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SOME PHYSIOLOGICAL AND BICHEMICAL RESPONSES TO CADMIUM IN SALICYLIC ACID APPLIED CUCUMBER (*CUCUMIS SATIVUS* L.) SEEDLINGS

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Abstract

In this study, the probability of salicylic acid (SA), serving as a mediator for protecting plants against cadmium (Cd) toxicity, was investigated. In cucumber seedlings exposed to increasing Cd concentrations (0, 25, 50 and 100 µM), the seedling and leaf elongation growth reduced, whereas the fresh and dry weight accumulation decreased. Furthermore, the levels of some important parameters regarding oxidative stress in the leaves of the seedlings, namely lipid peroxidation (MDA) increased, while reduced glutathione (GSH), fatty acid methyl esters (FAME) and the photosynthetic pigment content decreased. In the leaves of seedlings, which were pre-applied with salicylic acid (0.75 mM), alleviated the negative effects of Cd on MDA and GSH. The SA pre-application caused different effects due to the Cd concentration on GSH and FAME. The beneficial effect of SA on the oxidative damages caused by Cd was mainly found rather significant for 25 and 50 µM. Our data indicated that in reducing the negative results of oxidative stress caused by the toxicity of this heavy metal, SA may have beneficial effects.

Introduction

Cadmium (Cd) is a very toxic heavy metal which induces oxidative stress in plants (Hasan *et al.,* 2009). Cd is released from metals, phosphate fertilizers, industrial processes and rock mineralization processes. The high mobility of this metal in the soil-plant system enables it to easily enter into the food chain (Nogawa *et al.,* 1987). In different plants, cadmium toxicity is usually accompanied by the inhibition of growth and a decrease in pigment content and biomass production (Faizan *et al.,* 2011; Lopez-Millan *et al.,* 2009; Bavi *et al.,* 2011; Raziuddin *et al.,* 2011). In oxidative stress caused by cadmium, the production of oxygen free radicals (ROS, H_2O_2, OH^-, O_2^- etc) increases (Balaknina *et al.*, 2005; Dietz *et al.,* 1999). Functions of plasma membranes are negatively affected by environmental stresses, and lipid peroxidation increases. Malondialdehyde (MDA) is the final product of this lipid peroxidation in membranes, and its content indicates the velocity of the process (Choudhury & Kumar, 2004; Vassilev & Lidon, 2011). Studies conducted on different plants revealed that Cd toxicity decreased pigment and protein contents, while increasing proline and MDA contents (Hassan *et al.,* 2005; Costa & Spitz, 1997; Gonçalves *et al.,* 2009; Vassilev & Lidon, 2011). Cd ions can inhibit or sometimes stimulate the activity of several anti-oxidative factors (Hasan *et al.,* 2009). It was also reported that high concentrations of cadmium in plants affected the fatty acid composition in membranes (Malik *et al.,* 1992; Vassilev, 2004). Salicylic acid (SA) as a patent signaling molecule in plants, is involved in eliciting specific responses to biotic and abiotic stress. SA is frequently referred to as a plant hormone due to its numerous physiological and metabolic activates in plants (Hayat & Ahmad, 2007; Raskin, 1995). When SA is applied onto plants separately, depending on the concentration, it creates stimulative and inhibitive effects on physiological responses (Fariduddin *et al.,* 2003; Pancheva *et al.,* 1996). Recently, it has been noted that SA produces favorable effects against oxidative damage resulting from heavy metal pollution. The rapid elevation of organic acid amounts in plants under heavy metal stress may be associated with these acids having tolerance building effects upon plants against oxidative damage (Pál *et al.,* 2005; Anwer *et al.,* 2012). It was reported that salicylic acid pre-applications, in different plants, diminished the negative effects of cadmium on growth parameters (Guo *et al.,* 2009; Popova *et al.,* 2009), the photosynthesis rate, and chlorophyll content (Krantev *et al.,* 2008). It was further determined that in SA pre-applied plants MDA increased based on the Cd toxicity diminishing (Popova *et al.,* 2009; Krantev *et al.,* 2008; Guo *et al.,* 2009; Metwally *et al.,* 2003). Toxic or oxidative stress caused by Cd in different plants also affected the fatty acid composition in seedling leave membranes (Ben Ammar *et al.*, 2005; Nouairi *et al.*, 2006). In their study on pea (*Pisum sativum* L.) Popova *et al*., (2009) determined that high concentrations of Cd application not only caused an increase in linoleic acid (18:2) content in the plant, but also a decrease in its (18:3) content. In a study conducted on corn, (*Zea mays* L.), it was reported that in SA pre-applied leaves, the change caused by Cd in the fatty acid composition was lesser. It was suggested in this study that SA protected the lipid membranes of the leaves against cadmium (Ivanova *et al.,* 2008). ROS generated in cells are highy reactive in nature and destroy the normal cellular function and metabolism. Plants have various defense mechanisms by which they can scavenge these ROS. Reduced glutathione (GSH) is a non-enzymatic antioxidant which clears oxygen free radicals of plant cells (Mohan & Hosetti, 2006). The responses of GSH in plants is different against stress. Similarly content of GSH react differently to treatment with Cd in plants (Hasan *et al.,* 2009). In a study conducted on sunflower (*Helianthus annuus* L.) plant it was reported that reduced glutathione content in leaves increased proportionally with increasing Cd concentration (Hatata *et al.,* 2008). A study conducted on *Oryza sativa* L., revealed that SA pre-application increased GSH content in roots and shoots against Cd toxic effect and alleviated oxidative damage due to H_2O_2 and MDA level was

