Journal of Agriculture and Ecology Research International

19(1): 1-15, 2019; Article no.JAERI.31832
ISSN: 2394-1073

Salicylic Acid Phytohormone Decreased the Toxicity Damage of Exogenous Lead Absorption in Seedlings of an Oilseed Plant, *Brassica napus* L. Grown in Hydroponic Conditions

Mahdi Khozaei[1*] and Shiva Boroumand Jazi[2]

[1]Department of Biology, University of Isfahan, Isfahan, Iran.
[2]Department of Biology, Islamic Azad University Borujerd Branch, Borujerd, Iran.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAERI/2019/v19i130073
Editor(s):
(1) Dr. Ahmed Esmat Abdel Moneim, Department of Zoology, Helwan University, Egypt And Institute of Biomedical Research Center, University of Granada, Granada, Spain.
(2) Dr. N. Karunakaran, Department of Economics and Vice-Principal, EK Nayanar Memorial Govt. College, Elerithattu, India.
(3) Dr. Chandra Shekhar Kapoor, Department of Environmental Sciences, (New Campus), University College of Science, Mohan Lal Sukhadia University, Udaipur-313001, Rajasthan, India.
Reviewers:
(1) Hakan Sevik, Kastamonu University, Turkey.
(2) Otitoloju Kekere, Adekunle Ajasin University, Nigeria.
Complete Peer review History: http://www.sdiarticle3.com/review-history/31832

Original Research Article

Received 01 December 2017
Accepted 13 February 2018
Published 28 June 2019

ABSTRACT

Oilseed plant, *Brassica napus* L. seedlings grown in hydroponic condition with different concentrations of Pb were treated with salicylic acid (SA) to investigate the role of exogenous salicylic acid in alleviating lead toxicity on biochemical and physiological activities of the plant. The results showed that application of different concentrations of Pb increased soluble sugars and reduced carbohydrate levels significantly in roots and shoots of the plants. The stress induced by application of Pb triggered significant inhibitory effects on growth and chlorophyll synthesis induced on the production of protein and proline and enhanced the levels of antioxidant activity. Salicylic acid (SA) treated plants showed alleviation increasing total dry mass, leaf area, shoot and root length as well as leaf total chlorophyll content in responses to Pb stress. Results revealed the importance of salicylic acid (SA) activity in enabling plants to reduce the soluble sugars and increase of insoluble sugar in heavy -metal-stressed plants. The content of proline and proteins

**Corresponding author: E-mail: m.khozaei@sci.ui.ac.ir;*

were also reduced in plants were treated with salicylic acid. Our data provide evidence that salicylic acid treatment decreased the activity of antioxidant enzymes in plants were exposed to different levels of Pb.

Keywords: Salicylic acid; toxicity; Brassica naps L.; oilseed; hydroponic conditions.

1. INTRODUCTION

Lead is a highly toxic and dispersed heavy metal pollutant in both terrestrial and aquatic ecosystems [1]. Accumulation of Pb into the environment is mainly due to the anthropogenic sources or agricultural mismanagement [2,3]. Pb is not listed in the necessary nutrient elements for plants growth, and its biological significance is not fully understood, but several reports demonstrated the considerable impact of the excess amount of Pb at the organismal and cellular levels. An excessive level of Pb can damage an organism at the variety of levels. High level of Pb has resulted in reduction of photosynthetic capacity, respiration and nitrogen metabolisms and stimulates the formation of free radicals and reactive oxygen species (ROS) resulting from oxidative stress [4,5]. This causes disturbance of metabolic pathways and damage to macromolecules and cellular metabolism [6]. Furthermore, exogenous Pb showed significant changes in antioxidant enzyme activities and hormone levels in perennial aquatic plants [7,8]. The results from several studies demonstrated that lead stress triggered the activity of superoxide dismutase and increased the content of malondialdehyde (MDA) in leaves of wheat and cabbage plants [8,9]. In radish leaves, elevated levels of lead stimulated the activity of peroxidase, acid phosphatase and ribonuclease [4]. The growth performance of *Lythrum salicaria* dramatically reduced and the chlorosis phenotype emerged in the leaves of radish when both plants were exposed to different levels of Pb [4]. The high content of Pb also exerted an adverse effect on protein structure and inhibited photosynthetic electron transport and the photochemical activity of photosystem II [10].

Plant adaptation and survival in response to a variety of stress mainly, heavy metal depends on the defence system, detoxification and developing tolerance. The plant strategies for preventing the heavy metal build up at the cellular level are diverse. At the plasma membrane, the mechanism to avoid or reduce the toxic effect of heavy metal is to restrict the massive metal influx into the cell or by activation of the efflux pump outside the cell [11]. Within the protoplast, several mechanisms are applied to cope with increasing levels of heavy metal toxicity. Different species may have adopted different mechanisms to counter detrimental effects of an excessive amount of heavy metals. Some species tolerate with high content of metals by applying one or several approaches including synthesis of peptides and proteins to chelate the metals through their cysteine residues, or by the accumulation of organic acids such as citric or oxalic to convert the metals to the almost non-toxic forms. To minimise the deleterious impact of heavy metals other mechanisms are evident, including biosynthesis of ligands, metallothioneins (MTs), phyto-chelatins (PCs), compartmentation of heavy metals within the vacuoles and up-regulation of an antioxidant system [12].

Extensive microarray analysis showed that more than 1310 genes were expressed in Arabidopsis seedlings when plants were exposed to Pb [13]. This study also revealed that many of these genes were associated with heavy metal-inducible genes and the genes encoding enzymes or proteins involved in the biosynthesis of the plant hormone, signalling molecules and detoxification molecules.

Salicylic acid (SA) is a well-known phytohormone signaling molecule which has different effects on physiological and biochemical activities of plants and exerts all its regulatory functions on the growth and development. Salicylic acid is also involved in various biotic and abiotic stresses signaling [14]. Pretreatment of salicylic acid on rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.) seedlings reduced the toxicity effect of Pb, Hg and Cd [15]. Moreover, pretreatment of salicylic acid enhanced partial protection and alleviated the negative consequence impact of oxidative damage caused by paraquat, heat, cold, water deficit and NaCl [16]. In another study, it was shown that exposure to copper and oxidizing stresses such as hydrogen peroxide [17] and stimulated salicylic acid accumulation in roots and leaves of plants [18]. Furthermore, Freeman [19] reported that salicylic acid is a known

signaling molecule that accumulated in shoot tissue of the hyperaccumulator plant. All these reports suggest an existing link between heavy metal and oxidizing stresses with stress signaling molecules such as salicylic acid [20].

The knowledge about the interaction of Pb and salicylic acid and their effect on oilseed plants in hydroponic conditions is still scarce, hence, the physiological characters and metabolic compounds were determined in *Brassica napus* L. seedlings treated with salicylic acid under Pb toxicity. This is aimed at discovering the potentials of salicylic acid in mitigating Pb toxicity in the plant.

