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Hormopriming instigates defense mechanisms in Thyme (*Thymus vulgaris* L.) seeds under cadmium stress

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ABSTRACT

Seed germination and root development are vital phases of the plant growth process; however, these steps are strongly influenced by the seed characteristics and environmental conditions. Seed priming provides a condition for uniform and rapid germination and plant establishment. Limited studies are available on the effect of seed priming to ameliorate cadmium (Cd) stress in thyme plants. Hence, the effect of seed hormopriming (salicylic acid and jasmonic acid, 100 mg.l^{-1}) was evaluated. Hydropriming was done using double distilled water. Seed priming significantly increased the seed germination percentage (GP) and germination rate (GR) at all stress levels of Cd contamination. SA-primed seed showed the greatest increase in GP and GR at 30 mg.l^{-1} Cd by 26 and 37 %, respectively, compared with the control. Salicylic acid (SA) increased plumule and radicle length 29 and 43 %, respectively, at 30 mg.l^{-1} of Cd concentration compared with the control seeds. SA pretreatment increased plumule dry weight more than radicle dry weight. The leaf antioxidant enzyme activity was increased with increasing Cd concentration. Seed priming with SA increased superoxide dismutase (SOD) and peroxidase (POX) activity by 14 and 15 %, respectively. Leaf malondialdehyde (MDA) content was increased 15 % at 30 mg.l^{-1} Cd concentration compared with the control plants, but SA priming reduced leaf MDA content 18 % compared with the control seeds. The greatest leaf proline content was observed at 30 mg.l^{-1} Cd concentration and SA priming. Regardless of the priming treatment, the greatest leaf protein content was observed at 10 mg.l^{-1} Cd concentration compared with the control. Seed priming with SA diminished the adverse effects of Cd on germination process *via* enhancing the antioxidant activities and accumulation of osmolytes. Therefore, hormopriming of thyme seeds by SA can enhance the resistance of this valuable medicinal herb to cadmium stress.

1. Introduction

The role of medicinal plants in medical remediation has been proved worldwide. In addition to their use as traditional medicine, medicinal plants are also consumed as spices and flavors in food processing. Thyme (*Thymus vulgaris* L.) belongs to the mint family (*Lamiaceae*) and consists of more than 250 species and subspecies (Yadegari, 2018a). It is an aromatic perennial evergreen herb, which has several biologically active compounds including essential oils, chemical compounds, and secondary metabolites. Thyme is one of the most consumed and valuable medicinal plants in the world. It has a wide range of industrial, agricultural, and commercial consumption as fresh and dried herbs, oleoresins, landscape design, and biological control. Thyme extracts are used as antibacterial, antiseptic, and spasmolytic agent (Elhabazi et al., 2008; Yadegari, 2018a; Yadegari and Shakerian, 2014).

In an environment, plants may expose to different abiotic and biotic stresses, *e.g.* drought, salinity, heat, chilling, pathogens, pests, heavy metals, and environmental pollutants. These environmental constraints reduce crop yield and affect economic stability (Miller et al., 2008; Seki et al., 2007). The presence of different types of organic and mineral pollutants in the soil is one of the challenges that humans face. Among these pollutants; heavy metals, which in addition to creating inappro-

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Abbreviations: CAT, catalase; Cd, cadmium; EL, electrolyte leakage; GP, germination percentage; GR, germination rate; GRI, germination rate index; HP, hydropriming; JA, jasmonic acid; MDA, malondialdehyde; NP, non-primed (control); POX, peroxidase; PDW, plumule dry weight; RDW, root dry weight; RL, root length; R/S, root to shoot ratio; SA, salicylic acid; SL, shoot length; SOD, superoxide dismutase; ROS, reactive oxygen species; SVI, seed vigor index.

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priate soil conditions and making it inefficient for agricultural activities, kill microorganisms living in the soil (Clemente et al., 2005).

According to their hazard degree, cadmium (Cd) ranked as the fourth highly hazardous heavy metal elements after selenium (Se), titanium (Ti), and Antimony (Sb) in soils (Vodyanitskii, 2016; Yadegari, 2018b). High levels of cadmium (Cd) have been observed in many agricultural lands due to the long-term use of phosphate fertilizers, industrial and urban activities, and municipal wastewater. Cd is not essential for plant growth, although it is available in the environment. Plants have evolved many mechanisms; including mechanisms of cadmium homeostasis, uptake, transport, and accumulation, to cope with unfavorable conditions that make them capable to maintain their survival and productivity (Falourd et al., 2014; Yadegari, 2018b). Plants possess homeostatic cellular mechanisms to regulate the concentration of metal ions inside the cell to minimize the potential damage that could result from the exposure to nonessential metal ions (Benavides et al., 2005). Sensitive plants usually show greater metabolic changes than tolerant plants. The tolerant plants show stimulation in the antioxidative defense systems and accumulation of osmolytes, which help to maintain cell water status and to protect against the ROS during stress (Miller et al., 2008).

Cadmium alters the physiological, morphological, and biochemical processes in plants, such processes as the inhibition of seedling growth and the production of abnormal seedlings. A long-term exposure to Cd leads the plant roots to become brownish, mucilaginous, and decomposed, and eventually, a reduction in plant growth and plant death (Kalai et al., 2014). Cadmium binds to the cell membrane and enzymes and negatively affects the stability of their functions. Heavy metal stress decreases germination and length of seedlings. Proper germination is a key to the success of plants in agroecosystems (Yadegari, 2018a). The decrease in seed germination under Cd stress is not only due to a decrease in water uptake, but also a result of a decrease in the transfer of endospermic materials to the embryo (Kalai et al., 2014; Liu et al., 2011).

The ROSs are usually produced during the seed germination process, from imbibition to radicle protrusion, where for complete germination, there should be a scavenging antioxidative system to remove those ROS. ROSs damage plants through protein and lipid peroxidation (Eisvand et al., 2016). Cd damages DNA and this damage cause destruction of the cell membrane, nucleic acids, lipids and proteins, and photosynthetic reaction centers that eventually negatively affect plant growth and development (Kranner and Colville, 2011). Therefore, damage to the antioxidant defense system can prevent detoxification mechanisms and trigger a cascade of uncontrolled oxidation. Cadmium contamination stimulates the ROSs production in plants that interferes with the system of antioxidative defense (Benavides et al., 2005). A range of antioxidant enzymes is generated in plants to resist against and subsequent recovery from Cd toxicity (Mohamed et al., 2012).