relatively low (Guo *et al.,* 2009). As a result of the same study, GSH content in the roots of seedlings treated with Cd were higher compared to those not treated with Cd (Guo *et al.,* 2009*).* The objective of this study was to performed the protective effect of pre-application with SA on cucumber seedlings exposed to cadmium concentrations based an physiological and biochemical.

Materials and Methods

Growth conditions of seedlings and experimental design: Cucumber (*Cucumis sativus* L. cv. Beit Alpha) seeds were submerged in tap water for 5h, surface sterilized with 0.5% (m/v) hypocholorid sodium for 30 min, rinsed several times with distilled water and then germinated on moist paper placed in sterilized petri dishes. After that the germinated seeds with age of 3 days and which have similar radicle lengths were transferred to wet filter paper convolutions that lay in glass jars filled with nutrition solution. The glass jars were put into growth cabins, where they were kept under natural light night and day, having the photoperiod 16/8, temperature 27±2, 25±2°C and relative humidity 60-70%. From the seedlings those which showed abnormal growth were eliminated. Before the applications, roots of the seedlings were washed with deionised water and the seedlings were placed between the canals of the sponge lids of jars that contained basic nutrient solution (30%). Nutrient solution (mg/L): $Ca(NO_3)_2$. $4H_2O$ 708.45; $MgSO_4$.$7H_2O$ 492.94; KH_2PO_4 136.09; K_2SO_4 348.50; $(NH_4)_2SO_4$ 396.39; $CaCI_2$.$2H_2O$ 441.09; H_3BO_3 2.868; $MnSO_4$. H_2O 1.545; EDTA-Fe 33.0345; $ZnSO_4.7H_2O$ 0.220; $CuSO_4$. $5H_2O$ 0.080; Na_2MoO_4 . $2H_2O$ 0.0299. Nutrient solution was aerated twice a day. 0.75 mM SA concentration prepared in deionized water. $CdCI_2$ (0, 25, 50 and 100 µM) concentrations prepared in nutrient solution were used as test solutions. The study used fifteen-day old seedlings. The seedlings were divided into eight groups, each consisting of 10 seedlings. The solutions were applied to the roots of seedlings using hydroponic method. Four groups were placed into jars containing deionized water and four into jars containing 0.75 mM SA solution. SA pre-application to the seedlings was performed for 1 day. At the end of this period of time, the roots of the seedlings were washed and the seedlings were transferred into jars containing 0, 25, 50 and 100 µM cadmium solutions. As a result of this application, growth parameters such as seedling and leaf length, fresh-dry weight were determined in seedlings. Additionally, photosynthetic pigment, MDA, fatty acid methyl esters (FAME) and GSH contents were determined.

