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Abdenour KHELOUFI*^{1,2,3}, Thorayya GOUDJA¹, Lahouaria Mounia MANSOURI^{1,2}, Mohammed DJELILATE⁴

SALINITY EFFECTS ON SEED GERMINATION OF TWO POTENTIAL HORTICULTURAL SPECIES (PHYSALIS PERUVIANA AND ARTEMISIA HERBA-ALBA)

SUMMARY

Salinity stress presents a challenging encounter to seed germination, deeply impacting plant establishment, particularly in regions characterized by arid and semiarid conditions. This study investigates the effect of varying NaCl concentrations on the seed germination of two multipurpose plant species, Artemisia herba-alba and Physalis peruviana. Through controlled experimental protocols, we evaluated critical germination attributes, including final germination percentage (FGP), mean germination time (MGT), time to 50% germination (T_{50}) and germination tolerance index (GTI). After 22 days of saline treatment, our results revealed distinct responses for each species to salinity stress. A. herba-alba demonstrated a moderate sensitivity, with FGP declining from 67.3% at 0 mM NaCl to 10.7% at 200 mM NaCl, whereas P. peruviana exhibited remarkable tolerance, maintaining a consistently high FGP of 100% across all NaCl concentrations except for the concentration of 200 mM (36.7%). Statistical analysis employing two-way ANOVA underscore the significant main effects of salinity, species, and their interactions on seed germination parameters. This study highlights the imperative of understanding species-specific adaptive strategies to mitigate salinity-induced inhibitions on seed germination. These insights advance our comprehension of seed responses to environmental stress and hold implications for the conservation, cultivation, and management of A. herba-alba and P. peruviana in saline-affected ecosystems.

Keywords: Artemisia herba-alba, NaCl, germination attributes, salinity, Physalis peruviana

¹ Abdenour Kheloufi (corresponding author: a.kheloufi@univ-batna2.dz), Thorayya Goudja, Lahouaria Mounia Mansouri, Department of Ecology and Environment, University of Batna 2, Batna 05078, ALGERIA.

²Abdenour Kheloufi, Lahouaria Mounia Mansouri, Laboratory of Biodiversity, Biotechnology and Sustainable Development, University of Batna 2, Batna 05078, ALGERIA.

³Abdenour Kheloufi, Laboratory of Biotechnology for Food and Energy Security, University of Oran 1, Oran 31000, ALGERIA.

⁴Mohammed Djelilate, Department of Biology, University of Relizane, 48000 Relizane, ALGERIA. Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online. *Recieved:12/05/2024 Accepted:15/11/2024*

INTRODUCTION

An elevated concentration of soluble salts in soils has detrimental effects on agricultural lands, crops, and subsequently, the livelihoods of people worldwide. Over 100 nations are dealing with challenges related to soil salinity and the concurrent salinization of groundwater resources. Irrigation of agricultural crops with saline water indeed increases the concentration of soluble salts in soil, thereby reducing the productivity of crop plants (Srivastava et al., 2019). Seed germination is affected by salinity stress, particularly in regions characterized by arid and semiarid conditions (Kigel, 2017; Kheloufi et al., 2018; Christiansen *et al.*, 2022). Saline soils in arid rangelands of Algeria are primarily constituted by the accumulation of diverse chloride and sulfate salts, with NaCl predominating at over 50% (Halitim, 1988; Mansouri and Kheloufi, 2024). When salinity stress and drought interact, seeds are subjected to osmotic stress, reducing water uptake and metabolic processes needed to germinate. Salinity stress is intensified by drought by reducing soil moisture, which increases salt accumulation in root zones (Johal and Goyal, 2023). Consequently, the dual stress of drought and salinity hampers seed germination and negatively impacts crop growth, development, and productivity. Moreover, the accumulation of salts in the soil further reduces its fertility, leading to long-term degradation of agricultural land in arid and semiarid regions (Muhammad et al., 2024).