2. MATERIALS AND METHODS

2.1 Plant Growth Condition; growth Parameters and Estimation of Chlorophyll Content

Seeds of canola (*Brassica napus* L.) cultivar Opera was collected from Institute of Agriculture, Lorestan, Iran. The seeds were sterilized with 20% sodium hypochlorite solution and planted in mosharige skooge nutrient medium. The seedlings were transferred into plastic containers with 650 mL of Hogland's nutrient solution. [21]. After 24 hours, treatments started. The seedlings treated at different concentrations of Pb(NO3)2 at (0, 0.25, 0.5, 0.75, 1, 1.5 and 2 mM) and salicylic acid (0, 5 and 10 µM) with three replicates per treatment. The treated plants were transferred to germination under 20°C at 16/8-h (hours) light/dark period. Light was provided with 12 50W fluorescent tubes with humidity of 75%. 10 days after treatment, the plants were removed from the Hogland nutrient solution and roots were washed with distilled water and separated from the shoot. Dry mass of roots and shoots were measured with a digital scale in grams(g), root and stem lengths were measured using the graph paper in millimeters (mm), leaf area was also measured with millimeters graph paper in (mm^2). Chlorophyll a, b and a+b content was determined in fresh leaves according to Arnon et al. [22]. Samples (0.2 g leaves) were homogenized in chilled 80% acetone and centrifuged at 4800 (rounds per minute) rpm for 20 mins. Absorbance of the acetone extracts was measured at 663 and 645 nanometers (nm) by using SPECTRONIC® 20 GENESYS model 4001/4 spectrophotometer. Chlorophyll Contents was expressed as mg/g fresh weight of leaves.

2.2 Determination of Soluble Sugar Content

Soluble and insoluble sugar was determined in the dry root and shoot according to Kochert [23]. Dry root and shoot (0.1 g) were crushed in a mortar and homogenized in 10 ml of 70% ethanol and kept in refrigerator for a week. Ethanolic extract (1 ml) (millilitre) was built to 2ml by adding double distilled water and mixed with 1 ml 5% phenol and 5 ml of 72% sulphuric acid. Total soluble sugar content was determined using a spectrophotometer at 485 nanometers (nm) using SPECTRONIC® 20 GENESYS model 4001/4 spectrophotometer. Calculation of the total soluble sugar content was done by creating a standard curve using a standard glucose and was expressed in mg/g dry weight (mg/g DW).

2.3 Determination of Insoluble Sugar Content

The determination of insoluble sugar content was similar to soluble sugar with some differences. In this method, the ethanolic extract collect for collection and measurement of soluble sugar was mixed with 10 ml (millilitre) of distilled water, then placed in boiling water bath for 15 minutes and the final volume of the extract was built to 25 ml(millilitre) with double distilled water. The extract was taken and the content of soluble and insoluble sugar was calculated by the following formula: C = (OD + 3.985)/ (36.62).

The amount of soluble and insoluble sugar was determined by using glucose standard and expressed as mg/g dry weight.

2.4 Determination of Proline

Proline content was determined following Bates [24]. Leaf tissue 0.5 g' was homogenized with 10 ml (millilitre) of 3% aqueous sulfosalicylic acid. The homogenate was centrifuged at 10,000rounds per minute (rpm) for 10 min, 2 ml of the supernatant was taken and mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin. This mixture was then boiled at 100°C for 1 hours. The developed color was extracted in 4 ml toluene and the absorbance was measured with a spectrophotometer at 520 nanometers (nm). To determine the proline content, a standard curve was made using pure proline. The content of proline was expressed in units of mgg-1 fresh weight (mgg-1 FW).

2.5 Antioxidative Enzyme Assay

Fresh plant materials 1g from leaves and roots were homogenized in 5 millilitre (ml) of antioxidant enzyme extraction buffer, 1.2 g Tris (8-hydroxyquinolinato) aluminum, 0.1 g Ascorbic acid, 17.2 g Sucrose, 0.1 g Chloride acetyl-cysteine and 26.8ml of Hydrochloric acid 0.2N in 100 ml (pH7.5). The extract was then centrifuged at 10000 rounds per minute (rpm) for 30 minutes and the supernatant was collected and kept at 4°C for the enzyme activities assay. The Catalase (CAT) activity was determined following the consumption of H_2O_2 at 530 nanometers (nm) for 1 minute [25]. The reaction mixture contained 2.5 millilitre (ml) potassium phosphate buffer (pH 7.0), 0.3 millilitre (ml) H_2O_2 3% and 0.2 millilitre (ml) of enzyme extract in a 3 millilitre (ml) volume. The enzyme activity was calculated using the extinction coefficient (39.4mM cm^{-1}) and the enzyme activity was reported as following

($OD.gr^{-1}.F.W.min^{-1}$).OD: Optical density, F.W: Fresh weight, min: minutes.

Peroxidase (POD) activity was assayed according to Koroi [26]. 0.1 millilitre (ml) of enzyme extract was homogenized with 2 (millilitre) ml acetate buffer (pH 4.8), 0.2 millilitre (ml) of H_2O_2 3% and 0.1 millilitre (ml) Benzidine. The enzyme activity was measured by following the change in absorption at 530 nanometers (nm) and the enzyme activity was decomposition of ($OD.gr^{-1}.F.W.min^{-1}$). OD: Optical density, F.W: Fresh weight, min: minutes.

2.6 Estimation of Protein Content

The Protein content was extracted from 0.2 g plant materials from the leaves and root. The plant material was ground in 5-10 millilitre (ml) of buffer and was centrifuged at 8000 rounds per minute (rpm) for 40 minutes, the supernatant was decanted and proteins were determined according to Lowry [27]. Amount of protein was measured at 750 nanometers by using bovine serum albumin as the standard protein. Protein content was expressed az $mg.g^{-1}$.D.W. D.W: Dry Weight.

2.7 Statistical Analysis

Various experiments in completely randomized factorial design with three replicates were conducted and the presented data included means of three separate experiments ±SD. Statistical analysis was performed by analysis of variance (ANOVA) using the SAS software and correlative analysis used SPSS software (SPSS 13).

