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Original Article

Effect of Seed Priming with Silk Reeling Effluent and Saffron Petal Wastes on Enhancing Cadmium Stress Tolerance in Physalis Species

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ABSTRACT

Cadmium (Cd) is a highly mobile and soluble heavy metal that is easily taken up by plants, making it a significant source of contamination in agricultural produce. This study was carried out to evaluate the potential of selected natural substances in mitigating the adverse effects of Cd stress on two *Physalis* species at the research laboratory of University of Torbat Heydarieh, Iran. The experiment was arranged as a factorial based on a completely randomized design, with treatments including two levels of Cd (0 and 500 µM), two *Physalis* species (*Ph. peruviana* and *Ph. ixocarpa*), and seven priming methods (control (no priming), hydropriming (HP), salicylic acid (SA) (1 mM), vermicompost water extract (VE):10%, Silk reeling effluent (SRE): 100%, wood vinegar (WV): 100%, and saffron petal water extract (SPE): 10%). The results showed that Cd stress significantly reduced germination rate, seedling biomass, chlorophyll a content, and the activities of Ascorbate Peroxidase (APX) and Catalase (CAT), while it increased malondialdehyde (MDA) and proline levels in both species. Among the two species, *Ph. ixocarpa* exhibited a higher stress tolerance index (STI) than *Ph. peruviana*. Seed priming with some natural compounds, particularly SRE and SPE, meaningfully enhanced germination rate, seedling biomass, chlorophyll a content, and activities of APX and CAT, but reducing stress markers such as MDA and proline. Seed priming with WV not only failed to improve germination and seedling growth, but also resulted in lower values than the control in both species. Overall, seed priming with SRE and SPE proved more effective than SA in enhancing seed germination and growth of *Physalis* species in Cd stress condition. Notably, *Ph. ixocarpa* demonstrated greater tolerance.

Keywords: Antioxidant enzyme, Wood vinegar, Vermicompost, Stress tolerance index

INTRODUCTION

Environmental contamination by heavy metals has become a serious global concern [1]. Among these metals, Cd poses a particularly high risk to plants due to its high mobility and water solubility, making it more readily absorbed compared to other heavy metals [2]. The high toxicity of Cd can significantly threaten plant growth and yield [2]. Cd stress negatively affects plant proteins and enzyme activities by replacing essential metal ions in biomolecules, leading to membrane disruption and impairments in fundamental metabolic processes such as homeostasis, respiration, and photosynthesis [3].

To enhance plant tolerance or adaptation to Cd-contaminated conditions, several strategies have been reported. These include proper nutrient management [4], the use of beneficial microorganisms such as bacteria and fungi for metal stabilization [5], application of adsorbents like biochar and activated carbon [6, 7], genetic modification [8], agronomic and physical methods [9], and the use of plant growth regulators [10]. In addition, seed priming with various agents prior to sowing has been proven to induce Cd tolerance in plants [8].

Seed priming enhances the speed and uniformity of germination, contributing to improved seedling establishment and increased plant growth and productivity [11]. This technique is particularly beneficial under adverse environmental conditions, where it can revitalize weak or damaged seeds. Moreover, seed priming is recognized as a promising approach to enhance tolerance against both abiotic and biotic stresses [12]. It contributes to the maintenance and repair of vital cellular components such as DNA, RNA, and proteins under stress, thereby supporting seedling emergence and viability [13]. Priming can stimulate the plant defense system and improve its preparedness to cope with environmental challenges. Pre-sowing treatments can involve chemical agents like SA, organic compounds, or biological agents. In recent years, eco-friendly and sustainable priming methods using bio-based waste materials have gained attention in modern agriculture [14]. These methods utilize biodegradable plant-derived residues such as fruit and vegetable waste, compost, plant extracts, and even green-synthesized nanoparticles to improve seed physiological performance. Replacing synthetic chemicals with biodegradable materials not only reduces production costs and environmental pollution in soils and water sources but also contributes to waste recycling and value creation within a circular economy framework. Evidence suggests that priming with bio-waste-derived materials—such as pistachio residues, saffron petals, and sullage silk — offers an effective and environmentally friendly strategy to improve the sustainability of agricultural production sy shoot [15].

Today, approximately 757 tons of fresh silkworm cocoons are produced in Iran, with 62% (about 470 tons) originating from Khorasan Razavi Province. Among the counties in this province, Torbat Heydarieh contributed around 105 tons. This high level of silk production results in the generation of large quantities of Silk reeling effluent (SRE) annually, a by-product that is typically underutilized. Harnessing this material not only has the potential to reduce reliance on synthetic agrochemicals but may also create a new revenue stream for sericulturists. Given the presence of sericin and various nutritional and hormonal compounds in SRE [16], it is hypothesized that this substance could contribute to enhanced plant growth under Cd stress conditions.

Iran is the leading global producer of saffron, accounting for over 90% of the world's total production. In saffron flowers, only the stigma is harvested and considered the economically valuable part, while other floral components, such as petals, are typically discarded. However, saffron petals are rich in bioactive compounds such as anthocyanins, crocin, picrocrocin, carotenoids, and flavonoids [17, 18]. In addition to their pharmacological and cosmetic potential, the vivid purple pigments in saffron petals also suggest possible applications in the dye industry. Due to the presence of such valuable secondary metabolites, saffron petals may also be beneficial in mitigating the adverse effects of Cd stress in plants.

Wood vinegar (WV), a byproduct of smoke condensation during weight pyrolysis, is another fully organic compound that has gained attention as a natural alternative to certain synthetic inputs in agriculture [19]. Globally, an estimated 6,000 to 7,000 m-3 of WV is produced annually. This complex liquid contains various organic acids, ketones, aldehydes, alcohols, benzenes, phenols and their derivatives, alkyl phenyl ethers, and nitrogenous compounds [20, 21]. Research has confirmed the efficacy of WV not only for controlling fungal pathogens and insect pests but also for enhancing crop yield [22-25].

Vermicompost has also been shown to stimulate seed germination, promote plant growth, and improve tolerance to both biotic and abiotic stresses [13]. Due to its abundance of functional groups such as hydroxyl, carboxyl, and phenolic groups, vermicompost exhibits (VE) a strong affinity for binding heavy metals and has been reported as a promising agent for heavy metal remediation [26].

Physalis spp. is a member of the Solanaceae family and has garnered global attention for its bioactive compounds, broad cultivation potential, and multifunctional uses. Its fruit is valued for culinary, medicinal, and industrial applications [27, 28]. To date, approximately 130 *Physalis* varieties have been identified worldwide [29]. Among these, the purple (*Physalis ixocarpa*) and yellow (*Physalis peruviana*) species, both native to Mexico, are well known for their traditional medicinal uses [30, 31].

This study was conducted to compare the effects of seed priming using various organic and biowaste-based materials—including Pa, Ss, and saffron petal extract—alongside SA and vermicompost, on mitigating the adverse effects of Cd stress in two *Physalis species* (*Ph. ixocarpa* and *Ph. peruviana*).

MATERIALS AND METHODS

Experimental Design and Treatments

This experiment was conducted as a factorial experiment based on a completely randomized design (CRD) with four replications at the research laboratory of the University of Torbat Heydarieh. Prior to the main experiment, a preliminary study was performed to evaluate the response of different *Physalis* species to various Cd concentrations (0, 60, 120, 2270, 500, and 750 µM) to determine to determine the threshold level of stress [6].

The experimental factors included two levels of Cd stress (0 µM as control and 500 µM Cd), two *Physalis* species (*Physalis ixocarpa* and *Physalis peruviana*), and six seed priming treatments including no priming; hydropriming HP; salicylic acid (SA): 1 mM, vermicompost extract (VE): 10%, silk reeling effluent (SRE): 100%, wood vinegar (WV): 10%, and saffron petal extract (SPE): 10%. The concentrations of the applied substances were determined based on previous studies [7].

Table 1 Analysis eesults of the vermicompost

pH	EC	N	P	K	OC	OM
	ds/m	%	mg/kg	mg/kg		%
7.92	5.61	1.15	86	424	22.2	38.3

Table 2 Analysis eesults of the Silk reeling effluent

pH	EC	N	P	K	Ca	Mg	Na	Fe	Zn	B	OC
	ds/m	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%
7.91	5.38	0.0728	37.99	422.28	38.40	16.96	310	0.111	0.06	2.7	0.0923

Extraction of Substances

Extraction of vermicompost and saffron petal solutions was carried out using the aerobic method described by [19]. Organic samples (vermicompost and saffron petals) were first ground into a fine powder using an electric grinder. For extraction, 100 grams of each powdered sample were mixed with 1000 mL of distilled water. The mixtures were then incubated in a water bath with continuous stirring at 120 rpm for 30 minutes to 2 hours. After cooling to room temperature, the mixtures were first filtered through muslin cloth and then through Whatman No. 1 filter paper to remove solid particles. The clear extracts were stored at 4 °C until further use.

Seed Preparation and Planting

Seeds used in this study were purchased from Fardin Kesht Seed Company (Iran). Seeds were surface sterilized with 5% sodium hypochlorite solution for 60 seconds, then rinsed three times with distilled water. The sterilized seeds were soaked in each priming solution for 6 hours, as described by Feizi [32]. Subsequently, 25 seeds per treatment were placed in sterile Petri dishes lined with Whatman filter paper. Depending on the stress treatment, 5 mL of either distilled water or Cd sulfate (Merck, Germany; code 102027) solution (500 µM $CdSO_4$) was added to each dish. The Petri dishes were then incubated in a growth chamber set at 25 ± 0.5 °C with a 16/8-hour light/dark photoperiod for 15 days [33].