Seed germination and seedlings root development besides being vital plant growth stages, they are among the most sensitive to environmental changes (Liu et al., 2011). Indeed, efficient germination is important for a successful establishment of seedling, which in turn requires a uniform and rapid radicle emergence and growth. Generally, seed priming increases the seed vigor that improves the performance of the seeds in many environments (Miller et al., 2008; Mirmahmood et al., 2015). During priming, the seeds progress to the second stage of germination but do not enter the third stage, *i.e.* root emergence. The physiological conditions of plants and vitality of the seeds are affected by priming. Several economic and agronomic advantages have been reported using priming strategies, from improved rate and uniformity of germination to seedling growth and stress resistance (Ghassemi-Golezani et al., 2010; Yuan-Yuan et al., 2010).

To invigorate seeds to resist against the environmental stresses, several priming methods have been developed. Plant growth regulators have been widely used to improve the plant physiological and biochemical status and seedling establishment (Ahmadi-Lahijani et al., 2018a, 2018b; Espanany et al., 2016). Hormopriming is one of the priming techniques that seed imbibition occurs in the presence of plant hormones. It can directly affect seed metabolisms. Among the plant growth regulators, salicylic acid (SA) and jasmonates (JA) has commonly been used for hormopriming (Lutts et al., 2016; Qiu et al., 2014). Jasmonates are endogenous growth regulators that are present in plants and affect plant growth. Jasmonic acid protected wheat seedlings against salt stress by stimulating the antioxidant enzyme activity to remove ROSs (Qiu et al., 2014). Salicylic acid is a phenolic hormone and signaling molecule that plays a vital role in regulating growth and defense mechanisms against a wide range of stresses. Salicylic acid can limit the effects of environmental stresses by enhancing membrane stability and preventing ion absorption under the heavy metals contamination such as Cd (Popova et al., 2009; Shekari et al., 2010).

Given the economic and phytotherapeutic importance of thyme, increasing its production and cultivation is important. Besides, regarding fast-increasing demand for thyme products, further study is essential to reveal the mechanisms upon which the plant behaves in and adapts to different adverse environmental conditions (Stahl-Biskup and Sáez, 2003). On the other hand, heavy metal contaminated soil and water, especially Cd, is gradually increasing due to urban sludge and overusing fertilizers, which have subtractive effects on the germination and seedling establishment of plants. Therefore, investigating the effects of heavy metals; in this case Cd, could be very informative for the plant breeders and farmers.

Studies have documented the effects of seed priming on germination characteristics and physiochemical status of various plants under environmental stresses (Espanany et al., 2015, 2016; Fallah et al., 2018; Karalija and Selović, 2018; Rezai et al., 2017), however, to our knowledge, limited information has been released considering the effects of thyme seed priming exposed to Cd stress. Due to the positive effects of JA and SA on plants, we examined if seed hormopriming could improve seed germination process, physiochemical characteristics, and early seedlings growth of thyme under Cd contamination.

2. Material and methods

2.1. Experimental materials and seed priming

The experiment was carried out in the Department of Agronomy, Lorestan University, Khorramabad, in 2016. The seeds of thyme (*Thymus vulgaris* L.) were purchased from Pakan Seed Company, Isfahan, Iran. Seeds were surface sterilized using 1% sodium hypochlorite (*v*:*v*) for 15 min and were then immersed in 70 % alcohol for 30 s. After that, they were rinsed thoroughly using distilled water and were primed. For hormopriming, SA (100 mg.l^{-1}) and JA (100 mg.l^{-1}) were applied. Seeds were placed in SA and JA solutions for 24 h in a controlled environment under dark conditions at 15 °C, were then rinsed twice by deionized distilled water to wash off the hormones from the seed surface. Hydropriming (HP) was done as described above using double distilled water (dd H_2O). Seeds were then air-dried for 24 h at room temperature and immediately were used for the test. Control seeds were surface-sterilized as the primed seeds and considered as control (Espanany et al., 2016).

2.2. Cadmium contamination and germination process

Petri dishes were sterilized for 24 min at 120 °C. 150 primed and control seeds in three replications for each treatment (50 seeds per replication) were placed in Petri dishes (10 cm diameter) on filter paper soaked by $CdCl_2$ solution at concentrations of 0, 10, 20, and 30 mg.l^{-1}, and were then incubated inside a growth chamber at 25 ± 2 °C and relative humidity of 70 ± 5% (Espanany et al., 2016; Thanos et al., 1995). The Petri dishes were sealed using parafilm to reduce evaporation. Protruding a 2 mm long radicle through the seed coat was considered as a germinated seed (ISTA, 2013).

2.3. Germination characteristics

The seed germination parameters (germination percentage, germination rate, and seed vigor index(were measured daily for 21 consecutive days (Espanany et al., 2016; Thanos et al., 1995). Then, the normal radicles and plumules in each petri dish were separated and were individually oven-dried at 75 °C for 24 h to obtain the average seedling dry weight in each treatment (ISTA, 2013). Seeds and seedling samples of all treatments were stored at -75 °C for biochemical analysis. Germination rate (GR), germination percentage (GP), and the vigor index (VI) were measured using the following equations (Ikić et al., 2012):

$$GP = \frac{Total\ seeds\ germinated\ after\ 2\ weeks}{Total\ number\ of\ seeds} \tag{1}$$

The GR was calculated by Maguire's equation (Maguire, 1962):

$$GR = \sum ni/ti \tag{2}$$

Where, *ni* is the number of germinated seeds on a given day, and *ti* is the time in days from the sowing day (0) (Abdul-Baki and Anderson, 1973).

$$SVI = GP\,(\%) \times SL\,(mm) \tag{3}$$

Where *SVI* is vigor index, *GP* is standard germination, and *SL* is the seedling length.

2.4. Lipid peroxidation

The method of De Vos et al. (1991) was used to assay the level of lipid peroxidation. 0.25 g fresh samples were heated in a solution contained 0.25 % thiobarbituric acid (TBA) in 10 % trichloroacetic acid (TCA), as described by De Vos et al. (1991). The absorbance of the supernatant was read at 535 nm and corrected for the specific A600 nm.