3. RESULTS AND DISCUSSION

3.1 Growth Parameters

The analysis of variance indicated a significant effect of lead and salicylic acid ($p<0.01$) and their interaction ($p<0.01$, $p<0.05$) on growth parameters of 10 days *Brassica napus* L. (Opera) seedlings when grown in hydroponic conditions (Fig. 1) (Table 1). The data presented in Table 2 showed that increase in the concentration of lead caused a significant reduction in shoot and root lengths, shoot and root dry weight as well as leaf area of the plants. Conversely, increasing of salicylic acid concentration has a positive significant impact on the value of growth parameters compared to the control (Table 2). There was no significant difference between the effect of salicylic acid at 5 and 10 µm on the root length, shoot length and shoot dry weight compared to the control (Table 2). As reported in Fig. 2, the interaction effect of pb and salicylic acid (SA) on growth parameters showed that root length (A), root dry weight (B), shoot length (C) and shoot dry weight (D) followed a similar pattern of reduction under Pb stress and the application of salicylic acid reduced the adverse effect of Pb in all treatments. The beneficial effect of salicylic acid (SA) was less pronounced on shoot dry weight in exposure to different Pb concentrations (Fig. 2 D). The interaction effect of Pb and salicylic acid on leaf area also revealed that by increasing the concentration of pb, leaf area decreased drastically and salicylic acid treatment alleviated the negative effect of Pb about 10% to 35% in 10 days old rassica napus seedlings (Fig. 3). The valuable effect of SA to reduce toxic effect of Pb was observed in all growth parameters and was shown to be statistically significant. In numerous related studies on a range of terrestrial plants, single or joint effects of heavy metals on plant system were investigated and the results showed that sensitive plants developed visible symptom of phytotoxicity due to high concentrations of heavy metal [28,29]. The growth performance, physiological characters and biochemical processes of plants were affected due to heavy metal exposure [30,31,8]. There is very much study about the plant growth regulators [32,33, 34,35]. Similar studies can be done with the other plant hormones. Excess levels of Pb were

typically demonstrated by a drastic change in the physiological characters following by seed germination and growth rate [36]. Pre-treatment of barley and soybean with salicylic acid revealed the beneficial effect of salicylic acid (SA) on modification of toxicity of cadmium on the growth parameter and an increased level of tolerance toward high cadmium (Cd) concentrations was evident [37,38]. (Fig. 2)

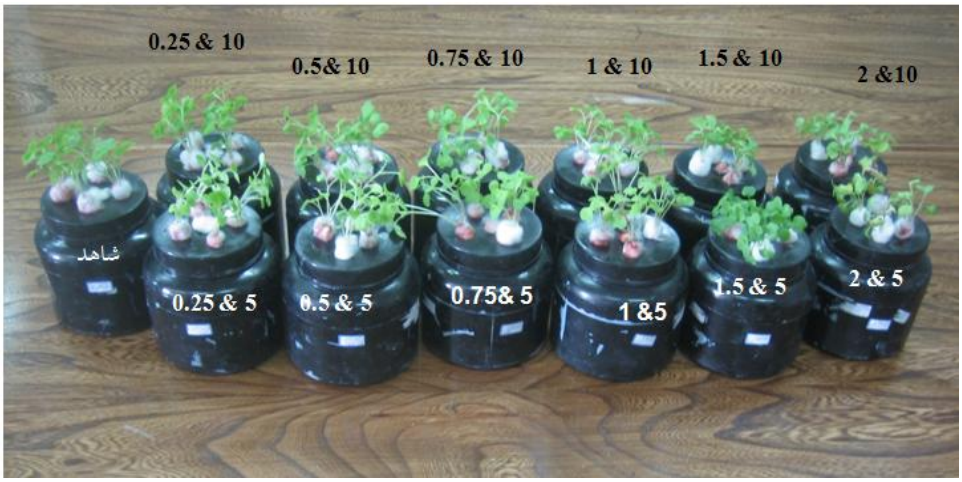

Fig. 1. The interaction effects of Pb and SA in hydroponic conditions in 10 days old rassica napus seedlings (Pb with different concentrations 0, 0.25,05,0.75.1,1.5 & 2 mM) and salicylic acid (0, 5 and 10 µM)

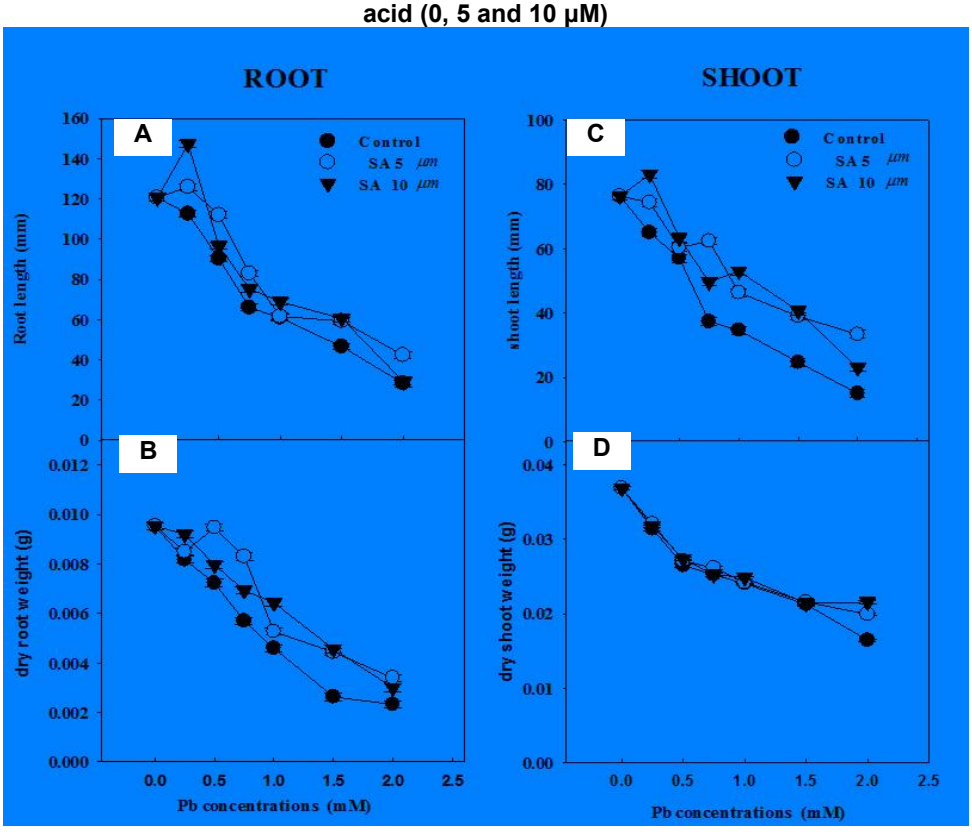

Fig. 2. The interaction effects of Pb and SA on root length (mm) (A), root dry weight (g) (B), shoot length (mm) (C) and shoot dry weight (g) (D)

Table 1. ANOVA analysis for root length, shoot length, leaf area, dry root and shoot weight of 10 days *Brassica napus* L. seedlings under lead and salicylic acid treatments

		Mean square				
Source of variation	**df**	**Root length (mm)**	**Shoot length (mm)**	**Leaf area (mm^2)**	**Dry root weight (g)**	**Dry shoot weight (g)**
Repeat	2	23.1111 ns	13.1905 ns	38.6229*	6.30159×10^{-8} ns	5.92063×10^{-8} ns
Pb	6	11148.0 **	3450.62 **	10012.8**	0.0000566885 **	0.000320188 **
Salicylic acid	2	836.245 **	926.714 **	1262.54**	0.00000946778**	0.00000239302**
Interaction Pb*SA	12	182.013 **	93.1958 **	200.23**	0 .00000121352**	5.44312×10^{-7} *
Total error	24	8.08466	4.33466	10.7218	6.41138×10^{-8}	1.16971×10^{-7}