Measured Items and Methods

Germination Percentage

Germination percentage (GP) was calculated using the following equation [34]:

$GP\ (\%) = \left(\frac{GS}{TS}\right) \times 100$ (eq. 1)

Where GS is the number of seeds germinated by the end of the test, and TS is the total number of seeds sown in each petri dish.

Germination Rate

GR was calculated as:

$GR = \sum_{i=1}^{n} \frac{S_i}{D_i}$ (eq. 2)

Where GR is the germination rate (seeds day^{-1}), S_i is the number of seeds germinated on the ith day, and D_i is the corresponding day of counting [35].

Seedling Growth Indexes

Hypocotyl weight, radicle weight and seedling weight were measured on day 15 using a digital balance with 0.0001 g precision. Hypocotyl and radicle length were also measured on day 15 using a ruler.

Proline Content Measurements

Proline content was determined following the method of Bates [36]. Fresh leaf tissue (100 mg) was homogenized with 1 mL of 1 N salicylic sulfuric acid and centrifuged at 3000 rpm for 5 minutes. A 0.2 mL aliquot of the extract was mixed with 0.2 mL of ninhydrin reagent and 0.2 mL of glacial acetic acid in microtubes, then incubated in a water bath at 100 °C for 1 hour. Samples were frozen at -20 °C for 24 hours. After thawing, 0.6 mL of toluene was added and vortexed for 20 minutes. Proline concentration was determined using a standard curve generated from L-proline over a concentration range of 0–100 µg^{-1}mL. The upper phase was collected, and absorbance was measured at 520 nm using a spectrophotometer. Proline concentration was calculated using a standard curve and expressed as µg/g FW.

Estimating Photosynthetic Pigments

Photosynthetic pigments (chlorophyll a (cha), chlorophyll b (chb), total chlorophyll (cht), and carotenoids (ca)) were quantified using spectrophotometry. Fresh leaves (0.2 g) were homogenized with 4 mL of 80% acetone. After centrifugation, absorbance was measured at 664, 647, and 424 nm. Pigment concentrations were calculated using the following equations [37]:

$\mathrm{Chl\ a\ (mg\ L^{-1}fw)} = (12.25 \times \mathrm{A}_{663}) - (2.79 \times \mathrm{A}_{647})$ (eq. 3)

$\mathrm{Chl\ b\ (mg\ L^{-1}fw)} = (21.50 \times \mathrm{A}_{647}) - (5.10 \times \mathrm{A}_{663})$ (eq. 4)

$\mathrm{Carotenoids\ (mg\ L^{-1}fw)} = \frac{(1{,}000 \times \mathrm{A}_{424}) - (1.82\ \mathrm{Chl\ a}) - (85.02\ \mathrm{Chl\ b})}{198}$ (eq. 5)

ChT= Cla+Clb+Clc (eq. 6)

MDA

MDA content was measured to assess lipid peroxidation [38]. Fresh leaf tissue (0.2 g) was homogenized in 5 mL of 0.1% trichloroacetic acid (TCA) and centrifuged. The supernatant was reacted with 24% TCA containing 0.5% Thio barbituric acid, incubated at 95°C for 15 minutes, and cooled. Absorbance was recorded at 532 and 600 nm.

Activity of antioxidant enzymes

CAT

The CAT enzyme activity was examined by mixing 0.5 g of milled fresh leaves in an ice-cold solution with 50 mmol L^{-1} KPO_4-buffer (pH=7) and 10 g L^{-1} polyvinylpyrrolidone, and then centrifuged at 4 °C for 10 min at 10,000 × g using a UV-vis spectrophotometer (UNICO model 2100, USA) [39]. CAT activity in leaves was determined by monitoring the decomposition rate of hydrogen peroxide (H_2O_2) using the spectrophotometer. Homogenized leaf tissue was added to the mentioned buffered H_2O_2 solution. The decrease in H_2O_2 concentration over time, measured by absorbance at 240 nm, was directly proportional to CAT activity, and expressed as unit per mg protein.

APX

Enzyme extract preparation followed the protocol by Hazrati. [40]. Leaf samples (0.1 g) were ground in liquid nitrogen and extracted with 1.5 mL of ice-cold potassium phosphate buffer (100 mM, pH 7.8). After centrifugation at 15,000 rpm for 20 minutes at 4°C, the supernatant was used for enzyme activity assays. APX activity was measured by adding 50 µL of enzyme extract to 2.5 mL of reaction buffer containing Tris (100 mM), hydrogen peroxide (5 mM), and pyrogallol (10 mM). Absorbance was recorded at 425 nm.

Seed vigor

The seed vigor was calculated using the following formula [41]:

$$SV = \mathbf{S}\text{eedling length} \times \text{germination percentage (eq. 7)}$$

Stress Tolerance Index (STI)

This index was calculated based on seedling weight using the following formula [42]:

$$STI = \frac{(Y_P)(Y_S)}{\bar{Y}_S^2} \qquad \text{(eq. 8)}$$

In the above equation, Ys is the seedling weight under stress conditions, Yp is the seedling weight under non-stress conditions, and the average seedling weight of species under each condition is considered accordingly.

Statistical Analysis

Data was analyzed using SAS software (version 9.4) based on the experimental design. Mean comparisons were performed based on slicing using Tukey's HSD test at a 5% probability level. Figures were drawn using Sigma Plot software (version 12.0).

RESULTS

Germination Indices

Germination Rate

In *Physalis ixocarpa*, Cd stress enhanced germination rate across all priming treatments (Figure 1). For instance, seed priming with SRE under Cd stress increased the germination rate of *Ph. ixocarpa* by 25% compared to the non-stress condition. Conversely, in *Physalis peruviana*, Cd stress reduced germination rate in all priming treatments except for priming with SA.

Under no Cd stress, the highest germination rate in *Ph. ixocarpa* was observed in seed priming with SRE (3.74 seeds/day) and SPE (3.49 seeds/day). Notably, seed priming with WV in *Ph. ixocarpa* not only failed to improve germination rate but also caused a significant decrease compared to non-primed seeds. In *Ph. peruviana*, HP resulted in the highest germination rate under non-stress conditions (4.47 seeds/day), representing approximately a 27% increase compared to non-primed seeds. Priming with SRE showed no substantial difference from the control in this species, whereas all other priming treatments led to a significant reduction in germination rate, with WV priming causing the most pronounced decrease (78% reduction compared to non-primed seeds).

Under Cd stress conditions, seed priming with SPE (5.60 seeds/day), VE (5.52 seeds/day), and SA (5.14 seeds/day) resulted in the highest germination rates in *Physalis ixocarpa*, with no statistically notable differences among these treatments. These priming methods increased germination rate by 57%, 55%, and 45%, respectively, compared to non-primed seeds under this condition. Under Cd stress, priming the *Ph. peruviana* seeds with SA led to the highest germination rate (4.11 seeds/day). WV consistently resulted in the lowest germination rates across both *Physalis species* and stress levels, showing a considerable negative effect compared to non-primed seeds.

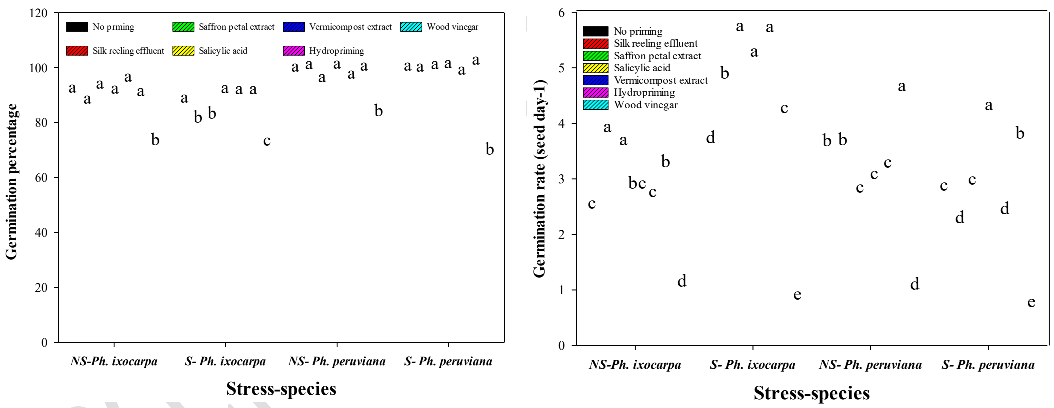


Fig. 1 Effect of Cd stress, *physalis* species and seed priming on germination rate and percentage of *Astragalus fasciculifolius* seed. Means with same letters as slicing are not significantly different by Tukey test ($p< 0.05$).

Germination Percentage

Under Cd stress, *Ph. Ixocarpa* seeds primed with SA (90%), VE (88.7%), and water (88.7%) had the highest germination percentages, with no significant difference from non-primed seeds (86.25%) (Figure 1). In *Ph. Peruviana*, all priming treatments except WV did not significantly differ from the control under Cd stress. Seed priming with WV resulted in the lowest germination percentages across both *Physalis* species and stress treatments.

Seedling Growth Indices

Hypocotyl Length

Overall, across both stress levels and different priming treatments, *Ph. ixocarpa* exhibited longer seedling Hypocotyl compared to *Ph. peruviana* (Figure 2). For example, under Cd stress without priming, the Hypocotyl length of *Ph. ixocarpa* (2.5 cm) was approximately triple that of *Ph. peruviana* (0.75 cm). Cd stress markedly decreased hypocotyl length in both *Ph. ixocarpa* and *Ph. peruviana* compared to non-stress conditions.