Growth parameters of seedlings and biochemical analysis: Growth parameters were examined in seedlings at the end of applications. In order to determine the seedling and leaf length (cm) were measured. Compared to the control seedlings, other groups were calculated as % fresh and dry weight of seedlings. To establish the dry weight of seedlings, the seedling, which were duly packed and marked, were kept in drying oven and their final weight was measured (Kacar, 1972). For the purpose of identifying the content of photosynthetic pigments, about 1 g of fresh leaf tissue was obtained from the seedlings and in 100 ml 80% acetone extracted. The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were measured by the spectrophotometric method by using 80% acetone extraction. After that, the absorbance of these extracts was separately read at 645, 663 and 440 nm wavelengths against a acetone blank. Using the absorbance values, chlorophyll (a+b) and carotenoid amounts were estimated by the equations of Witham *et al*., (1971). Lipid peroxidation was measured using TBARS method (Heath and Packer, 1968). For this purpose, 1 g leaf tissue was homogenized in 10 ml 0.1% TCA. The homogenate was centrifuged at 6000 g for 15 minutes. Then 1 ml of the surface phase was collected and added to 0.5% TBARS prepared in 4 ml 20% TCA and 0.04 ml butylated hydroxyl toluene (4% solution prepared in BHT-ethanol). The mixture was heated at 95°C for 30 minutes and cooled down in an ice bath. The samples were centrifuged at 6000 g for 15 minutes. The surface phases were taken and added 3 ml butanol. The mixture was centrifuged again, and the phases were separated. The surface phase was put into a spectra tube and its absorbance was recorded at 532 nm in a blind manner. Then the content of MDA in the sample was determined as nmol/g FW. For the analyses of fatty acid, 1 g of leaf tissue and 10 ml 1% TCA were homogenized. It is then added 1 ml 20% of TCA and centrifuged for 5 minutes at 6000 gravity. Its pellet was added a 10 ml of hexane/isopropanol/BHT mixture and vortexed, being centrifuged at 6000/5 minutes. Thereafter, 5 ml of surface phase was collected in a spectra tube, added with 5 ml of methanolic sulfuric acid and overturned after the caps were closed. It was left in the drying oven at 55°C for 15-17h. At the end of the given time, each tube were added with 5 ml 5% NaCl, 5ml hexane and those which were lacking were completed with hexane and overturned. After 1 hour, surface phase was collected. This was added with 5 ml 2% of KH_2CO_3 and another 3h was spent. Subsequently, surface phases were collected in another tube and left to fly at 40°C in the rotary evaporator under a stream of nitrogen. At the end of this period, tubes were added with 1 ml of heptane vials and analyzed in gas chromatography (Christie, 1990; Hara & Radin, 1978). Then the content of FAME (fatty acids methyl esters) in the sample was determined as mg/g FW. Additionally, 1 g of leaf tissue was placed in a erlenmeyer flask. It was homogenized by being added 30 ml 5% of TCA. This obtained homogenate was centrifuged at 6000 gravity and 4ºC' for 15 minutes. Supernatant's pH was set to 7.5 and its absorbance was recorded at 412 nm. Absorbance results were put in place and the GSH content in the tissue was determined as µg/g.FW. Tissue extraction and measurement of contents were carried out in a modified manner (Ellman, 1959; Griffith, 1980). Three replicates for each treatment were maintained. All physiological analyses were replicated three times for each treatment. Results were analyzed using one-way ANOVA (SPSS 15.0 Evaluation Version Production Mode Facility). The difference between treatments were considered significant at $p<0.01$-0.05. Duncan test was performed to compare means.

Results

Effect of SA on growth response to Cd toxicity: The present study revealed the regulatory effect of SA on the toxicity induced by cadmium in cucumber seedlings. Our data revealed that seedling and leaf elongation growth reduced (the data in parentheses shows that increase and decline/wrinkle in elongation growth between the initial and final length of seedling and leaf.), and the seedlings fresh and dry weight accumulation decreased proportionally with increasing Cd concentration (at $p<0.01$-0.05) (Tables 1 and 2). It was found that SA pre-application resulted in an alleviating of the inhibitive effect in leaf elongation growth, when compared to seedlings which were treated with 25 µM Cd without SA and 50 µM Cd without SA (at $p\geq0.05$). It even confirmed the negative effect on seedling elongation growth for 100 µM Cd without SA concentration (at $p<0.05$).