2.5. Malondialdehyde content

The extinction coefficient of 155 mM^{-1}.cm^{-1} was applied to measure the MDA concentration and indicated as nmol of MDA g^{-1} fresh weight (Eisvand et al., 2016).

2.6. Antioxidant enzyme assay

Samples were ground with liquid nitrogen to a fine powder. 0.5 g of samples were blended with 5 mL of potassium phosphate (100 mM) buffer (pH 7.0) and were then centrifuged at 12,000 × *g* for 20 min at 4 °C. The supernatant was kept at -20 °C to assay enzymes activity.

2.6.1. Peroxidase activity (POD) (EC 1.11.1.7)

The formation rate of 0.15 M Na-phosphate-citrate buffer in the oxidized DAB (3,3'-Diaminobenzidine) was considered as the increase in A465 nm for 3 min. The supernatant contained 0.6 % H_2O_2 and DAB solution. One enzyme unit was considered as mmol. ml^{-1} of destroyed H_2O_2 per min (Herzog and Fahimi, 1973).

2.6.2. Superoxide dismutase (SOD) (EC 1.15.1.1)

The method of Rosales et al. (2009) was used to assay SOD activity. 5 mL of reaction mixture containing 50 mmol.l^{-1} Na_2CO_3 (pH 10), 0.25 mL.l^{-1} Triton X-100, 1.3 mmol.l^{-1} riboflavin, 13 mmol.l^{-1} methionine, 63 mmol.l^{-1} NBT and an appropriate aliquot of enzyme extract were used. The SOD activity was defined by monitoring the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT). A PPFD (Photosynthetically Photon Flux Density) of 380 mmol.m^{-2}.s^{-1} was used to illuminate the reaction mixtures for 15 min. To correct the background absorbance, not illuminated mixtures were used. One unit activity of SOD was considered as the enzyme quantity needed to 50 % inhibition of the NBT reduction as recorded at 560 nm.

2.6.3. Catalase (CAT) (EC 1.11.1.6)

A total volume (3 mL) of the reaction mixture contained 20 mM H_2O_2, 0.8 mM Na-EDTA 25 mM, and Tris-acetate buffer (pH 7.0). The consumption of H_2O_2 at 240 nm for 5 min at 25 °C was considered as CAT activity (Nakano and Asada, 1981).

2.7. Proline content

L-proline was used to assay the free proline (Bates et al., 1973). Centrifugation of the extracts was performed in a matched quartz (10 mm) cell using a high-speed centrifuge. The absorbance was determined spectrophotometrically (A_{520}).

2.8. Protein content

The Bradford method was used to assay the content of protein using bovine serum albumin (BSA) as the standard (Bradford, 1976).

2.9. Statistical analysis

The experiment was carried out as a factorial scheme (4 priming treatments × 4 levels of Cd concentrations) based on a completely randomized design (CRD) with three replications. Analysis of variance (ANOVA) was performed at 0.05 probability using SAS *v.* 9.1 software. The Excel software was used to draw the figures. The mean comparison was made by the LSD test at 0.05 probability level.

3. Results

3.1. Germination parameters

The effect of Cd stress and hormonal priming and the interaction of Cd and hormonal priming on GP was significant (Table 1). The GP of thyme seed was significantly decreased due to Cd contamination and, in contrast, priming significantly increased the seed GP at all stress levels of Cd contamination (Table 1). Under the control conditions, the primed seeds with JA (100 mg.l^{-1}) showed the maximum GP, however, SA-primed seed showed the greatest GP at 20 and 30 mg.l^{-1} Cd by 26 and 27 %, respectively, compared with the control (Table 1).

Seed GR significantly affected by Cd stress and priming treatments (Table 1). Generally, seed GR was decreased by increasing Cd concentration, while the priming treatments increased seed GR (Table 1). Germination of primed seeds started faster than that of the control seeds at all Cd concentrations. The priming treatments significantly increased seed GR compared with the control seeds. JA-primed seeds showed the greatest GR under zero and 10 mg.l^{-1} Cd concentrations compared with the control seeds, however, the greatest GR under 30 mg.l^{-1} Cd concentrations was observed in SA-treated seeds that was 37 % higher than the control seeds (Fig. 4).

3.2. Seed vigor index

The effect of Cd stress and priming on thyme seed vigor index was significant (Table 1). Seed Vigor index decreased with an increase in Cd concentration in the primed and control seeds. According to Table 1, the greatest seed VI at zero cadmium level was observed in JA pre-

Table 1
ANOVA results and means comparison of cadmium and seed priming on germination characteristics and growth parameters of thyme.

Cadmium concentration (mg l^{-1})	Priming treatment	Germination (%)	Germination Rate (day^{-1})	Root length (cm)	Plumule length (cm)	Seedling length (cm)	Vigor index
0	NP	61.9 c	8.0 c	19.0 b	7.3 c	26.4 c	2822 d
	HP	76.0 b	9.5 bc	23.1 b	10.4 a	33.5 b	3341 c
	JA	93.5 a	12.6 a	27.7 a	8.4 b	36.1 ab	4029 a
	SA	88.3 ab	11.0 ab	32.7 a	9.1 b	41.8 a	3598 b
10	NP	61.7 c	7.9 c	17.5 c	6.8 c	24.3 c	2251 b
	HP	74.3 bc	9.2 bc	22.3 b	8.0 b	30.4 b	2644 b
	JA	86.7 a	12.4 a	26.1 b	8.7 b	34.8 ab	3175 ab
	SA	81.1 ab	10.3 b	31.3 a	10.2 a	41.5 a	3516 a
20	NP	58.7 c	7.8 b	17.0 c	6.8 c	23.8 c	1987 b
	HP	74.6 b	12.2 a	22.6 bc	7.9 b	30.5 bc	2275 b
	JA	74.9 b	10.6 ab	26.4 b	8.9 a	35.3 b	2394 ab
	SA	79.9 a	9.3 ab	30.6 a	9.4 a	40.0 a	2559 a
30	NP	54.1 c	7.5 c	17.2 b	6.6 b	23.8 c	1278 b
	HP	62.0 b	9.1 b	20.0 b	7.6 b	27.6 bc	1397 b
	JA	69.0 b	10.2 a	23.5 b	8.9 a	26.4 ab	1499 b
	SA	74.6 a	11.9 a	30.2 a	9.3 a	39.5 a	1828 a
ANOVA	df						
SOV							
Cadmium (Cd)	3	109.8*	1.09*	4.64^{ns}	0.84^{ns}	9.18^{ns}	187988*
Priming (P)	3	1818.6**	44.8**	394.4**	19.3**	578.2**	6177375**
Cd × P	9	14.0*	0.10 ^{ns}	0.37^{ns}	0.25*	0.97ns	19661^{ns}
Error	32	15.7	0.32	2.61	0.31	5.22	54112
CV (%)		8.45	7.5	7.0	6.7	6.96	12.40

ns, ** and * indicate no significant effect, significant at 1 % and 5 % probability levels, respectively. SOV: source of variance, df: degree of freedom, CV: coefficient variance. Means with the same letter in each column and each Cd concentration are not significantly different ($p \leq 0.05$) based on the LSD test (5 %).