Under non-stress conditions, seed priming with VE and water led to the greatest increases in Hypocotyl length for *Ph. ixocarpa*, with respective increases of 105% and 95% compared to non-primed seeds. In *Ph. peruviana*, seed priming with SA produced the longest seedling Hypocotyls under normal conditions, enhancing Hypocotyl length by 35.49% relative to the control. HP ranked second in this regard.

Under non-stress conditions, *Ph. peruviana* seeds primed with VE (2.55 cm), SPE (2.52 cm), and SRE (2.62 cm) showed no significant difference in Hypocotyl length compared to non-primed seeds (2.62 cm).

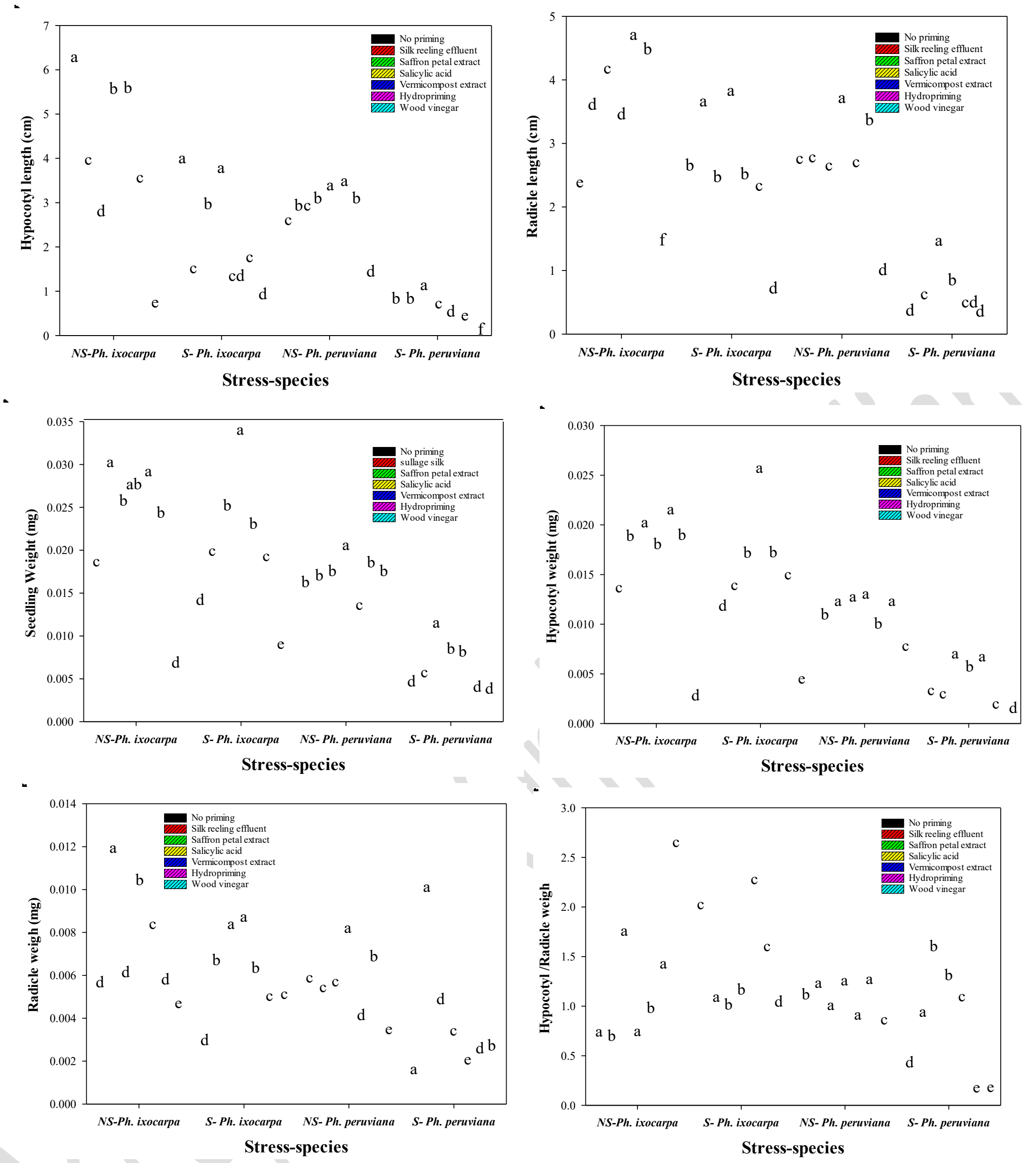


Fig. 2 Effect of Cd stress, physalis species and seed priming on seedling growth parameters of Astragalus fasciculifolius. Means with same letters as slicing are not significantly different by Tukey test ($p < 0.05$).

In Cd stress conditions, *Ph. ixocarpa* seeds primed with SA (3.62 cm) and SRE (3.5 cm) exhibited the highest hypocotyl lengths without sizable differences between them, increasing hypocotyl length by 45% and 42%, respectively, compared to non-primed seeds under stress. Conversely, priming with HP (2.2 cm), VE (2.3 cm), and SPE (2.3 cm) reduced hypocotyl length by 12%, 8%, and 8%, respectively, relative to non-primed seeds. In *Ph. peruviana*, priming with SPE (1.32 cm) under Cd stress resulted in the greatest hypocotyl length, approximately six times longer than that of non-primed seeds in the same condition. Seed priming with WV not only failed to improve hypocotyl length in *Physalis* under both stress and non-stress conditions, but also led to a reduction compared to the non-primed control in both cultivars.

Radicle Length

On average, *Ph. ixocarpa* exhibited longer radicle s than *Ph. peruviana* across all treatments. Cd stress reduced radicle length in both species and across all priming treatments, except for *Ph. ixocarpa* seeds primed with SPE (2.72 cm) (Figure 2).

For example, in *Ph. peruviana* without priming, Cd stress caused a 79% reduction in radicle length compared to non-stress conditions. Under non-stress conditions, seed priming with SRE resulted in the longest radicle s in *Ph. ixocarpa*, increasing radicle length by 76% compared to non-primed seeds. Priming with SA and VE followed, showing no significant difference.

In *Ph. peruviana*, the greatest radicle length s under non-stress were observed with VE (3.22 cm) and SA (3.33 cm).

Under Cd stress, priming *Ph. ixocarpa* seeds with SRE (3.75 cm) and SA (3.5 cm) significantly increased radicle length by 184% and 165%, respectively. In *Ph. peruviana*, Cd stress had the least impact on radicle length when seeds were primed with SPE (0.9 cm), which increased radicle length by approximately 80% compared to non-primed seeds under stress. Across all conditions and both species, seed priming with WV consistently decreased radicle length relative to non-primed controls.

Seedling Weight

On average, seedling weight of *Ph. ixocarpa* exceeded that of *Ph. peruviana*. For instance, under non-stress and non-primed conditions, the weight of Ph. ixocarpa seedlings was approximately 21% higher than that of *Ph. Peruviana* (Figure 2). Cd stress meaningfully reduced seedling weight in both species across all priming treatments compared to the non-stress control.

Under non-stress conditions, the highest seedling weight in *Ph. ixocarpa* was observed with priming using SRE (0.029 g) and VE (0.028 g), with no statistically significant difference between these treatments. In *Ph. peruviana*, seed priming with SA yielded the greatest weight (0.019 g), representing a 36% increase compared to non-primed seeds.

Under Cd stress, SA priming approximately doubled the seedling weight of *Ph. ixocarpa* relative to non-primed seeds, achieving the highest weight recorded for this species under stress. In *Ph. peruviana*, priming with SPE (0.010 g) produced the greatest seedling weight under Cd stress, increasing weight by 233% compared to non-primed seeds. Seed priming with Pa consistently decreased seedling weight in both species under all conditions compared to controls.

Hypocotyl Weight

Across all priming treatments and Cd levels, *Ph. ixocarpa* exhibited higher Hypocotyl weight than *Ph. peruviana*. Cd stress markedly reduced Hypocotyl weight in both species across all priming treatments compared to the non-stress control (Figure 2).

Under normal conditions, *Ph. ixocarpa* seedlings primed with SPE (1.8 mg) and VE (2 mg) showed the highest hypocotyl weight, with no significant difference between them. These treatments increased hypocotyl weight by 50% and 67%, respectively, compared to non-primed controls. In *Ph. peruviana* under this condition, the highest hypocotyl weights were observed in seeds primed with SRE (1.1 mg), SPE (1.1 mg), SA (1.1 mg), and HP (1 mg).

Under Cd stress, *Ph. ixocarpa* seeds primed with SA produced the highest hypocotyl weight (2.4 mg), corresponding to a 140% increase compared to non-primed seeds. In *Ph. peruviana*, priming with SPE (5.7 mg) and VE (5.4 mg) resulted in the greatest hypocotyl weight, with increases of 200% and 184%, respectively. WV priming consistently reduced hypocotyl weight in both species across all treatments.

Radicle Weight

The results showed that *Ph. ixocarpa* had higher radicle weight than *Ph. Peruviana* in both stress and no stress conditions (Figure 2). For example, under Cd stress without priming, radicle weight in *Ph. ixocarpa* was 108% higher than in *Ph. peruviana*.

Under non-stress conditions, the highest radicle weight (1.1 mg) in *Ph. ixocarpa* was achieved by priming with SRE, representing a 112% increase relative to non-primed seeds. The lowest radicle weight under non-stress irrigation was recorded in *Ph. ixocarpa* seeds primed with WV (0.43 mg). In *Ph. peruviana*, the maximum radicle weight (0.78 mg) under non-stress was observed with SA priming, which increased radicle weight by 37% compared to non-primed seeds. Under this condition, priming *Ph. peruviana* seeds with WV (0.50 mg) and SPE (0.53 mg) did not significantly differ from non-primed seeds (0.054 mg) in radicle weight.