*** and * significant at the 5% and 1% probability levels and ns not significant effect*

Table 2. Means comparison of lead levels and SA treatment on root length and shoot length, leaf area, root and shoot dry weight of 10 days *Brassica napus* L seedling

Experimental factor	**Root length (mm)**	**Shoot length (mm)**	**Leaf area (mm^2)**	**Root dry weight (g)**	**Shoot dry weight (g)**
Pb concentrations					
0 (mM)	128.667 a	76.3333 a	138.933 a	0.00953333 a	0.0368333a
0.25(mM)	120.667 b	74.1111b	131.978 b	0.00862222 b	0.0317111b
0.5(mM)	99.6667 c	60.2222 c	126.0 c	0.00822222 c	0.0269667c
0.75(mM)	74.7778 d	49.7778 d	106.556 d	0.00697778 d	0.0255444 d
1(mM)	63.8889 e	44.6667 e	102.667 e	0.00543333 e	0.0243778f
1.5(mM)	55.5556 f	34.7778 f	70 f	0.00387778 f	0.0214222e
2(mM)	33.3333 g	23.7778 g	49 g	0.00291111 g	0.0195556 g
SA concentrations					
0(µM)	75.0952 c	44.2857c	94.7905 c	0.00574286 c	0.0262429 c
5(µM)	86.4286 a	56 a	109.419 a	0.00698571 a	0.0267857 ab
10(µM)	85.5714 ab	55.5714ab	106.562 b	0.00680476 b	0.0268619 a

All means followed by the same letter in column are not significantly different at the 5% probability level

3.2 Effect of Pb and Salicylic Acid (SA) on Soluble and Insoluble Sugars

Two-way ANOVA showed a significant effect of Pb and salicylic acid (SA) ($p<0.01$) and their interaction ($p<0.01$) on the content of soluble and insoluble sugars in different parts of plants the shoot and root (Table 3). Table 4 showed that an increase in the concentration of lead caused a significant increase in shoot and root soluble sugars. Conversely, increasing of salicylic acid levels had negative significant impact on the value of shoot and root soluble sugars compared to that of the control (Table 4). No significant difference was evident between the effect of salicylic acid at 5 and 10 µm on the reduction of soluble sugars in shoot and root under lead stress (Table4). The pattern of accumulation of soluble sugars was similar in shoot and root of those plants exposed to Pb and salicylic acid (SA) treatment but in the case of salicylic acid 5 and 10 treatments the level of soluble sugars was lower than the control over the period of measurement (Fig. 4 A and B). The soluble sugar than that of the root by the salicylic acid content in the shoot was more affected in plants when grown under Pb stress. According to Fig. 4 (C and D), as Pb concentration increased, insoluble sugars content reduced significantly and salicylic acid treatment statistically limited the reduction of insoluble sugars in both shoot and root of plants in about 20%-40% (Fig. 4 C and D). These results showed that salicylic acid treatment exerted a certain effect on the reduction of soluble sugars and increase of insoluble sugars in 10 days old rassica napus' seedlings exposed to different levels of Pb. The reduction of insoluble sugars was in an opposite trend with increasing of soluble sugar concentration in plants exposed to Pb. This means that an increase in soluble sugars content was accompanied by a sharp decreased in levels of insoluble sugars. The metabolism of starch is sensitive to changes to the environmental conditions and stress generally leads to the degradation of starch level toward the

accumulation of soluble sugars in leaves [39,40] which then formed osmolytes to maintain cell growth and increase the adaptation and ability of plants from stress damage [41]. The findings indicate that high concentration of Pb unfavorably changed change the content of soluble and insoluble sugars in *Brassica napus* L. seedlings and salicylic acid treatment reduced the adverse effect of heavy metal stress and helped stressed-plants to keep their homeostasis balance and promote plant growth regulation.

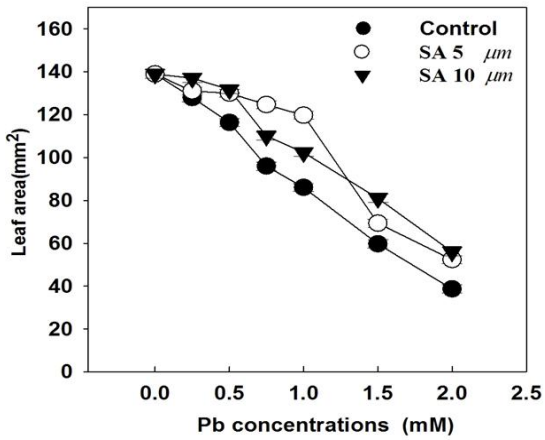

Fig. 3.The interaction effects of Pb and SA on Leaf area (mm^2)

3.3 Effect of Pb and Salicylic Acid (SA) on Leaf Total Chlorophyll

Total leaf chlorophyll (a+b) contents of 10 days old rassica napus' seedlings were significantly affected by lead ($p<0.01$) and salicylic acid (SA) ($p<0.05$) levels (Table 3). The interaction effect of Pb and salicylic acid (SA) was not significant on the content of total chlorophyll (Table 3). Total chlorophyll (a+b) contents gradually decreased with increasing Pb concentration (Table 4). In contrast, the content of total chlorophyll significantly increased with rising salicylic acid concentration in 10 days old rassica napus' seedlings (Table 4). Burzyński [36] reported that Pb and Cd damaged chlorophyll synthesis due to restriction in uptake and transport of essential elements such as Mg and Fe by plants. Numerous studies have also indicated that heavy metals induce inhibition of chlorophyll biosynthesis and a decline in the photosynthetic rate and an increase in the rate of chlorophyll. degradation [42]. The results of this study showed that Pb application damaged the chlorophyll synthesis and applications of salicylic acid avoided cumulative damage development in response to heavy metal.

3.4 Effect of Pb and Salicylic Acid (SA) on Root and Shoot Proline and Total Protein

Total protein and proline levels in shoot and root of 10 days old rassica napus seedlings were significantly afected by lead and salicylic acid ($p<0.05$) (Table 5). There was no significant interaction between Pb and salicylic acid (SA) on the content of proline in shoot and root (Table 5). This was the case for the content of protein in shoot, but a significant interaction ($p<0.05$) was evident between Pb and salicylic acid (SA) on the levels of protein in root (Table 5). Results also showed that increase in the concentration of Pb had a positive significant effect on synthesis and accumulation of protein and proline in root and shoot (Table 6). The results revealed that salicylic acid had a significant negative effect on the production of protein and proline and as the concentration of salicylic acid increased, the level of protein and proline were reduced (Table 6). A similar result was reported by Zhiqiang [8]; Kovacs [43] when the content of protein and proline was evaluated in seedlings of four Chinese cabbage cultivars treated with Pb and Cd. They showed that the content of proline and protein increased as Pb and Cd concentrations increased but they decreased significantly at concentrations above 18 mg/L. Numerous studies have confirmed that proline accumulated in many plant species in response to different abiotic stresses such as drought, high salinity, heavy metals. These studies considered proline as an osmolyte, to scavenge Reactive oxygen species (ROS) and binds to heavy metal to reduce their toxicity leading to protect cells from damage caused by stress [44,45].

