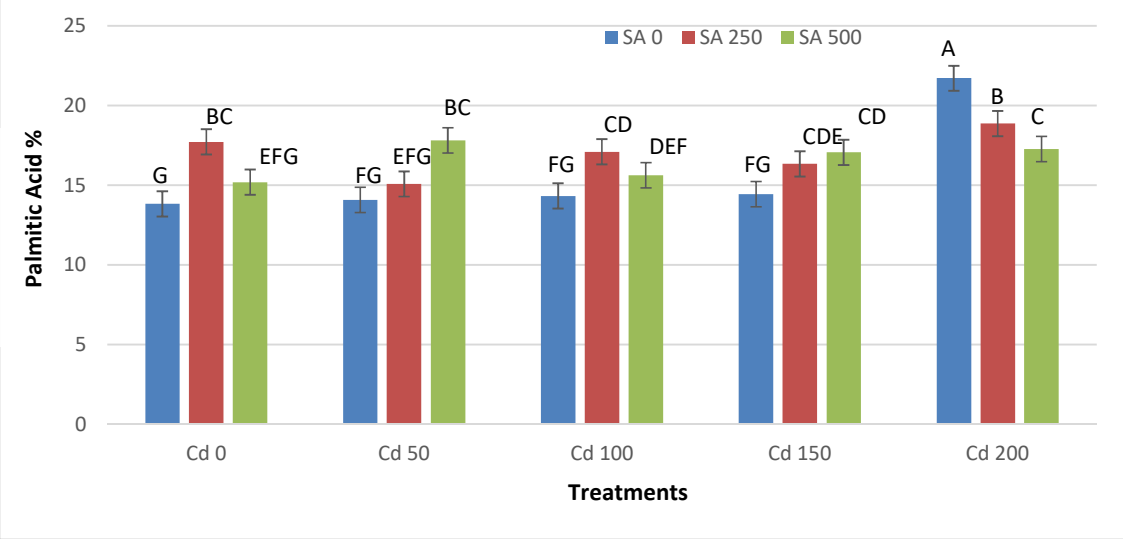

Fig. 5: Graphical representation of LSD test for percentage of palmitic Acid at Cd and SA treatments

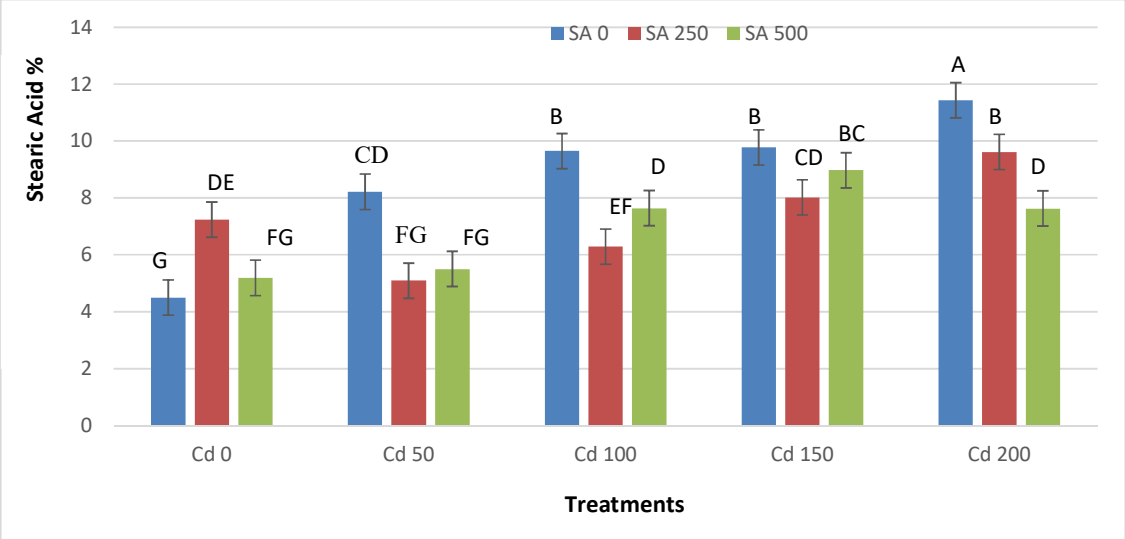

Fig. 6: Graphical representation of LSD test for percentage of stearic acid at Cd and SA treatments

3.2.2. Unsaturated Fatty Acids

The results in Figure 7 and Figure 8, expressed as percentages of unsaturated fatty acids, show that the key difference in fatty acid composition between control and Cd-contaminated sunflower leaves was a decrease in unsaturated fatty acids, with oleic acid was decreased more than half and linoleic acid by 5.15% under 200 µmol Cd compared to control plants. Treatment with 250 µmol SA, whether or not Cd was present, increased the levels of linoleic acid and 500 µmol SA enhanced oleic acid content.