Effect of SA on pigment and MDA contents: Datas has shown that chlorophyll (a+b) content decreases proportionally with increasing Cd concentration, in other groups compared to the leaves of the control seedlings (at $p<0.01$-0.05) (Table 2). In leaves, the decreasing effect on chlorophyll (a+b) and carotenoid contents caused by the Cd treatment was alleviated by the effect of SA pre-application, but was not found significant (at $p\geq0.05$). Also data has revealed that in all groups MDA content increased in parallel with increasing Cd concentration. It was found that the content of MDA was decreased in the leaves of seedlings pre-applied with SA at rates 27.35% and 26.63%, when compared to seedlings treated with 25 µM Cd without SA and 50 µM Cd without SA (at $p<0.05$). Compared to the control, SA alone caused an increased of rate 22.30% in the MDA level, and was found to be significant (at $p<0.05$). Compared to the control, SA alone caused an increased of rate 22.30% in the MDA level, and was found to be significant (at $p<0.05$).

Table 1. Growth parameters of Cucumber (*Cucumis sativus* L.) seedlings under Cd toxicity, without and with SA (0.75 mM) pre-application.

Cd(µM)	Seedling length (cm)		Seedling fresh weight (%)		Seedling dry weight (%)	
	SA pre- applied (0.75 mM)					
	without SA	with SA	without SA	with SA	without SA	with SA
0 (Control)	23.00 ± 0.13 [a] (+ % 0.30)	22.98 ± 0.20 [a] (+ % 0.26)	100 ± 3.24 [a]	95.13 ± 2.01 [a]	100 ± 5.04 [a]	93.28 ± 4.16 [a]
25	21.95 ± 0.28 [bc¤] (- % 0.09)	21.96 ± 0.45 [bc¤] (- % 0.07)	78.64 ± 2.53 [b¤]	79.28 ± 3.36 [b¤]	74.32 ± 3.94 [bc¤]	77.18 ± 2 88 [b¤]
50	22.01 ± 0.58 [b¤] (- % 0.18)	22.91 ± 0.25 [b¤] (- % 0.13)	72.93 ± 3.12 [b¤]	74.63 ± 2 77 [b¤]	67.11 ± 2.42 [c¤]	67.61 ± 2.85 [c¤]
100	21.40 ± 0.10 [c¤] (- % 0.29)	20.57 ± 0.40 [d¤*] (- % 0.24)	72.51 ± 4.18 [b¤]	71.45 ± 3.92b [c¤]	65.77 ± 2.61 [c¤]	63.08 ± 2.85 [c¤]

¤Compared to the control group, *Compared to the associated group; at $p<0.01$-0.05 probability levels. Data is means ± SE (n:10). The data in parentheses, increase (+) and decline/wrinkle (-) in elongation growth between the initial and final length of the seedling. The values given in the table are the final length of seedlings

Table 2. Leaf length (cm), chlorophyll (a+b) and carotenoid amounts in Cucumber (*Cucumis sativus* L.) seedling leaves under Cd toxicity, without and with SA (0.75 mM) pre-application.

Cd (µM)	Leaf length (cm)		Chlorophyl (a+b) (mg/g.FW)		Carotenoid (mg/g.FW)	
	SA pre- applied (0.75 mM)					
	without SA	with SA	without SA	with SA	without SA	with SA
0 (Control)	2.33 ± 0.05 [a] (+ % 2.90)	2.66 ± 0.03 [b¤] (+ % 1.75)	1.770 ± 0.15 [a]	1.733 ± 0.05 [a]	0.350 ± 0.05 [a]	0.323 ± 0.03 [a]
25	2.27 ± 0.08 [a] (+ % 0.35)	2.39 ± 0.04 [ab¤] (+ % 0.44)	1.467 ± 0.12 [b¤]	1.476 ± 0.21 [b¤]	0.253 ± 0.02 [b¤]	0.255 ± 0.06 [ab]
50	2.27 ± 0.07 [a] (- % 0.69)	2.58 ± 0.20 [ab¤] (- % 0.44)	1.350 ± 0.13 [b¤]	1.451 ± 0.12 [b¤]	0.236 ± 0.01 [b¤]	0.240 ± 0.05 [b¤]
100	2.36 ± 0.14 [a] (- % 0.85)	2.34 ± 0.11 [a] (- % 1.20)	1.287 ± 0.11 [b¤]	1.039 ± 0.07 [c¤]	0.210 ± 0.05 [b¤]	0.226 ± 0.04 [b¤]