3.5 Effect of Pb and Salicylic Acid on Peroxidase and Catalase

The activities of antioxidant enzymes including peroxidase and catalase were significantly affected by lead and salicylic acid in shoot and root of 10 days old rassica napus seedlings (Table 7). As shown in Table 8, antioxidant activity increased significantly as Pb concentration increased. The enzymes activities were negatively affected by salicylic acid and were reduced significantly with increasing salicylic acid concentration. The interaction effect of lead and salicylic acid was significant ($p<0.01$) on the activities of peroxidase in both root and

shoot whereas it was significant for catalase activity in shoot but not in root (Table 7). The results of from this study showed that peroxidase activity was accelerated in response to Pb treatment in both shoot and root whereas, salicylic acid treatment reduced the activity of the enzyme significantly over the period of the experiment (Fig. 5 A and B).

Catalase activity was a similar pattern as peroxidase in response to Pb and salicylic acid (SA) (Fig. 5 C and D). The results showed that increasing catalase activity was significantly affected by Pb and the different concentration of Pb gradually increased the enzyme activity (Fig. C). From the Fig. 5, it can be apparently seen that 10 days *Brassica napus* L. seedling exhibited the same trends in aspect of antioxidant activity changes under different treatment parameters. The data obtained from this study is in sharp contrast with what was reported by Song [9] when different genotypes of the wheat were exposed to varied concentrations of Pb and the activity of catalase reduced significantly. Typically, an antioxidant enzyme such as catalase is up-regulated in existing of Reactive oxygen species (ROS) leading to protect the cell from H_2O_2. The roles of salicylic acid in the transient production of Reactive oxygen species (ROS) are still a matter of debate. Several studies have reported different results the effect of salicylic acid on the activity of catalyse. Sanchez-Casas and Klessig [46] reported that salicylic acid did not inhibit catalase activities. Chan and Tian [47] reported that salicylic acid with the concentration of 0.5mM significantly inhibited catalase activity in sweet cherry fruit. Hayat [14], Ananieva [16] indicated that salicylic acid could enhance catalase activity in leaves of plants exposed to paraquat. Xu and Tian [48] also reported that salicylic acid at 2mM concentration increased the activity of catalyse and glutathione reroxidase in sweet cherry fruit and concluded that salicylic acid activated antioxidant enzymes leading to increasing in plant tolerance against pathogen. It was found that salicylic acid at 5 and 10 µM concentrations have a significant effect in reducing levels of catalase activity in 10 days old rassica napus seedlings in response to Pb stress. Results provide evidence that salicylic acid is a signaling molecule with a complex biochemical property which may have a direct regulatory effect on the antioxidant activity.

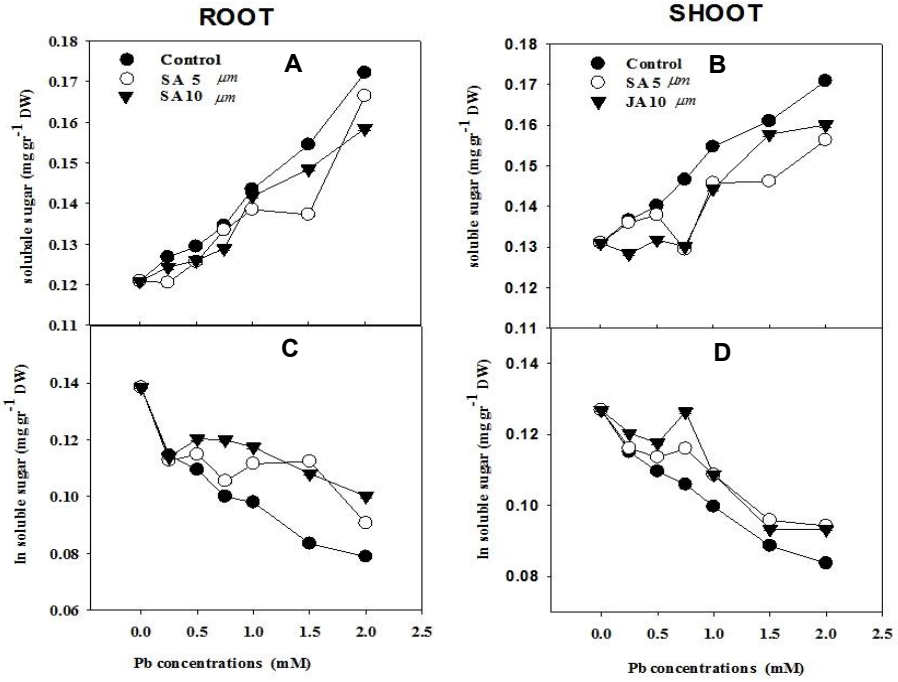

Fig. 4. The interaction effects of Pb and SA on soluble sugars content on root (mg. gr^{-1}DW) (A) and in shoot (mg. gr^{-1}DW) (B) and insoluble sugars content in root (mg. gr^{-1}DW) (C) and in shoot (mg. gr^{-1}DW) (D)

Table 3. ANOVA analysis for total clorophyll a+b, Soluble sugar of shoot and root, insoluble sugar of root and shoot of 10 days *Brassica napus* L seedling under lead and salicylic acid treatments

		Mean square				
Source of variation	**Df**	**Total clorophyll a+b ($mg.F^{-1}.w$)**	**Soluble sugar of shoot ($mg.gr^{-1}.D.w$)**	**Soluble sugar of root ($mg.gr^{-1}.D.w$)**	**Insoluble sugar of root ($mg.gr^{-1}.D.w$)**	**Insoluble sugar of shoot ($mg.gr^{-1}.D.w$)**
Repeat	2	3.48571×10^{-7} ns	0.00000161762 ns	0.00000169968 ns	0.00000163159 ns	0.00000463762*
Pb	6	0.000100159 **	0.00131843 **	0.00207041 **	0.00198374 **	0.00175542 **
Salicylic acid	2	0.00000152333 *	0.000479818 **	0.0000778997**	0.00104749 **	0.000311067**
Interaction Pb*SA	12	9.37037×10^{-8} ns	0.0000620643**	0.0000657689**	0.000154493**	0.0000458647 **
Total error	24	1.54299×10^{-7}	8.8496×10^{-7}	7.89497×10^{-7}	8.80238×10^{-7}	5.40291×10^{-7}

