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Original Article

Alleviation of Cadmium Toxicity on the Germination Characteristics of Balangu (*Lallemantia royleana* (Benth.)) and Basil (*Ocimum basilicom* L.) Seeds by using Ascorbic Acid

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ABSTRACT

The present research aimed to investigate the effect of different concentrations of cadmium and ascorbic acid on some characteristics of germination and early growth of balangu (*Lallemantia royleana* (Benth.)) and basil (*Ocimum basilicom* L.). This experiment was as a factorial layout based on a completely randomized design with two factors and three repetitions. The experimental treatments included four levels of cadmium sulfate 0, 20, 40, and 60 mg l^{-1} and three levels of ascorbic acid 0, 200, and 400 mg l^{-1}. The results showed that the increase in the concentration of cadmium caused a decrease in the length of the radicle, the number of normal seedlings, germination percentage, seed germination, seedling weight, and of course, an increase in the weight of the balangu plumule. In basil, root length decreased by 29.1, 18.6, and 35.2% in treatments with 20, 40, and 60 mg l^{-1} of Cd compared to the control. In other words, radicle length significantly declined with rising Cd concentration from 0 to 60 mg l^{-1}. Ascorbic acid improved the characteristics of the length of the radicle, germination rate, and percentage in balangu. In addition, ascorbic acid mitigated the negative effects of cadmium and increased the number of normal balangu seedlings, especially at concentrations of 40 and 60 mg l^{-1} of cadmium. Basil root length increased with 200 and 400 mg l^{-1} of ascorbic acid application. In general, it seems that seed pretreatment with ascorbic acid could be used as a strategy to moderate the negative effects of Cd stress.

Keywords: Heavy metals, Germination percentage, Early growth, Medicinal plants, Seed vigor

INTRODUCTION

With the advent of the Industrial Revolution, heavy metals started to pollute the biosphere to a great degree. The manipulations of nature by humans and the increase in activities such as metal casting, the use of fossil fuels, the construction of mines, the increased application of fertilizers and pesticides, and the ever-increasing production of sewage and urban wastes, all these environmental and atmospheric pollution by heavy metals has become a serious and exhausting problem for today's mankind. [1]. Heavy metals are defined as elements with metallic properties and atomic numbers greater than 20 and density greater than 5 $g.cm^{-3}$ [2]. These elements are naturally found in small amounts in soil, but when their concentration exceeds biological tolerance, heavy metals become extremely toxic and should be considered dangerous soil pollutants with adverse effects on plants and animals [3, 4]. Cadmium is a chemical element in the periodic table with atomic number 48. Cadmium is a bivalent metal with a bluish-white color and is mainly produced as a byproduct of the extraction and purification of urea sulfide from zinc core [5, 4]. Cadmium has no structural role in the human body and this element, and its compounds are toxic even in very small amounts.

The source of cadmium in the soil is mainly the urban sewage and phosphate fertilizers. Cadmium is not dynamic in soil; therefore, it mainly accumulates on the surface of soil [5]. Heavy metals can have negative effects on the osmotic potential of the soil solution, on seed water absorption and germination, and on early plant growth.

Adequate and uniform germination causes better establishment of plants in the soil. Research has shown that one of the most important defense mechanisms of plants against various stresses is increasing the amount of ascorbic acid, which plays a role in the detoxification of reactive oxygen species [6]. The presence of ascorbic acid for several millimoles in the leaves shows the important role of this substance as a part of the antioxidant defense system [7]. Ascorbic acid prevents oxidation and destruction of protein structures by directly and indirectly removing free radicals. Oxygen free radicals produced during stress destroy cell membranes, nucleic acids, and cell proteins due to their high affinity with proteins and lipids [6, 8]. In general, the conducted experiments have shown that soaking the seeds before planting causes better establishment, more vegetative growth, acceleration of flowering and ripening, and ultimately higher plant yield [9].

Balangu (*Lallemantia royleana* (Benth.)) is an important medicinal plant from the mint family (Lamiaceae) and native to Iran, Central Asia, and the Caucasus. Because it has valuable medicinal properties, it might have entered Europe from East Asia in ancient times [10]. Balangu is an herbaceous, annual, and cross-pollinating plant whose seeds have an economical yield. The seeds have mucilage and verbenone essence [11]. Its fruit is caryopsis and mucilage accumulates in the exocarp cells of the fruit. Mucilages are complex metabolites and are insoluble in water, but they are highly absorbent of moisture [12]. Because of their mucilage, balangu seeds help relieve coughs caused by colds, strengthen the heart, relieve joint pain, relieve colic, and treat dysentery [13, 14]. Evidence shows that exogenous application of ascorbic acid at different growth stages enhances the oxidative defense system, improves signaling to plant hormones, and ion transfer under stress conditions, being very effective in plant growth and development [15, 16].