Artemisia herba-alba Asso. (Asteraceae) (also called white wormwood) and Physalis peruviana L. (Solanaceae) (also called golden berry) possess a range of ecological, medicinal, and economic attributes. A. herba-alba is known for its anti-inflammatory, antimicrobial, and antioxidant properties, making it valuable in traditional medicine practices (Nedjimi and Beladel, 2015). P. peruviana is also valued for its medicinal properties, with various parts of the plant used to treat diseases such as inflammation, asthma, and gastrointestinal disorders (Ezzat and Salama, 2024). P. peruviana produces edible fruits enclosed in a papery husk, which are commonly consumed fresh or used in culinary applications such as jams, desserts, and salads (Cortés et al., 2012). The fruit is rich in vitamins, minerals, and antioxidants, contributing to its nutritional value and culinary versatility. It is cultivated commercially for its fruits, which are traded internationally and have economic value in fresh and processed forms (Obregón La Rosa, 2024). A. herba-alba possesses aromatic properties, with the plant emitting a distinctive fragrance due to its essential oil content. This aromatic quality has led to its use in perfumery, aromatherapy, and the production of essential oils (Fadel et al., 2023). Both species are perennial and play significant roles in their respective ecosystems. A. herba-alba is known to have allelopathic effects, influencing the composition and dynamics of plant communities in its habitat (Arroyo et al., 2016). P. peruviana, on the other hand, serves as a food source for various wildlife species and contributes to ecosystem biodiversity.

Unfortunately, both species are subjected to diverse environmental stresses, including salinity, which significantly affect their growth, development, and yield potential (Nedjimi and Zemmiri, 2019; Aydin, 2024). In previous studies, *A. herba-alba* and *P. peruviana* have demonstrated significant horticultural potential due to their tolerance to salinity and drought, making them suitable for cultivation

in arid regions. These characteristics position both species as valuable options for sustainable agriculture in areas facing water scarcity and soil salinity challenges (Nedjimi and Zemmiri, 2019; Muñoz et al., 2021). On the other hand, there is a scarcity of information regarding the ecophysiological factors influencing germination in these two species. Therefore, understanding the responses of key plant species, such as A. herba-alba and P. peruviana, to salinity stress is crucial for creating strategies to mitigate the adverse effects of salinity on crop production in these challenging environments. Indeed, previous studies have highlighted the detrimental impact of salinity on seed germination, attributing it to alterations in water uptake, osmotic potential, and ion imbalance within seeds (Nikolić et al., 2023; Khan et al., 2023). The sensitivity of seeds to salinity varies across species, with some revealing tolerance mechanisms such as osmotic adjustment, ion exclusion, and antioxidant defense systems (Johnson and Puthur, 2021). However, the comprehensive mechanisms underlying salinity tolerance during seed germination remain incompletely understood, requiring further investigation.

In this study, we aim to elucidate the salinity effects on seed germination of *P. peruviana* and *A. herba-alba*, focusing on key germination responses underlying their differential tolerance to salinity stress under varying salinity levels of sodium chloride. The findings from this study are expected to enhance our understanding of the adaptive mechanisms employed by these two species to cope with salinity stress during seed germination. Furthermore, the insights gained could have implications for the conservation, cultivation, and management of these species in salt-affected environments, contributing to sustainable agricultural practices and ecosystem resilience in the face of global environmental changes.

MATERIAL AND METHODS

Seed harvest and origin

Table 1 presents the provenances of the seeds used in this study for *Physalis peruviana* and *Artemisia herba-alba*. The table also presents seed biometric parameters for each species, including the 1000-seed weight, as well as seed length and width. The measurements were taken based on a sample of 100 seeds per species. Both seed species were collected on November 2023 from several individuals growing in apple orchard (Figure 1). For *P. peruviana*, the ripe fruits were selected, opened, and the seeds were extracted manually before being left to dry naturally for two weeks. Seeds of both species were then stored in paper bags at room temperature until their use on February 2024.

Table 1. Seed characteristics and origins of *Physalis peruviana* and *Artemisia herba-alba*.