¤Compared to the control group; at $p<0.01$-0.05 probability levels. Data are means ±SE (n:10 for leaf length, n:3 for pigment). The data in parentheses, increase (+) and decline/wrinkle (-) in elongation growth between the initial and final length of the leaf. The values given in the table are the final length of leaves

Effect of SA on GSH and FAME contents: Compared with the leaves of the control seedlings, different values in FAME content occurred due to SA pre-application and increasing Cd concentration. Compared to the control groups, its content decreased at rates of 13.08% for 100 µM Cd only (at $p<0.05$) (Table 3). Compared to the control, SA alone caused an increase in the FAME level, was not found significant (at $p\geq0.05$). In SA pre-applied groups, FAME contents differed (increased) from other groups, but they were found statistically insignificant (at $p\geq0.05$). It was found that the content of FAME was increased in the leaves of seedlings pre-applied with SA at rate 7.90%, when compared to seedlings treated with 100 µM Cd without SA (at $p<0.05$). Compared to the control, GSH content was found to be lower in other groups in our study. Compared to the control, SA alone caused a rate 24.74% decrease in the GSH content, and was found significant (at $p<0.05$) (Table 3). It was found that the content of GSH was increased in the leaves of seedlings pre-applied with SA at rate 48.03, when compared to seedlings treated with 50 µM Cd without SA (at $p<0.05$).

Table 3. MDA, FAME and GSH amounts in Cucumber (*Cucumis sativus* L.) seedling leaves under Cd toxicity, without and with SA (0.75 mM) pre-application.

Cd(µM)	MDA (nmol/g.FW)		FAME (mg / g FW)		GSH (µg /g.FW)	
	SA pre- applied (0.75 mM)					
	without SA	with SA	without SA	with SA	without SA	with SA
0 (Control)	33.22 ± 1.29 ^{a}	40.63 ± 4.47 ^{b*}	1.819 ± 0.12 ^{a}	1.835 ± 0.16 ^{a}	27.16 ± 2.91 ^{a}	20.44 ± 2.68 ^{b*}
25	47.02 ± 4.29$^{bc¤}$	34.16 ± 3.61^{ab*}	1.622 ± 0.14^{ab}	1.748 ± 0.13 ^{a}	19.25 ± 3.96$^{bc¤}$	21.46 ± 4.18 ^{ab}
50	54.11 ± 4.93 $^{c¤}$	39.70 ± 3.29$^{b¤*}$	1.810 ± 0.09 ^{a}	1.897 ± 0.12 ^{a}	22.38 ± 4.11 ^{ab}	33.13 ± 3.95 ^{a*}
100	50.13 ± 4.12$^{bc¤}$	47.10 ± 4.74$^{bc¤}$	1.581 ± 0.09$^{b¤*}$	1.706 ± 0.07^{a}	17.46 ± 3.43$^{bc¤}$	18.46 ± 5.40$^{bc¤}$

¤Compared to the control group,*Compared to the associated group; at $p<0.01$-0.05 probability levels. Data are means ± SE (n:3)