Basil (*Ocimum basilicom* L.) is one of the valuable medicinal plants from the Lamiaceae family, which is grown worldwide [17]. Basil is an herbaceous, annual, and aromatic plant and its leaves, which contain considerable essential oil content, generally account for the economic part of the plant [17]. Basil leaves are used as a tranquilizer because they contain a type of essential oil, called linalool, and can effectively regulate blood sugar and pressure due to the presence of an essential oil type, called estragole. This plant is even used in food, perfumery, and cosmetic-health industries [18].

Given the wide contamination of farmlands with heavy metals resulting from anthropogenic activities, the current study aims to explore the potential role of ascorbic acid application on balangu and basil seed traits and seedling growth parameters under Cd stress conditions in the laboratory experiment. We hypothesized that ascorbic acid could alleviate the negative impacts of Cd stress on two medicinal plant models, especially during seedling development. Our results are useful for designing ecotoxicological bioassays and planning phytoremediation projects in Cd soil pollution.

MATERIALS AND METHODS

This study was carried out in the seed laboratory of the University of Torbat Heydarieh to investigate the combined effects of cadmium sulfate and ascorbic acid on the germination characteristics of balangu and basil seeds in 2023. The experiment was conducted as factorial based on a randomized complete design with two factors and three repetitions. The treatments included four levels of cadmium sulfate ($CdSO_4$) with different concentrations of distilled water of (control) 20, 40, and 60 mg l^{-1}, and three levels of ascorbic acid with concentrations of distilled water (control) of 200, and 400 mg/liter (a total of 1600 Seed). ascorbic acid ($C_6H_8O_6$) was supplied by Sigma Company. After weighing the desired amount for each concentration, it was solved in distilled water and added to Petri dishes. Balangu seeds (provided by Pakan Bazr Co., Isfahan) were placed in a 5% sodium hypochlorite solution for 20 minutes after surface disinfection with distilled water and were finally washed several times with distilled water. Then, 25 seeds from each treatment were placed in a Petri dish on Whatman filter paper at room temperature, and 5 cc of cadmium sulfate solution was added to each Petri dish. Cadmium sulfate was provided by Sigma Company. Its purity was 99.99%. Based on each treatment of Cd, weighted and solved in distilled water. We tried to keep the moist on the surface of the filter paper with the prepared solution. The containers inside the terminator were at a temperature of about 25 degrees Celsius for 16 hours of light and 8 hours of darkness [19]. The germinated seeds were counted daily starting from the second day, and seeds with root lengths of more than two millimeters were counted as germinated seeds [20].

After 14 days, the studied parameters were measured by the following equations:

Germination percentage was calculated by equation 1 [20]:

Equation 1: $GP= (n/N) \times 100$

Where GP is the germination percentage, n is the number of germinated seeds and N is the total number of seeds.

Germination rate was calculated by equation 2 [20]:

Equation 2: $GR= \sum (ni/di)$

Where Gr is the germination rate, ni is the number of germinated seeds on day i and di is the day i.

The seed vigor was also calculated by equation 3 [20]:

Equation 3: $VI= SGP (\%) \times SL$

Where VI is vigor index, SGP is the seed germination percent, and SL is the seedling length (cm)

The number of normal seedlings was determined, a normal seedling is an embryo with the following parts:

1) Relatively developed root system, 2) axis of aerial part (plumule axis), 3) cotyledons, 4) terminal bud, 5) coleoptile (for the family of Gramineae) [19].

MGT was calculated by the Feizi et al., [20] formula as equation (4):

Equation 4: $MGT = \sum (F.X) \div \sum F$

In this equation, MGT: mean germination time (day), F: the number of new germinated seeds per day of the counting day X and X days of counting.

The number of normal seedlings was counted in each Petri dish on the last day of the experiment and reported as percent [19].

Radicle and plumule length traits were also calculated and the seedling weight was measured using a digital scale with an accuracy of 0.0001.

The data were analyzed by SAS software (9.2 version) and a comparison of means was done by LSD test at the probability level of 5%.

RESULTS AND DISCUSSIONS

Germination Percentage

The effect of cadmium sulfate on balangu germination percentage was significant (Table 1). The highest percentage of germination was observed in the control treatment (0 milligrams per liter of cadmium sulfate), followed by the treatments of 20, 40, and 60 mg l^{-1} of cadmium sulfate that were placed in separate statistical classes. In other words, by increasing cadmium sulfate concentration from 0 to 60 mg l^{-1}, the germination percentage decreased significantly and the lowest germination percentage was seen in 60 mg l^{-1} cadmium sulfate treatment. In this treatment, the germination percentage did not even reach 70% (Table 2). The decrease in seed germination rate can cause poor establishment of plants in the soil and ultimately, a decrease in the number of green plants [21]. It seems that heavy metals (such as cadmium) can disrupt seeds' water absorption and reduce the germination percentage by making the osmotic potential of the solution more negative. Because under stress conditions, the amount of ABA in plant tissues increases and this is effective in preventing seed germination and plant growth [22]. Heavy metals can hinder enzymatic activities involved in cellular respiration, where stored energy (carbohydrates) transforms into usable energy (ATP). Cadmium exposure, for instance, generates superoxide radicals and hydrogen peroxide, triggering a chain reaction of ROS production. Excessive ROS accumulation can damage cellular structures, including lipids, proteins, and DNA [23]. One of the primary mechanisms by which heavy metals disrupt membrane integrity is through lipid peroxidation. When heavy metals accumulate in plant tissues, they can stimulate the production of ROS, as discussed earlier. ROS can attack and degrade the lipids present in the cell membrane, leading to lipid peroxidation [24].