treatment, while the lowest was observed in control seeds (Table 1). By increasing the level of Cd contamination, SA pretreatment had a greater effect on seed VI; compared with the control seed, VI was increased 35, 21, and 27 % at 10, 20, and 30 mg.l^{-1} Cd concentration, respectively (Table 1 and Fig. 4). The greater seed VI and GP indicated that primed seeds had a better germination performance compared with the control seeds at all Cd concentrations (Table 2).

3.3. Plumule and radicle length

The effect of priming and Cd × seed priming on radicle and plumule length was significant (Table 1). Primed-seeds had greater radicle and plumule length than those of Cd-germinated. The maximum radicle and plumule length were observed in SA pretreatment and the absence of Cd. For instance, SA pretreatment increased radicle and plumule length 43 and 29 %, respectively, at 30 mg.l^{-1} of Cd concentration compared with the control seeds (Table 1). Although root to shoot ratio did not differ between the Cd treatments, seed priming increased root to shoot ratio (R/S) ratio compared with the control seeds (Table 2 and Fig. 1C). The greatest R/S ratio was observed in SA-treated seeds so that it was 24 and 20 % greater than the respective control seeds at 20 and 30 mg.l^{-1} Cd levels, respectively (Figs. 1C and 4).

Table 2
ANOVA results of cadmium and priming on growth parameters of thyme seedlings.

SOV	df	Radicle dry weight	Plumule dry weight	Root/Shoot
Cadmium (Cd)	3	0.000006*	0.00000078^{ns}	0.01^{ns}
Priming (P)	3	0.00002**	0.000026**	0.80**
Cd × P	9	0.0000005^{ns}	0.00000035^{ns}	0.02^{ns}
Error	32	0.000001	0.000001	0.03
CV (%)		13.4	13.5	6.5

ns, **, and * indicate no significant effect, significant at 1 % and 5 % probability levels, respectively. SOV: source of variance, df: degree of freedom, CV: coefficient variance.

3.4. Plumule and radicle dry weight

Plumule dry weight was affected by the priming treatments, while radicle dry weight was either affected by Cd or priming treatment (Table 2). Radicle dry weight of thyme seedling was decreased by increasing Cd stress both in the primed and control seeds, however, the primed seeds showed greater plumule and radicle dry weight (Fig. 1). At all cadmium concentrations, the highest and lowest radicle dry weight was observed in SA-primed and the control seeds, respectively. No positive effect of hydropriming was observed on the plumule and radicle dry weight of thymus seeds, however, SA and JA priming increased plumule and radicle dry weight compared with the control. Radicle dry weight was decreased 17 % exposed to 30 mg.l^{-1} Cd concentration compared with the control, while seed priming with SA improved radicle dry weight to 30 % compared with the control seeds at 30 mg.l^{-1} Cd level (Fig. 1A). The same trend was observed in plumule dry weight, where SA pretreatment increased plumule dry weight 58 and 90 % compared with the respective values of control seeds at the control and 30 mg.l^{-1} Cd concentration, respectively (Figs. 1B and 4).

3.5. Antioxidant enzymes activity

The results showed that the effects of treatments on the antioxidant enzyme activity were different; CAT and SOD were affected by priming treatments, and POX was affected by priming and priming × Cd interaction (Table 3). Generally, the activity of the antioxidant enzymes was stimulated by increasing the concentration of Cd to some extent (Fig. 2). The greatest activity of POX was observed at 10 mg.l^{-1} of cadmium concentration 16 and 14 % over the uncontaminated treatment when JA and SA applied, respectively, although the activity of POX decreased at the higher Cd concentration (Fig. 2A). The highest CAT activity was observed in HP treatment at 30 mg.l^{-1} of Cd that was 23 % over the control, however, SA suppressed CAT activity 15 % compared with HP at 30 mg.l^{-1} of Cd concentration (Fig. 2B). SA-primed seeds showed the highest SOD activity compared with the control seeds (Fig. 2C). SA pretreatment, however, stimulated SOD activity 15 % com-