Under Cd stress, *Ph. ixocarpa* seeds primed with SPE (0.8 mg) and SA (0.84 mg) exhibited the highest radicle weight without notable differences. Non-primed *Ph. ixocarpa* seeds showed the lowest radicle weight (0.25 mg) under Cd stress Seed priming with SRE (0.97 mg) resulted in a sevenfold increase in radicle weight compared with the non-primed control in *Physalis peruviana* under Cd stress.

Hypocotyl -to-Radicle Ratio (S/R)

Under non-stress, *Ph. Ixocarpa* WV priming produced the highest S/R (2.53); SRE and SA yielded values similar to controls (≈0.56–0.61) (Figure 2). In *Ph. Peruviana*, SRE, SA, and water priming resulted in the highest S/R (~1.00–1.13), with no significant difference from non-primed controls (0.98). Under Cd stress, WV priming in *Ph. Ixocarpa* gave the highest S/R (2.14), similar to controls (1.89). SRE, SPE, SA, and Pa treatments in *Ph. Ixocarpa* produced lower S/R (0.86–1.03). In *Ph. Peruviana* under stress, SPE priming gave the highest S/R (1.47), while WV produced the lowest.

Photosynthetic Pigments

Chlorophyll a Content

In *Ph. ixocarpa* under non-stress conditions, the highest chlorophyll a content (0.16 mg/g) was found in seeds primed with SRE, representing a 167% increase relative to non-primed seeds (Figure 3). SA (0.097 mg/g) and SPE (0.092 mg/g) also significantly increased chlorophyll a by 59% and 51% compared to control, respectively. WV priming resulted in the lowest chlorophyll a content (0.004 mg/g) under non-stress condition. In this condition, priming *Ph. peruviana* seeds with WV produced the highest chlorophyll a content (0.14 mg/g), corresponding to a 56% increase relative to non-primed seeds. The lowest chlorophyll a content under non-stress was observed in seeds primed with WV (0.006 mg/g), representing a 93.33% reduction.

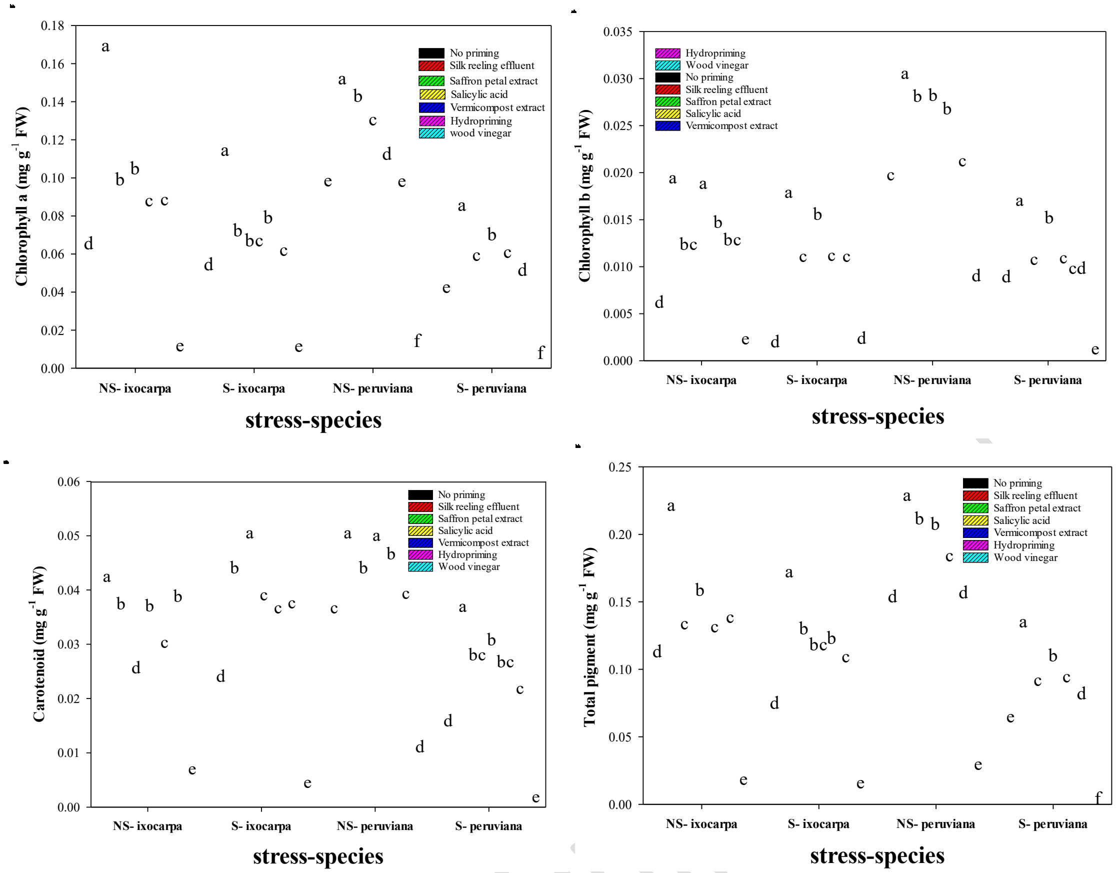


Fig. 3 Effect of Cd stress, physalis species and seed priming on photosynthesis piments contents of Astragalus fasciculifolius. Means with same letters as slicing are not significantly different by Tukey test ($p < 0.05$).

Under Cd stress, *Ph. ixocarpa* seeds primed with SRE exhibited the highest chlorophyll a content (0.10 mg/g). Chlorophyll a level in seeds primed with SPE (0.06 mg/g), SA (0.05 mg/g), and VE (0.07 mg/g) did not differ meaningfully. The lowest chlorophyll a content under Cd stress was observed with WV priming (0.002 mg/g). In *Ph. peruviana*, SRE priming under Cd stress increased chlorophyll a by 123% compared to non-primed seeds, yielding the highest chlorophyll a content in this condition.

Chlorophyll b Content

Under most seed priming treatments and across both *Physalis* species, Cd stress caused a notable reduction in chlorophyll b levels (Figure 3). In *Ph. ixocarpa*, the highest chlorophyll b content under Cd stress was observed with SRE priming (0.016 mg/g). Under non-stress conditions, *Ph. ixocarpa* showed the highest chlorophyll b content with both SRE (0.018 mg/g) and SA priming (0.033 mg/g), which represented increases of 2.5 and 2.4 times, respectively, compared to non-primed seeds. Conversely, priming with WV resulted in the lowest chlorophyll b content (0.001 mg/g) under non-stress conditions in *Ph. ixocarpa*. Under Cd stress, *Ph. ixocarpa* seeds primed with either no treatment or WV exhibited the lowest chlorophyll b content (0.001 mg/g).

In *Ph. peruviana*, the highest chlorophyll b content under non-stress conditions was recorded with priming treatments using SPE (0.027 mg/g), SA (0.027 mg/g), VE (0.025 mg/g), and SRE (0.029 mg/g), with no significant differences among them. Under Cd stress, SA (0.014 mg/g) and SRE (0.016 mg/g) priming resulted in the highest chlorophyll b contents in *Ph. peruviana*. WV priming caused the greatest reduction in chlorophyll b in this species, with decreases of 56% and 100% under stress and non-stress conditions, respectively, compared to non-primed seeds.

Carotenoid Content

In *Ph. ixocarpa*, the highest carotenoid content (0.041 mg/g) was observed under non-stress and no priming. Under non-stress condition, priming the seeds with SRE (0.036 mg/g), SA (0.034 mg/g), and HP (0.036 mg/g) without substantial differences were contained the highest carotenoid content (Figure 3). In *Ph. peruviana*, the maximum carotenoid content under non-stress was detected in seeds primed with SRE (0.049 mg/g) and SA (0.047 mg/g), representing increases of 40% and 34%, respectively, compared to non-primed seeds.

Under Cd stress, *Ph. ixocarpa* seeds primed with SPE exhibited the highest carotenoid content (0.048 mg/g), corresponding to a 54% increase relative to non-primed seeds. In *Physalis peruviana*, the highest carotenoid content under Cd stress was observed in the SRE priming treatment (0.035 mg/g), representing an approximately 40% increase relative to the non-stress condition. WV priming consistently resulted in the lowest carotenoid levels across both species and conditions; for example, in *Ph. ixocarpa* under non-stress, WV priming reduced carotenoid content by 91%.

Total Pigment Content

Under non-stress conditions, SRE priming led to the highest total pigment content in *Ph. ixocarpa* (0.21 mg/g), resulting in a 110% increase compared to non-primed seeds (Figure 3). In *Ph. peruviana*, the highest total pigment content under non-stress was also observed with SRE priming (0.22 mg/g), corresponding to a 57% increase over the control. Under Cd stress, *Ph. ixocarpa* seeds primed with SRE showed the highest total pigment content (0.16 mg/g), a 167% increase compared to non-primed seeds. Priming with SPE (0.12 mg/g) and SA (0.11 mg/g) also significantly elevated total pigment content under stress in *Ph. ixocarpa*, by 100% and 83%, respectively. For *Ph. peruviana*, SRE priming resulted in the highest pigment content (0.11 mg/g) under Cd stress. WV priming caused a significant decrease in total pigment content across all treatments, species, and stress levels.