The effect of ascorbic acid on balangu germination percentage was significant (Table 1). The highest percentage of germination was found in two treatments of 200 and 400 mg l^{-1} of ascorbic acid, and these two treatments were included in the same statistical group. The lowest germination percentage was observed in the treatment of non-applied ascorbic acid. In such a way the germination percentage in this treatment did not even reach 80% (Table 2). The results of the present study showed that the use of 200 and 400 mg l^{-1} of ascorbic acid increased germination percentage in balangu. Although there was no significant difference between these two levels of ascorbic acid. It has been reported that during the seed's pretreatment with the compounds effective on germination (such as ascorbic acid), protein synthesis and enzyme activation, especially hydrolase and alpha-amylase, occur in the embryo. This can accelerate the supply of water and sugar for the embryo and ultimately lead to an increase in germination percentage [25]. Ascorbic acid executes as a co-factor for certain enzymes and maintains the process of phytohormone mediating signaling [10] and several plant physiological processes [11]. Ascorbic acid also controls cell division and cell expansion, modulates plant sense, and is involved in photosynthesis, hormone biosynthesis, and regeneration of antioxidants. Ascorbic acid restrains the formation of tocopherol, which helps plants defend themselves against many environmental hazards and stresses (29).

Basil germination percent was significantly influenced by the interaction effect of Cd+ ascorbic acid application (Table 3). The Cd control treatment presented the highest germination percentage at different ascorbic acid levels. A germination percentage of >80% was obtained only in these three treatments. The germination percentage decreased significantly with rising $CdSO_4$ concentration from 0 to 60 mg l^{-1} at different ascorbic acid levels (Fig. 2). The different ascorbic acid levels were not significantly different in 20 mg l^{-1} of $CdSO_4$ treatment. However, the germination percentage rose significantly with 200 and 400 mg l^{-1} of ascorbic acid at 40 and 60 mg l^{-1} of $CdSO_4$ compared to the ascorbic acid -free control (Fig. 2). The lowest germination percentage was measured under the ascorbic acid-free conditions in treatments with 40 and 60 mg l^{-1} of $CdSO_4$, in which the germination percentage did not reach even 55% (Fig. 2). A decrease in seed germination percentage can lead to poor plant establishment in the soil and, ultimately, reduce the number of germinated plants [21]. The presence of Cd seems to impair seed water uptake and reduce germination through the more negative osmotic potential of the solution because ABA increases in plant tissues in stressful conditions, which can inhibit seed germination and plant growth [22]. The present investigation demonstrated that the negative Cd effects could be moderated by seed pretreatment with 200 and 400 mg l^{-1} of ascorbic acid, which prevented an excessive reduction in the germination rate under severe drought stress conditions. As shown in the literature, increasing the severity of environmental stresses and disruption in plant physiological processes will elevate ROS levels in plant tissues, inducing the plant to improve the level of antioxidant compounds in its tissues to scavenge ROS [27]. The same as catalase, ascorbate peroxidase is a powerful enzymatic antioxidant that catalyzes the H_2O_2 conversion process into water and oxygen (6) and can play a role in increasing the ascorbate peroxidase level. Ascorbic acid is also a powerful non-enzymatic antioxidant and can be effective in ROS scavenging [6].

Mean Germination Time (MGT)

The effect of cadmium sulfate on Balangu's MGT was significant, but the interaction effect of Cd and ascorbic acid was not significant on MGT (Table 1). Thus, the highest MGT was observed in the treatment of 60 mg l^{-1} cadmium sulfate, followed by 40 mg l^{-1} cadmium sulfate treatment. The lowest MGT was also found in two treatments of 0 and 20 mg l^{-1} of cadmium sulfate. In these two treatments, MGT did not even reach 1.64 days (Table 2). In general, MGT indicates the average number of germinated seeds per day, and the lower it is, the faster the seeds have germinated [20]. It seems that high amounts of cadmium sulfate (40 and 60 mg l^{-1}) increase the time required for seed germination.

It is probably because of the decrease in the rate of water absorption by the seeds exposed to high concentrations of cadmium sulfate. Because increasing the concentration of solutes can reduce the ability of the plant (the seed) to absorb water since it makes the osmotic potential of the solution more negative [28] and if it occurs during germination, it can slow down the germination rate.