*** and * significant at the 5 and 1% probability levels and ns not significant effect*

Table 4. Means comparison of lead levels and SA treatment on total clorophyll a+b, soluble sugar of root and shoot, insoluble sugar of root and shoot of 10 days *Brassica napus* L seedlings

Experimental factor	**Total clorophyll a+b ($mg.F^{-1}.w$)**	**Soluble sugar of shoot ($mg.gr^{-1}.D.w$)**	**Soluble sugar of root ($mg.gr^{-1}.D.w$)**	**Insoluble sugar of root ($mg.gr^{-1}.D.w$)**	**Insoluble sugar of shoot ($mg.gr^{-1}.D.w$)**
Pb concentrations					
0 (mM)	0.0104667 a	0.131 g	0.120933 g	0.138467 a	0.1268 a
0.25 (mM)	0.00974444b	0.133644 f	0.126689 ef	0.114856 b	0.117144 b
0.5(mM)	0.00644444 c	0.135344 e	0.127544 e	0.113756 c	0.116044 c
0.75(mM)	0.00482222 d	0.136578 d	0.132289 d	0.113044 cd	0.113533 d
1 (mM)	0.00343333 e	0.148256 c	0.141289 c	0.108967 e	0.105644 e
1.5(mM)	0.00307778 ef	0.154956 b	0.146089 b	0.101322 f	0.0906889 f
2(mM)	0.00191111 g	0.162422 a	0.165344 a	0.0899 g	0.0897111 g
SA concentration					
0(µM)	0.00542381 c	0.14869 a	0.13939 a	0.104033 c	0.104171 c
5(µM)	0.00571429 ab	0.140352 bc	0.135981 bc	0.1123 b	0.109843 b
10(µM)	0.0059619 a	0.140471 b	0.136133 b	0.118086 a	0.111514 a

All means followed by the same letter in column are not significantly different at the 5% probability level

Table 5. ANOVA analysis for proline of root and shoot, total protein of root and shoot of 10 days *Brassica napus* L seedlings under lead and salicylic acid treatments

		Mean square			
Source of variance	**Df**	**Proline shoot ($mg.gr^{-1}$.F.w)**	**Proline root ($mg.gr^{-1}$.F.w)**	**Total protein shoot ($mg.gr^{-1}$.D.w)**	**Total protein root ($mg.gr^{-1}$.D.w)**
Repeat	2	0.0000163968 ns	0.00000442857 ns	0.00000525397 ns	0.00000996825 ns
Pb	6	0.0127911 **	0.0130718 **	0.00572725 **	0.00618099 **
Salicylic acid	2	0.000106778 *	0.0000489048 *	0.0000703016 *	0.0000973492 *
Interaction Pb*SA	12	0.0000226852 ns	0.00000746032 ns	0.00000924603 ns	0.0000237566*
Total erorr	24	0.0000106534	0.0000133532	0.00000675794	0.0000088955

*** and * significant at the 5 and 1% probability levels and ns not significant effect*

Table 6. Means comparison of lead levels and SA tretment on Proline shoot and root, total protein shoot and root of 10 days old seedling of *Brassica napus* L

Experimental factor	**Proline shoot ($mg.gr^{-1}$.F.w)**	**Proline root ($mg.gr^{-1}$.F.w)**	**Total protein shoot ($mg.gr^{-1}$.D.w)**	**Total protein root ($mg.gr^{-1}$.D.w)**
Pb concentrations				
0 (mM)	0.0236667 g	0.0326667 g	0.0328889 fg	0.0342222 fg
0.25 (mM)	0.0382222 f	0.0453333 f	0.033 f	0.0353333 f
0.5 (mM)	0.0647778 e	0.0728889 e	0.046 e	0.0487778 e
0.75 (mM)	0.0742222 d	0.0892222 d	0.0671111 d	0.0656667 d
1 (mM)	0.102556 c	0.108333 c	0.0807778 c	0.0814444 c
1.5 (mM)	0.114333 b	0.125 b	0.0864444 b	0.0887778 b
2 (mM)	0.121444 a	0.130889 a	0.092 a	0.0995556 a
SA concentration				
0 (µM)	0.079 a	0.0879048 a	0.0647143 a	0.0672857 a
5 (µM)	0.0775238 ab	0.0862381 ab	0.0614762 bc	0.0639048 b
10 (µM)	0.0745714 c	0.0848571 bc	0.061619 b	0.0632857 bc

All means followed by the same letter in colum are not significantly different at the 5% probability level

Table 7. ANOVA analysis for peroxidase activity of root and shoot, catalase activity of root and shoot of 10 days old seedling of *Brassica napus* L. under lead and salicylic acid treatment

		Mean square			
Source of variance	**Df**	**Peroxidase activity of root (OD. gr^{-1}.F.W.min^{-1})**	**Peroxidase activity of shoot (OD.gr^{-1}.F.W.min^{-1})**	**Catalase activity of root (OD.gr^{-1} .F.W.min^{-1})**	**Catalase activity of shoot (OD.gr^{-1} .F.W.min^{-1})**
Repeat	2	0.0000389048 ns	0.0000652063 ns	0.0000203333 ns	0.00000315873 ns
Pb	6	0.0805879 **	0.120985 **	0.00599344 **	0.0140997 **
Salicylic acid	2	0.00741462**	0.00250221**	0.000036619 *	0.000843921 **
Interaction Pb*SA	12	0.000721638 **	0.00106471 **	0.00000641534 ns	0.000504754 **
Total error	24	0.0000727566	0.0000590635	0.00000861772	0.00000694841

*** and * significant at the 5 and 1% probability levels and ns not significant effect*

Table 8. Means comparison of lead levels and SA treatment on Peroxida activity of root and shoot, catalase activity of root and shoot in 10 days *Brassica napus* L. seedling

Experimental factor	**Peroxidase activity of root (OD. gr^{-1}.F.W.min^{-1})**	**Peroxidase activity of shoot (OD. gr^{-1}.F.W.min^{-1})**	**Catalase activity of root (OD.gr^{-1} .F.W.min^{-1})**	**Catalase activity of shoot (OD.gr^{-1} .F.W.min^{-1})**
Pb concentrations				
0 (mM)	0.418333 g	0.368667 g	0.00533333 g	0.0153333 g
0.25 (mM)	0.437667 f	0.407778 f	0.00866667 f	0.0283333 f
0.5 (mM)	0.479556 e	0.460556 e	0.0165556 e	0.0423333 e
0.75 (mM)	0.533667 d	0.513333 d	0.0321111 d	0.0686667 d
1(mM)	0.561111 c	0.563333 c	0.0444444 c	0.0716667 c
1.5 (mM)	0.634778 b	0.622778 b	0.053 b	0.0891111 b
2 (mM)	0.665222 a	0.690778 a	0.0758889 a	0.131667 a
SA concentrations				
0 (µM)	0.549714 a	0.530286 a	0.0352381 a	0.0711905 a
5 (µM)	0.536381 b	0.509095 c	0.0329048 bc	0.060381 b
10 (µM)	0.512619 c	0.515143 b	0.033 b	0.0600476 bc