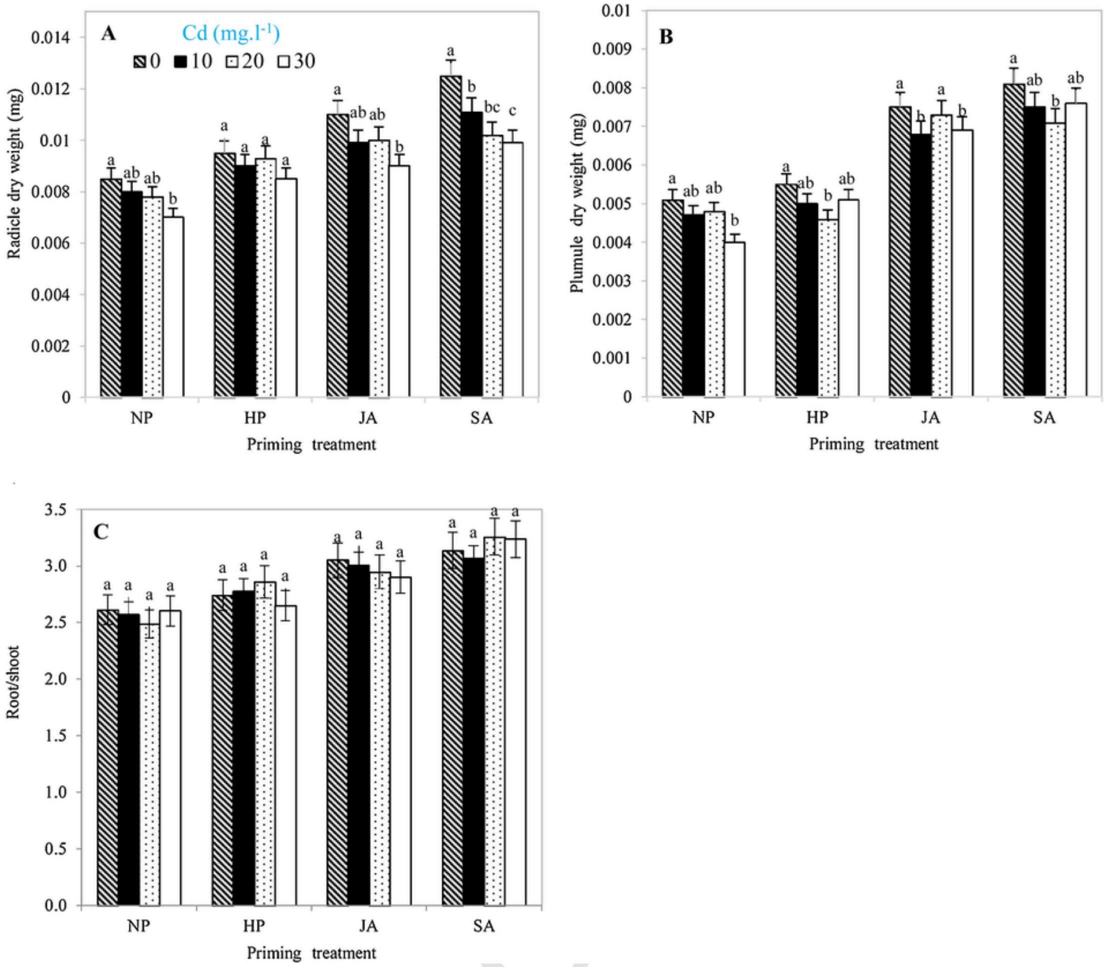

Fig. 1. Radicle dry weight (A), plumule dry weight (B), and root/shoot ratio (C) of control and primed seedling of thyme under different cadmium (Cd) concentrations (mg.l^{-1}). NP: non-primed (control), HP: hydropriming, JA: jasmonic acid, SA: salicylic acid. Seeds were primed with SA (100 mg.l^{-1}), JA (100 mg.l^{-1}), and water for 24 h. Data presented are means of four replicates with standard errors. Means with the same letter in each priming treatment are not significantly different ($p \leq 0.05$) based on the LSD test.

Table 3
ANOVA results of cadmium and priming on biochemical traits of thyme seedlings.

SOV	df	CAT	POX	SOD	MDA	Proline	Protein
Cadmium (Cd)	3	2.82 ns	0.29ns	1.07ns	0.14*	0.44ns	0.11**
Priming (P)	3	114.5**	39.03**	195.6**	9.37**	3422.5**	0.03*
Cd × P	9	1.85 ns	0.19*	0.73ns	0.02 ns	3.04ns	0.01ns
Error	32	2.38	0.40	1.38	0.11	805.7	0.009
CV (%)		10.4	12.4	5.7	10.8	3.7	12.2

ns, **, and * indicate no significant effect, significant effect at 1 % and 5 % probability levels, respectively. SOV: source of variance, df: degree of freedom, CV: coefficient variance, CAT: catalase, POX: peroxidase, SOD: superoxide dismutase, MDA: malondialdehyde.

pared with the control, under 30 mg.l^{-1} of Cd concentration (Figs. 2C and 4).

3.6. Malondialdehyde content

The effect of Cd and priming was significant on MDA content (Table 3). Malondialdehyde (MDA) accumulation was significantly lower in both control and primed seeds than those germinated under Cd stresses (Fig. 2D). The highest level of MDA was observed at 30 mg.l^{-1} Cd in the absence of priming treatments. MDA content was increased 15 % exposed to 30 mg.l^{-1} compared with the control. Exposure to 30 mg.l^{-1} Cd, MDA content of the seeds treated with HP and JA was decreased compared with non-primed seeds. Seed priming resulted in a decrease in the lipid peroxidation of cell membranes. The lowest value of MDA was obtained under the conditions of priming with 100 mg.l^{-1} SA under uncontaminated conditions (Fig. 2D). However, MDA increased by increasing Cd concentration. SA priming reduced MDA content 18 % compared with the control at 30 mg.l^{-1} Cd (Figs. 2D and 4).

3.7. Proline content

The proline content was affected by priming treatments (Table 3). The proline content of seedling showed an increasing trend with increasing Cd concentration (Fig. 3A). The proline content of primed seed was also significantly greater compared with the control seeds (Fig. 3A). Overall, the greatest proline content was observed at 30 mg.l^{-1} Cd concentration and SA priming. SA priming increased the proline content 40 % at 30 mg.l^{-1} Cd concentration compared with the control, however, the magnitude of increase was different in con-