The effect of ascorbic acid on Balangu MGT was significant (Table 1). Thus, the highest MGT was observed in the treatment of non- ascorbic acid applications. The lowest MGT was also found in the two treatments of 200 and 400 mg l^{-1} of ascorbic acid, and these two treatments were in the same statistical group (Table 2). All in all, lower MGT indicates faster seed germination [20].

In the current study, the use of 200 and 400 mg l^{-1} of ascorbic acid decreased MGT and improved the germination rate. Although there was no significant difference between these two levels of ascorbic acid. In the research conducted in this field, various pre-treatments have been introduced to enhance seed germination. These treatments shorten the time between planting and germination and protect the seeds from biotic and abiotic stresses during the critical stage of seedling establishment [29].

Basil MGT was significantly affected by $CdSO_4$ application (Table 3), and the highest MGT was recorded in 60 mg l^{-1} of $CdSO_4$ treatment, which was 20.6% more than the control. After this treatment, the next rank belonged to 40 mg of $CdSO_4$ treatment. The least MGT (< 2.43/day) was measured in 0 and 20 mg of $CdSO_4$ treatment (Table 4). MGT generally represents the mean number of germinated seeds per day, and a lower MGT means faster seed germination. Seed pretreatment with high $CdSO_4$ concentrations (40 and 60 mg l^{-1}) seems to increase the time needed for seed germination. This results from a reduction in water absorption rate by seeds exposed to high $CdSO_4$ concentrations because increasing salt concentration can reduce plant (here seeds) water uptake capacity as it results in more negative osmotic potential of the solution [28]. This event at germination time can decelerate the rate of germination.

Basil MGT was significantly influenced by ascorbic acid application (Table 3), and the utmost MGT was determined in the control treatment (without ascorbic acid application). The lowest MGT (< 2.43/day) was observed in 200 and 400 mg l^{-1} of ascorbic acid treatments, and these two treatments were assigned to one statistical group. In other words, MGT was lower in 200 and 400 mg l^{-1} of ascorbic acid treatments (4.13% and 5.64%, respectively) compared to the control (Table 4). A lower MGT generally indicates more rate of seed germination. In this study, using 200 and 400 mg l^{-1} of ascorbic acid reduced MGT and improved the rate of seed germination; however, these two ascorbic acid levels were not different significantly. According to the literature, different compounds as pretreatment are used to enhance seed germination. These treatments shorten the time between sowing and germination and even protect seeds from biotic and abiotic stresses during the critical stage of seedling establishment [29].

Seed Vigor

The effect of cadmium sulfate on balangu seed vigor was significant (Table 1). The highest seed vigor was observed in the control treatment (0 mg l^{-1} of cadmium sulfate) and it was only in this treatment that the seed vigor reached above five (Table 2). The treatments of 20, 40, and 60 mg l^{-1} of cadmium sulfate were placed in one statistical class and the next rank. In other words, using cadmium sulfate in different concentrations significantly reduced seed vigor. In these treatments, seed vigor did not even reach 4.33 (Table 2). Seed vigor is one of the most important physiological traits in assessing seed vitality [30]. It appears that the presence of cadmium can reduce seed vigor by disrupting seeds' water absorption and affecting the formation of oxygen free radicals (ROS). In this regard, Zhang et al. (31) after investigating the negative effects of heavy metals such as lead and cadmium on blackberry's seedlings, reported that these two heavy metals caused a significant increase of oxygen free radicals, especially superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) in blackberry's seedlings, and this caused excessive stimulation of chloroplasts and increased synthesis of the antioxidants of ascorbate peroxidase (APX) and superoxide dismutase (SOD) which allocated a part of photosynthetic products of the plant to cope with the stress of these heavy metals.

Basil seed vigor was significantly affected by $CdSO_4$ application (Table 3), and the maximum seed vigor was measured in 0 mg l^{-1} of $CdSO_4$ (control) treatment, the only treatment in which this trait reached more than 560 (Table 4). After this treatment, 40 and 60 mg l^{-1} of $CdSO_4$ treatments were in one statistical class in a lower rank (37.5% and 39.6% reductions, respectively) than the control. In these two treatments, seed vigor did not even reach 355 (Table 4). Seed vigor is an important physiological trait in the study of seed power and vitality [30]. Cd heavy metal seems to reduce seed vigor by disrupting the seed water uptake and affecting the formation of reactive oxygen species (ROS). This result agrees with those reported in other studies. A significant reduction in seed vigor of lentil seedlings from 831 to 93 was reported with increasing Cd concentration from 0 to 800 ppm [20]. In a study on the adverse effects of Pb and Cd, ROS (particularly superoxide anion ($O2^{\bullet-}$) and hydrogen peroxide (H_2O_2) increased significantly in black mulberry seedlings exposed to these two heavy metals, which in turn led to the partial allocation of plant photosynthesis to dealing with heavy metal stress [31].