Proline Content

Cd stress induced a significant increase in proline accumulation across all priming treatments and both species (Figure 4). For instance, in *Ph. ixocarpa* without priming, proline content under Cd stress were 2.5 times higher than under non-stress conditions. In *Ph. ixocarpa*, the highest proline content was recorded in seeds primed with WV under both stress (40.03 µg/g) and non-stress (27.36 µg/g) conditions. Under non-stress conditions in *Ph. peruviana*, priming with SPE (6.01 µg/g), VE (6.15 µg/g), and HP (5.2 µg/g) showed no significant difference compared to non-primed seeds (5.34 µg/g). The lowest proline content under non-stress in *Ph. ixocarpa* was observed with SRE (8.18 µg/g) and SA (7.98 µg/g) priming. Under Cd stress, SA priming included the lowest proline levels in *Ph. ixocarpa* (13.25 µg/g), representing a 59% reduction compared to non-primed stressed seeds.

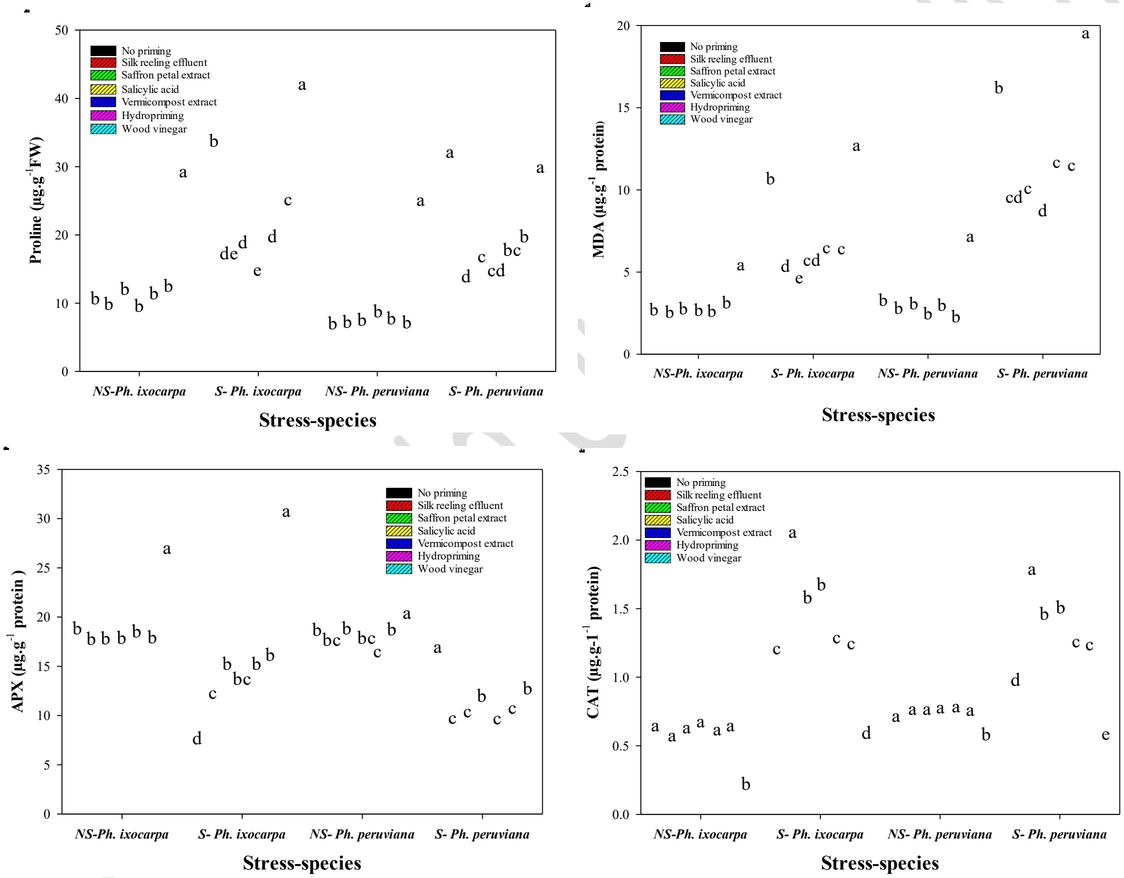


Fig. 4 Effect of Cd stress, physalis species and seed priming on enzymes activity, and malondialdehyde (MDA) and proline contents of Astragalus fasciculifolius. Means with same letters as slicing are not significantly different by Tukey test ($p< 0.05$).

In *Ph. peruviana*, the highest proline content under non-stress was observed in WV-primed seeds (23.57 µg/g), indicating a 3.4-fold increase relative to non-primed seeds. Priming with SRE (5.53 µg/g), SPE (6.01 µg/g), VE (6.15 µg/g), and HP (5.2 µg/g) did not significantly affect proline content compared to the control. Under Cd stress, the highest proline content in *Ph. peruviana* was recorded in WV primed seeds (28.53 µg/g) and non-primed seeds (29.95 µg/g). SRE (12.14 µg/g) and SA (13.24 µg/g) priming resulted in the lowest proline accumulation under stress in this species.

Malondialdehyde (MDA) Activity

In both *Physalis* species, MDA activity was higher under stress conditions than under non-stress conditions across all seed priming treatments (Figure 4). The highest MDA content in Ph. ixocarpa under non-stress conditions was recorded in the WV treatment (81.4 Unit mg-1 protein), indicating that seed priming with WV increased MDA activity by 152% compared to the control. Seed priming with all other treatments, except WV, exhibited the lowest MDA activity under non-stress conditions, with no statistically significant differences among them. In *Ph. ixocarpa*, the highest MDA content under Cd stress was observed in the WV seed priming treatment (111.2 Unit mg-1 protein). The lowest MDA level in this species under non-stress conditions was recorded in SPE priming (25.33 Unit mg-1 protein), indicating that seed priming with SPE increased MDA content by 46% compared to the control. Under stress conditions in *Ph. peruviana*, the highest MDA content was detected in the WV treatment (187.18 Unit mg-1 protein), whereas the lowest value (80.1 Unit mg-1 protein) was observed in the SA treatment.

Ascorbate Peroxidase (APX) Activity

In all treatments and in both *Physalis* species, Cd stress led to a reduction in APX activity compared with non-stress conditions (Figure 4). The highest APX activity (48.25 Unit mg-1 protein) under non-stress conditions in *Ph. ixocarpa* was observed in the WV seed priming treatment, which resulted in a 46% increase compared to the control. In *Ph. ixocarpa*, APX activity in the no-priming (33.44 Unit mg-1 protein), SRE (16.40 Unit mg-1 protein), SPE (16.56 Unit mg-1 protein), SA (16.60 Unit mg-1 protein), VE (33.25 Unit mg-1 protein), and HP (16.56 Unit mg-1 protein) treatments was significantly lower than in the WV treatment. In *Ph. peruviana*, the highest APX activity under non-stress conditions was recorded in the WV treatment (19.13 Unit mg-1 protein), which increased the enzyme activity by 10% compared to the control. The greatest reduction in APX activity under non-stress conditions in *Ph. peruviana* was observed in the VE priming treatment (15.21 Unit mg-1 protein). Under Cd stress conditions in *Ph. ixocarpa*, all seed priming treatments meaningly increased APX activity compared with the non-primed control. In this species, the lowest APX activity under Cd stress was observed in the non-primed seeds (6.41 Unit mg-1 protein), while the highest activity (29.41 Unit mg-1 protein) was recorded in the WV priming treatment, representing a 3.5-fold increase compared to the control. In *Ph. peruviana*, the highest APX activity under stress conditions was observed in the non-primed seeds (15.78 Unit mg-1 protein), and all seed priming treatments resulted in a reduction in APX activity under stress. Among these treatments, the lowest APX activity was recorded in the SRE (8.37 Unit mg-1 protein) and VE (8.42 Unit mg-1 protein) priming treatments**.**

Catalase (CAT) Activity

Cd stress increased CAT activity across all seed priming treatments in both *Physalis* species (Figure 4). Under non-stress conditions, no significant differences were observed among the non-primed control and the priming treatments with SRE, SPE, SA, VE, and HP, all of which showed the highest CAT activity in both species. Seed priming with WV reduced CAT activity by 76% and 19% compared with the non-primed control under non-stress conditions in *Ph. ixocarpa* and *Ph. peruviana*, respectively. Under Cd stress conditions, the highest CAT activity in *Ph. ixocarpa* was observed in the SRE priming treatment (1.98 Unit mg-1 protein), where priming with SRE increased CAT activity by 74% compared to the control. In *Ph. peruviana*, the highest CAT activity under Cd stress was also recorded in the SRE priming treatment (1.72 Unit mg-1 protein). In both *Physalis* species, the lowest CAT activity under stress was observed in the WV priming treatment, which reduced CAT activity by 43% and 55% in *Ph. peruviana* and *Ph. ixocarpa*, respectively, compared with the control.

Seedling Vigor

Across all priming treatments and in both *Physalis* species, seed vigor was lower under cadmium stress compared to non-stress conditions (Figure 5). For instance, in *Ph. Peruviana*, the seed vigor of non-primed seeds under stress was 98% lower than under non-stress conditions. The highest seed vigor in *Ph. Ixocarpa* under non-cadmium stress was observed with VE priming (11.26), representing a twofold increase compared to the non-primed control. In *Ph. Peruviana*, the highest seed vigor under non-stress conditions was obtained with SA priming (5.56). Under non-stress conditions, WV priming resulted in the lowest seed vigor in both species. Under cadmium stress, the highest seed vigor in *Ph. Ixocarpa* was recorded with SRE (5.08) and SA (5.72) priming, with no statistically significant difference between them. These treatments increased seed vigor by 257% and 303%, respectively, compared to the non-primed control. The lowest seed vigor for *Ph. Ixocarpa* under stress was found with WV priming (0.14). In *Ph. Peruviana*, SPE priming resulted in the highest seed vigor under cadmium stress (0.59).