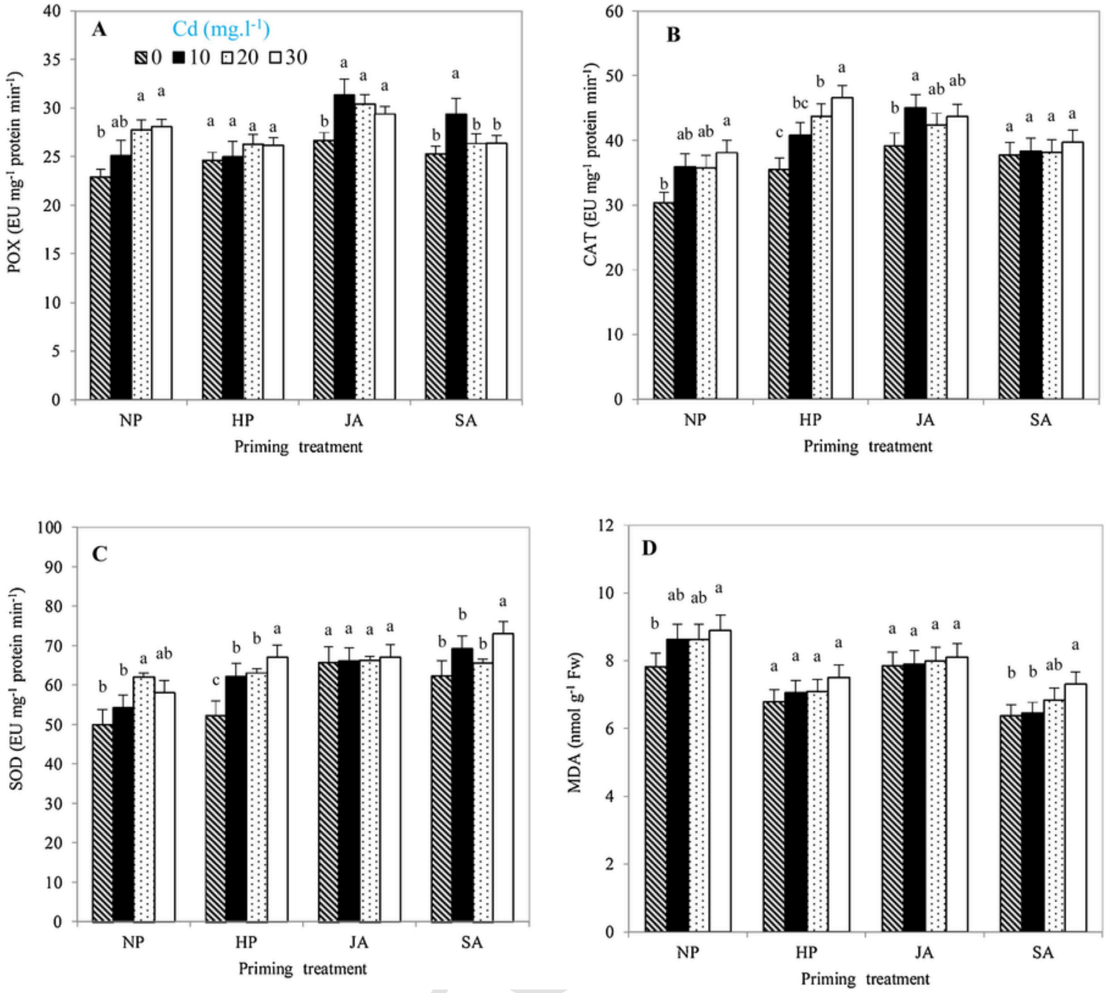

Fig. 2. Peroxidase (A), catalase (B), superoxide dismutase (C) activity, and malondialdehyde content of control and primed seedling of thyme under different cadmium (Cd) concentrations ($mg.l^{-1}$). NP: non-primed (control), HP: hydropriming, JA: jasmonic acid, SA: salicylic acid. Seeds were primed with SA (100 $mg.l^{-1}$), JA (100 $mg.l^{-1}$), and water for 24 h. Data presented are means of four replicates with standard errors. Means with the same letter in each priming treatment are not significantly different ($p \leq 0.05$) based on the LSD test.

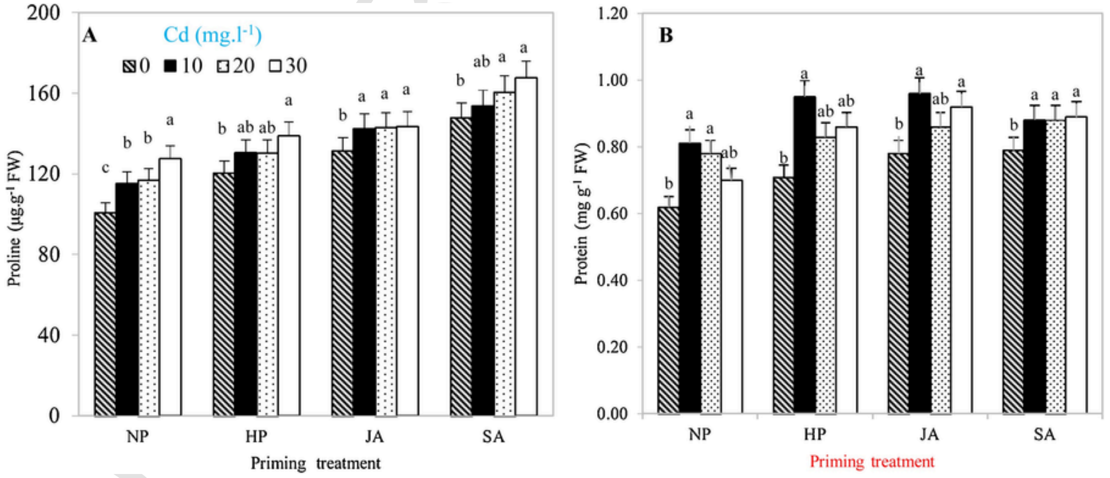

Fig. 3. Proline (A) and protein (B) content of control and primed seedling of thyme under different cadmium (Cd) concentrations ($mg.l^{-1}$). NP: non-primed (control), HP: hydropriming, JA: jasmonic acid, SA: salicylic acid. Seeds were primed with SA (100 $mg.l^{-1}$), JA (100 $mg.l^{-1}$), and water for 24 h. Data presented are means of four replicates with standard errors. Means with the same letter in each priming treatment are not significantly different ($p \leq 0.05$) based on the LSD test.

trol and primed seeds. For instance, the proline accumulation was increase 13, 14, and 22 % in HP, JA, and SA treatments under 30 $mg.l^{-1}$ Cd contamination compared with control seeds, while in primed seeds it was increased with 4, 7 and 11 % in HP, JA, and SA, respectively, after 10 days (Figs. 3A and 4).

3.8. Protein content

Cadmium contamination and seed priming affected protein content; however, the effect of their interaction was not significant (Table 3). Seedling protein content was promoted by Cd concentration. The protein content of control seeds was increased 23, 20, and 11 % at 10, 20, and 30 mg.l^{-1}, respectively, compared with the control (Fig. 3B). Protein content at Cd concentration of 30 mg/l^{-1} was decreased in both primed and control seed, although that was still higher than the control. The greatest increase in the protein content was obtained in 10 mg.l^{-1} Cd concentration under all priming treatments over the control. For instance, 30, 34, 23, and 11 % increase were observed in NP, HP, JA, and SA priming treatments, respectively, compared with the uncontaminated treatment. However, the protein content tended to decrease at the highest Cd concentration. Seed priming significantly affected the protein content of seedlings at all Cd contamination levels. At 10 mg.l^{-1} Cd concentration, JA-primed seeds had the greatest protein content followed by HP compared with the control. JA and SA increased protein content of seedlings by 23 and 21 %, respectively, compared with the control seeds, and 15 and 11 %, respectively, compared with the control, at 30 mg.l^{-1} Cd concentration (Figs. 3B and 4).