All means followed by the same letter in colum are not significantly different at the 5% probability level

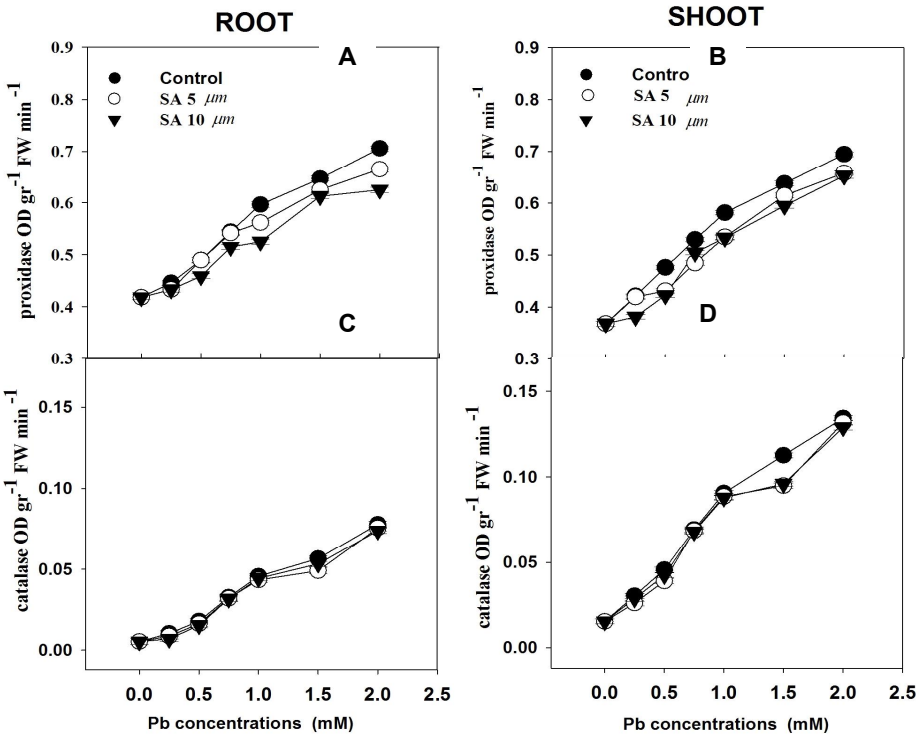

Fig. 5. The interaction effects of Pb and SA on proxidase activity in root (OD gr $^{-1}$ FW min^{-1}) (A) and in shoot (OD gr $^{-1}$ FW min^{-1}) (B) and Catalse activity in root (OD gr $^{-1}$ FW min^{-1}) (C) and in shoot (OD gr $^{-1}$ FW min^{-1}) (D)

4. CONCLUSION REMARKS

Pb caused a toxic condition for plant growth and affected the physiological and biochemical activities of 10 days oil seeds plant, *Brassica napus* L. seedlings. The significant negative effect of Pb was observed growth, metabolite synthesis and antioxidant activities. This investigation provides evidence that application of exogenous salicylic acid could reduce the toxicity effect of heavy metal stress in 10 days old rassica napus seedlings. The application of salicylic acid caused improvement in activities of antioxidant enzymes, reduction of soluble sugar content and increased the level of insoluble sugars in lead-stressed plants. Salicylic acid treatment increased chlorophyll content and improved the growth rate of the plant. The protein content and proline levels were reduced by exogenous salicylic acid. Salicylic acid alleviated Pb toxicity which may be assumed that salicylic acid exerts its favourable effect through some mechanisms that inactivate Pb stress, possibly through phytochelatins or by activating Pb relocation into vacuoles or by promoting growth rate. This study may suggest that plant hormones stabilize intracellular redox homeostasis and exert their growth-promoting effects through inhibition of Reactive oxygen species (ROS) accumulation and detoxifying oxidative agents on heavy metals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sharma P, Dubey RS. Lead toxicity in plants. Brazilian Journal of Plant Physiology. 2005;17:35–52.
2. Kaur G, Singh HP, Batish DR, Kohli RK. Lead (Pb) induced biochemical and ultrastructural changes in Wheat (*Triticum aestivum*) roots. Protoplasma. 2013; 250(1):53- 62.
3. Alkhatib R, Maruthavanan J, Ghoshroy S, Steiner R, Sterliny T, Creamer R. Physiological and ultrastructural effects of Lead on tobacco. Biologia Plantarum. 2011;56(4):711-716.

4. Gopal R, Rizvi AH. Excess lead alters growth, metabolism and translocation of certain nutrients in radish. Chemosphere. 2008;9(70):1539-1544.
5. Dogan M, Saygideger SD, Colak U. Effect of lead toxicity on aquatic macrophyte *Elodea canadensis* michx. Bulletin of Environmental Contamination and Toxicology. 2009;83:249–254.
6. Singh I, Shah K. Evidences for suppression of Cadmium induced oxidative stress in presence of sulphosalicylic acid in rice seedlings. Plant Growth Regulation. 2015;76:99-110.
7. Ling Q, Hong FS. Effects of Pb^{2+}on the structure and function of photosystem II of *Spirodela polyrrhiza*. Biological Trace Element Research. 2009;129:251–260.
8. Zhiqiang X, Qixing Z, Weitao L. Joint effects of cadmium and lead on seedlings of four Chinese cabbage cultivars in northeastern China. Journal of Environmental Sciences. 2009;21:1598–1606.
9. Song W, Zheng A, Shao H, Chu L, Brestic M, Zhang Z. The alleviative effect of salicylic acid on the physiological indices of the seedling leaves in six different wheat genotypes under lead stress. POJ. 2012; 5(5):486-493.
10. Wu X, Hong FS, Liu C, Su MY, Zheng L, Gao FQ, Yang F. Effects of Pb^{2+}on energy distribution and photochemical activity of spinach chloroplast**.** Spectrochimica Acta Part A: Molecular and Biomolecular. 2008; 69:738–742.
11. Manara A. Plant responses to heavy metal toxicity. In plants and heavy metals. A. Furini (ed). Springer Briefs in Biometals. 2012;27-53.
12. Sharma S, Dietz KJ. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. Journal of Experimental Botany. 2006;57:711–726.
13. Liu T, Liu S, Guan H, Ma L, Chen Z, Gu H, Qu LJ. Transcriptional profiling of Arabidopsis seedlings in response to heavy metal lead (Pb). Environmental and Experimental Botany. 2009;67:377–386.
14. Hayat Q, Hayata S, Irfan M, Ahmad A. Effect of exogenous salicylic acid under changing environment: A Review Environmental and Experimental Botany. 2010;68: 14–25.
15. Rivas-San Vicente M, Plasencia J. Salicylic acid beyond defence its role in plant growth and development. Journal of Experimental Botany. 2011;62(10):3321-3338.
16. Ananieva EA, Christov KN, Popova LP. Exogenous treatment with salicylic acid leads to increased antioxidant capacity in leaves of barley plants exposed to paraquat. Journal of Plant Physiology. 2004;161:319–28.
17. Mostofa MG, Fujita M. Salicylic acid alleviates copper toxicity in rice (*Oryza sativa* L.) seedlings by Up- regulating antioxidaitive and glyoxalase systems. Ecotoxicology. 2013;22(6):959-973.
18. Maksymiec W. Signaling responses in plants to heavy metal stress. Acta Physiologiae Plantarum. 2007;29:177-187.
19. Freeman JL, Garcia D, Kim D, Hopf A, Salt DE. Constitutively elevated salicylic acid signals glutathione-mediated nickel tolerance in Thlaspi nickel hyperaccumulators. Plant Physiology. 2005;137: 1082–1091.
20. Tao S, Sun L, Ma C, Li L, Li G, Hao L. Reducing basal salicylic acid enhances Arabidopsis tolerance to Lead or Cadmium. Plant Soil. 2013;372:309-318.
21. Hogland DR, Arnon DI. The water – culture for growing plants without soil. University of California Agei Experiment Station. 1957;347.
22. Arnon DI. Copper enzymes in isolated chloroplasts, polyphenol oxidases in *Beta vulgaris* L. Plant Physiology. 1957;24: 115.
23. Kochert G. Carbohydrate determination by the phenol sulfuric acid method, In Helebust JA, Craig JS (ed). Handbook physiological methods. Cambridge University. 1978;96-97.
24. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies. Plant Soil. 1973;39:205-207.
25. Chance B, Maehly C. Assay of catalase and peroxidase, Method Enzymol. 1995; 11:764-775.
26. Koroi SAA. Gelektrophers tissue and spectral photometrischon under change zomeinfluss tempetature and structure peroxidase isoenzyme. Chemistry of