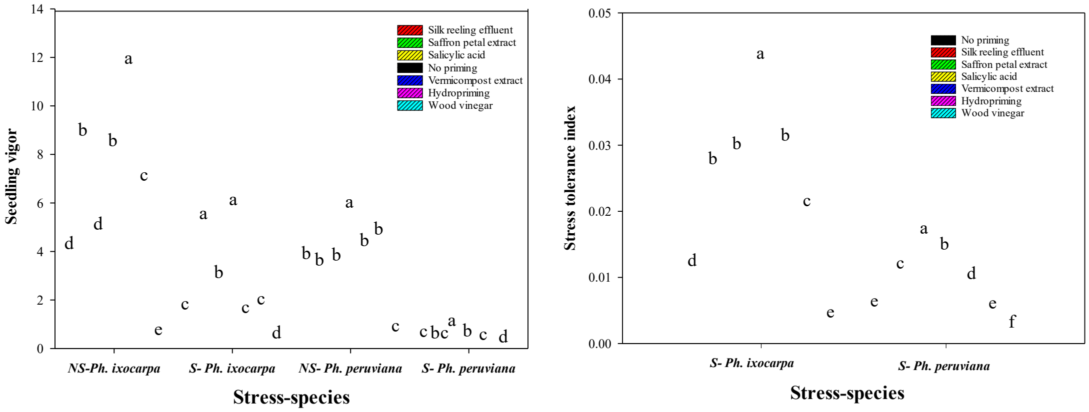


Fig. 5 Effect of Cd stress, physalis species and seed priming on seed vigor and stress tolerance index (STI) of Astragalus fasciculifolius. Means with same letters as slicing are not significantly different by Tukey test (p< 0.05).

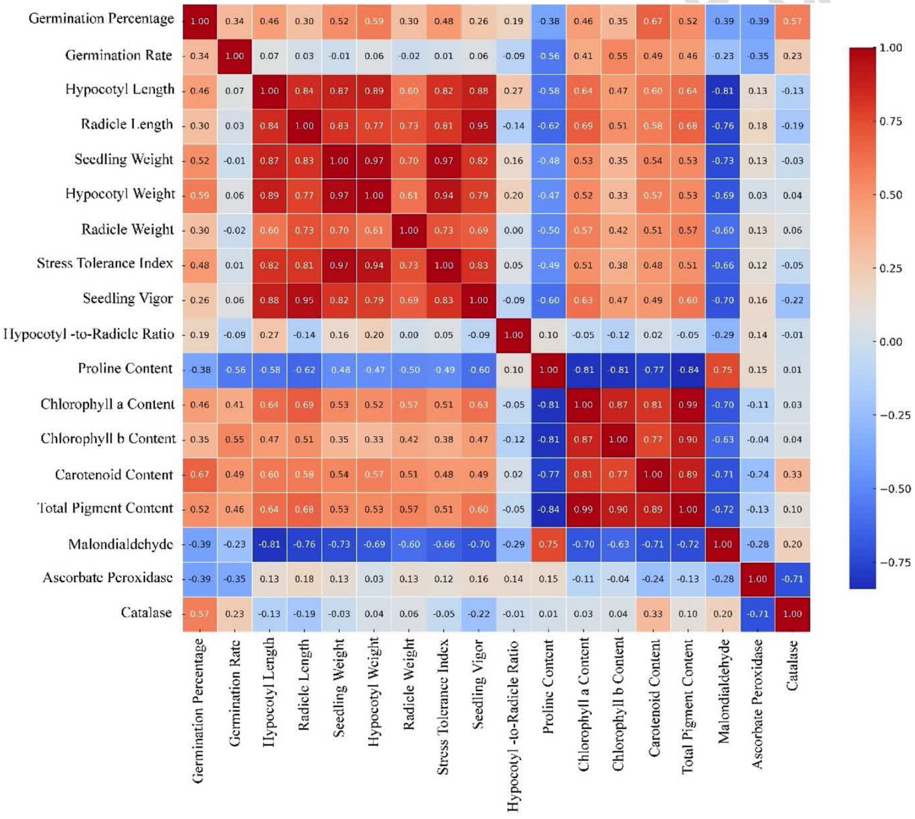
Stress Tolerance Index (STI)

Generally, the STI in *Ph. ixocarpa* was higher than in *Ph. peruviana* under all seed priming conditions. In *Ph. ixocarpa*, seed priming with SA resulted in a threefold increase in the STI compared to non-primed seeds (Figure 5). The lowest STI in *Ph. ixocarpa* was observed with WV priming (0.033). The application of SPE (0.021), VE (0.021), and SRE (0.016) enhanced the cadmium stress tolerance of *Ph. ixocarpa* by 268%, 268%, and 180%, respectively, compared to the control (non-primed seeds). In *Ph. peruviana*, priming with SPE (0.013) showed the highest STI value, leading to approximately a threefold increase in the index under stress conditions compared to non-primed seeds. The lowest STI in *Ph. peruviana* under stress conditions was recorded with WV priming (0.0008). The cadmium stress tolerance of *Ph. peruviana* seedlings under seed priming with SPE and SRE was increased by 4-fold and 3-fold, respectively, compared to the non-primed condition.

Pearson Correlation Analysis

Pearson correlation analysis revealed that seed vigor exhibited the strongest positive correlations with germination rate (r = 0.88) and radicle length (r = 0.95) (Figure 6). In contrast, negative correlations were observed between seed vigor and radicle-to-hypocotyl weight ratio (r = –0.09), proline content (r = –0.60), MDA content (r = –0.24), and CAT activity (r = –0.22). Seedling fresh weight was also negatively correlated with proline (r = –0.48) and MDA levels (r = –0.73), indicating that higher accumulation of these stress markers coincided with reduced growth under stress conditions. Strong positive correlations were observed between the stress tolerance index and seedling weight (r = 0.97), as well as hypocotyl weight (r = 0.97). while proline (r = –0.49), MDA (r = –0.68), and CAT activity (r = –0.05) were negatively correlated with STI. These findings suggest that greater accumulation of stress-related compounds occurred in plants experiencing more severe stress and lower tolerance.

Among biochemical traits, proline showed the strongest positive correlation with MDA (r = 0.75), confirming their co-accumulation under stress. Moreover, proline displayed significant negative correlations with key growth and physiological traits, including germination percentage (r = –0.38), germination rate (r = –0.56), hypocotyl length (r = –0.58), radicle length (r = –0.62), seedling weight (r = –0.48), hypocotyl weight (r = –0.47), radicle weight (r = –0.50), STI (r = –0.49), seed vigor (r = –0.60), chlorophyll a (r = –0.81), chlorophyll b (r = –0.81), carotenoids (r = –0.77), and total pigments (r = –0.84). Interestingly, MDA content exhibited a positive correlation only with proline (r = 0.75), and negative correlations with most other traits, highlighting its role as a marker of oxidative damage and stress severity.



	Germination Percentage	Germination Rate	Hypocotyl Length	Radicle Length	Seedling Weight	Hypocotyl Weight	Radicle Weight	Stress Tolerance Index	Seedling Vigor	Hypocotyl -to-Radicle Ratio	Proline Content	Chlorophyll a Content	Chlorophyll b Content	Carotenoid Content	Total Pigment Content	Malondialdehyde	Ascorbate Peroxidase	Catalase
Germination Percentage	1.00	0.34	0.46	0.30	0.52	0.59	0.30	0.48	0.26	0.19	-0.38	0.46	0.35	0.67	0.52	-0.39	-0.39	0.57
Germination Rate	0.34	1.00	0.07	0.03	-0.01	0.06	-0.02	0.01	0.06	-0.09	-0.56	0.41	0.55	0.49	0.46	-0.23	-0.35	0.23
Hypocotyl Length	0.46	0.07	1.00	0.84	0.87	0.89	0.60	0.82	0.88	0.27	-0.58	0.64	0.47	0.60	0.64	-0.81	0.13	-0.13
Radicle Length	0.30	0.03	0.84	1.00	0.83	0.77	0.73	0.81	0.95	-0.14	-0.62	0.69	0.51	0.58	0.68	-0.76	0.18	-0.19
Seedling Weight	0.52	-0.01	0.87	0.83	1.00	0.97	0.70	0.97	0.82	0.16	-0.48	0.53	0.35	0.54	0.53	-0.73	0.13	-0.03
Hypocotyl Weight	0.59	0.06	0.89	0.77	0.97	1.00	0.61	0.94	0.79	0.20	-0.47	0.52	0.33	0.57	0.53	-0.69	0.03	0.04
Radicle Weight	0.30	-0.02	0.60	0.73	0.70	0.61	1.00	0.73	0.69	0.00	-0.50	0.57	0.42	0.51	0.57	-0.60	0.13	0.06
Stress Tolerance Index	0.48	0.01	0.82	0.81	0.97	0.94	0.73	1.00	0.83	0.05	-0.49	0.51	0.38	0.48	0.51	-0.66	0.12	-0.05
Seedling Vigor	0.26	0.06	0.88	0.95	0.82	0.79	0.69	0.83	1.00	-0.09	-0.60	0.63	0.47	0.49	0.60	-0.70	0.16	-0.22
Hypocotyl -to-Radicle Ratio	0.19	-0.09	0.27	-0.14	0.16	0.20	0.00	0.05	-0.09	1.00	0.10	-0.05	-0.12	0.02	-0.05	-0.29	0.14	-0.01
Proline Content	-0.38	-0.56	-0.58	-0.62	-0.48	-0.47	-0.50	-0.49	-0.60	0.10	1.00	-0.81	-0.81	-0.77	-0.84	0.75	0.15	0.01
Chlorophyll a Content	0.46	0.41	0.64	0.69	0.53	0.52	0.57	0.51	0.63	-0.05	-0.81	1.00	0.87	0.81	0.99	-0.70	-0.11	0.03
Chlorophyll b Content	0.35	0.55	0.47	0.51	0.35	0.33	0.42	0.38	0.47	-0.12	-0.81	0.87	1.00	0.77	0.90	-0.63	-0.04	0.04
Carotenoid Content	0.67	0.49	0.60	0.58	0.54	0.57	0.51	0.48	0.49	0.02	-0.77	0.81	0.77	1.00	0.89	-0.71	-0.24	0.33
Total Pigment Content	0.52	0.46	0.64	0.68	0.53	0.53	0.57	0.51	0.60	-0.05	-0.84	0.99	0.90	0.89	1.00	-0.72	-0.13	0.10
Malondialdehyde	-0.39	-0.23	-0.81	-0.76	-0.73	-0.69	-0.60	-0.66	-0.70	-0.29	0.75	-0.70	-0.63	-0.71	-0.72	1.00	-0.28	0.20
Ascorbate Peroxidase	-0.39	-0.35	0.13	0.18	0.13	0.03	0.13	0.12	0.16	0.14	0.15	-0.11	-0.04	-0.24	-0.13	-0.28	1.00	-0.71
Catalase	0.57	0.23	-0.13	-0.19	-0.03	0.04	0.06	-0.05	-0.22	-0.01	0.01	0.03	0.04	0.33	0.10	0.20	-0.71	1.00