Discussion

The present study was performed to analyze the mechanisms of the beneficial effect of SA on cucumber plants exposed to toxic Cd concentrations. In our study, we could not detect significant effect of SA on inhibitive effect (Hassan *et al.,* 2005; Farooqi *et al.,* 2009) of growth parameters formed by Cd on cucumber seedlings. The effort of alleviating and supporting the inhibitive effect of lower and higher Cd concentration on growth by SA pre-application was not found to be significant (Popova *et al.*, 2009; Krantev *et al.*, 2008; Metwally *et al.*, 2003). This may be a result of complex behaviors of SA, which it is considered as a plant hormone (Fariduddin *et al.,* 2003; Hayat & Ahmad, 2007). Liu et al. (2003) advanced that the inhibiting effect of Cd on plant growth may result from its preventative effect on cell growth and division. We macroscopically observed an increase in chlorosis associated with pigment destruction and necrotic stains resulting from cell destruction in leaves at varying levels in all groups. The increase in photosynthetic pigment destruction is a typical consequence of heavy metal toxicity in plants. The decrease in the chlorophyll content of plant tissues treated with a heavy metal may be caused by a problem in the synthesis, or by an increase in the destruction of this pigment. The data showed that the chlorophyll content was reduced in Cd treated plants. We do not think that reducing effect of SA on pigment destruction based on toxic effect of Cd on cucumber seedling leaves (Costa and Spitz, 1997; Lopez-Millan *et al.,* 2009) as significant. The MDA content in Cd treated cucumber plants was observed to be greater than the control. This study showed that Cd toxicity in cucumber plants was linked to lipid peroxidation. This condition indicates that membrane integrity and membrane structure has been impaired. The elevation of MDA contents indicates that Cd treated seedlings are exposed to more stress, relative to their control seedlings. In addition to this, it was found that SA pre-applied decreased MDA accumulation caused by Cd, indicating its involvement in the protection against oxidative damage. This data corresponds to those reported by researchers (Krantev *et al.*, 2008; Metwally *et al.*, 2003; Popova *et al.*, 2009; Guo *et al.*, 2009). Free radicals increasing with oxidative stress, increase lipid peroxidation in plant cells (Quariti *et al.*, 1997; Dietz *et al.*, 1999). In this study, we also investigated the effects of oxygen free radicals caused by oxidative stress on the fatty acid composition of cucumber seedling leaves. Oxidative stress caused decreasing in FAME content in the leaves (Ivanova *et al.,* 2008). Our data shows that cadmium caused a decrease in the amount of the FAME. Similar data on the effect of Cd were reported for different plant species, wheat (Malik *et al.,* 1992), barley (Vassilev, 2004), tomato (Ben Ammar *et al.,* 2005) and mustard (Nouairi *et al.,* 2006). Pre-applying with SA exerted a protective effect on the membrane stability judging by the increased FAME level and by changes in their FA composition. Also, it was suggested that the change in the ratio of fatty acids was a reaction to the damaging effect of Cd (Popova *et al.*, 2009; Ivanova *et al.,* 2008). lt was suggested in another study that heavy metal treatment induced an alteration in the fatty acid desaturation processes (Quariti *et al.*, 1997). In general, regarding the effect of damage caused by cadmium on fatty acids, the alleviating effect of SA pre-application was not found significant. Therefore, no data on fatty acids is

reported. In order to repair the damage initiated by ROS, plants evolve complex antioxidant metabolism. In our study, the non-enzymic antioxidant glutathione showed different responses to increasing Cd concentration in other groups compared with the leaves of control group seedlings. In a study conducted on rice (*Oryza sativa* L.) it was found that compared to seedling roots pretreated with SA, GSH content increased for low Cd concentrations, and decreased for high Cd concentrations (Choudhury & Kumar, 2004). While cadmium treatment caused a decrease in GSH content in pea (*Pisum sativum* L.) roots and leaves (Dixit *et al.,* 2001), it caused reactions in corn (*Zea mays* L.) where a remarkable increase in root reduced glutathione (GSH) content occurred (Rellán-Álvarez *et al.,* 2006). However, after leaf segments were taken from sunflower seedlings, it was reported that generally speaking, Cd resulted in decreasing chlorophyll and GSH contents while increasing lipid peroxidation (Gallego *et al.,* 1996). In our study, it was determined that pre-application of SA decrease GSH destruction for 50 µM Cd which is intermediary dose (Choudhury & Kumar, 2004). In our study, GSH content generally decreased when compared to the control. Due to its membrane transmittance regulating and membrane integrity protecting characteristics in cells (El-Tayep, 2005), SA may have reduced the toxic damage of Cd in cucumber seedlings. This decrease in GSH levels is suggestive of SA playing a particular role in glutathione biosynthesis (Freeman *et al.,* 2005). Therefore, differences in glutathione content can be placed in an enzymatic context (Yoshida *et al.,* 2009). Being able to logically discuss the increase of GSH contents despite the toxic damage of Cd requires studies on all antioxidant enzymes. Additionally, these parameters must also be used in relation to the roots.

Conclusion

Although there are numerous studies examining the effects of cadmium on plants in isolation, we have not found any literature study addressing it together with SA. Thus, we shall discuss the effects of SA pre-application on cadmium toxicity only on the basis of the studies conducted with other plants. When used on cucumbers, SA concentration alone caused toxic effects most of the time in some parameters. The study has suggested that the beneficial effect of pre application with SA during growth period may help seedlings to avoid cumulative damage upon exposure to Cd. SA may activate Cd tolerance mechanisms which differ from Cd distribution and antioxidant defenses. Alternatively, SA could enhance repair processes. However, further studies need to be conducted with cucumber seedlings in order to gain deeper insights into this topic.

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