Basil seed vigor was significantly affected by ascorbic acid application (Table 3), and the uppermost seed vigor was observed in 200 and 400 mg l^{-1} of ascorbic acid treatments, which were placed in a single statistical group. Seed vigor was lowermost in the ascorbic acid -free (control) treatment in which this trait did not reach even 375 (Table 4). Compared to the control, basil seed vigor increased by 19% and 13.9% with 200 and 400 mg l^{-1} of ascorbic acid application, respectively. However, these two ascorbic acid levels did not differ significantly in this respect. Seed pretreatment with compounds, such as ascorbic acid, can reportedly increase protein synthesis and activation of enzymes, particularly hydrolase and α-amylase, in the fetus [25], which seems to elevate seed vigor.

Basil seed health was significantly influenced by $CdSO_4$ application (Table 3), and seed health was maximized in 0 mg l^{-1} of $CdSO_4$ (control) treatment, the only treatment in which this trait reached more than 58 (Table 4). After this treatment, 40 and 60 mg l^{-1} of $CdSO_4$ treatments were assigned to a single statistical class in a lower rank (64.1% and 76.7% reductions, respectively) than the control (Table 4). This trait is referred to as another indicator of seed vigor in some studies; in other words, seed vigor and seed health in this research are referred to as seed vigor indicators 1 and 2 in some investigations [20]. To better distinguish between these two indicators, however, they should be named seed vigor and seed health indicators. Similarly, Feizi et al. [20] reported a significant decrease in this indicator from 4531 to 2920 in lentil seedlings with increasing Cd concentration from 0 to 500. As reported by Hussain et al. [32], free radicals formed in the plant by different environmental stresses impose oxidative stress, which disrupts cell membrane function and destroys membrane selective permeability, thereby increasing seed substance leakage and lowering seed vigor.

Basil seed health was significantly affected by ascorbic acid application (Table 3), and the utmost seed health was recorded in 200 and 400 mg l^{-1} of ascorbic acid treatments, which were in one statistical group. The lowest seed health belonged to the ascorbic acid -free (control) treatment in which this trait did not exceed even 27 (Table 4). Compared to the control, basil seed health rose by 29.2% and 45.2% with 200 and 400 mg l^{-1} of ascorbic acid application, respectively, but no significant difference was found between these two ascorbic acid levels. Seed pretreatment with ascorbic acid can seemingly elevate basil seed health. The exogenous application of ascorbic acid can improve plant growth ability by promoting soluble protein levels and improving the antioxidant defense system.

Plumule Length

The effect of cadmium sulfate was significant on plumule length of balangu (Table 1). The highest length of the plumule was observed in two treatments of 40 and 60 mg l^{-1} of cadmium sulfate, and in these two treatments, the plumule length reached above three centimeters. The two other treatments (control and 20 mg l^{-1} of cadmium sulfate) ranked next and were in the same statistical class. In these treatments, the length of the plumule did not even reach 2.8 cm (Table 2). These results were beyond the expectations of the researchers because by increasing cadmium concentration from 0 to 60 mg l^{-1}, the length of the plumule also increased. However, by increasing cadmium concentration, radicle length and seedling weight traits decreased. But this increase in the length of the plumule, by the increase in the concentration of cadmium, was not expected. It seems that this is caused by the effect of cadmium on gibberellin hormone activity. Research has shown that environmental stress can disrupt the activity of the gibberellin hormone and thereby affect the length of the plumule. [33].

Basil shoot length was significantly influenced by $CdSO_4$ application (Table 3), and the maximum shoot length (> 2.85) was measured in 40 and 60 mg l^{-1} of $CdSO_4$ treatments. The other two treatments (control and 20 mg l^{-1} of $CdSO_4$) were placed in a lower rank in one statistical class in this trait. In these two treatments, the shoot length did not even reach 2.74 cm (Table 4). However, the different Cd treatments were not significantly different in the shoot length trait in comparison with the effect of Cd treatment on the other traits. Nonetheless, an increase in Cd concentration led to a slight increase in shoot length, which was not expected. This observation seems to result from the effect of Cd on gibberellin activity, which is disrupted by environmental stresses, thereby affecting shoot length [33]. Cd may increase photosynthetic pigment contents in plant shoots and thereby improve plant growth [34]. Cd application at a low concentration (100 ppm) resulted in a significant increase in the shoot length of lentil seedlings compared to the control, but this trait decreased significantly by Cd application at high concentrations (250 and 500 ppm) [20].