Fig. 6 Pearson Correlation Matrix (heatmap) of morphological, physiological, and biochemical characteristics of *Physalis* species in Cd and priming treatments

Principal Component Analysis (PCA)

The results of principal component analysis (PCA) indicated that the combined effects of seed priming treatments and Cd stress led to a clear separation of samples along the principal component axes. The first two components together explained 69.4% of the total variance (Figure 7). The first principal component (PC1), which accounted for the largest portion of variance (52.8%), was primarily associated with seedling growth traits and stress-responsive physiological indicators, including antioxidant enzyme activities (CAT, APX), MDA content, and proline accumulation. Under non-stress conditions, seed priming with SRE, WV, SPE, SA, VE, and HP had positive effects on traits Hypocotyl length, radicle length, Hypocotyl weight, radicle weight, seedling weight, STI and seed vigor in *Ph. ixocarpa*. In contrast, in *Ph. peruviana*, the priming treatments of VE, SPE, and SA predominantly influenced traits Germination percentage, Germination rate Hypocotyl length, radicle length, Hypocotyl weight, radicle weight, seedling weight, STI, seed vigor, H/R, Proline**,** cha and MDA. HP exhibited a pattern similar to that of the unprimed control, with limited impact on most traits. Among all treatments, seed priming with WV displayed a distinct effect across both species and stress levels. It had minimal influence on most traits and predominantly affected only proline content and, to a lesser extent, APX activity.

Under Cd stress, seed priming with SA positively influenced the growth parameters of *Ph. ixocarpa*. Meanwhile, traits Germination percentage**,** Germination rate, Chlorophyll a, Chlorophyll b, Carotenoid and MDA in this species were most affected by priming with VE, SPE, and SRE. APX activity in *Ph. peruviana* under Cd stress responded to all priming treatments except WV, while in *Ph. ixocarpa*, the enzyme's activity was more prominently influenced by HP and the unprimed control. The distribution of ecotypes within the PCA biplot also revealed that *Ph. ixocarpa* exhibited higher tolerance to Cd stress compared to *Ph. peruviana*.

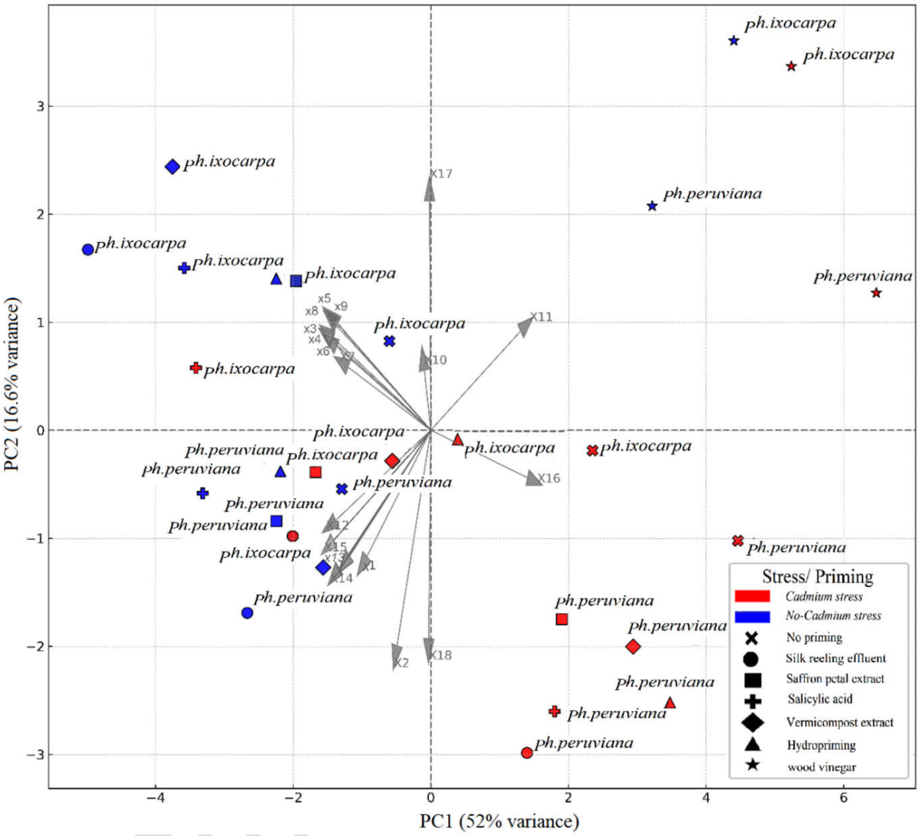


Fig. 7 Biplot of the PCA results in discrimination of treatments and variables

(X1: Germination percentage, X2: Germination rate; X3: Hypocotyl length, X4: radicle length, X5: seedling weight, X6: Hypocotyl weight, X7: radicle weight, X8: STI, X9: SVI, X10: Hypocotyl/ radicle weight, X11: Proline content, X12: chlorophyll a, X13: chlorophyll b, X14: carotenoids, X15: MDA, X16: APX, X33: CAT, X18: Total Pigment Content)

DISCUSSION

The results indicated that exposing *Physalis* seeds to 500 µM Cd stress had no significant effect on the germination percentage of either *Ph. ixocarpa* or *Ph. peruviana*. However, other parameters such as germination rate, seedling growth, and biochemical characteristics were significantly affected. In other words, although the seeds successfully initiated germination under Cd stress, their subsequent growth was impaired. This discrepancy may be attributed to the differing physiological requirements between the initial germination phase and later stages of seedling development [33]. Germination percentage, typically measured based on radicle emergence, largely depends on the activity of hydrolytic enzymes and the mobilization of pre-stored reserves within the seed. These processes are generally less sensitive to direct interference by heavy metals [15]. Hence, the embryo is often capable of completing germination even under Cd stress through reliance on its intrinsic resources and early defense mechanisms. Nevertheless, post-germination development encompassing cell division and differentiation, protein synthesis, and elongation of radicle and Hypocotyl tissues requires the coordinated activity of enzymatic system and the maintenance of ionic and osmotic balance. Cd can disrupt these processes, thereby hindering normal seedling growth and altering biochemical indices, even when germination has occurred successfully [15].

The findings also demonstrated species-specific responses to Cd stress. In *Ph. peruviana*, Cd exposure led to a reduction in germination rate, whereas *in Ph. ixocarpa*, a considerable increase in germination rate was observed. This suggests genetic variation in tolerance or physiological responses to heavy metal stress between the two *Physalis* species [43, 44]. The reduced germination rate in *Ph. peruviana* may be attributed to its higher sensitivity to Cd toxicity, potentially due to disruptions in enzyme activity, water uptake, or ionic homeostasis [45]. Conversely, the enhanced germination rate in *Ph. ixocarpa* may indicate the activation of compensatory or more efficient defense mechanisms. This response could involve stimulation of antioxidant pathways, improved regulation of signaling networks, or restricted translocation of Cd to sensitive tissues [46]. Similar findings by Lux [45] emphasize that interspecific variation in Cd tolerance may arise from differences in cellular architecture, metabolic capacity, expression of metal tolerance-related genes, or ion transport efficiency.

Moreover, Cd stress significantly reduced seedling growth traits such as Hypocotyl length, radicle length, Hypocotyl weight, and radicle weight in both *Physalis* species compared to the control. This reduction may result from physiological disruptions such as inhibited cell division and elongation, ionic imbalance, reduced enzymatic activity, and damage to cellular structures [44]. Previous studies have also reported the adverse effects of Cd on seed germination and early plant development. For instance, several investigations have shown that Cd accumulation in plant tissues can induce the generation of reactive oxygen species (ROS), triggering oxidative stress and subsequently inhibiting seedling organ growth [47].