Parameters	Physalis peruviana	Artemisia herba-alba	
1000-seed weight (g)	0.11	0.21	
Length (cm)	0.20 ± 0.01	0.11 ± 0.01	
Width (cm)	0.15 ± 0.01	0.05 ± 0.01	
Region in Algeria	Thniet El Abed (Batna, Algeria)		
GPS coordinates	35°20' N ; 6°20' E		

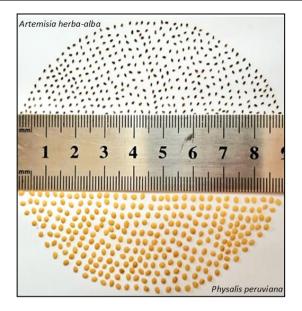


Figure 1. Seeds of Artemisia herba-alba and Physalis peruviana.

Experimental design and application of salt stress

The germination test was performed in plastic Petri dishes (9 cm \emptyset) included one Whatman filter paper moistened of the different saline concentrations (0, 50, 75, 100 and 200 mM) of NaCl (Table 2). For each salinity level, three replicate Petri dishes, each with 50 seeds, were wrapped in aluminum foil (continuous dark) and incubated under 25 °C (±2 °C). Maintaining a specific humidity level for the seeds was a critical aspect of the experiment. The papers were replaced every three days to prevent salt accumulation during the 22 days of the experiment. A complete randomized design was used to conduct the germination test (Kheloufi and Mansouri, 2019).