4. Discussion

The results of our study indicated that cadmium adversely affected growth in a concentration-dependent way that was shown by a decrease in GR and GP, and radicle and plumule length and dry weight. Cd stress had a negative impact on the radicle and plumule length of some plants (Mahmood et al., 2007). In the experiment by Ahmad et al. (2012), wheat GP decreased with increasing cadmium concentration. A decrease in the germination rate of plants under heavy metal stress can be due to reduced effective water uptake and disruption of elements uptake (Islam et al., 2015). The decrease in GR is probably due to a delay in the onset of germination in mature seeds. Increased duration of germination in the stressed seeds has also been reported in other studies, which results in a slower GR (Espanany et al., 2016; Mirmahmood et al., 2015). The reason for the delay is probably because the seeds require repairing the damaged membrane and other parts of the cell, also, the re-initiation of the antioxidant system and the prevention of oxidative stress take time, and the damage can only be repaired after the water is absorbed by the seed.

The decrease in seed VI under Cd stress is probably because of a decrease in its components, including GP and seedling length, both of which decrease under seed aging conditions. Kalsa and Abebie (2012) showed that one of the methods used to increase VI, germination, and thus, seedling growth is seed priming by the plant hormones. It has been reported seed germination, seedling emergence, and root growth of rice were improved by seed priming (Yuan-Yuan et al., 2010). In a work by Ghassemi-Golezani et al. (2010), it has been shown that seed priming increased the seed VI of pinto beans. Improvement of germination indices by priming has been reported in different plants (Farooq et al., 2013; Parmoon et al., 2015). In general, seed priming improves GR and germination uniformity and reduces seed susceptibility to environmental factors (Espanany et al., 2016). Priming accelerates germination and increases protein and DNA synthesis. It also affects membrane cell phospholipids. Salicylic acid appears to reduce the toxic and destructive effects of Cd stress and to increase germination by affecting the antioxidant system.

In our study, the radicle and plumule length and dry weight were increased by seed priming with SA. It has been reported that some of the plant hormones, *e.g.* cytokinin and auxin were enhanced by SA, while ion leakage from the plant cells and accumulation of toxic ions in plants were reduced by SA pretreatment. Due to a better activity of some enzymes in seed primed with the plant hormones, access to nutrients is easier during the germination and it improves germination (Shekari et al., 2010). Increased root and shoot length at an appropriate level of priming were probably due to the stimulation of metabolic activities within the embryo. For example, during priming, DNA replication, stimulation of RNA activity and, consequently, protein synthesis, cell membrane repair, and increase in the concentration of germination stimulating hormones were stimulated (Popova et al., 2009; Rouhollah et al., 2013).

Cadmium stress increased membrane damage in seeds as estimated by MDA content. It seems that Cd by over-generation of ROSs caused membrane damage and lipid peroxidation. Seedlings exposed to different concentrations of Cd showed an increase in MDA production that is an indicator of lipid peroxidation. Our results are consistent with those of others who observed MDA content in cucumber and cumin seedlings was increased under Cd stress (Espanany et al., 2015; Goncalves et al., 2007). Peroxidation of lipids begins with the release of oxygen free radicals. In the presence of oxygen, the free radicals do auto-oxidization of the lipids in the seed. The production of free radicals dam-

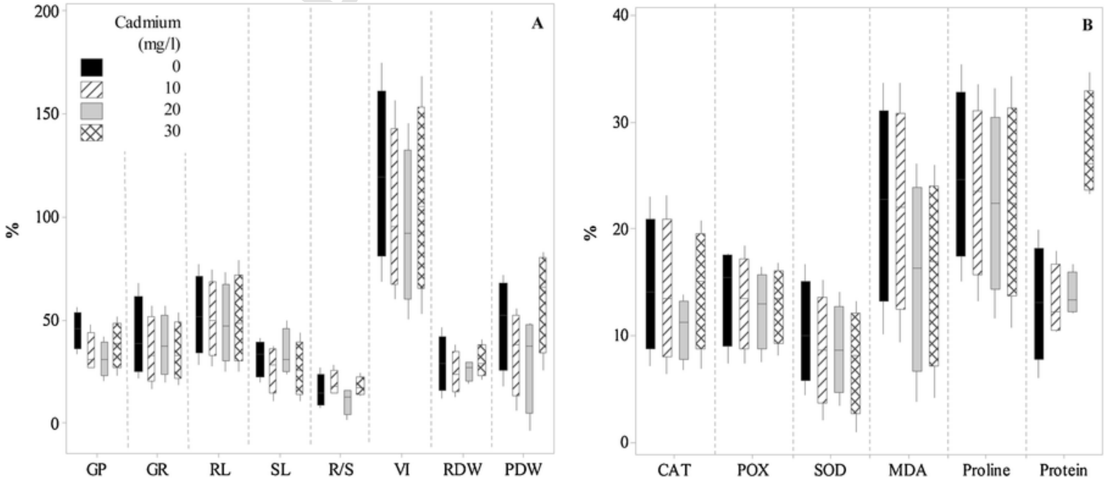

Fig. 4. Percent changes of germination and growth parameters (A) and biochemical traits (B) of thyme affected by a combination of cadmium concentrations and priming treatments compared to the control. GP: Germination percentage, GR: Germination rate, RL: Root length, SL: Shoot length, R/S: Root to shoot ratio, VI: Vigor index, RDW: Root dry weight, PDW: Plumule dry weight, CAT: Catalase, POX: Peroxidase, SOD: Superoxide dismutase, MDA: Malondialdehyde.

ages cellular contents, such as lipid membranes, proteins, and nucleic acids during seed dormancy and germination (McDonald, 1999). MDA accumulation and electrolyte leakage of SA-treated seeds were decreased under Cd stress, which supports the role of MDA to protect the cells against oxidation damage (Krantev et al., 2008). Lower values of MDA was observed in primed seeds with the plant growth regulators, possibly due to reduced detrimental effects of free radicals and greater protection of the membrane, thereby preventing unsaturated fatty acid damage and reducing membrane permeability (Qi et al., 2010). The lower MDA accumulation could be attributed to the membrane reorganization of cells improving membrane repairment, and stimulation of antioxidant enzymes during priming, which provide protection against oxidative damage (Nawaz et al., 2012).