Radicle Length

The effect of cadmium sulfate on balangu radicle length was significant (Table 1). The highest radicle length, 11.3 cm, was observed in the control treatment (zero mg l^{-1} of cadmium sulfate). The treatments of 20, 40, and 60 mg l^{-1} of cadmium sulfate were placed in separate statistical classes and ranked next. In other words, by increasing the concentration of cadmium sulfate from zero to 60 mg l^{-1}, radicle length decreased significantly, and the lowest radicle length was found in 60 mg l^{-1} cadmium sulfate treatment. The length of the radicle did not even reach 1.75 cm in this treatment (Table 2). Studies have shown that as the osmotic potential becomes more negative, the root absorbs less water [28] and it seems that, in the current research, this has harmed the length of the radicle. Moreover, under stress conditions, the amount of ABA in plant tissues increases and this might prevent plant growth [22]. These results agree with the findings of other researchers. Feizi et al. [20], while studying the effect of cadmium on the germination and growth of lentil seedlings, reported that with the increase of cadmium concentration from zero to 500 ppm, the radicle length decreased significantly. From 5.6 cm in the control treatment to 0.417 cm in the 250 ppm treatment; and in the 500 ppm treatment, no radicle had emerged from the seeds. The effect of ascorbic acid on balangu radicle length was significant (Table 1). The highest radicle length, 2.47 and 2.39 cm was found in treatments of 200 and 400 mg l^{-1} of ascorbic acid, respectively, and these two treatments were included in the same statistical group. The lowest radicle length was also observed in the control treatment. In this treatment, the length of the radicle did not even reach 1.2 cm (Table 2). The results of the present research showed that the use of 200 and 400 mg l^{-1} of ascorbic acid increased the length of the radicle in Balangu. Although there was no significant difference between these two levels of ascorbic acid. It has been reported that seed pretreatment with compounds effective on germination, stimulates the synthesis and initial activation of hydrolytic enzymes such as α and β amylase, which provide the energy needed for seedling growth by oxidizing the nutrients stored in the seed [23]. Different experiments have shown that osmotic pretreatment of seeds with 4% mannitol caused elongation of the radicle and plumule of the chickpea [35].

The effect of $CdSO_4$ application was significant on basil rootlet length (Table 3), and the treatment with 0 mg l^{-1} of $CdSO_4$ (control) presented the highest rootlet length (3.81 cm). Compared to the control, rootlet length decreased by 29.1, 18.6, and 35.2% in treatments with 20, 40, and 60 mg of $CdSO_4$. In other words, rootlet length declined significantly with rising $CdSO_4$ concentration from 0 to 60 mg l^{-1} (Table 4). Root water uptake is reported to be lower with more negative osmotic potential [28]. This seems to have adversely affected rootlet length in the current research. Moreover, ABA increases in plant tissues under stressful conditions, which preclude plant growth [22]. These results correspond to those of Feizi *et al.* [20], who reported a decrease in rootlet length with increasing Cd concentration. Contrarily, Carvalhoa *et al.* [34] claimed that Cd might improve rootlet growth.

Basil rootlet length was significantly affected by ascorbic acid application (Table 3), and the uppermost rootlet length was noticed in 200 and 400 mg l^{-1} of ascorbic acid treatments (3.16 and 3.11 cm, respectively), which were in a single statistical group. The minimum rootlet length was obtained in the control treatment without ascorbic acid application in which this trait did not reach even 2.8 cm (Table 4). Basil rootlet length increased with 200 and 400 mg l^{-1} of ascorbic acid application, but these two ascorbic acid levels did not differ significantly in this respect. Seed pretreatment with ascorbic acid can elevate seed root formation ability [36]. Seed osmotic pretreatment with 4% mannitol elongated rootlet and plumule in the chickpea plant [35].

Heavy metals have a high degree of persistence in the environment. The contamination of soil with heavy metals stands as a significant environmental concern with wide-ranging implications for crop production and food protection. Crops can absorb heavy metals from contaminated soils through their root systems. High levels of metals in the soil can lead to reduced plant productivity, stunted growth, and lower yields. Additionally, crops exposed to heavy metals may show a decline in nutritional quality, with decreased levels of essential oil [23]. On the other hand, cultivation of medicinal plants is extending, hence it can be one of the important challenges for medicinal plant production.

Seedling Weight

The effect of cadmium sulfate on the weight of balangu seedlings was significant (Table 1). The highest seedling weight, 1.78 g.plant^{-1}, was observed in the control treatment (zero mg.l^{-1} of cadmium sulfate). Followed by the treatment of 20 mg.l^{-1} cadmium sulfate with a seedling weight of 1.37 g.plant^{-1}. The 40 and 60 mg.l^{-1} cadmium sulfate treatments were placed in one statistical class and ranked next in the chart. In these two treatments, the weight of the seedling did not even reach 0.825 g.plant^{-1}. In other words, by increasing the concentration of cadmium sulfate from zero to 60 mg.l^{-1}, the weight of the seedling decreased significantly, and the lowest weight of the seedling was seen in two treatments of 40 and 60 mg.l^{-1} of cadmium sulfate (Table 2).

It appears that cadmium has caused a decrease in the photosynthetic capacity of the plant due to its effect on the osmotic potential of the solution becoming more negative and subsequently, reducing the water content of the cells, as well as forcing a part of the plant's energy to be used to initiate stress coping mechanisms [37]. This has led to a decrease in dry matter production and finally a decrease in the seedling weight. In this regard, Wang et al. [38] while investigating the effects of cadmium contamination of corn seeds in Qingdao, China, have discovered that adding 5 mg/kg of cadmium to the corn growth medium inhibited plant growth and reduced seedling dry weight, and at the same time, high accumulation of cadmium in corn's tissues. In recent years, there has been an increasing interest in using medicinal plants for different purposes. Hence cultivation area of medicinal plants is extended, especially basil and balangu. One of these purposes is to decrease the negative effects of different heavy metals on plants by applications of ascorbic acid. Although there are some studies on the effect of cadmium on basil and balangu, few studies have been done on the positive effects of ascorbic acid on cadmium pollution.