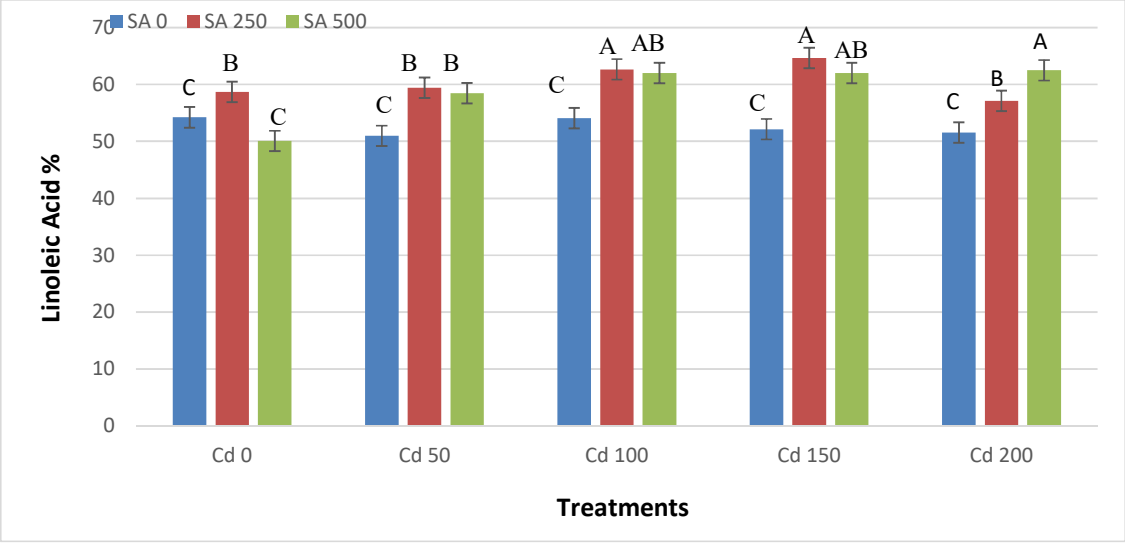

Fig. 7: Graphical representation of LSD test for percentage of linoleic acid at Cd and SA treatments

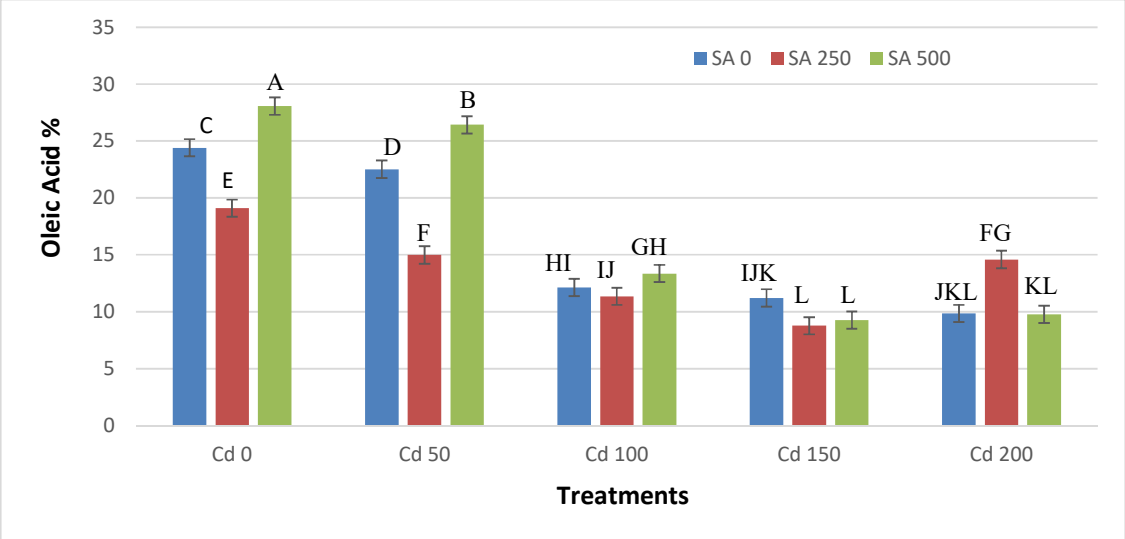

Fig. 8: Graphical representation of LSD test for percentage of oleic acid at Cd and SA treatments

4. Conclusions

From this experiment it can be concluded that the growth parameters like number of leaves, fresh weight of leaves, stem and root length were decreased significantly under the influence of Cd. In contrast, after foliar application of 500 µM SA in plants exposed to Cd resulted in increased number of leaves, higher fresh weight of leaves and enhanced stem and root elongation in both Cd-exposed and non-exposed plants. Foliar application of SA alleviated this oxidative stress caused by Cd toxicity. Results showed that content of unsaturated fatty acids were reduced while content of saturated fatty acids were enhanced in percentage on cadmium exposure as compared to controls. However, the presence of an antioxidant like SA, whether or not Cd was present, content of unsaturated fatty acids was enhanced and that of saturated fatty acids content were reduced.

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