Concentrations (mM NaCl)	pH at 19°C	Electrical conductivity (EC) (mS.cm ⁻¹) at 23.3°C	
		``````````````````````````````````````	
0 (Control)	9.11	0.03	
50	8.80	5.08	
75	7.81	7.55	
100	8.09	9.52	
200	8.04	18.7	

Table 2. Saline solutions and corresponding pH and electrical conductivity

## **Germination parameters**

*Final germination percentage (FGP):* The FGP designates the seeds that successfully germinated relative to the total number of seeds sown in each Petri dish. This parameter was determined using the formula:

$$FGP (\%) = \frac{\sum ni}{N} \times 100$$

where FGP is the final germination percentage, ni is the number of germinated seeds on the last day of the test, and N is the total number of seeds incubated per test (Côme, 1970).

*Mean Germination Time (MGT):* The MGT indicates the rate at which seeds germinate within a population. A reduced MGT value reflects a faster germination rate, while a higher value signifies a slower rate. MGT was calculated using the following formula:

MGT (days) = 
$$\frac{\sum(ti.ni)}{\sum ni}$$

where MGT is the mean germination time, ti is the number of days since the beginning of the test, ni is the number of germinated seeds recorded at time t(i), and  $\Sigma$ ni is the total number of germinated seeds (Orchard, 1977).

*Time to 50% germination* ( $T_{50}$ ): The T₅₀ was designed to determine the time needed for 50% of the seeds to germinate. It is calculated using the following formula:

T50 (days) = 
$$\frac{\text{ti} + (N/2 - ni)(\text{tj} - \text{ti})}{(nj - ni)}$$

where N final number of seeds emerged, nj and ni are the cumulative numbers of seeds emerged after adjacent counts during tj and ti, when ni < N/2 > Nj (Coolbear et al., 1984).

*Germination Tolerance Index (GTI):* The GTI is a quantitative parameter used to evaluate the capacity of seeds to germinate under varying salinity levels. The calculation follows the formula provided by Khan and Ungar (1997):

$$GTI (\%) = \frac{FGP \text{ under stress condition}}{FGP \text{ under non} - \text{stress condition}} \times 100$$

#### **Statistical analyses**

The effects of different NaCl concentrations on the four variables studied were tested by a one-way and two-way analysis of variance (ANOVA). Differences between treatments following ANOVA were made by means comparison. Multiple comparisons of means were carried out using Tukey's test ( $p \le 0.05$ ). A repeated measures analysis of variance was carried out for the germination kinetics. All statistical analyses were performed using SAS software Version 9.0 (Statistical Analysis System) (2002).

## **RESULTS AND DISCUSSION**

The data presented in Figure 2 displays the overall germination rates for seeds of *Artemisia herba-alba* and *Physalis peruviana* over a period of 22 days as a function of increasing NaCl concentrations (mM). The figure highlights three distinct phases: an initial phase of seed imbibition resulting in a latency period,

followed by an exponential phase of rapid germination, and, finally, a plateau phase indicating a cessation in germination (stationary phase). Notably, the two species were able to germinate at all NaCl concentrations during the 22-day experimental period.

For *A. herba-alba*, the control group shows 17.3% germination by the  $6^{th}$  day with a stationary phase starting on the  $21^{st}$  day. Seeds treated with 50, 75, and 100 mM have a low initial germination rate at the 6th day, which improves starting of the 14th day with a stationary phase not exceeding 40% of germination (Figure 2).

For *P. peruviana*, the stationary phase begins on the 6th day in the control and 50 mM NaCl group with 100% germination. As salinity increases, the stationary phase starts at around the  $10^{\text{th}}$  day for 75 mM NaCl and 100 mM NaCl and the germination rate decreases with increasing NaCl concentration. As the NaCl concentration increases by 200 mM, the exponential phase begins with a lower germination rate, reaching 6% germination at the 13th day and reaching the maximum at the 21st day with 36.7% germination.

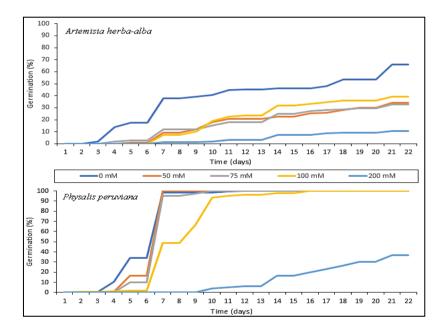


Figure 2. Cumulative germination percentages of *Artemisia herba-alba* and *Physalis peruviana* seeds treated with NaCl for a 22-day period.

According to Figure 2, the germination rates of all the species examined decrease with increasing salinity stress, and NaCl has a significant effect on this reduction especially at 200 mM (p<0.001). In addition, it is evident that the length of the latency period varies among species and increases as the concentration of NaCl increases. A repeated measures analysis of variance (performed over a 22-day period with daily evaluations) indicates that there is a

highly significant effect (p<0.001) between various factors and variables, such as salinity concentration, species, and time, with both between-subject and within-subject effects and their correlation.

The delay in germination and inhibition of growth induced by salinity is caused by various factors such as reduced external water potential, ion imbalance, and specific ion toxicity (Soni *et al.*, 2023). In such conditions, there is a reduction in water uptake alongside an over-absorption of ions. The salinity tolerance of seeds includes both their duration in the soil, during which they may encounter elevated salinity levels and extreme temperatures, as well as their germination phase (Haider *et al.*, 2023).