The Number of Normal Seedlings

The interaction effect of cadmium sulfate and ascorbic acid on the number of normal balangu seedlings was significant ($p \leq 1\%$) (Table 1). The highest number of normal seedlings at different levels of ascorbic acid was observed in the control treatment of cadmium sulfate. By increasing the concentration of cadmium sulfate from zero to 60 mg l^{-1}, at different levels of ascorbic acid, the number of normal seedlings decreased significantly (Fig. 1). There was no significant difference between different levels of ascorbic acid in the two levels of control and 20 mg l^{-1} cadmium sulfate; however, at two levels of 40 and 60 mg/l of cadmium sulfate, the use of 200 and 400 mg l^{-1} of ascorbic acid caused a significant increase in the number of normal seedlings compared to not using ascorbic acid (Fig. 1).
The lowest number of normal seedlings without application of ascorbic acid was observed in two treatments of 40 and 60 mg l^{-1} of cadmium sulfate. In these two treatments, the number of normal seedlings did not even reach 15 (Fig. 1). In general, the number of normal seedlings has an important role in plant density in the field. Because the seeds might have germinated in the field, but the number of normal seedlings is low, which affects the canopy because the abnormal seedlings may not come out of the soil [20]. In the present research, it was found that in the conditions of severe cadmium stress, seeds with 200 and 400 mg l^{-1} of ascorbic acid can be effective, to some extent, in balancing the negative effects of cadmium stress and preventing excessive reduction of normal seedlings.
Other research has shown that with the increase in the intensity of environmental stress and disturbances in the physiological processes of the plant, the concentration of reactive oxygen species (ROS) increases in plant tissues, and this makes the plant increase the level of antioxidant compounds in its tissues to scavenge ROS [27].

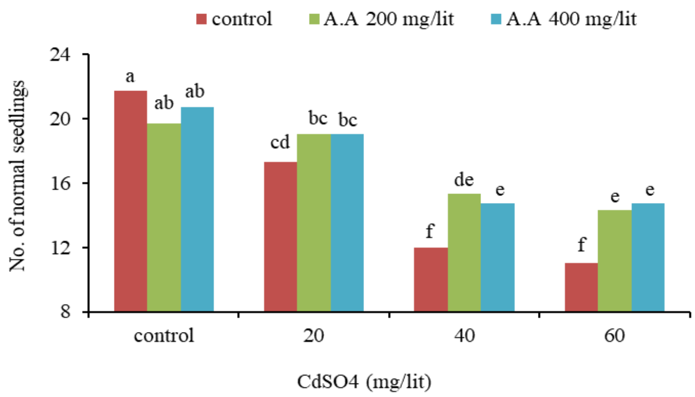

Fig. 1 Interaction effect of $CdSO_4$ and ascorbic acid (A.A) on number of normal seedlings of *Lallemantia royleana* (Benth.). This means that have a common letter and have no significant difference at the 5% probability based on the LSD test.

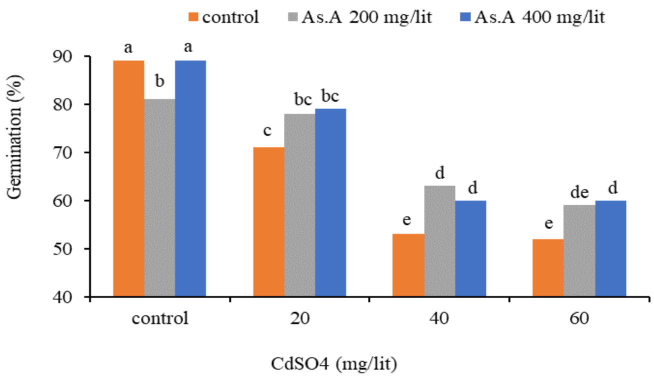

Fig. 2 Mean comparison of interaction of $CdSO_4$ and ascorbic acid (A.A) on germination percentage of *Ocimom basilicom* L. This means that have a common letter and have no significant difference at the 5% probability based on the LSD test.

Table 1 ANOVA of investigated traits ($CdSO_4$ and ascorbic acid) in *Lallemantia royleana* (Benth.)