Vegetable Physiology and agriculture. 1989;20:15-22.

27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. Biological Chemistry. 1951;193:256-257.
28. Malec P, Maleva MG, Prasad MNV, Strzałka K. Identification and characterization of Cd-induced peptides in Egeria densa (water weed): Putative role in Cd detoxification. Aquatic Toxicology. 2009;95: 213–221.
29. Wan G, Najeeb U, Jilani G, Naeem MS, Zhou W. Calcium invigorates the cadmium-stressed *Brassica napus* L. plants by strengthening their photosynthetic system. Environmental Science and Pollution Research. 2011;18:1478–86.
30. Shu X, Yin L, Zhang Q, Wang W. Effect of Pb toxicity on leaf growth, antioxidant enzyme activities, and photosynthesis in cuttings and seedlings of *Jatropha curcas* L. Environmental Science and Pollution Research. 2012;19:893–902.
31. Yusuf M, Fariduddin Q, Varshney P, Ahmad A. Salicylic acid minimizes nickel and/or salinity-induced toxicity in Indian mustard (*Brassica juncea*) through an improved antioxidant system. Environmental Science and Pollution Research. 2012;19:8–18.
32. Guney K, Cetin M, Sevik H, Guney KB. Influence of germination percentage and morphological properties of some hormones practice on *Lilium martagon* L. seeds. Oxidation Communications. 2016a; 39(1-II):466-474.
33. Guney K, Cetin M, Sevik H, Guney KB. Effects of some hormone applications on germination and morphological characters of endangered plant species *Lilium artvinense* L. seeds, new challenges in seed biology- basic and translational research driving seed technology. Dr. Susana Araujo (Ed.), In Tech. 2016b;4:97-112.
34. Sevik H, Cetin M. Effects of some hormone applications on germination and morphological characters of endangered plant species *Lilium artvinense* L. Onion scales. Bulgarian Chemical Communi-cations. 2016;48(2): 256-260.
35. Topacoglu O, Sevik H, Guney K, Unal C, Akkuzu E, Sivacioglu A. Effect of rooting hormones on the rooting capability of *Ficus benjamina* L. cuttings. Sumarski List. 2016a;140(1-2):39- 44.
36. Burzyński M, Grabowski A. Influence of lead on nitrate uptake and reduction in cucumber seedlings. Acta Societatis Botanicorum Poloniae. 1984;53:77–86.
37. Szalai G, Krantev A, Yordanova R, Popova LP, Janda T. Influence of salicylic acid on phytochelatin synthesis in *Zea mays* during Cd Stress. Turkish Journal of Botany. 2013;37:708-714.
38. Drazic G, Mihailovic N. Modification of cadmium toxicity in soybean seedlings by salicylic acid. Plant Science. 2005;168: 511–517.
39. Basu PS, Ali M, Chaturvedi SK. Osmotic adjustment increases water uptake, remobilization of assimilates and maintains photosynthesis in chickpea under drought. Indian Journal of Experimental Biology. 2007;45:261–67.
40. Kempa S, Krasensky J, Dal Santo S, Kopka J, Jonak C. A central role of abscisic acid in stress-regulated carbohydrate metabolism. PLoS One. 2008;3: e3935.
41. Krasensky J, Jonak C. Drought salt, and temperature stress-induced metabolic rearrangements and regulatory networks. Journal of Experimental Botany. 2012;63: 1593–1608.
42. López-Millán AF, Sagardoy R, Solanas M, Abadı´a A, Abadı´a J. Cadmium toxicity in tomato (*Lycopersicon esculentum* L.) plants grown in hydroponics. Environmental and Experimental Botany. 2009;65: 376–385.
43. Kovacs V, Gondor Ok, Szalai G, Darko E, Majlath I, Janda T, et al. Synthesis and role of salicylic acid in wheat varieties with different levels of *Cadmium tolerance*. Journal of Hazardous Materials. 2014a; 280:12-19.
44. Verbruggen N, Hermans C. Proline accumulation in plants: A review amino acids. 2008;35:753–759.
45. Szabados L, Savoure A. Proline: A multifunctional amino acid. Trends Plant Sciences. 2010;15:89–97.
46. Sánchez-Casas P, Klessig DF. A salicylic acid-binding activity and a salicylic acid-inhibitable catalase activity are present in a

variety of plant species. Plant Physiology. 1994;106: 1675–1679.

47. Chan Zh, Tian Sh. Induction of H_2O_2-metabolizing enzymes and total protein synthesis by antagonistic yeast and salicylic acid in harvested sweet cherry fruit. Postharvest Biology and Technology. 2006;39:314–20.

48. Xu X, Tian S. Salicylic acid alleviated pathogen-induced oxidative stress in harvested sweet cherry fruit. Postharvest Biology and Technology. 2008;49:379-385.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/31832