Species	Salinity	FGP	MGT	T ₅₀	GTI
Species	(NaCl)	(%)	(days)	(days)	(%)
Artemisia herba-alba	0 mM	67.3ª	3.81 ^a	6.01 ^{ab}	100 ^a
	50 mM	39.3 ^b	4.14 ^a	5.73 ^{ab}	58.5 ^b
	75 mM	32.7 ^b	3.89 ^a	5.99 ^{ab}	48.5 ^b
	100 mM	34.1 ^b	4.42 ^a	8.21 ^a	50.9 ^b
	200 mM	10.7°	1.26 ^a	1.91 ^b	16.2°
	F-value	24.76	1.92	4.71	22.73
	<i>p</i> -value	< 0.001	0.183	0.021	< 0.001
Physalis peruviana	0 mM	100 ^a	5.61 ^a	5.19 ^a	100 ^a
	50 mM	100 ^a	5.82ª	5.39 ^a	100 ^a
	75 mM	100 ^a	5.97ª	5.47 ^a	100 ^a
	100 mM	100 ^a	6.51ª	6.12 ^a	100 ^a
	200 mM	36.7 ^b	1.45 ^b	2.00 ^a	36.7 ^b
	F-value	59.77	32.34	3.26	59.77
	<i>p</i> -value	< 0.001	< 0.001	< 0.001	< 0.001

Table 3. Effect of NaCl concentrations on germination traits of *Artemisia herbaalba* and *Physalis peruviana* 

FGP-final germination percentage; MGT-mean germination time; T₅₀-time to 50% germination; GTI-germination tolerance index.

Table 3 summarizes the effects of varying NaCl concentrations on the final germination percentage (FGP), mean germination time (MGT), time to 50% germination ( $T_{50}$ ), and germination tolerance index (GTI) for *A. herba-alba* and *P. peruviana*, along with the results of a one-way ANOVA for each species. In addition to the results presented, the statistical analysis conducted through a two-way ANOVA provides further insights into the effects of salinity, species, and their interactions on seed germination parameters (Table 4).

*A. herba-alba* exhibited varying responses to increasing NaCl concentrations. At lower concentrations (0 mM and 50 mM), the FGP was relatively high, with values of 67.3% and 39.3% respectively. However, as salinity levels increased to 75 mM and 100 mM, the FGP declined substantially to 32.7% and 34.1% respectively. Notably, the highest NaCl concentration (200 mM) resulted in a significant reduction in FGP to 10.7%, indicating a pronounced

inhibitory effect on germination (Table 3). These findings suggest that *A. herbaalba* is moderately sensitive to salinity stress during seed germination, with higher concentrations exerting greater inhibitory effects. Similar findings were reported by Nedjimi and Zemmiri (2019), demonstrating a significant decrease in final germination percentage (FGP) with increasing salinity levels. The highest FGP of 80% was observed in the distilled water control group. Salinity can influence germination by promoting the uptake of toxic ions, which in turn can lead to alterations in certain enzymatic or hormonal activities within the seed (Martínez-Ballesta *et al.*, 2020). Salinity has been reported to cause substantial reductions in both the rate and final percentage of germination and emergence across various vegetable crops. Consequently, this may lead to uneven stand establishment and reduced crop yields (Gul *et al.*, 2022).

In terms of germination timing, *A. herba-alba* seeds exposed to different NaCl concentrations exhibited comparable MGT values, ranging between 3.81 to 4.42 days. However, the time to 50% germination (T50) showed slight variations across treatments, with seeds exposed to 200 mM NaCl requiring significantly less time (1.91 days) compared to other concentrations. This accelerated germination rate at higher salinity levels may be attributed to osmotic adjustment mechanisms triggered by salt stress, aiming to mitigate the adverse effects on seedling establishment.

The germination tolerance index (GTI) provides a comprehensive measure of seedling performance under salinity stress, considering both germination percentage and germination timing. *A. herba-alba* seeds exhibited the highest GTI (58.5%) when exposed to 50 mM NaCl, indicating relatively better tolerance to moderate salinity levels. However, as salinity increased, the GTI declined progressively, reaching the lowest value of 16.2% at 200 mM NaCl (Table 3). This decline in GTI underscores the detrimental impact of high salinity on seedling vigor and overall germination performance in *A. herba-alba*.

In contrast, *P. peruviana* demonstrated remarkable tolerance to salinity stress during seed germination. *P. peruviana* seeds consistently achieved a high FGP of 100%, indicating minimal inhibitory effects on germination even at elevated salinity levels. This high germination percentage suggests intrinsic physiological adaptations that enable *P. peruviana* seeds to tolerate salt stress during germination, thus ensuring successful establishment under adverse environmental conditions. Furthermore, both MGT and  $T_{50}$  values remained relatively consistent across different NaCl concentrations for *P. peruviana*, indicating that salinity did not significantly influence the timing of germination. This consistent germination timing suggests efficient physiological processes involved in seed imbibition and embryo development, unaffected by salt stress (Dey and Bhattacharjee, 2023).

The GTI values for *P. peruviana* remained consistently high across all salinity treatments, maintaining optimal seedling performance irrespective of NaCl concentration. This remarkable germination tolerance underscores the species' resilience to salinity stress during the critical germination stage,