The state of cell membranes can be reflected by solute and ion leakage from plant material. High leakage from imbibing seeds, which results in low conservation of solutes, might be due to incomplete or damaged membranes. The detrimental effects of an oxidative injury caused by heavy metal can increase lipid peroxidation and change the membrane structure (Agrawal and Mishra, 2009). Increased generation of ROS in *Hordeum vulgare* under heavy metal stress conditions increased cell fatality and electrolyte leakage (Tamas et al., 2006), which might be related to Cd-induced higher EC of growth medium. Compared with the control seeds, greater amounts of soluble sugars, potassium, and zinc nutrients leaked from the tissues in Cd-contaminated treatments (Tamas et al., 2006). Somashekaraiah et al. (1992) observed that peroxidative injury to the membranes by the polyunsaturated fatty acid degradation increased Cd-induced production of free radicals, which further led to an increase in lipoxygenase activity.

Oxidative stresses inhibit growth through the disruption of cell division. Therefore, the protection of plants against oxidative stress is crucial for their germination. The final stress tolerance of the seedlings depends on the persistence of the activated antioxidative system after germination (Lutts et al., 2016). The plant defense mechanisms such as antioxidant enzymes can diminish Cd toxicity. The plant cell metabolism can be dangerously affected by Cd. Cadmium binds to the sulfhydryl groups, which are essential for structural proteins and enzymes, and damage the structure of proteins and prevent enzymatic activity. In the present study, the control seeds also indicated an increase in the antioxidant enzyme activities. This indicated that the seedlings could probably adapt to some levels of Cd through developing an antioxidative defense system (Alscher et al., 2002). Despite an increase in antioxidant enzyme activity under Cd-stressed conditions, lipid peroxidation was also increased, which shows that an increase in antioxidant enzyme activity might have not been adequate to prevent Cd-induced membrane lipid peroxidation (Antonio et al., 2002). In response to abiotic stress, tolerant plants typically increase their antioxidative activities to reestablish an equilibrium between the production and scavenging of ROS, which can decrease Cd toxicity (Alscher et al., 2002).

During the first phase of the priming process, many parts of the ROS-mediated signaling pathway are activated. In our study, the primed seeds treated with Cd showed an increase in POX activity. It has been shown that POX activity can improve cell wall status and maintain cell membrane integrity in seedlings of stressed plants (Alscher et al., 2002; Antonio et al., 2002). Here, POX activity differed between the Cd concentrations, and the highest activity was observed at 10 mg.l^{-1} Cd concentration. It indicated that cellular H_2O_2 was scavenging through the POX enzyme (Vranová et al., 2002). The higher activity of antioxidative enzymes in primed seeds has also been previously reported by others in cumin, lettuce, and *Brassica juncea* under heavy metals and saline stresses (Espanany et al., 2015; Mohamed et al., 2012; Nasri et al., 2011). Bailly et al. (2000) found that the activity of SOD and CAT were stimulated and MDA content was decreased during priming in safflower seeds. The higher activity of POX in the primed seeds shows that POX activity improves the cell membrane integrity and the cell wall mechanical properties under Cd stress.

An effective response of plants to stressful conditions is an accumulation of compatible osmolytes like proline, which can be a good representative of heavy metal contamination. Furthermore, proline can have an antioxidative activity to protect the cells from the damage of ROSs against Cd contamination by providing a favorable environment for Cd sequestration and phytochelatin synthesis (Siripornadulsil et al., 2002). The seedlings proline content of SA-primed seeds was significantly higher than that of control seedlings (Krantev et al., 2008). Salicylic acid could form a complex with Cd during the earlier growth period that might help the plants to tolerate Cd stress. In addition, the expression of defense-related enzymes and specific proteins could also be stimulated by SA. The greater protein production when the seeds exposed to Cd contamination showing that stress protein production induced by heavy metal stress. An increase in protein content was also observed in *Phaseolus aureus* and cumin under arsenic and cadmium stresses, respectively (Espanany et al., 2015; Kaur et al., 2012). It is possible that exposure to metals increases the protein content by stimulating *de novo* synthesis from stress proteins (Verma et al., 2003).

5. Conclusions

Increased human activity has had negative effects on the environment. Heavy metals pollution such as Cd in water resources has been increased and is rapidly spreading out in the soil and water. The morphological, physiological, and biochemical processes, *e.g.* seed germination and plant growth have been affected by the application of contaminated water in the agricultural systems of the arid and semi-arid areas. Cadmium contamination stimulated the production of ROS, which caused an increase in MDA content of Cd-stressed seedlings. Morpho-physiological and biochemical traits of thyme were also adversely affected by Cd contamination. In the present study, although all germination parameters were decreased when exposed to Cd contamination, the toxicity of Cd was alleviated by seed priming treatments. Seed priming with the growth regulators improved the germination and growth attributes of thyme seedlings under Cd-induced oxidative stress. SA was almost the most effective treatment with the greatest alleviation effects in the GR, GP, and dry weight and length of plumule and radicle, compared with the control. The greatest proline, protein, and MDA content, and antioxidant enzyme activity were recorded under different Cd concentrations compared with the control, however, SA pretreatment decreased MDA content. The SA-treated seed showed the greatest proline and SOD at 30 mg.l^{-1} Cd. As a final result, it can be concluded that under Cd contamination, SA priming is the most appropriate hormopriming treatment. Therefore, priming of thyme seeds with the growth regulators can be considered as a simple method to improve the physiological and biochemical status of seeds and seedlings and provide a strategy to decrease adverse effects of Cd on plants and to enhance tolerance of plants to environmental pollutions.

CRediT authorship contribution statement

Saeed Moori: Conceptualization, Methodology, Software, Project administration, Writing - original draft. **Mohammad Javad Ahmadi-Lahijani:** Software, Formal analysis, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing for financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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