S.O.V	Df	No. of normal seedlings	MGT	Seed vigor	Germination percentage	Length of radicle	Length of plumule	Dry weight of seedling
$CdSO_4$ (A)	3	112 **	0.462 **	1.65 *	895 **	3.30 **	1.46 *	2.28 **
Ascorbic acid (B)	2	11.2 **	0.076 **	1.14 ns	118 **	0.517 *	0.827 ns	0.172 ns
A×B	6	5.42 **	0.018 ns	0.087 ns	17.1 ns	0.165 ns	0.155 ns	0.124 ns
Error	24	1.47	0.012	0.0571	10.7	0.107	0.437	0.107
CV (%)	-	7.30	6.13	5.36	3.99	14.1	21.7	27.9

ns, * and **: non-significance, significance at 5 and 1 percent probability level, respectively

Table 2 Effect of $CdSO_4$ and ascorbic acid on traits of MGT, seed vigor, germination percentage, length of radicle, length of plumule, and weight of seedling in *Lallemantia royleana* (Benth.)

Treatment	Concentration	MGT (day)	Seed vigor	Germination percentage (%)	Length of radicle (cm)	Length of plumule (cm)	Dry weight of seedling (mg/plant)
$CdSO_4$ (mg/l)	0 (Control)	1.59 c	5.09 a	93.2 a	3.11 a	2.77 b	1.78 a
	20	1.63 c	4.26 b	86.0 b	1.98 c	2.77 b	1.37 b
	40	1.84 b	4.32 b	78.9 c	2.42 b	3.02 ab	0.823 c
	60	2.08 a	4.15 b	69.9 d	1.74 c	3.62 a	0.699 c
Ascorbic acid (mg/l)	0 (Control)	1.87 a	-	78.6 b	2.08 b	-	-
	200	1.76 b	-	82.7 a	2.49 a	-	-
	400	1.72 b	-	84.7 a	2.37 a	-	-

This means that have a common letter and have no significant difference at the 5% probability based on the LSD test.

Table 3 ANOVA of investigated traits ($CdSO_4$ and ascorbic acid) in *Ocimom basilicom* L.

S.O.V	df	MGT	Seed health	Seed vigor	Rate of germination	Length of radicle	Length of plumule	Dry weight of seedling
$CdSO_4$ (A)	3	0.465 **	3733 **	94668 **	1765 **	3.08 **	1.15 *	0.354 **
Ascorbic acid (B)	2	0.076 **	457 **	16404 *	95.6 *	0.501 *	0.456 ns	0.068 **
A×B	6	0.017 ns	14.3 ns	1757 ns	51.4 *	0.144 ns	0.143 ns	0.001 ns
Error	24	0.012	27.1	3112	19.4	0.096	0.384	0.005
CV (%)	-	4.24	15.4	13.4	6.34	10.2	21.1	15.5

ns, * and **: non-significance, significance at 5 and 1 percent probability level, respectively

Table 4 Mean comparison of $CdSO_4$ and ascorbic acid concentration on traits of MGT, seed vigor, seed health, length of radicle, length of plumule, and weight of seedling in *Ocimom basilicom* L.

Treatment	Concentration	MGT (day)	Seed vigor	Seed health	Length of radicle (cm)	Length of plumule (cm)	Dry weight of seedling (mg/plant)
$CdSO_4$ (mg/lit)	0 (Control)	2.38 c	562 a	58.5 a	3.81 a	2.73 b	0.681 a
	20	2.42 c	408 b	41.7 b	2.70 c	2.66 b	0.546 b
	40	2.63 b	351 c	21.0 c	3.10 b	2.87 ab	0.353 c
	60	2.87 a	339 c	13.6 d	2.47 c	3.45 a	0.235 d
Ascorbic acid (mg/lit)	0 (Control)	2.66 a	374 b	27.0 b	2.78 b	-	0.371 b
	200	2.55 b	445 a	34.9 a	3.16 a	-	0.474 a
	400	2.51 b	426 a	39.2 a	3.11 a	-	0.517 a

This means that have a common letter and have no significant difference at the 5% probability based on the LSD test.

CONCLUSION

In general, the results of this research showed that by increasing the concentration of cadmium from zero to 60 mg.l^{-1}, the amount of radicle length, the number of normal seedlings, germination percentage, seed germination, and the weight of balangu seedlings decreased. However, ascorbic acid moderated the negative effects of cadmium and increased the number of normal seedlings, especially at concentrations of 40 and 60 mg of cadmium. Although this increase in the number of normal seedlings was still significantly lower compared to control conditions, it seems that ascorbic acid can be used to moderate the negative effects of cadmium. In basil, radicle length, shoot length, germination percentage and rate, seed vigor, and seedling weight decreased with increasing Cd concentration from 0 to 60 mg l^{-1}. Seed pretreatment with ascorbic acid could moderate the negative Cd effects and increase the germination percent and rate, particularly at 40 and 60 mg l^{-1} of Cd; however, this increase in the germination percent was significantly lower than the control conditions. Since there was no significant difference between the two levels of 200 and 400 mg l^{-1} of ascorbic acid in most of the studied traits; therefore, 200 mg l^{-1} of ascorbic acid solution can be recommended to improve germination and early growth characteristics of balangu seedlings.

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Conflict of Interests
The authors declare that they have no conflict of interests.

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