highlighting its potential for cultivation in salt-affected soils. Several authors have described a decrease in germination attributed to elevated salinity levels (Alkharabsheh *et al.*, 2021). The present study revealed significant differences in all observations concerning salinity. These findings are consistent with earlier observations made for several cultivars of golden berry (Miranda *et al.*, 2010; Yildirim *et al.*, 2011; Cebeci and Hanci, 2015).

For FGP, both salinity (F=67.95, p<0.001) and species (F=424.97, p<0.001) showed significant main effects, indicating their individual contributions to variations in germination percentage. Additionally, the interaction between salinity and species (S×SP) was also significant (F=12.86, p<0.001), suggesting that the effects of salinity on FGP varied between *A. herba-alba* and *P. peruviana*.

Table 4. Two-way ANOVA of salinity and species effects on germination traits of *Artemisia herba-alba* and *Physalis peruviana*.

Variables	Factors	df	Mean square	<b>F-value</b>	<i>p</i> -value
	S	4	3062.13	67.95	< 0.001
FGP	SP	1	19152.13	424.97	< 0.001
	$S \times SP$	4	579.46	12.86	< 0.001
	S	4	16.56	11.29	< 0.001
MGT	SP	1	18.39	12.53	< 0.001
	$S \times SP$	4	0.93	0.64	ns
	S	4	22.51	7.84	< 0.001
T ₅₀	SP	1	4.05	1.41	ns
	$S \times SP$	4	1.02	0.36	ns
GTI	S	4	4391.15	55.16	< 0.001
	SP	1	7933.82	99.66	< 0.001
	$S \times SP$	4	719.41	9.04	< 0.001

FGP-final germination percentage; MGT-mean germination time;  $T_{50}$ -time to 50% germination; GTI-germination tolerance index; S-salinity; SP-species; df-degree of freedom; ns-non significant at p<0.05.

Similarly, MGT exhibited significant main effects of both salinity (F=11.29, p<0.001) and species (F=12.53, p<0.001), indicating their influence on the timing of germination. However, the interaction between salinity and species was not significant (p>0.05), suggesting that the effect of salinity on MGT did not differ significantly between the two species. For  $T_{50}$ , salinity demonstrated a significant main effect (F=7.84, p<0.001), indicating its impact on the time required for 50% germination. However, the effect of species and the interaction between salinity and species were not significant (p>0.05), suggesting that both *A. herba-alba* and *P. peruviana* responded similarly to salinity in terms of  $T_{50}$ . Regarding GTI, significant main effects of salinity (F=55.16, p<0.001) and species (F=99.66, p<0.001) were observed, indicating their influence on seedling vigor under different salinity levels. Additionally, the interaction between salinity and species (S×SP) was significant (F=9.04, p<0.001), suggesting differential

responses of *A. herba-alba* and *P. peruviana* to salinity stress in terms of GTI (Table 4).

Despite the harmful effects of NaCl, this study shows that seeds of *A*. *herba-alba* and *P. peruviana* can germinate under 200 mM (Table 3). Such a salt concentration is considered to correspond to a significantly high level of salinity. This level of salinity exceeds the salinity tolerance level of the majority of cultivated vegetable species, as well as several halophytes (Bayuelo-*Jiménez et al.*, 2002; Malcolm *et al.*, 2003). As noted by Ungar (1982) and more recently by Suleiman *et al.* (2023), seeds of many perennial species possess the ability to preserve their viability for prolonged periods of exposure to harsh conditions, especially salinity and drought, and then to propagate when the ecological conditions are favorable. The maturation of seeds common to arid climates takes place during the autumn and the seeds begin to germinate within a few days of the first precipitation of the spring season. The seeds are typically found in the surface layers of the soil and propagate when high salt concentrations are leached away by rainfall.

#### CONCLUSIONS

Contrasting responses of A. herba-alba and P. peruviana to salinity stress during seed germination highlight species-specific adaptive mechanisms influencing germination performance under adverse conditions. Our analysis of germination attributes and statistical assessments reveals species-specific reactions to varying NaCl concentrations, offering valuable insights into their adaptive strategies under saline conditions. A. herba-alba showed moderate sensitivity to salinity, with decreasing germination percentages and tolerance indices as NaCl levels increase. In contrast, P. peruviana displayed remarkable resilience, maintaining high germination rates and vigorous tolerance across all salinity treatments. These distinct responses underscore the importance of species-specific adaptations in mitigating salinity stress effects on seed germination. These findings develop our knowledge of seed responses to salinity stress, relevant for conservation, cultivation, and management of these economically and ecologically vital species in saline-affected areas. Further exploration of molecular and physiological mechanisms behind salinity tolerance is essential for developing resilient crop varieties and sustainable agricultural practices in saline environments. Cultivating these species for horticultural purposes supports biodiversity while providing effective strategies to enhance agricultural productivity in challenging environments.

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