The results of this study revealed that Cd stress significantly increased the levels of proline, MDA, and CAT activity in both *Physalis ixocarpa* and *Physalis peruviana*. These changes reflect the activation of plant defense mechanisms in response to oxidative stress induced by heavy metal accumulation. Elevated MDA concentration is considered a key indicator of lipid peroxidation and membrane damage. The increase in MDA observed here suggests oxidative damage caused by Cd stress, likely due to excessive generation of ROS [44]. This finding supports the notion that Cd adversely affects membrane integrity and induces oxidative stress. On the other hand, the accumulation of proline, a well-known Osmo protectant amino acid, represents a recognized adaptive response to abiotic stress. Proline contributes to osmotic adjustment, stabilization of proteins and membranes, and scavenging of ROS, thereby helping protect plant cells [48, 67]. The increased proline levels observed are consistent with previous studies that reported enhanced proline accumulation in plants under Cd stress [44]. Moreover, plants attempted to mitigate ROS-induced damage by upregulating antioxidant enzymes such as CAT. As a key antioxidant enzyme, CAT decomposes hydrogen peroxide (H_2O_2) into water and oxygen, and its increased activity under Cd exposure is regarded as a protective response to oxidative stress [44, 48].

Seed priming, particularly with SRE, SPE, and SA, improved germination rate and seedling growth compared to non-primed controls. These findings highlight the beneficial role of priming in enhancing Cd tolerance during early developmental stages. Priming with these bioactive agents may exert protective effects by physiologically conditioning the seeds, enhancing antioxidant defense Syseedling and maintaining oxidative balance [49, 50]. Improved seedling growth in primed treatments, accompanied by reduced levels of proline, CAT activity, and MDA content, suggests a lower level of oxidative stress in these treatments. Since proline, MDA, and CAT are commonly used as indicators of oxidative stress responses [51, 52], their decline may indicate a more stable and healthier physiological status in the primed seedlings.

A negative correlation between these oxidative markers and both seedling growth and APX activity suggests that enhanced growth was associated with reduced need for intense antioxidant defense activation. In essence, the improved physiological condition of primed seeds reduced the necessity for stress-responsive pathways, leading to a decline in stress biomarkers [53, 54]. Rather than reacting to Cd stress post-exposure, priming likely prevented stress perception, allowing seedlings to maintain near-normal physiological functioning. Among the tested priming agents, Ss and saffron petal extracts appeared particularly effective, likely due to their natural content of phenolic compounds, flavonoids, and antioxidants. These effects are in line with previous studies on the protective roles of plant-based extracts [55; 56]. In addition, SA a known plant growth regulator and signaling molecule played a vital role in priming-induced stress tolerance by enhancing plant readiness and reducing Cd-induced damage [54].

The findings of this study demonstrated that seed priming with SRE extract had significant positive effects on improving growth and physiological characteristics of seedlings in both *Physalis ixocarpa* and *Physalis peruviana* under Cd stress and non-stress conditions. It appears that SRE extract, through its bioactive constituents such as alkaloids, flavonoids, and polysaccharides, stimulated metabolic activities and enhanced the antioxidant capacity of the plants, ultimately promoting higher germination rate, seedling vigor, and seedling weight [57, 58]. The observed increase in APX activity in response to Ss priming indicates an enhanced antioxidant defense shoot against Cd-induced oxidative stress. This response was accompanied by a reduction in MDA accumulation and proline content, both of which are considered indicators of reduced stress intensity in primed seedlings [49, 59]. Furthermore, elevated levels of photosynthetic pigments such as chlorophylls in the Ss treatment may contribute to maintaining chloroplast structure and improving photosynthetic efficiency under unfavorable conditions [44]. These findings align with previous reports highlighting the role of plant-based bio stimulants in enhancing plant responses to environmental stress [60, 61].

Similarly, the beneficial effects of SPE priming on improving germination parameters, seedling growth, and chlorophyll a content in both *Physalis* species were confirmed in this study. The bioactive components of saffron petals, including flavonoids, crocin, picrocrocin, and saffranal, appear to modulate physiological processes and strengthen the plant's antioxidant defense. These compounds mitigate oxidative stress, support membrane integrity, and stabilize photosynthetic pigments, thereby contributing to improved seedling growth and early performance [62, 63]. Under Cd stress, substantial reductions in MDA, proline, and CAT activity in saffron-primed treatments compared to the control indicate lowered oxidative stress levels. This is consistent with previous studies emphasizing the role of natural antioxidants in minimizing lipid peroxidation and regulating osmolytes such as proline [64, 65] The reduced levels of these stress markers in primed treatments suggest more efficient antioxidant responses and decreased cellular damage. The observed increase in chlorophyll a may reflect protection of chloroplast structures and stabilization of chlorophyll biosynthetic enzymes under stress, ultimately enhancing photosynthesis and dry matter accumulation [66, 44]. Therefore, it can be concluded that SPE acts as an effective bio stimulant, not only improving plant performance under optimal conditions but also enhancing tolerance to abiotic stressors such as Cd toxicity.

The findings of this study revealed that seed priming with WV did not enhance seedling growth traits in *Ph. ixocarpa* and *Ph. peruviana*. In fact, it exerted significant negative effects on most of the evaluated parameters. A notable reduction in seedling weight, radicle and plumule weight, along with a marked decline in chlorophyll a content under both stress and non-stress conditions in both species, indicates an inhibitory impact of this substance on physiological processes associated with plant growth. The high content of phenolic compounds, relatively strong acidity, and presence of phytotoxic substances such as phenols and aldehydes in WV may disrupt ionic balance, interfere with pigment synthesis, and impair photosynthetic efficiency [67]. Moreover, the sharp decline in the activity of key antioxidant enzymes such as CAT and APX in response to WV priming suggests that this treatment not only failed to mitigate oxidative stress but may have weakened the plant's defense system. A compromised antioxidant system can render the plant more vulnerable to oxidative damage caused by reactive oxygen species (ROS) [64]. Among the tested priming treatments, WV exhibited the least favorable effects on germination indices and seed vigor. These findings are consistent with previous studies reporting that improper use or high concentrations of organic amendments like WV can have phytotoxic effects, potentially inducing secondary stress instead of enhancing seedling performance [68].

Additionally, the results indicated that *Ph. ixocarpa* exhibited greater tolerance to Cd stress compared to *Ph. peruviana*. This difference may stem from inherent physiological, biochemical, and genetic distinctions between the two species that influence Cd uptake, distribution, and toxicity. Under Cd stress, *Ph. ixocarpa* outperformed *Ph. peruviana* in key growth indicators such as seedling weight, radicle and plumule weight, chlorophyll a content, and stress tolerance index. These observations point to a more stable photosynthetic apparatus and better growth maintenance in *Ph. ixocarpa*. Furthermore, lower levels of stress-related compounds such as MDA and proline in *Ph. ixocarpa* suggest more effective antioxidant responses and greater capacity to limit lipid peroxidation under heavy metal stress. These findings align with previous research indicating that plant species exhibit varying responses to metal stress, including Cd [69; 53;70]. It is likely that *Ph. ixocarpa* benefits from more efficient mechanisms such as limiting Cd translocation to aerial tissues, enhancing the production of chelating agents like phytochelatins or glutathione, and upregulating enzymatic defense pathways, which collectively contribute to its higher stress resilience [71].

Overall, the results showed that *Ph. ixocarpa* was more responsive to seed priming treatments than *Ph. peruviana*. This disparity may reflect genetic and physiological differences that influence the perception and utilization of priming stimuli. In *Ph. ixocarpa*, seed priming, especially with bio stimulant-based treatments such as silk extract, SA, and saffron petal extract, significantly enhanced seedling growth parameters, chlorophyll content, and antioxidant activity. Conversely, *Ph. peruviana* exhibited a more limited response to these treatments, with improvements generally restricted to specific traits such as radicle length or germination rate. This variation may be attributed to differences in hormonal receptor sensitivity, activation of defense signaling pathways, or the capacity to absorb and utilize bioactive compounds present in the priming agents [72, 73].

CONCLUSION

The results demonstrated that exposure of *Physalis* seeds to 500 µM Cd stress had no significant effect on germination percentage in either *Ph. ixocarpa* or *Ph. peruviana*. However, other parameters such as germination rate, seedling growth, and biochemical traits were adversely affected by Cd toxicity. This study revealed that seed priming with certain biotic compounds can serve as an effective strategy to enhance Cd stress tolerance in *Physalis* seedlings. Priming with compounds such as SRA, SPE, and SA significantly improved physiological and biochemical indicators, including increased chlorophyll content, reduced proline and MDA accumulation, and enhanced antioxidant enzyme activities. These improvements reflect better oxidative balance, protection of cellular structures, and maintenance of photosynthetic activity in primed seedlings. Conversely, seed treatment with WV not only lacked beneficial effects but also significantly impaired growth and physiological performance in most evaluated traits. Moreover, comparative analysis between the two species indicated that *Ph. ixocarpa* exhibited greater tolerance to Cd stress and responded more favorably to priming treatments. This species consistently showed superior performance in growth and antioxidant activity, likely due to genetic differences, enhanced capacity to limit Cd uptake, more effective ROS scavenging, and better utilization of biotic compounds. These attributes position *Ph. ixocarpa* as a more suitable candidate for cultivation in heavy metal-contaminated soils. Overall, the findings underscore the potential of targeted seed priming with biotic compounds as a promising approach to improve stress tolerance in medicinal and underutilized crops.

Consent for Publication

Not Applicable.

Data Availability

Not Applicable.

Conflict of Interests

The authors have not declared any conflict of interests.

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